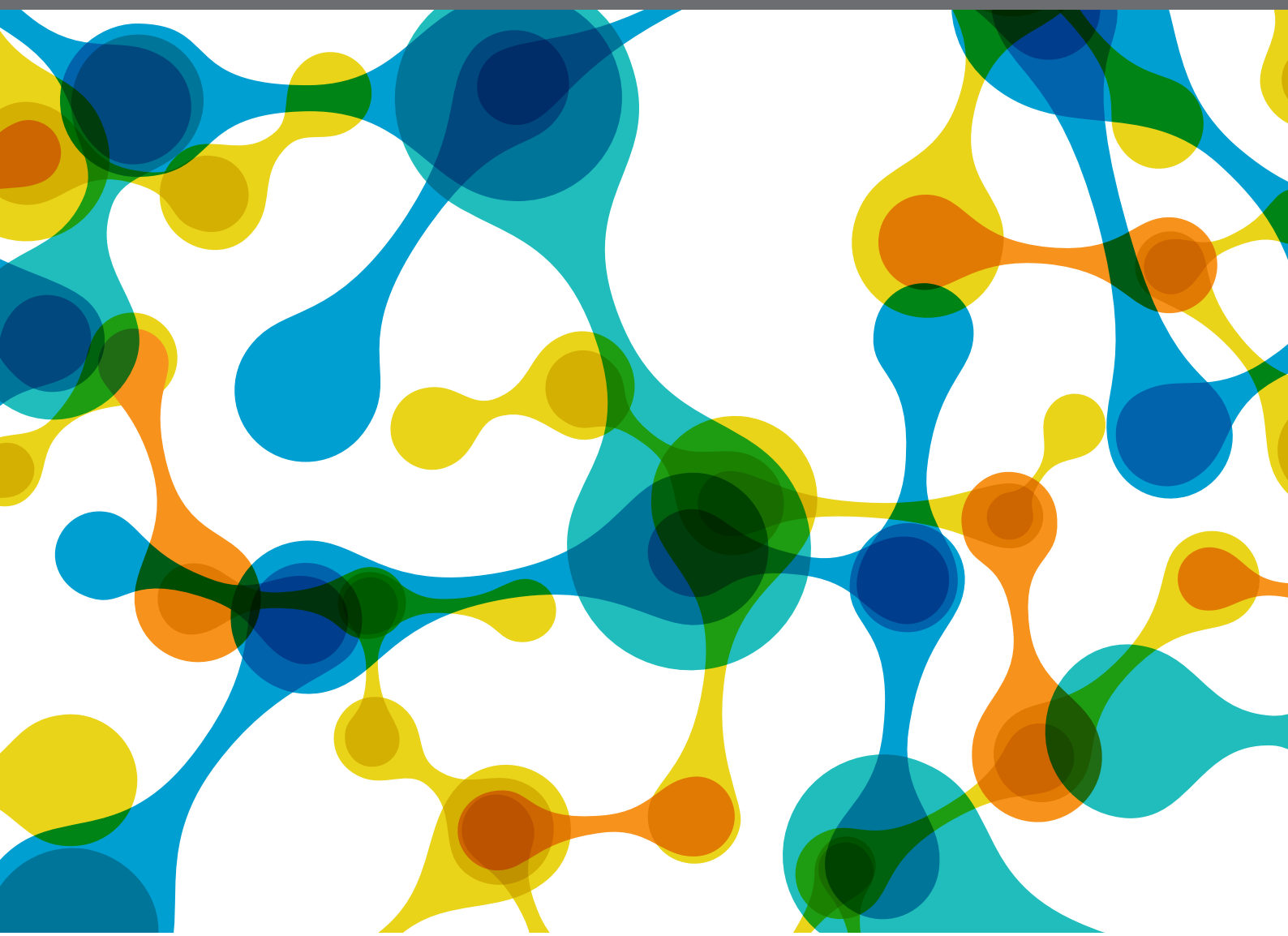
THE TRIBUTE OF PHYSIOLOGY FOR THE UNDERSTANDING OF COVID-19 DISEASE

EDITED BY: Georges Leftheriotis, Denis Noble, Susan Wray, Emmanuelle Vidal-Petiot, Adriana Castello Costa Girardi, Matthew A. Bailey, Deborah Schechtman and Ravi Nistala

PUBLISHED IN: Frontiers in Physiology and Frontiers in Public Health



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88971-654-8
DOI 10.3389/978-2-88971-654-8

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

THE TRIBUTE OF PHYSIOLOGY FOR THE UNDERSTANDING OF COVID-19 DISEASE

Topic Editors:
Georges Leftheriotis, Université Côte d'Azur, France
Denis Noble, University of Oxford, United Kingdom
Susan Wray, University of Liverpool, United Kingdom
Emmanuelle Vidal-Petiot, Assistance Publique Hopitaux De Paris, France
Adriana Castello Costa Girardi, University of São Paulo, Brazil
Matthew A. Bailey, University of Edinburgh, United Kingdom
Deborah Schechtman, University of São Paulo, Brazil
Ravi Nistala, University of Missouri, United States

Citation: Leftheriotis, G., Noble, D., Wray, S., Vidal-Petiot, E., Girardi, A. C. C., Bailey, M. A., Schechtman, D., Nistala, R., eds. (2021). The Tribute of Physiology for the Understanding of COVID-19 Disease. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88971-654-8

Table of Contents

07 ***Editorial: The Tribute of Physiology for the Understanding of COVID-19 Disease***
Georges Lefthériotis, Susan Wray, Adriana Castello Costa Girardi, Emmanuelle Vidal-Petiot, Matthew A. Bailey, Deborah Schechtman, Nistala Ravi and Denis Noble

09 ***ACE2, COVID-19 Infection, Inflammation, and Coagulopathy: Missing Pieces in the Puzzle***
Zaid Abassi, Abd Al Roof Higazi, Safa Kinaneh, Zaher Armaly, Karl Skorecki and Samuel N. Heyman

17 ***Pathophysiology of Cardiovascular Complications in COVID-19***
Vladimir Petrovic, Dina Radenkovic, Goran Radenkovic, Vukica Djordjevic and Maciej Banach

28 ***Heparin Therapy Improving Hypoxia in COVID-19 Patients – A Case Series***
Elnara Marcia Negri, Bruna Mamprim Piloto, Luciana Kato Morinaga, Carlos Viana Poyares Jardim, Shari Anne El-Dash Lamy, Marcelo Alves Ferreira, Elbio Antonio D'Amico and Daniel Deheinzelin

35 ***SARS-CoV-2 Aiming for the Heart: A Multicenter Italian Perspective About Cardiovascular Issues in COVID-19***
Matteo Briguglio, Mauro Porta, Francesca Zuffada, Alberto R. Bona, Tiziano Crespi, Fabio Pino, Paolo Perazzo, Marco Mazzocchi, Riccardo Giorgino, Giuseppe De Angelis, Alfonso Ielasi, Giuseppe De Blasio and Maurizio Turiel

46 ***Biological Context Linking Hypertension and Higher Risk for COVID-19 Severity***
Caio A. M. Tavares, Matthew A. Bailey and Adriana C. C. Girardi

60 ***Covid-19 Mortality: A Matter of Vulnerability Among Nations Facing Limited Margins of Adaptation***
Quentin De Larochelambert, Andy Marc, Juliana Antero, Eric Le Bourg and Jean-François Toussaint

71 ***Study of Clinical and Biological Characteristics of Moroccan Covid-19 Patients With and Without Olfactory and/or Gustatory Dysfunction***
Hasnae Benkirane, Jaafar Heikel, Fatima Zahra Laamiri, Amina Bouziani, Houria Lahmam, Ayoub Al-Jawaldeh, Noureddine El Haloui, Khalid Ennibi, Naim Akhtar-Khan, El Mostafa El Fahime, Majdouline Obtel, Amina Barkat and Hassan Aguenaou

82 ***Pathophysiology of SARS-CoV-2 in Lung of Diabetic Patients***
Tales Lyra Oliveira, Igor Santana Melo, Léia Cardoso-Sousa, Igor Andrade Santos, Mohamad Bassim El Zoghbi, Caroline Gusson Shimoura, Renata Pereira Georjutti, Olagide Wagner Castro, Luiz Ricardo Goulart, Ana Carolina Gomes Jardim, Thúlio Marquez Cunha and Robinson Sabino-Silva

98 ***COVID-19-Associated Hyper-Fibrinolysis: Mechanism and Implementations***
Giris Jacob, Anat Aharon and Benjamin Brenner

108 ***Physiology of Midkine and Its Potential Pathophysiological Role in COVID-19***

Giulia Sanino, Martino Bosco and Giuseppe Terrazzano

116 ***Pathophysiological Processes Underlying the High Prevalence of Deep Vein Thrombosis in Critically Ill COVID-19 Patients***

Sebastian Voicu, Chahinez Ketfi, Alain Stépanian, Benjamin G. Chousterman, Nassim Mohamedi, Virginie Siguret, Alexandre Mebazaa, Bruno Mégarbane and Philippe Bonnin

124 ***Stimulating the Resolution of Inflammation Through Omega-3 Polyunsaturated Fatty Acids in COVID-19: Rationale for the* COVID-Omega-F *Trial***

Hildur Arnardottir, Sven-Christian Pawelzik, Ulf Öhlund Wistbacka, Gonzalo Artiach, Robin Hofmann, Ingalill Reinholdsson, Frieder Braunschweig, Per Tornvall, Dorota Religa and Magnus Bäck

131 ***The Impact of Polyphenols-Based Diet on the Inflammatory Profile in COVID-19 Elderly and Obese Patients***

Juliana Carvalho Santos, Marcelo Lima Ribeiro and Alessandra Gambero

143 ***Serum Renin Levels Increase With Age in Boys Resulting in Higher Renin Levels in Young Men Compared to Young Women, and Soluble Angiotensin-Converting Enzyme 2 Correlates With Renin and Body Mass Index***

Lars Jehpsson, Jiangming Sun, Peter M. Nilsson, Andreas Edsfeldt and Per Swärd

152 ***Hyperventilation: A Possible Explanation for Long-Lasting Exercise Intolerance in Mild COVID-19 Survivors?***

Justina Motiejunaite, Pauline Balagny, Florence Arnoult, Laurence Mangin, Catherine Bancal, Marie-Pia d'Ortho and Justine Frija-Masson

160 ***A Potential Role of the Renin-Angiotensin-System for Disturbances of Respiratory Chemosensitivity in Acute Respiratory Distress Syndrome and Severe Acute Respiratory Syndrome***

Swen Hülsmann, Sepideh Khabbazzadeh, Konrad Meissner and Michael Quintel

168 ***COVID 19-Induced Smell and Taste Impairments: Putative Impact on Physiology***

Nicolas Meunier, Loïc Briand, Agnès Jacquin-Piques, Laurent Brondel and Luc Pénicaud

180 ***Pathogenesis of Multiple Organ Injury in COVID-19 and Potential Therapeutic Strategies***

Miquéias Lopes-Pacheco, Pedro Leme Silva, Fernanda Ferreira Cruz, Denise Battaglini, Chiara Robba, Paolo Pelosi, Marcelo Marcos Morales, Celso Caruso Neves and Patricia Rieken Macedo Rocco

203 ***Pain in Covid Era***

Heloísa Alonso-Matielo, Victória Regina da Silva Oliveira, Victhor Teixeira de Oliveira and Camila Squarzoni Dale

209 ***Determinants of Health and Physical Activity Levels Among Breast Cancer Survivors During the COVID-19 Pandemic: A Cross-Sectional Study***

Aline Rachel Bezerra Gurgel, Pedro Mingroni-Netto, Jose Carlos Farah, Christina May Moran de Brito, Remama Group, Anna S. Levin and Patricia Chakur Brum

216 ***Corrigendum: Determinants of Health and Physical Activity Levels Among Breast Cancer Survivors During the COVID-19 Pandemic: A Cross-Sectional Study***
Aline Rachel Bezerra Gurgel, Pedro Mingroni-Netto, Jose Carlos Farah, Christina May Moran de Brito, Remama Group, Anna S. Levin and Patricia Chakur Brum

219 ***The Physiological Mechanisms of the Sex-Based Difference in Outcomes of COVID19 Infection***
Susan Wray and Sarah Arrowsmith

231 ***Is There an Effect of Fetal Mesenchymal Stem Cells in the Mother–Fetus Dyad in COVID-19 Pregnancies and Vertical Transmission?***
Athina Samara and Eric Herlenius

244 ***SARS-COV-2 and Ocular Surface: From Physiology to Pathology, a Route to Understand Transmission and Disease***
Dalton de Freitas Santoro, Luciene Barbosa de Sousa, Niels O. S. Câmara, Denise de Freitas and Lauro Augusto de Oliveira

253 ***Estimating COVID-19 Pneumonia Extent and Severity From Chest Computed Tomography***
Alysson Roncally Silva Carvalho, Alan Guimarães, Thiego de Souza Oliveira Garcia, Gabriel Madeira Werberich, Victor Fraga Ceotto, Fernando Augusto Bozza, Rosana Souza Rodrigues, Joana Sofia F. Pinto, Willian Rebouças Schmitt, Walter Araujo Zin and Manuela França

265 **In vitro** ***and*** **In silico** ***Models to Study SARS-CoV-2 Infection: Integrating Experimental and Computational Tools to Mimic "COVID-19 Cardiomyocyte"***
Rafael Dariolli, Chiara Campana, Amy Gutierrez and Eric A. Sobie

275 ***The Fundamentals of Respiratory Physiology to Manage the COVID-19 Pandemic: An Overview***
Edem Allado, Mathias Poussel, Simon Valentin, Antoine Kimmoun, Bruno Levy, Duc Trung Nguyen, Cécile Rumeau and Bruno Chenuel

287 ***COVID-19-Associated Neurological Manifestations: An Emerging Electroencephalographic Literature***
Geoffroy Vellieux, Romain Sonneville, Sérafima Vledouts, Pierre Jaquet, Anny Rouvel-Tallec and Marie-Pia d'Ortho

301 ***Controversial Roles of the Renin Angiotensin System and Its Modulators During the COVID-19 Pandemic***
Simon B. Gressens, Georges Leftheriotis, Jean-Claude Dussaule, Martin Flamant, Bernard I. Levy and Emmanuelle Vidal-Petiot

321 ***Correlation of Coagulation Parameters With Clinical Outcomes During the Coronavirus-19 Surge in New York: Observational Cohort***
Morayma Reyes Gil, Jesus D. Gonzalez-Lugo, Shafia Rahman, Mohammad Barouqa, James Szymanski, Kenji Ikemura, Yungtai Lo and Henny H. Billett

336 ***Metabolic Alterations in SARS-CoV-2 Infection and Its Implication in Kidney Dysfunction***
Magaiver Andrade Silva, Ana Ruth Paolinetti Alves da Silva, Mariana Abrantes do Amaral, Matheus Garcia Fragas and Niels Olsen Saraiva Câmara

344 ***Proteinuria as a Biomarker for COVID-19 Severity***
Hajar Ouahmi, Johan Courjon, Lucas Morand, Juliette François, Vincent Bruckert, Romain Lombardi, Vincent Esnault, Barbara Seitz-Polski, Elisa Demonchy, Jean Dellamonica and Sonia Boyer-Suavet

352 ***Olfactory Dysfunction in Frontline Health Care Professionals During COVID-19 Pandemic in Brazil***
Mariana Ferreira Sbrana, Marco Aurélio Fornazieri, Alexandre Bruni-Cardoso, Vivian I. Avelino-Silva, Deborah Schechtman, Richard Louis Voegels, Bettina Malnic, Isaias Glezer and Fabio de Rezende Pinna

363 ***The Good Treatment, the Bad Virus, and the Ugly Inflammation: Pathophysiology of Kidney Involvement During COVID-19***
Marie-Bénédicte Le Stang, Jordan Desenclos, Martin Flamant, Benjamin G. Chousterman and Nahid Tabibzadeh

372 ***Alteration of Diffusion Capacity After SARS-CoV-2 Infection: A Pathophysiological Approach***
Justine Frija-Masson, Catherine Bancal, Laurent Plantier, Hélène Benzaquen, Laurence Mangin, Dominique Penaud, Florence Arnoult, Martin Flamant and Marie-Pia d'Ortho

379 ***The Affinity of Hemoglobin for Oxygen is Not Altered During COVID-19***
Thomas Gille, Lucile Sesé, Eric Aubourg, Emmanuelle E. Fabre, Florence Cymbalista, Kayaththiry Caroline Ratnam, Dominique Valeyre, Hilario Nunes, Jean-Paul Richalet and Carole Planès

388 ***Supplemental Learning in Respiratory Physiology for Healthcare Professionals Towards Successful Treatment of COVID-19***
Helen Wallace and Robert Angus

394 ***How and to What Extent Immunological Responses to SARS-CoV-2 Shape Pulmonary Function in COVID-19 Patients***
Pierantonio Laveneziana, Christian Straus and Silke Meiners

frontiers in Physiology

EDITORIAL
published: 27 September 2021
doi: 10.3389/fphys.2021.761644

Editorial: The Tribute of Physiology for the Understanding of COVID-19 Disease

Georges Lefthériotis[1]*, *Susan Wray*[2], *Adriana Castello Costa Girardi*[3], *Emmanuelle Vidal-Petiot*[4], *Matthew A. Bailey*[5], *Deborah Schechtman*[6], *Nistala Ravi*[7] and *Denis Noble*[8]

[1] Université Côte d'Azur, CNRS, LP2M, Nice, France, [2] University of Liverpool, Liverpool, United Kingdom, [3] Laboratory of Genetics and Molecular Cardiology, Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil, [4] Assistance Publique Hopitaux De Paris, Paris, France, [5] Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom, [6] University of São Paulo, São Paulo, Brazil, [7] University of Missouri, Columbia, KY, United States, [8] University of Oxford, Oxford, United Kingdom

Keywords: editorial, physiology, COVID-19, pandemia, collection

Editorial on the Research Topic

The Tribute of Physiology for the Understanding of COVID-19 Disease

While the specter of the COVID-19 pandemic appears to be gradually receding, the lessons learned from the pandemic are still relevant today. During the year 2021, a collective of physiologists belonging to different national (French, UK, Brazil,...) and European societies (federation of the European Society of Physiology—FEPS), joined forces to make their contribution to the scientific knowledge accumulated during this pandemic.

More than 50 articles have been submitted for publication, and viewed more than 450,000 times, demonstrating the interest of the physiological community in the subject. The articles published in this collection have provided new information or reflections in all areas of physiology, from immunity to respiratory, cardiovascular function, including hemostasis, neurophysiology or even certain related aspects to epidemiology.

The objective of the collection was to provide a better understanding of the interaction between COVID-19 and physiological functions at different stages of organization, from genes to the whole living organism in different disciplinary fields, including cardiovascular, renal, gastrointestinal, endocrine, respiratory and pulmonary, immune and neuronal systems and their physiological functions.

It is important to note that the roles of environmental factors, including age, gender, smoking, metabolic imbalances (e.g., diabetes), as well as immuno-allergic status were taken into account in the selection of articles. The symptoms of the COVID-19 infection have uniquely revealed a richness and diversity focused on a limited time and affecting different populations of cultures and ethnicities. Symptomatology has illustrated interactions and relationships between different physiological functions (e.g., anosmia and neurological symptoms).

The entire invited editorial team is also engaged in the evaluation of interdisciplinary opinions and points of view, as well as data from original work of the different physiological systems targeted by SARS-Cov-2.

The virus is still there with its share of unknowns and uncertainties, and the scientific community too, in an attempt to answer questions from the medical community and human populations in general. The anti-COVID vaccination and its procession of sometimes unusual manifestations is in itself another

OPEN ACCESS

Edited and reviewed by:
Geoffrey A. Head, Baker Heart and Diabetes Institute, Australia

***Correspondence:**
Georges Lefthériotis
leftheriotis@unice.fr

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *20 August 2021*
Accepted: *30 August 2021*
Published: *27 September 2021*

Citation:
Lefthériotis G, Wray S, Girardi ACC, Vidal-Petiot E, Bailey MA, Schechtman D, Ravi N and Noble D (2021) Editorial: The Tribute of Physiology for the Understanding of COVID-19 Disease. Front. Physiol. 12:761644. doi: 10.3389/fphys.2021.761644

chapter that opens in the history of this pandemic. There is still significant scientific work to be done in these areas in order to improve our understanding of the mechanisms of this pandemic and to better care for patients and protect us from the next waves.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the editorial board of the collection.

ACKNOWLEDGMENTS

The invited editorial committee would like to thank all the contributors to this collection, authors and reviewers for their availability and their investment in the editorial process.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

PERSPECTIVE
published: 06 October 2020
doi: 10.3389/fphys.2020.574753

ACE2, COVID-19 Infection, Inflammation, and Coagulopathy: Missing Pieces in the Puzzle

Zaid Abassi[1,2], Abd Al Roof Higazi[3], Safa Kinaneh[1], Zaher Armaly[4], Karl Skorecki[5] and Samuel N. Heyman[6]*

[1]Department of Physiology and Biophysics, Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel, [2]Laboratory Medicine, Rambam Medical Center, Haifa, Israel, [3]Department of Clinical Biochemistry, Hadassah Medical Center, Hadassah Hebrew University Hospital, Mt. Scopus, Jerusalem, Israel, [4]Department of Nephrology, Nazareth Hospital, EMMS, Nazareth and Azrieli Faculty of Medicine in Safed, Safed, Israel, [5]The Bar-Ilan University Azrieli Faculty of Medicine in Safed, Safed, Israel, [6]Department of Medicine, Hadassah Hebrew University Hospital, Mt. Scopus, Jerusalem, Israel

Engulfed by the grave consequences of the coronavirus disease 2019 (COVID-19) pandemic, a better understanding of the unique pattern of viral invasion and virulence is of utmost importance. Angiotensin (Ang)-converting enzyme (ACE) 2 is a key component in COVID-19 infection. Expressed on cell membranes in target pulmonary and intestinal host cells, ACE2 serves as an anchor for initial viral homing, binding to COVID-19 spike-protein domains to enable viral entry into cells and subsequent replication. Viral attachment is facilitated by a multiplicity of membranal and circulating proteases that further uncover attachment loci. Inherent or acquired enhancement of membrane ACE2 expression, likely leads to a higher degree of infection and may explain the predisposition to severe disease among males, diabetics, or patients with respiratory or cardiac diseases. Additionally, once attached, viral intracellular translocation and replication leads to depletion of membranal ACE2 through degradation and shedding. ACE2 generates Ang 1-7, which serves a critical role in counterbalancing the vasoconstrictive, pro-inflammatory, and pro-coagulant effects of ACE-induced Ang II. Therefore, Ang 1-7 may decline in tissues infected by COVID-19, leading to unopposed deleterious outcomes of Ang II. This likely leads to microcirculatory derangement with endothelial damage, profound inflammation, and coagulopathy that characterize the more severe clinical manifestations of COVID-19 infection. Our understanding of COVID-ACE2 associations is incomplete, and some conceptual formulations are currently speculative, leading to controversies over issues such as the usage of ACE inhibitors or Ang-receptor blockers (ARBs). This highlights the importance of focusing on ACE2 physiology in the evaluation and management of COVID-19 disease.

Keywords: COVID-19 pandemic, angiotensin converting enzyme 2, SARS-CoV-2, RAS inhibition, inflammation, coagulopathy

OPEN ACCESS

Edited by:
Georges Leftheriotis, Université Côte d'Azur, France

Reviewed by:
Nirmal Parajuli, Henry Ford Health System, United States
Huijing Xia, Louisiana Stete University Health Sciences Center, United States
Marta Riera, Mar Institute of Medical Research (IMIM), Spain

****Correspondence:***
Zaid Abassi
abassi@tx.technion.ac.il

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *21 June 2020*
Accepted: *09 September 2020*
Published: *06 October 2020*

Citation:
Abassi Z, Higazi AAR, Kinaneh S, Armaly Z, Skorecki K and Heyman SN (2020) ACE2, COVID-19 Infection, Inflammation, and Coagulopathy: Missing Pieces in the Puzzle. Front. Physiol. 11:574753. doi: 10.3389/fphys.2020.574753

BACKGROUND

Coronavirus disease 2019 (COVID-19), caused by the highly contagious coronavirus 2 (SARS-CoV-2), is initiated by invasion into host cells through viral attachment to angiotensin (Ang)-converting enzyme (ACE) 2. ACE2, expressed in numerous different tissues, serves as an anchor for specific domains on the viral spikes (Hamming et al., 2004; Hoffmann et al., 2020; Zou et al., 2020).

Additionally, ACE2, through the modulation of the renin-Ang-aldosterone system (RAS), plays an important physiologic role in the homeostasis of tissue microcirculation and inflammation (Crackower et al., 2002; Hamming et al., 2007; Santos et al., 2008; Clarke and Turner, 2012; Datta et al., 2020). This minireview will address the role of ACE2 within the RAS, and the inter-association of ACE2 and SARS-CoV-2, with their plausible combined impact on the clinical manifestations of COVID-19 disease. We shall further address knowledge gaps that require elucidation in order to better understand the pathophysiology and clinical features of COVID-19 in order to develop effective means for disease prevention and management.

ACE2: AN IMPORTANT COMPONENT OF RAS

Figure 1A illustrates our current understanding of the complexity of the RAS. Until recently, most clinicians were familiar with only one axis, namely renin-mediated proteolysis and conversion of angiotensinogen to the 10-amino-acid peptide Ang I, followed by a further cleavage by ACE, principally present in the lungs to form the bioactive 8-amino-acid compound Ang II (Crackower et al., 2002; Hamming et al., 2007; Santos et al., 2008; Clarke and Turner, 2012). The COVID-19 pandemic shifted our attention to another component of RAS, namely ACE2, which plays a role in SARS-CoV-2 virulence. Ang II could be further cleaved by ACE2 to form the bioactive 7-amino-acid peptide Ang 1-7. In addition, ACE2 converts Ang I into Ang (1-9), which can be further converted to Ang 1-7 by ACE. A third pathway of Ang 1-7 generation involves neprilysin (neural endopeptidase-NEP), which converts Ang I directly into Ang 1-7 (Tipnis et al., 2000; Crackower et al., 2002; Vickers et al., 2002; Hamming et al., 2007; Santos et al., 2008; Clarke and Turner, 2012). An alternative degradation pathway with conversion of Ang I to Ang II takes place by the proteolytic enzyme, chymase, explaining ongoing generation of Ang II in patients on ACE inhibitors (Miyazaki and Takai, 2006).

Importantly, Ang derivatives differ by their downstream physiologic properties and are mediated by diverse signal transduction mechanisms (**Figure 1A**). Ang II acts principally as a potent vasoconstrictor, pro-inflammatory, pro-fibrotic, and anti-diuretic agent. These actions are mediated by Ang II binding to Ang T_1 receptors (AT1R) on affected cell membranes. Opposing activities may be initiated *via* attachment of Ang II to Ang T_2 receptors (AT2R; Li et al., 2017). Indeed, Ang II-mediated vasoconstriction or vasodilation at the renal cortex and medulla, respectively, reflects diverse receptor distribution and activity, predominantly AT1R in the cortex and AT2R in the medulla (Duke et al., 2003). As also shown in **Figure 1A**, unlike Ang II, Ang 1-7 exerts unequivocal vasodilatory, anti-inflammatory, anti-fibrotic, and natriuretic actions by binding to a G-protein-coupled Mas receptor (MasR; Li et al., 2003; Santos et al., 2008).

Thus, a tight physiologic balance exists by the opposing effects of Ang derivatives whenever this system undergoes perturbations, with the aim of preventing extreme vasoactive deviations or uncontrolled inflammation and remodeling, with Ang 1-7 serving to counterbalance the undesired adverse effects of unbridled Ang II action.

ACE2 AND SARS-CoV-2 ASSOCIATION

Angiotensin-converting enzyme is expressed on the plasma membranes of various cell types, including alveolar and intestinal epithelia, vascular endothelial cells in the heart, kidney, and

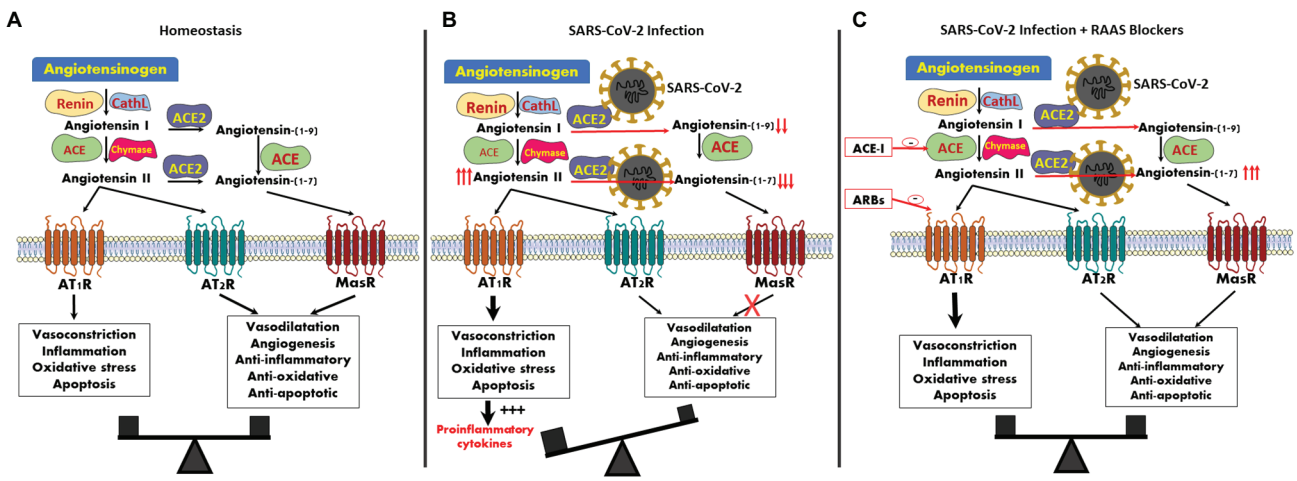


FIGURE 1 | Angiotensin derivatives, their targets and downstream action: **(A)** Balanced impact of angiotensin (Ang) II and Ang 1-7 on vascular tone and control of inflammation. **(B)** SARS-CoV2 infection generates Ang 1-7 depletion, likely leading to unopposed vasoconstriction and inflammation. **(C)** Concomitant renin-Ang-aldosterone system (RAAS) inhibition with Ang-converting enzyme (ACE) inhibitors or Ang-receptor blockers (ARBs) may restore the balance, with parallel suppression of signals mediated by Ang T_1 receptors (AT1R) and Mas receptor (MasR).

testis, and on macrophages, where it catalyzes the production of Ang 1-7 and its likely paracrine activity (Crackower et al., 2002; Hamming et al., 2007; Santos et al., 2008; Clarke and Turner, 2012; Abassi et al., 2020c). Unfortunately, cell-membrane-bound ACE2 also serves as a binding site for the viral spike proteins of SARS-CoV-1 and SARS-CoV-2 (Li et al., 2003; Hamming et al., 2004; Hoffmann et al., 2020; Walls et al., 2020; Wan et al., 2020; Wu et al., 2020; Zou et al., 2020). The viral attachment to ACE2 with subsequent internalization is facilitated by additional modifications and cleavage of the S1/S2 spike proteins by convertases, such as transmembrane protease serine (TMPRSS 2) and related proteases (Furin and Corin; Heald-Sargent and Gallagher, 2012; Coutard et al., 2020; Hoffmann et al., 2020; Shang et al., 2020; Walls et al., 2020), and probably by activated factor X (Xa), which was shown to cleave recombinant and pseudoviral S protein into S1 and S2 subunits (Du et al., 2007), all exposing the fusion sites in the viral spike protein (**Figure 2**).

Two principal sites of SARS-CoV-2 invasion include the gastrointestinal and respiratory tracts, which express abundant ACE2. While intestinal homing is clinically more pronounced in children, manifested by gastrointestinal symptoms, the lungs conceivably serve as the principal port of entry, with viral attachment to type II alveolar cells (AT2), and to alveolar macrophages coated by membranal ACE2 (Abassi et al., 2020c,d). Interestingly, conditions identified as predisposing to severe COVID-19 disease are characterized by enhanced pulmonary expression of ACE2. First, chronic airway disease, smoking, and pollution are associated with expansion of the population of alveolar macrophages expressing ACE2 (Abassi et al., 2020d). Furthermore, ACE2 expression is increased in males (La Vignera et al., 2020; Papadopoulos et al., 2020). Indeed, bioinformatics analyses revealed higher abundance of ACE2-expressing AT2 cells in men than women (Wei et al., 2020), potentially enhancing viral susceptibility among men. In this context, testosterone has been described to induce ACE2 expression, the receptor entry of the SARS-CoV-2 infection, but also exerts protective effect against lung injury (Kuba et al., 2005). Enhanced ACE2 is also found in diabetes (Muniyappa and Gubbi, 2020) and heart failure (Zisman et al., 2003; Goulter et al., 2004; Chen et al., 2020), and possibly with the administration of RAS inhibitors (Li et al., 2017). Diabetes is also associated with increased expression of furin (Fernandez et al., 2018). Thus, while testosterone levels decline with aging among men (Harman et al., 2001; Feldman et al., 2002), the presence of comorbidities like obesity, diabetes mellitus, and cardiovascular diseases, possibly counterbalance the decline in viral homing capacity related to age-dependent testosterone drop (Camacho et al., 2013; Rastrelli et al., 2015). In addition, testosterone enhances AT1R expression in male, whereas estrogen preferentially upregulates AT2R expression in females (Chanana et al., 2020). Finally, hypogonadal males are characterized by low T cell count which may provide unrestrained environment for severe responses to SARS-CoV-2 infection (Papadopoulos et al., 2020). In sum, it is tempting to assume that enhanced expression of ACE2 in target organs and also of other molecules permissive to viral binding to ACE2 facilitate viral invasion and augment viral load (**Figure 3**), although the details of this formulation require validation in further studies.

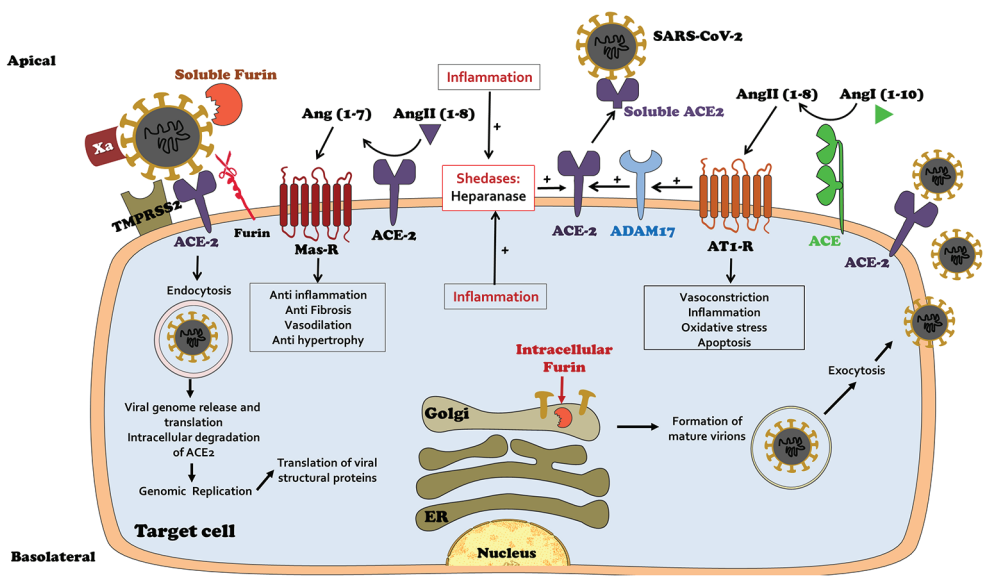


FIGURE 2 | Physiology of coronavirus disease 2019 (COVID 19) homing to target host cells expressing ACE2: viral spike-domains enable attachment to cell-membrane-bound ACE2. Attachment is further enabled by furin, corin, TMPRSS2, and Factor Xa. Following attachment the virus undergoes internalization and replication in host cells, a process associated with degradation of internalized ACE2. Ang 1-7 synthesis consequently declines. Unopposed Ang II action triggers inflammation which activates ADAM 17, leading to shedding of membranal ACE2, further depleting cell-bound ACE2 and local Ang 1-7 synthesis. Viral attachment to target host cells may be attenuated by its competitive binding with rising titers of circulating ACE2.

UNBALANCED RAS IN SARS-CoV 19 DISEASE

As illustrated in **Figures 1, 2**, SARS-CoV-2 invasion unbalances the RAS. Viral cellular internalization is coupled with degradation of membranal ACE2. Furthermore, circulating Ang II, combined with internalized ACE2 activates a sheddase named ADAM metallppeptidase domain 17 (ADAM 17) also called tumor necrosis factor-α-converting enzyme (TACE; Lambert et al., 2005), which in turn triggers shedding of membranal ACE2 into the circulation with the formation of soluble ACE2 (sACE2), further depleting membranal ACE2 along enhanced TNF-α production (**Figure 2**). Thus, viral cellular invasion and replication, initially facilitated by ACE2 and in particular under conditions characterized by enhanced ACE2 expression, later lead to diminution of cell membrane-attached ACE2, and likely increase circulating sACE2 (**Figures 2, 3**). At the microcirculatory and tissue level, this is expected to result in unbalanced paracrine action of Ang compounds, with a local depletion of Ang 1-7 leaving Ang II activity unopposed (**Figure 1B**). Likely, this has a role in microcirculatory dysfunction, intense inflammation, hypercoagulability, tissue damage, and fibrosis (**Figure 3**). Lung inflammation in SARS CoV-19 disease exemplifies the outcome of Ang II/Ang 1-7 imbalance: Ang II enhances vascular permeability along infiltration of neutrophils into alveolae and indirectly *via* induction of interleukin 8 (IL-8; Diamond, 2020). Accumulation of neutrophils and their accompanied prooxidative role lead to loss of alveolar epithelial cells and the development of ARDS. Nevertheless, this Ang II-derived lung injury is prevented by Ang 1-7 as was evident in ACE2 deficient mice (Zou et al., 2014).

Additional adverse aspect of unrestricted Ang II action during SARS-CoV-2 infection is the increased tendency of thrombosis documented in large number of hospitalized COVID-19 patients (Bikdeli et al., 2020; Klok et al., 2020). Although this phenomenon is multifactorial, as outlined below, AT1R activation plays an important role where it leads to enhancement of tissue factor (TF) expression on endothelial cells and sequentially initiation of clotting cascade along increased permeability and neutrophils mobilization (Dielis et al., 2005).

KNOWLEDGE GAPS: THE MISSING PIECES IN THE PUZZLE

Many sections in the preceding paragraphs are based on *in vitro* and animal studies, some with inconsistent and even conflicting interpretations. Furthermore, some fundamental concepts are currently being re-evaluated. For instance, previously reported ACE2 expression on vascular endothelial cells (Hamming et al., 2004) has recently been questioned, based on the measurement of single-cell RNA (Batlle et al., 2020). Human data based on patients infected by SARS-CoV-2 are sparse and are now being intensively studied as we write these lines. It is evident that the foregoing statements should be further examined in the human clinical scenario of COVID-19 disease.

Second, the role of altered ACE2 physiology detailed above in subsequent clinical features of the disease requires in-depth evaluation (Essig et al., 2020). There are several hypothetical mechanisms, outlined in **Figure 3**, that warrant consideration. Possibly, unopposed Ang II due to depletion of cell membrane-bound ACE2 results in altered regional microcirculation and hypoxia, with the generation of reactive oxygen species and endothelial damage, glycocalyx degradation, and disseminated coagulopathy (Abassi et al., 2020a). This may further compromise

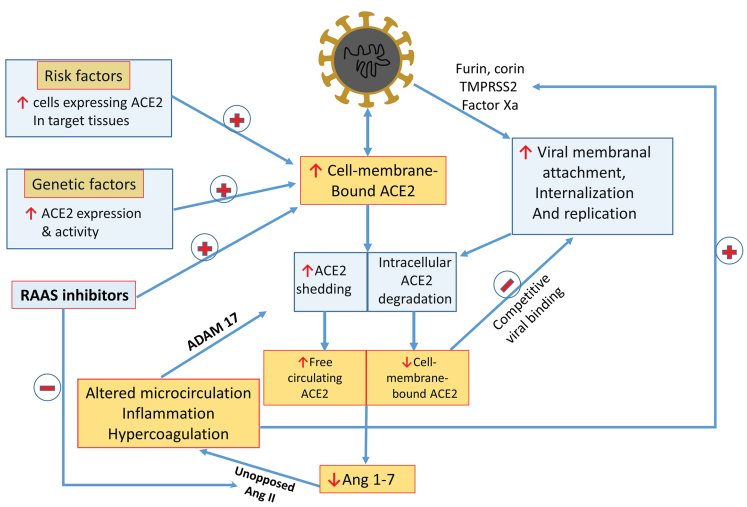


FIGURE 3 | A summarizing scheme of suggested COVID-17/RAS interactions: see text for details. Highlighted are factors enhancing ACE2 expression and viral binding to target host-cells, as are mechanisms leading to declining membranal ACE2 and Ang 1-7 synthesis. The impact of shedded sACE2 on tissue Ang 1-4 production and on inhibiting viral homing to target cells expressing ACE2 by means of competition require further elucidation. RAS inhibitors potentially can enhance viral invasion by enhancing ACE2 expression, yet they may attenuate the unfavorable outcome of Ang 1-7 depletion by a parallel inactivation of functionally opposing Ang II activity. Potential hazardous feed-forward loops are AT1R-mediated enhanced ACE2 shedding and intensification of viral attachment *via* proteases activated by vasoconstriction and ischemia, inflammation, and coagulopathy.

the regional microcirculation with a feed forward loop, leading to organ failure including the heart (Abassi et al., 2020b), lungs (Abassi et al., 2020d), and kidneys (Batlle et al., 2020). Furthermore, intense inflammation and coagulopathy may result from unopposed Ang II and by ADAM 17–mediated activation of TNF-α/IL-6/STAT-3 pathways (Hirano and Murakami, 2020) as well as uncontrolled heparanase activity (Li and Vlodavsky, 2009) together with the induction of defensins (Abu-Fanne et al., 2019). Concerning the latter, preliminary findings from our group indicate that alpha-defensin-1, released from polymorphonuclear cells as a part of the inflammatory response, plays a pivotal role in the hypercoagulopathy associated with COVID-19 disease, as its rising titers parallel increasing plasma levels of D-dimers (Higazi AAR, submitted manuscript). Regarding the interplay between Ang II and ADAM 17/TNF-α/IL-6/STAT-3 pathways, it was found that Ang II activates NF-κB and release of proinflammatory cytokines (Dandona et al., 2007; Benigni et al., 2010). Specifically, induction of ADAM17 by Ang II initiates the conversion of interleukin-6 (IL-6Rα) to the soluble form (sIL-6Rα) along activation of signal transducer and activator of transcription 3 (STAT3) *via* the sIL-6Rα-IL-6 complex in various nonimmune cells including fibroblasts, endothelial cells, and epithelial cells (Hirano and Murakami, 2020). Moreover, STAT3, essential for the NF-κB pathway, is principally stimulated by IL-6 during inflammation (Murakami et al., 2019). Since IL-6 plays a key role in the recruitment of lymphoid cells and myeloid cells, including activated T cells and macrophages (Murakami et al., 2019), and likely enhances defensin release (Higazi AAR, unpublished data), its elevated levels during senescence may contribute to the enhanced COVID-19 mortality in aged people and to coagulopathy. Interestingly, AT1R density is increased, while AT2R abundance declines under inflammatory conditions (Diamond, 2020). Collectively, these results may explain proinflammatory cytokine release and hypercoagulopathy during SARS-CoV-2 infection *via* the associated Ang II pathway and a possible therapeutic target *via* the IL-6-STAT3 axis (Diamond, 2020).

Reduced inherent expression of ACE2 in the lungs with aging, as demonstrated in rats (Xie et al., 2006; Alghatrif et al., 2020) may reduce the risk for SARS-CoV-2 infection on the one hand, whereas its further suppression to very low levels during viral infection, on the other hand, could amplify Ang II/Ang 1-7 imbalance, leading to more profound deleterious pulmonary consequences. Conversely, younger individuals with higher inherent ACE2 expression may have a higher incidence, yet less severe SARS-CoV-2 infection, since ACE2 depletion would not be as severe as in aged patients, with Ang 1-7 generation sufficient to counteract Ang II (Alghatrif et al., 2020). Deranged vascular reactivity will likely be affected by other mediators, such as iNOS- activation and intense nitric oxide production (plausibly with abundant formation of the toxic-free radical peroxynitrite), and by altered endothelial production of endothelin and prostaglandins. Notably, there are additional plausible inherent feed-forward loops in the scheme of SARS-CoV-2 infection and inflammation, including hypoxia-driven perpetuation of endothelial damage and tissue damage. Furthermore, as illustrated in **Figures 2, 3**, Ang II suppresses Ang 1-7 generation secondary to downregulation of membranal ACE2 *via* ADAM 17 activation. Moreover, Factor Xa, generated during disseminated coagulation, is expected to expose attachment sites on viral spikes and enhance viral attachment to target cells expressing ACE2 (Du et al., 2007). Interestingly, *in vitro* studies illustrate that heparin interferes with ACE2 binding to the S1 viral spike protein, reducing viral internalization (Mycroft-West et al., 2020). Thus, enhanced heparanase activity in infected patients might damage endothelial cover by heparin-like proteoglycans and further facilitate viral endothelial invasion.

Third, discussions regarding the potential impact of medications affecting RAS are currently based on inconsistent observations and educated guesses (Essig et al., 2020). We really do not know for sure if blocking steps in the RAS cascade indeed results in enhanced ACE2 expression in humans, and whether this promotes viral attachment and invasion. On the other hand, discontinuation of RAS inhibitors might further intensify the uncontrolled action of Ang II, shown in **Figure 1B**, leaving it unopposed once Ang 1-7 generation is hampered. Those in favor of uninterrupted administration of RAS inhibitors would argue that, as illustrated in **Figure 1C**, depleting Ang II or blocking its action on AT1R [by ACE inhibitors or Ang-receptor blockers (ARBs), respectively] would balance the exhaustion of Ang 1-7 caused by viral invasion and might prevent consequent vasoconstriction (South et al., 2020). Furthermore, it is also likely that the profile of Ang derivatives may differ in patients treated by ARBs, by ACE inhibitors or by spironolactone (Malha et al., 2020). That is why blanket reassurance regarding continuation of RAS inhibitors during the current pandemic (Vaduganathan et al., 2020) should be regarded with caution. A cautious approach might consider the avoidance of ACE inhibitors or ARBs during an active epidemic in non-infected and hemodynamically-stable patients in order to reduce ACE2 expression, permissive to viral attachment, but consideration of ACE inhibitors, or ARBs at advanced stages of COVID-19 disease to prevent Ang II predominance due to depleted Ang 1-7. Most of the clinical trials and data analysis are performed on adults, however potential differences between adults and children may exist, thus coronavirus-related research should be undertaken in children as well, including the impact of ACE-I and ARBs on COVID-19 evolvement among this subpopulation. Hopefully, this may provide clues for the question why children are at decreased risk of severe COVID-19 disease (South et al., 2020). Furthermore, we have no idea about the function or malfunction of circulating sACE2 following its shedding from cell membranes. Does it exert systemic vasodilation or improve the microcirculation? Can it compete with cell-membrane-bound ACE2 (Ciaglia et al., 2020) and reduce viral attachment to target cells as suggested in **Figure 3**? Nor can we tell if diverse inherent expression and activity of circulating or cell-bound ACE2 or its capacity to attach to viral spike proteins affects infection, infectivity, or susceptibility to severe and complicated disease. We also are not sufficiently knowledgeable of plausible changes in ACE2 transcription in various tissues in response to SARS-CoV-2 infection. Indeed, Rice et al. (2006) reported that up to 67% of the phenotypic variation in circulating ACE2 could be accounted for by genetic factors. These findings may

partially explain the different mortality rate among the various ethnic groups, and strongly support studies of genetic analysis of ACE2 polymorphisms as a reliable approach for precision medicine in the prevention, diagnosis, and therapy of COVID-19 disease. Evidence is currently lacking as to whether levels of circulatory sACE2 may have diagnostic and prognostic implication when monitoring patients infected by SARS-CoV-2, as it does in patients with heart failure (Epelman et al., 2008; Ortiz-Perez et al., 2013). With so many pieces of data missing, the need for vigorous clinical studies guided by physiology-based questions and hypotheses are most urgent. Such a question includes the continuation or even introduction, rather than cessation of RAS inhibitors in patients infected by SARS-CoV-2 (Kai and Kai, 2020), or can we inhibit binding of SARS spike proteins to ACE2, for instance by antibodies, without hampering its catalytic capacities to generate Ang 1-7? Is there a role for the application of Ang 1-7 or MasR agonists or for the administration of intravenous sACE2, with an available proof of concept for such postulated approaches (Yang et al., 2014; Hemnes et al., 2018)?

It is likely that many of the above options will be considered and examined in the near future. Meanwhile, we are challenged by epidemiologic aspects, by issues of supportive and critical care for very sick individuals, and by minimizing the risk to healthcare providers. The ultimate solution probably will be effective vaccination. Yet, until we reach this goal, studying and manipulating ACE2-viral association is a plausible approach, along with the development of effective anti-viral agents.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

ZAb, AH, ZAr, KS, and SH equally participated in the design, execution of the view of point, drafted the manuscript, participated in critical discussions, and revised the manuscript. SK, SH, and ZAb prepared the figures. SH supervised the project. All authors contributed to the article and approved the submitted version.

FUNDING

ZAb acknowledges research support from the Israel Science Foundation Grant 544/18.

REFERENCES

Abassi, Z., Armaly, Z., and Heyman, S. N. (2020a). Glycocalyx degradation in ischemia-reperfusion injury. *Am. J. Pathol.* 190, 752–767. doi: 10.1016/j.ajpath.2019.08.019

Abassi, Z., Assady, S., Khoury, E. E., and Heyman, S. N. (2020b). Letter to the editor: angiotensin-converting enzyme 2: an ally or a trojan horse? Implications to SARS-CoV-2-related cardiovascular complications. *Am. J. Physiol. Heart Circ. Physiol.* 318, H1080–H1083. doi: 10.1152/ajpheart.00215.2020

Abassi, Z., Knaney, Y., Karram, T., and Heyman, S. N. (2020c). The lung macrophage in SARS-CoV-2 infection: a friend or a foe? *Front. Immunol.* 11:1312. doi: 10.3389/fimmu.2020.01312

Abassi, Z. A., Skorecki, K., Heyman, S. N., Kinaneh, S., and Armaly, Z. (2020d). Covid-19 infection and mortality: a physiologist's perspective enlightening clinical features and plausible interventional strategies. *Am. J. Phys. Lung Cell. Mol. Phys.* 318, L1020–L1022. doi: 10.1152/ajplung.00097.2020

Abu-Fanne, R., Stepanova, V., Litvinov, R. I., Abdeen, S., Bdeir, K., Higazi, M., et al. (2019). Neutrophil alpha-defensins promote thrombosis in vivo by altering fibrin formation, structure, and stability. *Blood* 133, 481–493. doi: 10.1182/blood-2018-07-861237

Alghatrif, M., Cingolani, O., and Lakatta, E. G. (2020). The dilemma of coronavirus disease 2019, aging, and cardiovascular disease: insights from cardiovascular aging science. *JAMA Cardiol.* 5, 747–748. doi: 10.1001/jamacardio.2020.1329

Batlle, D., Soler, M. J., Sparks, M. A., Hiremath, S., South, A. M., Welling, P. A., et al. (2020). Acute kidney injury in COVID-19: emerging evidence of a distinct pathophysiology. *J. Am. Soc. Nephrol.* 31, 1380–1383. doi: 10.1681/ASN.2020040419

Benigni, A., Cassis, P., and Remuzzi, G. (2010). Angiotensin II revisited: new roles in inflammation, immunology and aging. *EMBO Mol. Med.* 2, 247–257. doi: 10.1002/emmm.201000080

Bikdeli, B., Madhavan, M. V., Jimenez, D., Chuich, T., Dreyfus, I., Driggin, E., et al. (2020). COVID-19 and thrombotic or thromboembolic disease: implications for prevention, antithrombotic therapy, and follow-up: JACC state-of-the-art review. *J. Am. Coll. Cardiol.* 75, 2950–2973. doi: 10.1016/j.jacc.2020.04.031

Camacho, E. M., Huhtaniemi, I. T., O'neill, T. W., Finn, J. D., Pye, S. R., Lee, D. M., et al. (2013). Age-associated changes in hypothalamic-pituitary-testicular function in middle-aged and older men are modified by weight change and lifestyle factors: longitudinal results from the European male ageing study. *Eur. J. Endocrinol.* 168, 445–455. doi: 10.1530/EJE-12-0890

Chanana, N., Palmo, T., Sharma, K., Kumar, R., Graham, B. B., and Pasha, Q. (2020). Sex-derived attributes contributing to SARS-CoV-2 mortality. *Am. J. Physiol. Endocrinol. Metab.* 319, E562–E567. doi: 10.1152/ajpendo.00295.2020

Chen, L., Li, X., Chen, M., Feng, Y., and Xiong, C. (2020). The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. *Cardiovasc. Res.* 116, 1097–1100. doi: 10.1093/cvr/cvaa078

Ciaglia, E., Vecchione, C., and Puca, A. A. (2020). COVID-19 infection and circulating ACE2 levels: protective role in women and children. *Front. Pediatr.* 8:206. doi: 10.3389/fped.2020.00206

Clarke, N. E., and Turner, A. J. (2012). Angiotensin-converting enzyme 2: the first decade. *Int. J. Hypertens.* 2012:307315. doi: 10.1155/2012/307315

Coutard, B., Valle, C., De Lamballerie, X., Canard, B., Seidah, N. G., and Decroly, E. (2020). The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antivir. Res.* 176:104742. doi: 10.1016/j.antiviral.2020.104742

Crackower, M. A., Sarao, R., Oudit, G. Y., Yagil, C., Kozieradzki, I., Scanga, S. E., et al. (2002). Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 417, 822–828. doi: 10.1038/nature00786

Dandona, P., Dhindsa, S., Ghanim, H., and Chaudhuri, A. (2007). Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. *J. Hum. Hypertens.* 21, 20–27. doi: 10.1038/sj.jhh.1002101

Datta, P. K., Liu, F., Fischer, T., Rappaport, J., and Qin, X. (2020). SARS-CoV-2 pandemic and research gaps: understanding SARS-CoV-2 interaction with the ACE2 receptor and implications for therapy. *Theranostics* 10, 7448–7464. doi: 10.7150/thno.48076

Diamond, B. (2020). The renin-angiotensin system: an integrated view of lung disease and coagulopathy in COVID-19 and therapeutic implications. *J. Exp. Med.* 217:e20201000. doi: 10.1084/jem.20201000

Dielis, A. W. J. H., Smid, M., Spronk, H. M. H., Hamulyak, K., Kroon, A. A., Cate, H. T., et al. (2005). The prothrombotic paradox of hypertension. *Hypertension* 46, 1236–1242. doi: 10.1161/01.HYP.0000193538.20705.23

Du, L. Y., Kao, R. Y., Zhou, Y. S., He, Y. X., Zhao, G. Y., Wong, C., et al. (2007). Cleavage of spike protein of SARS coronavirus by protease factor Xa is associated with viral infectivity. *Biochem. Biophys. Res. Commun.* 359, 174–179. doi: 10.1016/j.bbrc.2007.05.092

Duke, L. M., Eppel, G. A., Widdop, R. E., and Evans, R. G. (2003). Disparate roles of AT2 receptors in the renal cortical and medullary circulations of anesthetized rabbits. *Hypertension* 42, 200–205. doi: 10.1161/01.HYP.0000083341.64034.00

Epelman, S., Tang, W. H., Chen, S. Y., Van Lente, F., Francis, G. S., and Sen, S. (2008). Detection of soluble angiotensin-converting enzyme 2 in heart failure: insights into the endogenous counter-regulatory pathway of the renin-angiotensin-aldosterone system. *J. Am. Coll. Cardiol.* 52, 750–754. doi: 10.1016/j.jacc.2008.02.088

Essig, M., Matt, M., and Massy, Z. (2020). The COVID-19 outbreak and the angiotensin-converting enzyme 2: too little or too much? *Nephrol. Dial. Transplant.* 35, 1073–1075. doi: 10.1093/ndt/gfaa113

Feldman, H. A., Longcope, C., Derby, C. A., Johannes, C. B., Araujo, A. B., Coviello, A. D., et al. (2002). Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *J. Clin. Endocrinol. Metab.* 87, 589–598. doi: 10.1210/jcem.87.2.8201

Fernandez, C., Rysa, J., Almgren, P., Nilsson, J., Engstrom, G., Orho-Melander, M., et al. (2018). Plasma levels of the proprotein convertase furin and incidence of diabetes and mortality. *J. Intern. Med.* 284, 377–387. doi: 10.1111/joim.12783

Goulter, A. B., Goddard, M. J., Allen, J. C., and Clark, K. L. (2004). ACE2 gene expression is up-regulated in the human failing heart. *BMC Med.* 2:19. doi: 10.1186/1741-7015-2-19

Hamming, I., Cooper, M. E., Haagmans, B. L., Hooper, N. M., Korstanje, R., Osterhaus, A. D. M. E., et al. (2007). The emerging role of ACE2 in physiology and disease. *J. Pathol.* 212, 1–11. doi: 10.1002/path.2162

Hamming, I., Timens, W., Bulthuis, M. L., Lely, A. T., Navis, G., and van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203, 631–637. doi: 10.1002/path.1570

Harman, S. M., Metter, E. J., Tobin, J. D., Pearson, J., and Blackman, M. R. (2001). Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore longitudinal study of aging. *J. Clin. Endocrinol. Metab.* 86, 724–731. doi: 10.1210/jcem.86.2.7219

Heald-Sargent, T., and Gallagher, T. (2012). Ready, set, fuse! The coronavirus spike protein and acquisition of fusion competence. *Viruses* 4, 557–580. doi: 10.3390/v4040557

Hemnes, A. R., Rathinasabapathy, A., Austin, E. A., Brittain, E. L., Carrier, E. J., Chen, X., et al. (2018). A potential therapeutic role for angiotensin-converting enzyme 2 in human pulmonary arterial hypertension. *Eur. Respir. J.* 51:1702638. doi: 10.1183/13993003.02638-2017

Hirano, T., and Murakami, M. (2020). COVID-19: a new virus, but a familiar receptor and cytokine release syndrome. *Immunity* 52, 731–733. doi: 10.1016/j.immuni.2020.04.003

Hoffmann, M., Kleine-Weber, H., Krüger, N., Müller, M., Drosten, C., and Pöhlmann, S. (2020). The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. bioRxiv [Preprint]. doi: 10.1101/2020.01.31.929042v1

Kai, H., and Kai, M. (2020). Interactions of coronaviruses with ACE2, angiotensin II, and RAS inhibitors-lessons from available evidence and insights into COVID-19. *Hypertens. Res.* 43, 648–654. doi: 10.1038/s41440-020-0455-8

Klok, F. A., Kruip, M. J. H. A., van Der Meer, N. J. M., Arbous, M. S., Gommers, D., Kant, K. M., et al. (2020). Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: an updated analysis. *Thromb. Res.* 191, 148–150. doi: 10.1016/j.thromres.2020.04.041

Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., et al. (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.* 11, 875–879. doi: 10.1038/nm1267

La Vignera, S., Cannarella, R., Condorelli, R. A., Torre, F., Aversa, A., and Calogero, A. E. (2020). Sex-specific SARS-CoV-2 mortality: among hormone-modulated ACE2 expression, risk of venous thromboembolism and hypovitaminosis D. *Int. J. Mol. Sci.* 21:2948. doi: 10.3390/ijms21082948

Lambert, D. W., Yarski, M., Warner, F. J., Thornhill, P., Parkin, E. T., Smith, A. I., et al. (2005). Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *J. Biol. Chem.* 280, 30113–30119. doi: 10.1074/jbc.M505111200

Li, W. H., Moore, M. J., Vasilieva, N., Sui, J. H., Wong, S. K., Berne, M. A., et al. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 426, 450–454. doi: 10.1038/nature02145

Li, J. P., and Vlodavsky, I. (2009). Heparin, heparan sulfate and heparanase in inflammatory reactions. *Thromb. Haemost.* 102, 823–828. doi: 10.1160/TH09-02-0091

Li, X. C., Zhang, J., and Zhuo, J. L. (2017). The vasoprotective axes of the renin-angiotensin system: physiological relevance and therapeutic implications in cardiovascular, hypertensive and kidney diseases. *Pharmacol. Res.* 125, 21–38. doi: 10.1016/j.phrs.2017.06.005

Malha, L., Mueller, F. B., Pecker, M. S., Mann, S. J., August, P., and Feig, P. U. (2020). COVID-19 and the renin-angiotensin system. *Kidney Int. Rep.* 5, 563–565. doi: 10.1016/j.ekir.2020.03.024

Miyazaki, M., and Takai, S. (2006). Tissue angiotensin II generating system by angiotensin-converting enzyme and chymase. *J. Pharmacol. Sci.* 100, 391–397. doi: 10.1254/jphs.cpj06008x

Muniyappa, R., and Gubbi, S. (2020). COVID-19 pandemic, coronaviruses, and diabetes mellitus. *Am. J. Physiol. Endocrinol. Metab.* 318, E736–E741. doi: 10.1152/ajpendo.00124.2020

Murakami, M., Kamimura, D., and Hirano, T. (2019). Pleiotropy and specificity: insights from the interleukin 6 family of cytokines. *Immunity* 50, 812–831. doi: 10.1016/j.immuni.2019.03.027

Mycroft-West, C. J., Su, D., Pagani, I., Rudd, T. R., Elli, S., Guimond, S. E., et al. (2020). Heparin inhibits cellular invasion by SARS-CoV-2: structural dependence of the interaction of the surface protein (spike) S1 receptor binding domain with heparin. bioRxiv [Preprint]. doi: 10.1101/2020.04.28.066761

Ortiz-Perez, J. T., Riera, M., Bosch, X., De Caralt, T. M., Perea, R. J., Pascual, J., et al. (2013). Role of circulating angiotensin converting enzyme 2 in left ventricular remodeling following myocardial infarction: a prospective controlled study. *PLoS One* 8:e61695. doi: 10.1371/journal.pone.0061695

Papadopoulos, V., Li, L., and Samplaski, M. (2020). Why does COVID-19 kill more elderly men than women? Is there a role for testosterone? *Andrology* doi: 10.1111/andr.12868 [Epub ahead of print]

Rastrelli, G., Carter, E. L., Ahern, T., Finn, J. D., Antonio, L., O'neill, T. W., et al. (2015). Development of and recovery from secondary hypogonadism in aging men: prospective results from the EMAS. *J. Clin. Endocrinol. Metab.* 100, 3172–3182. doi: 10.1210/jc.2015-1571

Rice, G. I., Jones, A. L., Grant, P. J., Carter, A. M., Turner, A. J., and Hooper, N. M. (2006). Circulating activities of angiotensin-converting enzyme, its homolog, angiotensin-converting enzyme 2, and neprilysin in a family study. *Hypertension* 48, 914–920. doi: 10.1161/01.HYP.0000244543.91937.79

Santos, R. A. S., Ferreira, A. J., and Silva, A. C. E. (2008). Recent advances in the angiotensin-converting enzyme 2-angiotensin(1-7)-mas axis. *Exp. Physiol.* 93, 519–527. doi: 10.1113/expphysiol.2008.042002

Shang, J., Wan, Y., Luo, C., Ye, G., Geng, Q., Auerbach, A., et al. (2020). Cell entry mechanisms of SARS-CoV-2. *Proc. Natl. Acad. Sci. U. S. A.* 117, 11727–11734. doi: 10.1073/pnas.2003138117

South, A. M., Brady, T. M., and Flynn, J. T. (2020). ACE2 (angiotensin-converting enzyme 2), COVID-19, and ACE inhibitor and Ang II (angiotensin II) receptor blocker use during the pandemic. *Hypertension* 76, 16–22. doi: 10.1161/HYPERTENSIONAHA.120.15291

Tipnis, S. R., Hooper, N. M., Hyde, R., Karran, E., Christie, G., and Turner, A. J. (2000). A human homolog of angiotensin-converting enzyme-cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* 275, 33238–33243. doi: 10.1074/jbc.M002615200

Vaduganathan, M., Vardeny, O., Michel, T., McMurray, J. J. V., Pfeffer, M. A., and Solomon, S. D. (2020). Renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. *N. Engl. J. Med.* 382, 1653–1659. doi: 10.1056/NEJMsr2005760

Vickers, C., Hales, P., Kaushik, V., Dick, L., Gavin, J., Tang, J., et al. (2002). Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J. Biol. Chem.* 277, 14838–14843. doi: 10.1074/jbc.M200581200

Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T., and Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 181:e286. doi: 10.1016/j.cell.2020.02.058

Wan, Y. S., Shang, J., Graham, R., Baric, R. S., and Li, F. (2020). Receptor recognition by the novel coronavirus from Wuhan: an analysis based on

decade-long structural studies of SARS coronavirus. *J. Virol.* 94, e00127–e00220. doi: 10.1128/JVI.00127-20

Wei, X., Xiao, Y. -T., Wang, J., Chen, R., Zhang, W., Yang, Y., et al. (2020). Sex differences in severity and mortality among patients with COVID-19: evidence from pooled literature analysis and insights from integrated bioinformatic analysis. arXiv [Preprint]. Available at: https://arxiv.org/abs/2003.13547

Wu, C., Zheng, S., Chen, Y., and Zheng, M. (2020). Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCoV, in the nasal tissue. medRxiv [Preprint]. doi: 10.1101/2020.02.11.20022228

Xie, X., Chen, J., Wang, X., Zhang, F., and Liu, Y. (2006). Age- and gender-related difference of ACE2 expression in rat lung. *Life Sci.* 78, 2166–2171. doi: 10.1016/j.lfs.2005.09.038

Yang, P., Gu, H., Zhao, Z., Wang, W., Cao, B., Lai, C., et al. (2014). Angiotensin-converting enzyme 2 (ACE2) mediates influenza H7N9 virus-induced acute lung injury. *Sci. Rep.* 4:7027. doi: 10.1038/srep07027

Zisman, L. S., Keller, R. S., Weaver, B., Lin, Q. S., Speth, R., Bristow, M. R., et al. (2003). Increased angiotensin-(1-7)-forming activity in failing human heart ventricles-evidence for upregulation of the angiotensin-onverting enzyme homologue ACE2. *Circulation* 108, 1707–1712. doi: 10.1161/01.CIR.0000094734.67990.99

Zou, X., Chen, K., Zou, J., Han, P., Hao, J., and Han, Z. (2020). Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front. Med.* 14, 185–192. doi: 10.1007/s11684-020-0754-0

Zou, Z., Yan, Y., Shu, Y., Gao, R., Sun, Y., Li, X., et al. (2014). Angiotensin-converting enzyme 2 protects from lethal avian influenza a H5N1 infections. *Nat. Commun.* 5:3594. doi: 10.1038/ncomms4594

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 09 October 2020
doi: 10.3389/fphys.2020.575600

Pathophysiology of Cardiovascular Complications in COVID-19

Vladimir Petrovic[1], Dina Radenkovic[2], Goran Radenkovic[1], Vukica Djordjevic[3] and Maciej Banach[4]*

[1] Department of Histology and Embryology, Faculty of Medicine, University of Nis, Nis, Serbia, [2] Guy's and St. Thomas' Hospital and King's College London, London, United Kingdom, [3] Institute of Public Health, Department of Virology, Faculty of Medicine, University of Nis, Nis, Serbia, [4] Polish Mother's Memorial Hospital Research Institute (PMMHRI), Lodz, Poland

Numerous recent studies have shown that patients with underlying cardiovascular disease (CVD) are at increased risk of more severe clinical course as well as mortality of COVID-19. Also, the available data suggests that COVID-19 is related to numerous *de novo* cardiovascular complications especially in the older population and those with pre-existing chronic cardiometabolic conditions. SARS-CoV-2 virus can cause acute cardiovascular injury, as well as increase the risk of chronic cardiovascular damage. As CVD seem to be the major comorbidity in critically unwell patients with COVID-19 and patients often die of cardiovascular complications, we review the literature and discuss the possible pathophysiology and molecular pathways driving these disease processes: cytokine release syndrome, RAAS system dysregulation, plaque destabilization and coagulation disorders with the aim to identify novel treatment targets. In addition, we review the pediatric population, the major cause of the cardiovascular complications is pediatric inflammatory multisystem syndrome that is believed to be associated with COVID-19 infection. Due to the increasingly recognized CVD damage in COVID-19, there is a need to establish clear clinical and follow-up protocols and to identify and treat possible comorbidities that may be risk factors for the development of cardiovascular complications.

Keywords: COVID-19, CVD, cytokine release syndrome, thrombosis, Pediatric Inflammatory Multisystem Syndrome

OPEN ACCESS

Edited by:
Deborah Schechtman, University of São Paulo, Brazil

Reviewed by:
Pasquale Pagliaro, University of Turin, Italy
Juhani Airaksinen, University of Turku, Finland

****Correspondence:***
Dina Radenkovic
dina.radenkovic@gmail.com

Specialty section:
This article was submitted to Clinical and Translational Physiology, a section of the journal Frontiers in Physiology

Received: *23 June 2020*
Accepted: *17 September 2020*
Published: *09 October 2020*

Citation:
Petrovic V, Radenkovic D, Radenkovic G, Djordjevic V and Banach M (2020) Pathophysiology of Cardiovascular Complications in COVID-19. Front. Physiol. 11:575600. doi: 10.3389/fphys.2020.575600

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a novel infection first documented in December 2019 in Wuhan, China (Zhou P. et al., 2020). The disease carries the risk of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its pathogenesis is still unknown. The spectrum of COVID-19 seems to lie from asymptomatic or mild viral illness to a systemic disease characterized by pneumonia, fever, dry cough, breathing difficulties (dyspnea), headache, anosmia and occasional diarrhea (Chen N. et al., 2020; Guan et al., 2020; Huang et al., 2020).

Numerous recent studies have shown that patients with underlying cardiovascular disease (CVD) are at increased risk of more severe clinical course of the disease, as well as mortality. Reports from Wuhan, Lombardy and New York showed that hypertension is the most common cardiovascular comorbidity among the patients admitted for hospital care (The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020; Grasselli et al., 2020;

Richardson et al., 2020). Diabetes and obesity, although not strictly CVD, were also identified as the predictors of severe clinical course in patients with COVID-19, regardless of age and sex (Kass et al., 2020; Kassir, 2020; Li et al., 2020). Also, the patients without the history of CVD were reported to develop cardiovascular complications during COVID-19 that may contribute to the bad outcome of the disease (Guo et al., 2020; Wang D. et al., 2020; Wu and McGoogan, 2020) and COVID-19 infection can raise cardiac biomarkers and cause direct cardiac and vascular injury (Bavishi et al., 2020). As CVD seem to be the major comorbidity in critically unwell patients with COVID-19 and patients often die of cardiovascular complications, we review the possible pathophysiology of these disease processes (Palmieri et al., 2020).

COVID-19 AND THE CARDIOVASCULAR SYSTEM

It is well documented that the influenza infections, as well as SARS and MERS viruses can cause cardiovascular complications that are most often represented in the form of myocarditis, acute myocardial infarction, acute heart failure, arrhythmia, subclinical diastolic impairment and cardiac arrest (Ferrari, 2020; Xiong et al., 2020). Like in previous coronavirus outbreaks, the available data suggests that COVID-19 is related to numerous cardiovascular complications especially in the older population and those who already have chronic conditions. SARS-CoV-2 virus can cause acute cardiovascular injury, as well as increase the risk of chronic cardiovascular damage (Zheng S. et al., 2020). Also, the patients with pre-existing cardiovascular conditions face increased risk of mortality when affected with the SARS-CoV-2 (Zheng Y.Y. et al., 2020). According to the analysis of Emami et al., that included 76993 patients presented in 10 studies, hypertension, CVDs, diabetes, kidney disease, smoking, and chronic obstructive pulmonary disease were among the most prevalent underlying diseases among hospitalized patients with COVID-19. Of these, CVD had the highest prevalence among diseases that put patients at higher risk from COVID-19 and was 12,11% (Emami et al., 2020). Based on a meta-analysis performed in China on 72,314 patient records, mortality in the group of the patients with CVD was shown to be 10.9% (The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020). Initial reports from China pointed to the cardiovascular complications arising in patients affected with SARS-Cov-2. In a cohort study performed on first 41 admitted patients in Wuhan, China, Huang et al. report that 5 out of 41 patients (12%), with proven SARS-CoV-2 infection, have developed acute cardiovascular injury (ACI) during the hospital treatment. Four of these patients were admitted to the intensive care unit, due to the severe clinical picture. All of the patients with ACI had an increase in high-sensitivity cardiac troponin I. Further laboratory analyses, performed on all 41 patients, showed that prothrombin time and D-dimer level on admission were higher in ICU patients (median prothrombin time 12,2 s; median D-dimer level 2,4 mg/L) than non-ICU patients (median prothrombin time 10,7 s; median D-dimer level 0,5 mg/L) (Huang et al., 2020). In another study, performed on 138 hospitalized patients in Wuhan, China, 10 patients developed ACI, of which eight were transferred to ICU. Also, arrhythmia was evidenced in 23 patients of which 16 were transferred to ICU. The laboratory findings in patients with ICU showed a similar profile as in a previous study. All patients with ACI had increase in high-sensitivity cardiac troponin I. Laboratory analyses showed that prothrombin time and D-dimer levels were higher in ICU patients (respectively, 13,2 s and 414) compared to the non-ICU patients (12.9 s and 166, respectively) (Wang D. et al., 2020).

In a study performed on 416 patients in Wuhan, Shi et al. (2020) report that 82 patients (19.7%) had cardiac injury. Compared to the patients without cardiac injury, these patients were older, had more comorbidities, had higher leukocyte counts and levels of C-reactive protein, procalcitonin, creatine kinase–myocardial band, myohemoglobin, high-sensitivity troponin I, N-terminal pro-B-type natriuretic peptide, aspartate aminotransferase and creatinine. Greater proportions of patients with cardiac injury required non-invasive mechanical ventilation (46.3 vs 3.9%) or invasive mechanical ventilation (22.0 vs 4.2%) than those without cardiac injury. Complications were also more common in patients with cardiac injury than those without cardiac injury and included acute respiratory distress syndrome (58.5 vs 14.7%), acute kidney injury (8.5 vs 0.3%), and coagulation disorders (7.3 vs 1.8%). Patients with cardiac injury showed higher mortality rate than those without cardiac injury (51.2 vs 4.5%) (Shi et al., 2020).

Cardiovascular complications mostly occur in admitted severe or fatal cases of COVID-19, that have already developed ARDS. The risk of heart injury was higher in severe cases, approximately 22.2–31%, than in mild cases, approximately 2–4% (Zhao et al., 2020). Myocardial injury and acute coronary syndrome were seen as cardiovascular complications in patients with severe or fatal respiratory infection caused by SARS-CoV-2 and were the signs of poor prognosis (Driggin et al., 2020). Also, the cardiac arrythmias were commonly present as a cardiovascular complication in COVID-19 patients, especially in severe cases and in patients in ICU compared to the non-ICU cases (44,4 vs 6,9%) (Wang D. et al., 2020). Wang reports that 16,7% of patients develop cardiac arrythmias as a part of COVID-19 symptomatology (Wang D. et al., 2020). However, studies from China report that 7,3% of hospitalized patients (10/137) had heart palpitations as a first symptom of COVID-19 (Liu et al., 2020). In the restrospective cohort study that included 191 patients, Zhou reports that 23% of patients developed heart failure, which was more common in fatal cases compared to survivors (52 vs 12%) (Zhou F. et al., 2020). Interestingly, Dong reports the development of four end-stage heart failure as a cardiovascular complication in two male patients with mild COVID-19 infections (Dong et al., 2020).

However, studies performed in pediatric patients with COVID-19 with cardiovascular complications, report the laboratory profile (cardiac enzymes, coagulation status) being within the normal reference range (Chen J. et al., 2020; Wang Z. et al., 2020).

AGE AND SEX DIFFERENCES RELATED TO CARDIOVASCULAR COMPLICATIONS IN COVID-19

The overall data suggest that the patients above 60 years of age are at higher risk from development of cardiovascular complications. The data from China reveals that only 0,5% of patients in their 40 s died from COVID-19, while the death rate increases with age (3,6% in 60 s, 8% in 70 s and 15% in 80 s). The data from Italy show that the lethal outcome was seen in 25% of patients in their 70 s and 31% in their 80 s (Márquez et al., 2020). Reports from Italy showed that cardiovascular comorbidities were the most commonly associated with risk of death of COVID-19, most notably hypertension (70%), ishaemic heart disease (30%), atrial fibrillation (20%), and heart failure (15%) (Penna et al., 2020). It is interesting to notice that the male/female ratio of lethality is above 1.1, going to 1.7 in some countries such as Spain, Italy, England, Belgium, Greece, Denmark, and Netherlands (Márquez et al., 2020). There have been many speculative theories to explain for such differences including that women in middle age tend to have preferential lipid profile as compared to men of same age partly due to the protective effects of female hormones. Consequently, it is possible that CVD are more prevalent in men, and that patients with already existing CVD have higher risk of complications during COVID-19 infection (Penna et al., 2020). In addition, basic science research has shown that estrogens can up regulate the expression of ACE2 in the female heart tissue. This may increase the port of entry for the virus, but can significantly limit the subsequent inflammatory response and cytokine storm (Bukowska et al., 2017).

CYTOKINE RELEASE SYNDROME AND HAEMODYNAMIC INSTABILITY

Cytokine release syndrome (CRS) represents systemic inflammatory response that was described after the administration of the anti-T-cell antibody muromonab-CD3 (OKT3), a medication that was used as an immunosuppressant after organ transplantation (Chatenoud et al., 1990; Shimabukuro-Vornhagen et al., 2018). CRS, as a form of innate immune response, can also occur as a complication of viral infections and is responsible for ARDS (acute respiratory distress syndrome) and multiple organ failure (Shimabukuro-Vornhagen et al., 2018). The cases of CRS outbreaks were reported during previous MERS and SARS epidemics, and there are also reports evidencing CRS in patients affected with SARS-CoV-2 (Hu et al., 2017; Xu et al., 2020).

Clinically, CRS can present in mild or more severe forms. Mild forms include fever, fatigue, rash, myalgia, artralgia, that are often seen at the onset of many infectious diseases and cannot be easily distinguished from other viral illnesses. However, CRS can progress to a more severe form, severe inflammatory response (SIRS), that manifests with hemodynamic compromise resulting in circulatory shock, vascular leakage, DIC (disseminated intravascular coagulopathy), and multisystem organ failure – cardiac dysfunction, renal and hepatic failure and ARDS (Murthy et al., 2019). The laboratory parameters usually show cytopenia, elevated levels of C-reactive protein, deranged markers of coagulation and thrombosis (D-dimer, prothrombin time) and the deranged levels of organ specific markers (Shimabukuro-Vornhagen et al., 2018).

Cardiac function is also affected in CRS which manifests mostly as a cardiomyopathy that resembles the one seen in sepsis and stress cardiomyopathy (Takotsubo cardiomyopathy). The cardiovascular complications during CRS arise as a result of acute cardiac toxicity and their pathogenesis is not completely understood (Lee et al., 2014). The ejection fraction can be reduced, and the other symptoms of cardiac dysfunction include the arrythmias, hypotension and tachycardia. The acute cardiac failure and elevated levels of troponin indicating acute cardiac injury can also appear as a complication of CRS (Lee et al., 2014; Shimabukuro-Vornhagen et al., 2018). Laboratory analyses of COVID-19 patients show that lymphopenia is connected with the severe clinical presentation. The number of T lymphocytes (CD4+, CD8+), B lymphocytes, NK cells, as well as the eosinophils, basophils and monocytes, is reduced, and the number of neutrophils usually shows higher percentage value (Cao, 2020).

CRS represents dysregulated and excessive immune response that fails to defend the organism against the infection, and instead damages the body. In their detailed review on CRS in SARS and COVID-19, Ye et al. (2020) discuss that the respiratory epithelial cells, dendritic cells and macrophages, after infection with SARS-CoV, firstly produce low levels of cytokines and chemokines, and in the later stages these cells secrete low levels of the antiviral factors interferons (IFNs) and high levels of proinflammatory cytokines: IL-1β, IL-6, and tumor necrosis factor (TNF), as well as the chemokines CCL-2, CCL-3, and CCL-5 (**Figure 1A**). The increased levels of cytokines and chemokines attract inflammatory cells, such as neutrophils and monocytes that convert to macrophages, which results in massive infiltration of the lung tissue causing lung injury, but also the parenchyma of other organs may be affected as well (Ye et al., 2020). In the lungs, the massive infiltration of neutrophils and macrophages causes diffuse alveolar damage with the formation of hyaline membranes and a diffuse thickening of the alveolar wall, that leads to ARDS (Cao, 2020). IFN-α/β (interferon-α/β) or macrophage-derived proinflammatory cytokines induce the apoptosis of T cells, which promotes further rapid viral replication, and cause the apoptosis of respiratory epithelial and endothelial cells (Hogner et al., 2013; Channappanavar et al., 2016). Apoptosis of endothelial cells and epithelial cells damages the pulmonary microvascular and alveolar epithelial cell barriers and causes increased endothelial permeability and alveolar edema.

Patients with COVID-19 have shown to have increased levels of IL-1B, IFN-γ, IP-10, and monocyte chemoattractant protein 1 (MCP-1) that are part Th1 immune response responsible the activation of specific immunity, but also higher levels of IL-4 and IL-10 that represent the part of the TH2 immune response that shows the anti-inflammatory effects (Ye et al., 2020). Also, IL-1β and TNFα (highly expressed by TH17 and TH1 cells), both promote TH17 responses and increased endothelial permeability leading to the vascular leakage (Wu and Yang, 2020).

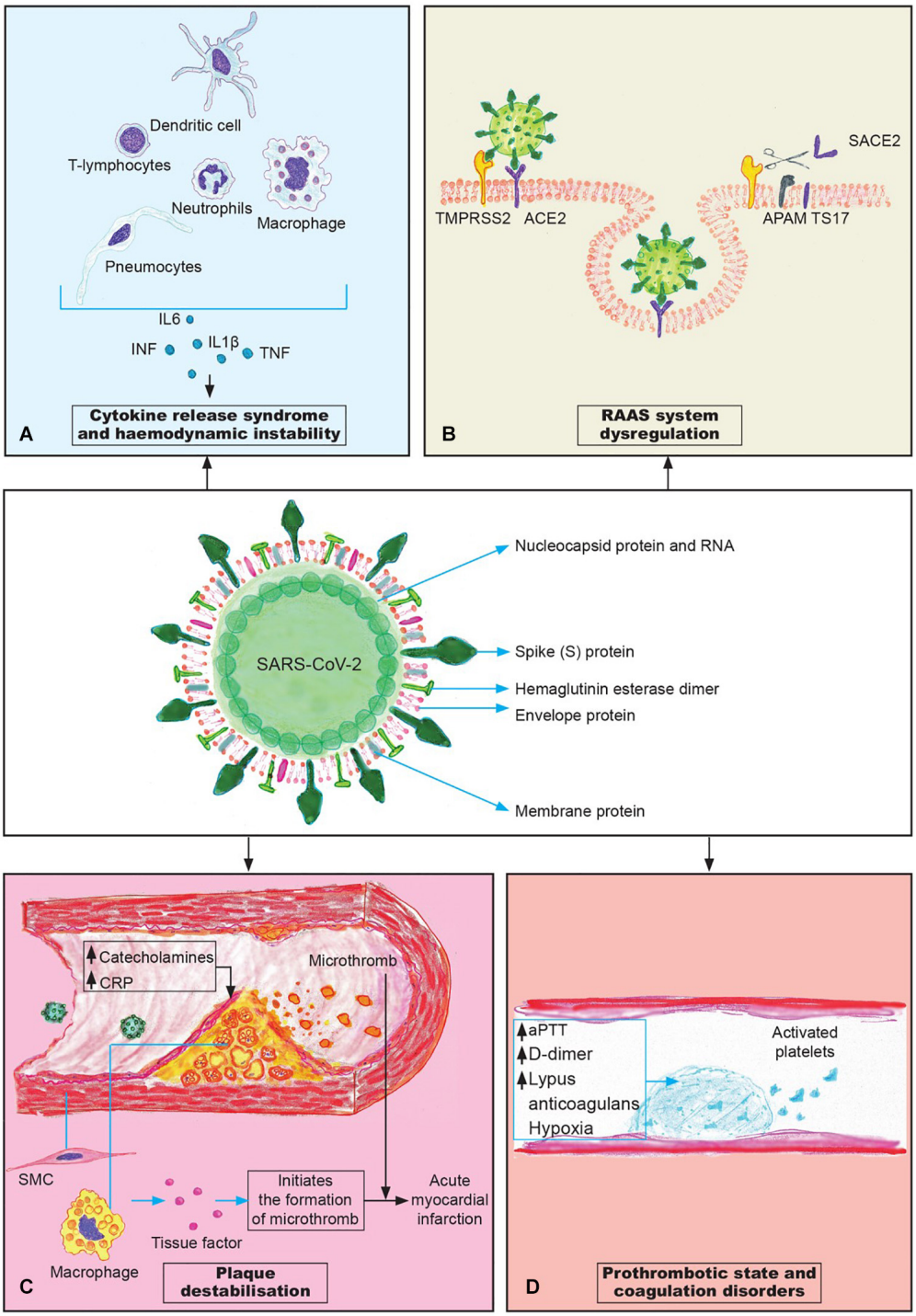


FIGURE 1 | The pathogenesis mechanisms of cardiovascular complications in COVID-19. **(A)** Cytokine release syndrome ("cytokine storm") is a systemic inflammatory response that plays the crucial role in the development of cardiovascular complications; **(B)** The downregulation of ACE2 receptors; **(C)** Plaque destabilization can lead to the plaque rupture and consequent formation of microthrombi; **(D)** Coagulation disorders contribute to the cloth formation and microthrombosis in different organs.

The studies have shown that the level of IL-6 correlates positively with the severity of the disease and the outcome, and that the use of drugs that inhibit the secretion of IL-6 may have an important role in the treatment of CRS (Henderson et al., 2020; McGonagle et al., 2020). The other potential targets in the treatment of CRS could be IL-1 and IL-17, and the future

clinical trials are needed to show the effectiveness and benefit of developing the drugs targeting their receptors and molecules involved in their signaling pathways (Cao, 2020).

RAAS SYSTEM DYSREGULATION

The mechanisms underlying the cardiovascular complications in patients with SARS-CoV-2 infection are still not completely elucidated. ACE2 (angiotensin-converting enzyme-2), as the point of entry of SARS-CoV-2 virus into the cell, is currently in the focus of researchers, especially bearing in mind their wide distribution on pneumocytes type 2 and endothelial cells of the arteries, arterioles and venules in various organs, including the lungs and the heart (Oudit et al., 2009).

ACE2 is a single-pass type I transmembrane metallocarboxypeptidase, with its enzymatically active domain exposed on the cell surface (Hamming et al., 2004). ACE2 functions as a negative regulator of the RAAS (renin-angiotensin-aldosteron system) and has the role in degradation of angiotensin-2 which results in the production of the heptapeptid called angiotensin 1–7 (Crackower et al., 2002). Angiotensin 1–7 binds to G-protein coupled mas oncogene receptor, exerting the vasodilatator, anti-hypertrophic and anti-inflammatory effects on the cardiovascular system (Crackower et al., 2002; Der Sarkissian et al., 2008; Zhong et al., 2010). On the other side, angiotensin-2, as a part of RAAS, binds to its receptors – angiotensin-2 receptors type 1 (AT1) and causes vasoconstriction that leads to the rise of blood pressure. If overstimulated, angiotensin-2 can negatively impact the cardiovascular system, by causing hypertension, inflammation, myocardial fibrosis and hypertrophy that all eventually lead to heart failure (Mehta and Griendling, 2007). The loss of ACE2 has been documented to have a negative impact on the cardiovascular system, leading to the cardiac hypertrophy and contractility disorders (Kassiri et al., 2009; Patel et al., 2012). Patel et al. (2014) reported that higher concentrations of angiotensin-2 suppress ACE2 by increasing TNF-α converting enzyme (TACE) activity which leads to the cleavage of the extracellular portion of ACE2, thus rendering the rest of the protein ineffective (**Figure 1B**). The cleavage of active sites results in the elevated plasma levels of ACE2 which is considered to be a marker of disease and poor prognosis (Bitker and Burrell, 2019). The downregulation of ACE2 was also documented in lungs especially in the men during ageing, in diabetes mellitus and is considered to be one of the factors causing hypertension (Verdecchia et al., 2020).

Upon the entrance into the cell via ACE2 receptor, SARS-CoV-2 leads to the downregulation of these receptors (Zhang H. et al., 2020). This newly established imbalance between ACE2 and angiotensin-2 may cause the appearance of cardiovascular complications in patients with no previous history of CVD or worsen the existing CVD in patients with COVID-19. The downregulation of ACE2 in mice experimental model that occurs after the infection with SARS-CoV worsens acute lung failure *in vivo* that can be attributed to the effects of angiotensin-2. The use of angiotensin-2 receptor blockers attenuates this situation by inhibiting the activity of RAAS (Kuba et al., 2005). Study performed on a murine model by Oudit et al. showed that SARS-CoV can affect the heart, leading to the partial down-regulation of *Ace2* mRNA expression with a complete loss of myocardial ACE2 protein levels (Oudit et al., 2009). The results obtained from the autopsies of the hearts of patients affected with SARS-CoV-2 showed the pronounced decrease in the ACE2 protein levels and macrophage infiltration. These data may suggest that the downregulation of protective function of ACE2 might cause myocardial inflammation and damage leading to the myocardial dysfunction (Oudit et al., 2009).

Although ACE2 serves as a receptor for SARS-CoV-2, its blockage would have a negative impact on the health of the patient due to its array of biological roles. However, in the study performed by Bertram et al. (2012) it was reported that the modest ACE2 expression in the upper respiratory tract might limit SARS-CoV transmissibility. Although SARS-CoV and SARS-CoV2 share many similarities, it is important to notice that SARS-CoV-2 has a furin cleavage site at the S_1/S_2 boundary, which is believed to increase its transmissibility and/or altering its pathogenicity compared to SARS-CoV that does not possess this sequence (Walls et al., 2020). At this moment, when the vaccine is still in development, one of the possible treatment options would be camostate mesylate, an inhibitor of enzyme TMPRSS2 that plays a crucial role beside ACE2 in the entry of the virus into the cell (Hoffmann et al., 2020). Also, the sera from the patients who recovered from SARS infections showed the ability to cross-neutralize SARS-CoV-2 entry into the cell (Hoffmann et al., 2020).

ACE inhibitors and ARBs are the widely used medications in the treatment of arterial hypertension and in prevention of heart remodeling. There is still not sufficient data concerning the effects of ACE inhibitors and ARBs on the levels of ACE2 in humans (Park et al., 2020; Vaduganathan et al., 2020). Although there were some suggestions that the use of ACE inhibitors and angiotensin receptor blockers (ARB) may facilitate the entry of the virus in the cells, the current recommendation of European Society of Cardiology is that the patients using these drugs in the therapy of hypertension should not discontinue their usage (European society of cardiology). Some authors discuss the potential beneficial effects of ARBs in the therapy lung injury in COVID-19 patients with hypertension (Gurwitz, 2020; Phadke and Saunik, 2020; Verdecchia et al., 2020). This is based on the ability of these medications to suppress angiotensin-2 effects on worsening the inflammation of the lung parenchyma during SARS-CoV-2 infection and to elevate the levels of downregulated ACE2 that produces the angiotensin 1–7. There are currently announced trials aiming to examine the role of losartan as a supporting therapy in patients with COVID-19. Observational studies until now show a survival benefit in patients with ACE2 inhibitors (Verdecchia et al., 2020).

PLAQUE DESTABILISATION

The increased level of catecholamines, which was shown to occur in COVID-19, as part of systemic inflammation may lead to the

plaque rupture and destabilization, thus causing acute coronary syndrome (Basu-Ray et al., 2020). Also, the levels of C-reactive protein were shown to be in the direct correlation with the risk of the onset of myocardial infarction due to plaque rupture (Ridker et al., 1997). Wang reported that C-reactive protein is elevated in patients with COVID-19, and that its levels correlate with the severity of clinical presentation (Wang, 2020).

The rupture of atheromatous plaque leads to the exposure of foamy macrophages, located under the endothelium, to the bloodstream. These macrophages express the tissue factor that, in contact with the blood, initiates the formation of microthrombi. Also, the rupture of the plaque exposes vascular smooth muscle cells to the blood flow, which also expresses tissue factor that facilitates the process of thrombogenesis (Libby and Simon, 2001; **Figure 1C**). Additionally, smooth muscle cells may undergo inflammatory activation which results in the excessive production of IL-6 that can induce the acute phase response. Therefore, some of the commonly prescribed drugs for lipid-lowering therapy like statins that are also believed to have plaque stabilizing properties might prove to be beneficial in a subgroup of COVID-19 patients with pre-existing atherosclerotic disease (Bahrami et al., 2018).

PROTHROMBOTIC STATE AND COAGULATION DISORDERS

The systemic inflammation caused by different infectious agents is also known to be associated with the disturbances in the hematopoietic system (Connors and Levy, 2020). It has the pro-coagulant effect thus facilitating the formation of microthrombi, which consecutively may cause the infarction of different organs. The disturbances in the coagulation were evidenced in the early reports from Wuhan, China. In a study that included 99 patients in Wuhan, China, Chen N. et al. (2020) reported significantly higher values for activated partial thromboplastin time (6% of the patients), prothrombin time (5%) and higher levels of D-dimer (36%). However, studies performed by Wang D. et al. (2020) (138 patients) and Huang (41 patients), showed minimal increase of values for prothrombin, prothrombin time and D-dimer (Huang et al., 2020).

In a study performed in Wuhan on 189 patients, it was reported that 21 patients (11,5%), who died during the hospital treatment, showed significantly higher levels of D-dimer and fibrin degradation products, and longer prothrombin time and activated partial tromboplastin time (aPTT) compared to survivors. Also, they reported that 71,4% of non-survivors developed disseminated intravascular coagulation (Tang et al., 2020).

In a study comprising 216 COVID-19 positive patients, Bowles et al. (2020) measured the coagulation status parameters. They reported that 44 (20%) patients had prolonged aPTT time. Further analyses showed that 34 out of these 44 patients were positive on lupus anticoagulant assays (Bowles et al., 2020). Lupus anticoagulant are antibodies that belong to the group of antiphospholipid antibodies and are associated with a thrombotic tendency within the antiphospholipid syndrome. They are directed against the anionic phospholipids or other membrane particles that are exposed to the immune system after membrane remodeling that occurs under different infectious, inflammatory or autoimmune stimuli (Gebhart et al., 2019; Helms et al., 2020). The role in pathogenesis if COVID-19 needs to be further elucidated (Bowles et al., 2020).

Helms et al. (2020) in a study performed on 150 patients diagnosed with COVID-19, report that more than 95% of patients had elevated D-dimer and fibrinogen. Results of their analysis show that Von Willebrand (vWF) activity, vWF antigen and FVIII (factor VIII) were considerably increased, and that 50/57 tested patients (87.7%) had positive lupus anticoagulant. 16,7% of patients developed pulmonary embolism during their hospital treatment, and there were no cases of DIC.

High D-dimer in admitted patients was usually associated with poor prognosis and a high mortality rate. Also, worsening lymphopenia over time with increased levels of D-dimer were the findings mostly seen in non-survivors (Basu-Ray et al., 2020).

The activation of coagulation during the systemic inflammation can occur through several mechanisms. Polyphosphates derived from microorganisms activate platelets, mast cells and factor XII of coagulation. Also, the system of complement and components of NETs (neutrophil extracellular traps), as well as the pathogen associated molecular mechanisms are involved in the activation of coagulation cascade (Connors and Levy, 2020). All the above-mentioned shows that the activation of coagulation cascade during the systemic inflammatory response is highly complex and involves several mechanisms that simultaneously lead to the formation of microthrombi and possible development of DIC. The procoagulant effects of hypoxemia should be also considered in the pathogenesis of coagulation disorders in COVID-19, bearing in mind that patients affected with this disease may develop a drop in oxygen saturation. The low levels of oxygen activate the transcription factor Egr-1 (early growth response-1) that leads to the transcription and translation of tissue factor in mononuclear phagocytes and smooth muscle cells, which results in the vascular fibrin deposition. Also, hypoxia upregulates plasminogen activator inhibitor-1 which suppresses fibrinolysis (Yan et al., 1999). The induction of hypoxia induced factor and endothelial inflammation during hypoxia and systemic inflammatory response may contribute to the formation of microthrombi (Chang, 2019; Gupta et al., 2019; **Figure 1D**).

PEDIATRIC INFLAMMATORY MULTISYSTEM SYNDROME IN CHILDREN

The pediatric population seems to be less affected with COVID-19 compared to the older individuals. The report show that only 1–2% of children were diagnosed with COVID-19 and that the risk of lethal outcome is 500 times lower than in adults (Fischer, 2020). There is still ongoing discussion concerning the mild clinical picture of COVID-19 in children. Several factors are being accounted for in the discussion, including

the prevalence of antibodies against seasonal coronaviruses in children, expression of ACE2 and the recent previous vaccination with a live vaccine such as BCG vaccine for Tuberculosis (Felsenstein and Hedrich, 2020). However, we still don't know the exact molecular mechanisms protecting children from severe outcomes of COVID-19 infection. Pagliaro proposes that the macrophage heterogeneity might be one of the protective key elements in children, discussing that the macrophages enter the lungs in three developmental waves of which two occur *in utero*, while the third one happens after birth. He further discusses that these three waves give rise to the three different lineages that differ among themselves by the expression of cell surface markers including ACE2 and that children most probably have more of macrophages that came through the first two waves of migration (Pagliaro, 2020). The role of macrophages is important for the initiation of innate immune response after infection with SARS-CoV-2, which occurs trough the activation of the NF-kappaB pathway (Chang et al., 2020). These three lineages of macrophages populate human lungs and their relation changes over time with ageing. Each one of these three types of macrophages may elicit different responses to virus infection thus possibly giving the different clinical pictures in children and in adults (Pagliaro, 2020).

There have been recent reports of children and adolescents developing Paediatric Inflammatory Multisystem Syndrome (PIMS) associated with COVID-19, that has a similar clinical picture to Kawasaki disease (Cheung et al., 2020; Jones et al., 2020; Riphagen et al., 2020; Whittaker et al., 2020). Kawasaki disease (KD) is a medium-vessel disease vasculitis that often affects the coronary arteries and is characterized by an acute onset, cardiac complications and self-limiting course (World Health Organization, 2020). The disease is more common in East Asian pediatric population (Newburger et al., 2004). As KD might affect coronary arteries, it can cause arrhythmia, acute heart failure and hemodynamic instability, a condition known as Kawasaki disease shock syndrome (KDSS) (Kanegaye et al., 2009).

The exact pathogenesis of KD is still unknown. There are reports that viruses from coronavirus family might be associated with the development of KD, however these results need further verification (Shirato et al., 2014; Verdoni et al., 2020). The studies report that the majority of children affected with PIMS were not having active COVID-19 infection, but IgG and IgM antibodies against SARS-CoV-2 were elevated in laboratory findings (Whittaker et al., 2020). This may lead to the assumption that the possible cause of PIMS might be the aberrant delayed immune response triggered by COVID-19 infection (Cheung et al., 2020; Whittaker et al., 2020). Also, that IL-6, IL-10 and interferon-gamma that are elevated in KD as well as in COVID-19, where these are shown to exert an inflammatory-mediated lung injury (Li et al., 2019; Cheung et al., 2020).

The treatment of PIMS has been mostly with aspirin and intravenous immunoglobulin (IVIG) sometimes co-administered with antibiotics to prevent super-infection, which is the treatment for KD, mostly due to the similar clinical picture. Although IVIG was widely used in the treatment of severe cases of SARS-CoV-2 in adults, there is still not enough evidence about its effectiveness in the treatment of children with PIMS (Zhang J. et al., 2020).

COVID-19 PANDEMIC SOCIETAL EFFECT AS A CARDIOVASCULAR RISK FACTOR

Lastly, with the COVID-19 pandemic response lasting for longer than 6 months in many countries all over the globe, one cannot exclude the indirect effects such as social isolation, chronic stress, change in eating habits and, for many, reduced physical activity with stay-at-home measures potentially contributing to cardiovascular risk in patients with preexisting conditions.

In the aim of dealing with the COVID-19 pandemic, the majority of countries have at some point implemented the total or partial quarantine of the population. Many mental health specialists have alerted already that the "lock down" phenomenon led to the raise of the number of patients with anxiety and higher stress levels, but also caused the changes in the lifestyle especially concerning the eating habits and lower physical activity (Mattioli et al., 2020). The chronic stress, perhaps arising as a consequence of the quarantine and social isolation or on the other hand, fear of political and financial aftermath, could lead to increase of the activity of the sympathetic nervous system which may cause increased risk of mortality in patients with pre-existing CVD (Steptoe and Kivimäki, 2012; Mattioli et al., 2020). In a study that was following a cohort of 1267 patients older than 65 years in a period of 10 years, it was shown that the social isolation was highly connected with the increased risk of mortality in these patients (Yu et al., 2020).

The "2019 ACC/AHA Guideline on the Primary Prevention of CVD" recommended that the adults should engage in at least 150 min per week of accumulated moderate-intensity or 75 min per week of vigorous-intensity aerobic physical activity, but those who cannot meet the required minimum should be anyway engaged in the moderate or vigorous physical activity even if it is less than recommended (Arnett et al., 2019). The physical activity plays a crucial role in reduction of inflammation and oxidative stress, but it is also important in maintaining the normal weight, thus decreasing the risk of diabetes mellitus, CVD and metabolic disorders (World Health Organization, 2018). Bearing in mind that the gyms and public spaces were closed during the quarantine hours it is recommended that the people should be involved in in-house physical activity in order to prevent the possible negative health impacts caused by the sedentary way of life. These considerations have rightly so not been the top priority in the immediate pandemic response. Nevertheless, as the world continues to battle this pandemic while awaiting for treatments and a vaccine, it is important to take consideration the general health and wellbeing of the population should there be reinstitution of quarantine measures especially in the autumn/winter months with often worse weather conditions for outdoor exercise in the Northern hemisphere. Although healthy lifestyle promotion is unfortunately often missed in the busy medical consultations, public health interventions for the

prevention of CVD should become a fundamental tool in the pandemic response.

PERSPECTIVE AND FUTURE DIRECTIONS

There is a need to define the most important comorbidities related to the cardiovascular system that can contribute to the development of the disease, in order to address the attention of medical personnel on possible complications. Also, the psychological counzelling should be provided to patients in order to make sure that they are aware of the reality of the situation and to prepare them to better cope with their hospital staying and post COVID-19 follow-up (Dolinski et al., 2020).

Patients with hypertension, diabetes and/or obesity represent high-risk group that should be closely monitored in order to prevent or properly treat the possible complications due to the SARS-CoV-2 infection. Of special interest are men and patients older than 60 years with severe disease, who were shown to have a longer duration of virus in stool, serum and respiratory samples (Zheng S. et al., 2020). As thromboembolic events tend to occur at higher frequency in patients with pre-existing atherosclerosis in COVID-19 infections, higher risk groups should be appropriately given anticoagulation therapy if in the hospital. Potentially high risk groups with pre-existing CVD disease even in milder confirmed COVID-19 could be evaluated by blood tests including cardiac specific markers and lipid levels, as well as electrocardiography and echocardiography to further risk stratify patients who would benefit from lipid lowering, plaque stabilizing and anti-platelet or anti-coagulation medications without an unacceptable risk of bleeding.

The regular therapy for CVD should not be discontinued during COVID-19 infection (Guzik et al., 2020). As it is increasingly recognized there is a direct damage to the blood vessels and the heart, there have been reports of the use of standard drugs for CVD prevention in COVID-19. ACE inhibitors may have a potential beneficial role. Statins and lipid lowering drugs have been suggested for the treatment of patients with COVID-19 due to their ability to disrupt lipid rafts in the cell membrane and prevent the binding of the virus on the cell. In patients with CVD their usage should not be discontinued. However, it remains unclear whether starting these medications prophylactically or during COVID-19 has any clinical benefit (Banach et al., 2020; Katsiki et al., 2020; Reiner et al., 2020).

Currently, we have limited knowledge on the possible cardiovascular complications that may arise in the aftermath of COVID-19 infection. There has only so far been speculative work about the molecular effects on the cardiomyocytes and endothelial cells and a possible increased risk of heart failure that might represent later. However, due to the increasingly recognized CVD damage in this disease, we will need to have a longer term follow up of severe COVID-19 patient survivors to answer this question.

AUTHOR CONTRIBUTIONS

DR conceived the concept of the manuscript. All authors contributed to the literature review and writing of the manuscript and approved the final version.

FUNDING

This manuscript was funded from Internal project number 38/20 of Faculty of Medicine, University in Nis, Serbia.

REFERENCES

Arnett, D. K., Blumenthal, R. S., Albert, M. A., Buroker, A. B., Goldberger, Z. D., Hahn, E. J., et al. (2019). 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: a report of the American college of cardiology/American heart association task force on clinical practice guidelines. *Circulation* 140, 596–646.

Bahrami, A., Parsamanesh, N., Atkin, S. L., Banach, M., and Sahebkar, A. (2018). Effect of statins on toll-like receptors: a new insight to pleiotropic effects. *Pharmacol. Res.* 135, 230–238. doi: 10.1016/j.phrs.2018.08.014

Banach, M., Penson, P. E., Fras, Z., Vrablik, M., Pella, D., Reiner, Ž, et al. (2020). Brief recommendations on the management of adult patients with familial hypercholesterolemia during the COVID-19 pandemic. *Pharmacol. Res.* 158:104891. doi: 10.1016/j.phrs.2020.104891

Basu-Ray, I., Almaddah, N. K., Adeboye, A., and Soos, M. P. (2020). *Cardiac Manifestations Of Coronavirus (COVID-19)*. Treasure Island, FL: StatPearls Publishing.

Bavishi, C., Bonow, R. O., Trivedi, V., Abbott, J. D., Messerli, F. H., and Bhatt, D. L. (2020). Acute myocardial injury in patients hospitalized with COVID-19 infection: a review. *Prog. Cardiovasc. Dis.* doi: 10.1016/j.pcad.2020.05.013 [Epub ahead of print].

Bertram, S., Heurich, A., Lavender, H., Gierer, S., Danisch, S., Perin, P., et al. (2012). Influenza and SARS-coronavirus activating proteases TMPRSS2 and HAT are expressed at multiple sites in human respiratory and gastrointestinal tracts. *PLoS One* 7:e35876. doi: 10.1371/journal.pone.0035876

Bitker, L., and Burrell, L. M. (2019). Classic and nonclassic renin-angiotensin systems in the critically Ill. *Crit. Care Clin.* 35, 213–227. doi: 10.1016/j.ccc.2018.11.002

Bowles, L., Platton, S., Yartey, N., Dave, M., Lee, K., Hart, D. P., et al. (2020). Lupus anticoagulant and abnormal coagulation tests in patients with COVID-19. *N. Engl. J. Med.* 383, 288–290. doi: 10.1056/NEJMc2013656

Bukowska, A., Spiller, L., Wolke, C., Lendeckel, U., Weinert, S., Hoffmann, J., et al. (2017). Protective regulation of the ACE2/ACE gene expression by estrogen in human atrial tissue from elderly men. *Exp. Biol. Med.* 242, 1412–1423. doi: 10.1177/1535370217718808

Cao, X. (2020). COVID-19: immunopathology and its implications for therapy. *Nat. Rev. Immunol.* 20, 269–270. doi: 10.1038/s41577-020-0308-3

Chang, J. C. (2019). Sepsis and septic shock: endothelial molecular pathogenesis associated with vascular microthrombotic disease. *Thromb J.* 17:10. doi: 10.1186/s12959-019-0198-4

Chang, Y. S., Ko, B. H., Ju, J. C., Chang, H. H., Huang, S. H., and Lin, C. W. (2020). SARS unique domain (SUD) of severe acute respiratory syndrome coronavirus induces NLRP3 inflammasome-dependent CXCL10-mediated pulmonary inflammation. *Int. J. Mol. Sci.* 21:3179. doi: 10.3390/ijms21093179

Channappanavar, R., Fehr, A. R., Vijay, R., Mack, M., Zhao, J., and Meyerholz, D. K. (2016). Dysregulated Type I Interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-Infected Mice. *Cell Host Microbe* 19, 181–193. doi: 10.1016/j.chom.2016.01.007

Chatenoud, L., Ferran, C., Legendre, C., Thouard, I., Merite, S., Reuter, A., et al. (1990). In vivo cell activation following OKT3 administration. Systemic cytokine release and modulation by corticosteroids. *Transplantation* 49, 697–702. doi: 10.1097/00007890-199004000-00009

Chen, J., Zhang, Z. Z., Chen, Y. K., Long, Q. X., Tian, W. G., Deng, H. J., et al. (2020). The clinical and immunological features of pediatric COVID-19 patients in China. *Genes Dis.* doi: 10.1016/j.gendis.2020.03.008 [Epub ahead of print].

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395, 507–513. doi: 10.1016/S0140-6736(20)30211-7

Cheung, E. W., Zachariah, P., Gorelik, M., Boneparth, A., Kernie, S. G., Orange, J. S., et al. (2020). Multisystem inflammatory syndrome related to COVID-19 in previously healthy children and adolescents in New York city. *JAMA* 324, 294–296. doi: 10.1001/jama.2020.10374

Connors, J. M., and Levy, J. H. (2020). COVID-19 and its implications for thrombosis and anticoagulation. *Blood* 135, 2033–2040. doi: 10.1182/blood.2020006000

Crackower, M. A., Sarao, R., Oudit, G. Y., Yagil, C., Kozieradzki, I., Scanga, S. E., et al. (2002). Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 417, 822–828. doi: 10.1038/nature00786

Der Sarkissian, S., Grobe, J. L., Yuan, L., Narielwala, D. R., Walter, G. A., Katovich, M. J., et al. (2008). Cardiac overexpression of angiotensin converting enzyme 2 protects the heart from ischemia-induced pathophysiology. *Hypertension* 51, 712–718. doi: 10.1161/HYPERTENSIONAHA.107.100693

Dolinski, D., Dolinska, B., Zmaczynska-Witek, B., Banach, M., and Kulesza, W. (2020). Unrealistic optimism in the time of coronavirus pandemic: may it help to kill, if so-whom: disease or the person? *J. Clin. Med.* 9:E1464. doi: 10.3390/jcm9051464

Dong, N., Cai, J., Zhou, Y., Liu, J., and Li, F. (2020). End-stage heart failure with COVID-19. strong evidence of myocardial injury by 2019-nCoV. *JACC Heart Fail.* 8, 515–517. doi: 10.1016/j.jchf.2020.04.001

Driggin, E., Madhavan, M. V., Bikdeli, B., Chuich, T., Laracy, J., and Biondi-Zoccai, G. (2020). Cardiovascular considerations for patients, health care workers, and health systems during the COVID-19 pandemic. *J. Am. Coll. Cardiol.* 75, 2352–2371.

Emami, A., Javanmardi, F., Pirbonyeh, N., and Akbari, A. (2020). Prevalence of underlying diseases in hospitalized patients with COVID-19: a systematic review and meta-analysis. *Arch. Acad. Emerg. Med.* 8:35.

Felsenstein, S., and Hedrich, C. M. (2020). COVID-19 in children and young people. *Lancet Rheumatol.* 2, e514–e516.

Ferrari, F. (2020). COVID-19: updated data and its relation to the cardiovascular system. *Arq. Bras. Cardiol.* 114, 823–826. doi: 10.36660/abc.20200215

Fischer, A. (2020). Resistance of children to Covid-19. How? *Mucosal. Immunol.* 13, 563–565. doi: 10.1038/s41385-020-0303-9

Gebhart, J., Posch, F., Koder, S., Quehenberger, P., Perkmann, T., Kuessel, L., et al. (2019). High risk of adverse pregnancy outcomes in women with a persistent lupus anticoagulant. *Blood Adv.* 3, 769–776. doi: 10.1182/bloodadvances.2018026948

Grasselli, G., Zangrillo, A., Zanella, A., Antonelli, M., Cabrini, L., Castelli, A., et al. (2020). Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region, Italy. *JAMA* 323, 1574–1581. doi: 10.1001/jama.2020.5394

Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W. H., Ou, C. Q., He, J. X., et al. (2020). Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* 382, 1708–1720. doi: 10.1056/NEJMoa2002032

Guo, T., Fan, Y., Chen, M., Wu, X., Zhang, L., He, T., et al. (2020). Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 5, 811–818. doi: 10.1001/jamacardio.2020.1017

Gupta, N., Zhao, Y. Y., and Evans, C. E. (2019). The stimulation of thrombosis by hypoxia. *Thromb. Res.* 181, 77–83. doi: 10.1016/j.thromres.2019.07.013

Gurwitz, D. (2020). Angiotensin receptor blockers as tentative SARS−CoV−2 therapeutics. *Drug Dev. Res.* 81, 537–540. doi: 10.1002/ddr.21656

Guzik, T. J., Mohiddin, S. A., Dimarco, A., Patel, V., Savvatis, K., Marelli-Berg, F. M., et al. (2020). COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. *Cardiovasc. Res.* 116, 1666–1687. doi: 10.1093/cvr/cvaa106

Hamming, I., Timens, W., Bulthuis, M. L., Lely, A. T., Navis, G., and van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203, 631–637. doi: 10.1002/path.1570

Helms, J., Tacquard, C., Severac, F., Leonard-Lorant, I., Ohana, M., Delabranche, X., et al. (2020). High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med.* 46, 1089–1098. doi: 10.1007/s00134-020-06062-x

Henderson, L. A., Canna, S. W., Schulert, G. S., Volpi, S., Lee, P. Y., Kernan, K. F., et al. (2020). On the alert for cytokine storm: immunopathology in COVID-19. *Arthritis Rheumatol.* 72, 1059–1063. doi: 10.1002/art.41285

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271–280. doi: 10.1016/j.cell.2020.02.052

Hogner, K., Wolff, T., Pleschka, S., Plog, S., Gruber, A. D., and Kalinke, U. (2013). Macrophage-expressed IFN-β contributes to apoptotic alveolar epithelial cell injury in severe influenza virus pneumonia. *PLoS Pathog.* 9:e1003188. doi: 10.1371/journal.ppat.1003188

Hu, B., Zeng, L. P., Yang, X. L., Ge, X. Y., Zhang, W., Li, B., et al. (2017). Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLoS Pathog.* 13:e1006698. doi: 10.1371/journal.ppat.1006698

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Jones, V. G., Mills, M., Suarez, D., Hogan, C. A., Yeh, D., Bradley Segal, J., et al. (2020). COVID-19 and kawasaki disease: novel virus and novel case. *Hosp. Pediatr.* 10, 537–540. doi: 10.1542/hpeds.2020-0123

Kanegaye, J. T., Wilder, M. S., Molkara, D., Frazer, J. R., Pancheri, J., Tremoulet, A. H., et al. (2009). Recognition of a Kawasaki disease shock syndrome. *Pediatrics* 123, 783–789. doi: 10.1542/peds.2008-1871

Kass, D., Duggal, P., and Cingolani, O. (2020). Obesity could shift severe COVID-19 disease to younger ages. *Lancet* 395, 1544–1545. doi: 10.1016/S0140-6736(20)31024-2

Kassir, R. (2020). Risk of COVID-19 for patients with obesity. *Obes. Rev.* 21:e13034. doi: 10.1111/obr.13034

Kassiri, Z., Zhong, J., Guo, D., Basu, R., Wang, X., Liu, P. P., et al. (2009). Loss of angiotensin converting enzyme 2 accelerates maladaptive left ventricular remodeling in response to myocardial infarction. *Circ. Heart Fail.* 2, 446–455. doi: 10.1161/CIRCHEARTFAILURE.108.840124

Katsiki, N., Banach, M., and Mikhailidis, D. P. (2020). Lipid-lowering therapy and renin-angiotensin-aldosterone system inhibitors in the era of the COVID-19 pandemic. *Arch. Med. Sci.* 16, 485–489. doi: 10.5114/aoms.2020.94503

Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., et al. (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. *Nat. Med.* 11, 875–879. doi: 10.1038/nm1267

Lee, D. W., Gardner, R., Porter, D. L., Louis, C. U., Ahmed, N., Jensen, M., et al. (2014). Current concepts in the diagnosis and management of cytokine release syndrome. *Blood* 124, 188–195. doi: 10.1182/blood-2014-05-552729

Li, B., Yang, J., Zhao, F., Zhi, L., Wang, X., Liu, L., et al. (2020). Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clin. Res. Cardiol.* 109, 531–538. doi: 10.1007/s00392-020-01626-9

Li, Y., Zheng, Q., Zou, L., Wu, J., Guo, L., Teng, L., et al. (2019). Kawasaki disease shock syndrome: clinical characteristics and possible use of IL-6, IL-10 and IFN-γ as biomarkers for early recognition. *Pediatr. Rheumatol. Online J.* 17:1. doi: 10.1186/s12969-018-0303-4

Libby, P., and Simon, D. I. (2001). Inflammation and thrombosis: the clot thickens. *Circulation* 103, 1718–1720. doi: 10.1161/01.cir.103.13.1718

Liu, K., Fang, Y. Y., Deng, Y., Liu, W., Wang, M. F., and Ma, J. P. (2020). Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. *Chin. Med. J. (Engl)* 133, 1025–1031. doi: 10.1097/cm9.0000000000000744

Márquez, E. J., Trowbridge, J., Kuchel, G. A., Banchereau, J., and Ucar, D. (2020). The lethal sex gap: COVID-19. *Immun. Ageing* 17:13.

Mattioli, A. V., Sciomer, S., Cocchi, C., Maffei, S., and Gallina, S. (2020). Quarantine during COVID-19 outbreak: changes in diet and physical activity increase the

risk of cardiovascular disease. *Nutr. Metab. Cardiovasc. Dis.* 30, 1409–1417. doi: 10.1016/j.numecd.2020.05.020

McGonagle, D., Sharif, K., O'Regan, A., and Bridgewood, C. (2020). The role of cytokines including interleukin-6 in COVID-19 induced Pneumonia and macrophage activation syndrome-like disease. *Autoimmun. Rev.* 19:102537. doi: 10.1016/j.autrev.2020.102537

Mehta, P. K., and Griendling, K. K. (2007). Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am. J. Physiol.* 292, 82–97. doi: 10.1152/ajpcell.00287.2006

Murthy, H., Iqbal, M., Chavez, J. C., and Kharfan-Dabaja, M. A. (2019). Cytokine release syndrome: current perspectives. *Immunotargets Ther.* 8, 43–52. doi: 10.2147/ITT.S202015

Newburger, J. W., Takahashi, M., Gerber, M. A., Gewitz, M. H., Tani, L. Y., Burns, J. C., et al. (2004). Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the committee on rheumatic fever, endocarditis and Kawasaki disease, council on cardiovascular disease in the young, American Heart Association. *Circulation* 110, 2747–2771. doi: 10.1161/01.CIR.0000145143.19711.78

Oudit, G. Y., Kassiri, Z., Jiang, C., Liu, P. P., Poutanen, S. M., Penninger, J. M., et al. (2009). SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur. J. Clin. Invest.* 39, 618–625. doi: 10.1111/j.1365-2362.2009.02153.x

Pagliaro, P. (2020). Is macrophages heterogeneity important in determining COVID-19 lethality? *Med. Hypotheses* 143:110073. doi: 10.1016/j.mehy.2020.110073

Palmieri, L., Andrianou, X., Barbariol, P., Bella, A., Bellino, S., and Benelli, E. (2020). *Characteristics of SARS-CoV-2 Patients dying in Italy Report based on available data on May 21st, 2020*. Available online at: https://www.epicentro.iss.it/en/coronavirus/bollettino/Report-COVID-2019_23_april_2020.pdf (accessed April 23, 2020).

Park, S., Lee, H. Y., Cho, E. J., Sung, K. C., Kim, J., Kim, D. H., et al. (2020). Is the use of RAS inhibitors safe in the current era of COVID-19 pandemic? *Clin Hypertens.* 26:11. doi: 10.1186/s40885-020-00144-0

Patel, V. B., Bodiga, S., Basu, R., Das, S. K., Wang, W., Wang, Z., et al. (2012). Loss of angiotensin converting enzyme-2 exacerbates diabetic cardiovascular complications and leads to systolic and vascular dysfunction: a critical role of the angiotensin II/AT1 receptor axis. *Circ. Res.* 110, 1322–1335. doi: 10.1161/CIRCRESAHA.112.268029

Patel, V. B., Clarke, N., Wang, Z., Fan, D., Parajuli, N., Basu, R., et al. (2014). Angiotensin II induced proteolytic cleavage of myocardial ACE2 is mediated by TACE/ADAM-17: a positive feedback mechanism in the RAS. *J. Mol. Cell. Cardiol.* 66, 167–176. doi: 10.1016/j.yjmcc.2013.11.017

Penna, C., Mercurio, V., Tocchetti, C. G., and Pagliaro, P. (2020). Sex−related differences in COVID−19 lethality. *Br. J. Pharmacol.* 177, 4375–4385. doi: 10.1111/bph.15207

Phadke, M., and Saunik, S. (2020). Use of angiotensin receptor blockers such as Telmisartan, Losartsan in nCoV Wuhan Corona Virus infections—Novel mode of treatment. *BMJ* 368:406. doi: 10.1136/bmj.m406

Reiner, Ž, Hatamipour, M., Banach, M., Pirro, M., Al-Rasadi, K., Jamialahmadi, T., et al. (2020). Statins and the COVID-19 main protease: in silico evidence on direct interaction. *Arch. Med. Sci.* 16, 490–496. doi: 10.5114/aoms.2020.94655

Richardson, S., Hirsch, J. S., Narasimhan, M., Crawford, D. M., McGinn, T., Davidson, K. W., et al. (2020). Presenting characteristics, Comorbidities, and outcomes among 5700 patients hospitalized With COVID-19 in the New York City area. *JAMA* 323, 2052–2059. doi: 10.1001/jama.2020.6775

Ridker, P. M., Cushman, M., Stampfer, M. J., Tracy, R. P., and Hennekens, C. H. (1997). Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N. Engl. J. Med.* 336, 973–979. doi: 10.1056/NEJM199704033361401

Riphagen, S., Gomez, X., Gonzalez-Martinez, C., Wilkinson, N., and Theocharis, P. (2020). Hyperinflammatory shock in children during COVID-19 pandemic. *Lancet* 395, 1607–1608. doi: 10.1016/S0140-6736(20)31094-1

Shi, S., Qin, M., Shen, B., Cai, Y., Liu, T., Yang, F., et al. (2020). Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China. *JAMA Cardiol.* 5, 802–810. doi: 10.1001/jamacardio.2020.0950

Shimabukuro-Vornhagen, A., Gödel, P., Subklewe, M., Stemmler, H. J., Schlößer, H. A., Schlaak, M., et al. (2018). Cytokine release syndrome. *J. Immunother. Cancer* 6:56. doi: 10.1186/s40425-018-0343-9

Shirato, K., Imada, Y., Kawase, M., Nakagaki, K., Matsuyama, S., and Taguchi, F. (2014). Possible involvement of infection with human coronavirus 229E, but not NL63, in Kawasaki disease. *J. Med. Virol.* 86, 2146–2153. doi: 10.1002/jmv.23950

Steptoe, A., and Kivimäki, M. (2012). Stress and cardiovascular disease. *Nat. Rev. Cardiol.* 9, 360–370.

Tang, N., Li, D., Wang, X., and Sun, Z. (2020). Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J. Thromb. Haemost.* 18, 844–847. doi: 10.1111/jth.14768

The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team (2020). Vital surveillances: the epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) — China, 2020. *China CDC Wkly.* 2, 113–122. doi: 10.46234/ccdcw2020.032

Vaduganathan, M., Vardeny, O., Michel, T., McMurray, J. J. V., Pfeffer, M. A., and Solomon, S. D. (2020). Renin-angiotensin-aldosterone system inhibitors in patients with COVID-19. *N. Engl. J. Med.* 382, 1653–1659. doi: 10.1056/NEJMsr2005760

Verdecchia, P., Cavallini, C., Spanevello, A., and Angeli, F. (2020). The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur. J. Intern. Med.* 76, 14–20. doi: 10.1016/j.ejim.2020.04.037

Verdoni, L., Mazza, A., Gervasoni, A., Martelli, L., Ruggeri, M., Ciuffreda, M., et al. (2020). An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet* 395, 1771–1778. doi: 10.1016/S0140-6736(20)31103-X

Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T., and Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 181, 281–292. doi: 10.1016/j.cell.2020.02.058

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. (2020). Clinical characteristics of 138 hospitalized patients With 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA* 323, 1061–1069. doi: 10.1001/jama.2020.1585

Wang, L. (2020). C-reactive protein levels in the early stage of COVID-19. *Med. Mal. Infect.* 50, 332–334. doi: 10.1016/j.medmal.2020.03.007

Wang, Z., Zhou, Q., Wang, C., Shi, Q., Lu, S., Ma, Y., et al. (2020). Clinical characteristics of children with COVID-19: a rapid review and meta-analysis. *Ann. Transl. Med.* 8:620. doi: 10.21037/atm-20-3302

Whittaker, E., Bamford, A., Kenny, J., Kaforou, M., Jones, C. E., Shah, P., et al. (2020). Clinical Characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated With SARS-CoV-2. *JAMA* 324, 259–269. doi: 10.1001/jama.2020.10369

World Health Organization (2018). *WHO Global Action Plan on Physical Activity 2018–2030: More Active People for a Healthier World*. Geneva: World Health Organization.

World Health Organization (2020). *Multisystem Inflammatory Syndrome in Children and Adolescents with COVID-19*. Available online at: https://www.who.int/news-room/commentaries/detail/multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19 (accessed June 23, 2020).

Wu, D., and Yang, X. O. (2020). TH17 responses in cytokine storm of COVID-19: an emerging target of JAK2 inhibitor Fedratinib. *J. Microbiol. Immunol. Infect.* 53, 368–370. doi: 10.1016/j.jmii.2020.03.005

Wu, Z., and McGoogan, J. M. (2020). Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. *JAMA* 323, 1239–1242. doi: 10.1001/jama.2020.2648

Xiong, T. Y., Redwood, S., Prendergast, B., and Chen, M. (2020). Coronaviruses and the cardiovascular system: acute and long-term implications. *Eur. Heart J.* 41, 1798–1800. doi: 10.1093/eurheartj/ehaa231

Xu, X., Han, M., Li, T., Sun, W., Wang, D., Fu, B., et al. (2020). Effective treatment of severe COVID-19 patients with tocilizumab. *Proc. Natl. Acad. Sci. U.S.A.* 117, 10970–10975. doi: 10.1073/pnas.2005615117

Yan, S. F., Mackman, N., Kisiel, W., Stern, D. M., and Pinsky, D. J. (1999). Hypoxia/Hypoxemia-Induced activation of the procoagulant pathways and the pathogenesis of ischemia-associated thrombosis. *Arterioscler. Thromb. Vasc. Biol.* 19, 2029–2035. doi: 10.1161/01.atv.19.9.2029

Ye, Q., Wang, B., and Mao, J. (2020). The pathogenesis and treatment of the 'Cytokine Storm' in COVID-19. *J. Infect.* 80, 607–613. doi: 10.1016/j.jinf.2020.03.037

Yu, B., Steptoe, A., Chen, L. J., Chen, Y. H., Lin, C. H., and Ku, P. W. (2020). Social isolation, loneliness, and all-cause mortality in patients with cardiovascular disease: a 10-year follow-up study. *Psychosom. Med.* 82, 208–214. doi: 10.1097/psy.0000000000000777

Zhang, H., Penninger, J. M., Li, Y., Zhong, N., and Slutsky, A. S. (2020). Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med.* 46, 586–590. doi: 10.1007/s00134-020-05985-9

Zhang, J., Yang, Y., Yang, N., Ma, Y., Zhou, Q., Li, W., et al. (2020). Effectiveness of intravenous immunoglobulin for children with severe COVID-19: a rapid review. *Ann. Transl. Med.* 8:625. doi: 10.21037/atm-20-3305

Zhao, M., Wang, M., Zhang, J., Ye, J., Xu, Y., Wang, Z., et al. (2020). Advances in the relationship between coronavirus infection and cardiovascular diseases. *Biomed. Pharmacother.* 127:110230. doi: 10.1016/j.biopha.2020.110230

Zheng, S., Fan, J., Yu, F., Feng, B., Lou, B., Zou, Q., et al. (2020). Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. *BMJ* 369:m1443. doi: 10.1136/bmj.m1443

Zheng, Y. Y., Ma, Y. T., Zhang, J. Y., and Xie, X. (2020). COVID-19 and the cardiovascular system. *Nat. Rev. Cardiol.* 17, 259–260.

Zhong, J., Basu, R., Guo, D., Chow, F. L., Byrns, S., Schuster, M., et al. (2010). Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. *Circulation* 122, 717–728. doi: 10.1161/CIRCULATIONAHA.110.955369

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395, 1054–1062. doi: 10.1016/S0140-6736(20)30566-3

Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273. doi: 10.1038/s41586-020-2012-7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

PERSPECTIVE
published: 19 October 2020
doi: 10.3389/fphys.2020.573044

Heparin Therapy Improving Hypoxia in COVID-19 Patients – A Case Series

Elnara Marcia Negri[1,2], Bruna Mamprim Piloto[1,3†], Luciana Kato Morinaga[1,3†], Carlos Viana Poyares Jardim[1,3†], Shari Anne El-Dash Lamy[4†], Marcelo Alves Ferreira[2†], Elbio Antonio D'Amico[5†] and Daniel Deheinzelin[1†]*

[1] Sirio-Libanes Hospital, São Paulo, Brazil, [2] Cell Biology Laboratory (LIM 59), Hospital das Clinicas, University of Sao Paulo Medical School, São Paulo, Brazil, [3] Department of Pulmonary, Heart Institute, University of Sao Paulo Medical School, São Paulo, Brazil, [4] CriticalCare Unit, A. C. Camargo Cancer Center, São Paulo, Brazil, [5] Department of Hematology and Hemotherapy, University of Sao Paulo Medical School, São Paulo, Brazil

Introduction: Elevated D-dimer is a predictor of severity and mortality in COVID-19 patients, and heparin use during in-hospital stay has been associated with decreased mortality. COVID-19 patient autopsies have revealed thrombi in the microvasculature, suggesting that hypercoagulability is a prominent feature of organ failure in these patients. Interestingly, in COVID-19, pulmonary compliance is preserved despite severe hypoxemia corroborating the hypothesis that perfusion mismatch may play a significant role in the development of respiratory failure.

Methods: We describe a series of 27 consecutive COVID-19 patients admitted to Sirio-Libanes Hospital in São Paulo-Brazil and treated with heparin in therapeutic doses tailored to clinical severity.

Results: PaO2/FiO2 ratio increased significantly over the 72 h following the start of anticoagulation, from 254(±90) to 325(±80), p = 0.013, and 92% of the patients were discharged home within a median time of 11 days. There were no bleeding complications or fatal events.

Discussion: Even though this uncontrolled case series does not offer absolute proof that micro thrombosis in the pulmonary circulation is the underlying mechanism of respiratory failure in COVID-19, patient's positive response to heparinization contributes to the understanding of the pathophysiological mechanism of the disease and provides valuable information for the treatment of these patients while we await the results of further prospective controlled studies.

Keywords: COVID-19, respiratory failure, thrombosis, perfusion mismatch, heparin

OPEN ACCESS

Edited by:
Georges Leftheriotis, Université Côte d'Azur, France

Reviewed by:
Melissa L. Bates, The University of Iowa, United States
Marli Maria Knorst, Federal University of Rio Grande do Sul, Brazil

****Correspondence:***
Elnara Marcia Negri
emnegri@yahoo.com.br
orcid.org/0000-0002-6428-6066

†ORCID:
Bruna Mamprim Piloto
orcid.org/0000-0002-8756-0400
Luciana Kato Morinaga
orcid.org/0000-0002-0900-2737
Carlos Viana Poyares Jardim
orcid.org/0000-0003-0425-5548
Shari Anne El-Dash Lamy
orcid.org/0000-0003-2915-4014
Marcelo Alves Ferreira
orcid.org/0000-0003-4181-760X
Elbio Antonio D'Amico
orcid.org/0000-0003-1069-1469
Daniel Deheinzelin
orcid.org/0000-0002-0253-4124

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *15 June 2020*
Accepted: *28 September 2020*
Published: *19 October 2020*

Citation:
Negri EM, Piloto BM, Morinaga LK, Jardim CVP, Lamy SAE-D, Ferreira MA, D'Amico EA and Deheinzelin D (2020) Heparin Therapy Improving Hypoxia in COVID-19 Patients – A Case Series. Front. Physiol. 11:573044. doi: 10.3389/fphys.2020.573044

INTRODUCTION

Since the beginning of the COVID-19 pandemic, disease severity has been linked to markers of coagulation disturbances such as prothrombin time prolongation, elevated fibrin degradation products, reduced platelet count, and specially to elevated D dimer (Chen G. et al., 2020, Chen T. et al., 2020; Han et al., 2020; Tang et al., 2020; Wang D. et al., 2020; Zhang et al., 2020; Zhou et al., 2020). Higher levels of D dimer and the presence of other coagulation disturbances

have been independently associated with development of respiratory failure and death in patients with COVID-19 (Wu et al., 2020). The use of heparin, particularly in those patients with more pronounced elevations of D dimer and in those with elevated sepsis induced coagulopathy (SIC) score, has been associated with a better prognosis (Tang et al., 2020; Wu et al., 2020). Diabetic patients, whose levels of D dimer are greater than those of non-diabetic patients, have also been shown to have a worse prognosis regarding COVID-19 (Guo et al., 2020). Moreover, hypercoagulative features can differentiate severe COVID-19 associated pneumonia from that caused by other viruses (Yin et al., 2020).

Over the last months it has been consistently shown that SARS-Cov-2 causes a cytokine storm, endothelial and epithelial dysfunction, which ultimately lead to the activation of the coagulation cascade, causing thrombotic phenomena (Mehta et al., 2020; Tang et al., 2020; Teuwen et al., 2020; Wu et al., 2020). Similarly to what happens in severe sepsis, the widespread deposition of intravascular clots compromises adequate blood supply, contributing to organ failure (Burzynski et al., 2019).

Disseminated intravascular coagulation (DIC) secondary to severe infection is classically associated with gram-positive or gram-negative bacteria, malaria and haemorrhagic fevers, but other viruses, such as dengue (an hemorrhagic virus), SARS-CoV and MERS-CoV, can also be responsible for systemic activation of intravascular coagulation (de Wit et al., 2016; Giannis et al., 2020).

Furthermore, in contrast to the characteristic stiffening of the lung usually seen in acute respiratory distress syndrome (ARDS), in COVID-19 patients the severe hypoxemia observed is accompanied by near normal pulmonary compliance, especially in early stages (Gattinoni et al., 2020). Autopsy findings from COVID-19 patients show microthrombi in the pulmonary microvasculature (Dolhnikoff et al., 2020; Tian et al., 2020; Yao et al., 2020) suggesting that ventilation-perfusion mismatch due to capillary obstruction could be a pivotal feature in the refractory hypoxemia presented by these patients. The anatomical distribution of this peripheral vascular bed mirrors the predominantly distal and patchy distribution of the radiological infiltrates (Ye et al., 2020).

In one of our first COVID-19 patients we noticed a concomitance of peripheral ischaemia (acro-ischemia) with the onset of respiratory distress, an observation that led us to consider the hypothesis that the normal compliance respiratory failure might actually be due to extensive pulmonary capillary obstruction, and that an intense process of intravascular coagulation might be playing a significant role in hypoxemia and outcome of COVID-19 patients.

The treatment of DIC consists in slowing down the coagulation cascade by using low doses of anticoagulation, alongside vigorous specific treatment of the underlying disorder. We therefore considered adding early heparin therapy to our standard care (Chen G. et al., 2020). The present study is a description of the outcome, particularly regarding oxygenation, of the first 27 COVID-19 patients we treated with anticoagulation in the course of the disease.

METHODS

This study is a case series of 27 consecutive COVID-19 patients seen by our team in Sirio-Libanês Hospital – São Paulo, Brazil, between March 21st and April 12th, 2020. The study was approved by the Sirio-Libanês Hospital Institutional Review Board under the number 3993056 and informed consent was waived.

All patients received initially enoxaparin 0.5 mg/kg SC every 24 h. Patients with a creatinine clearance under 30 mL/min received subcutaneous unfractionated heparin at a dose of 5,000 units every 8 h. If an abrupt decrease in oxygenation or an increase in D Dimer levels was observed, enoxaparin dose was raised to 0.5 mg/kg SC every 12 h and, in the event of thrombotic phenomena or worsening hypoxia, the dose was further increased to 1 mg/kg SC every 12 h. Patients with a BMI (body mass index) of 35 or higher were also considered for the higher dose regimen. Patients in shock or intubated were treated from the beginning with intravenous heparin, targeting an APTT ratio around 1.5 to 2.0 times the normal range. If a patient presented any acute thrombotic event, heparin dosing was increased to obtain an APTT approximately 2.0 to 2.5 times the normal range.

All patients received a 10-day course of azithromycin (500 mg on day 1, then 250 mg daily) (Cramer et al., 2017). Methylprednisolone 40 mg daily was initiated if a worsening in the radiological pattern accompanied by an increase in serum LDH levels was observed. If the patient presented subsequent rise in C-reactive protein, we actively searched for secondary infection and promptly initiated antibiotics.

To evaluate severity of disease during hospital stay, we used the ordinal scale for clinical improvement proposed by the World Health Organization (WHO score): 0. – no clinical or virological evidence of infection; 1. no limitation of activities; 2. limitations of activities; 3. hospitalized, no oxygen therapy; 4. oxygen by mask or nasal prongs; 5. non-invasive ventilation or high-flow oxygen; 6. intubation and mechanical ventilation; 7. ventilation plus additional organ support (pressors, renal replacement therapy, ECMO); 8. death (World Health Organization [WHO], 2020).

RESULTS

We followed a total of 27 hospitalized patients with a diagnosis of COVID-19, all confirmed by PCR. Seventy percent were male, their mean age was 56 ± 17 years, mean BMI was 28.8 ± 6 kg/m^2, and comorbidities were present in 67% of them. Individual data from all patients are presented at **Table 1**. The mean WHO score at admittance was 4.0 ± 1.2 (and the mean maximum score achieved during hospitalization was 4.6 ± 1.6). Entry CT scans showed radiologic infiltrates compromising up to 25% of lung area in 22% of patients, 25–50% of lung area in 48% of patients, and 30% of patients presented infiltrates in over half of lung parenchyma. Symptoms started at an average of 9.6 ± 4.0 days prior to hospitalization, and the anticoagulation protocol was initiated at an average of 3.4 ± 4.0 days after admission. Nineteen patients received methylprednisolone in the course of the disease.

TABLE 1 | Individual data from all patients included at admission and during evolution.

Subject	Age (years)	Gender	Comorbidities	WHO score	D-dimer (ng/mL FEU)	LDH (U/L)	Platelet count (*10^3/mm^3)	Antibiotics	Corticosteroids	Anticoagulation	In-hospital days	Bleeding	Macro-thrombosis	Outcome
1	52	M	None	Adm – 3 Max – 4	Adm – 480 Max – 20000	Adm – 271 Max – 271		Yes	Yes	Enoxaparin 2 mg/kg/d	9	No	No	Discharged
2	45	M	None	Adm – 4 Max – 4	Adm – 1360 Max – 780	Adm – 653 Max – 653	Adm – 247 Min – 247 Max – 514	Yes	Yes	Enoxaparin 1 mg/kg/d	4	No	No	Discharged
3	46	F	Breast cancer	Adm – 3 Max – 3	Adm – 561 Max – 561	Adm – 549 Max – 635	Adm – 175 Min – 232 Max – 175	Yes	Yes	Enoxaparin 0.5 mg/kg/d	4	No	No	Discharged
4	46	M	None	Adm – 3 Max – 3	Adm – 1053 Max – 1053	Adm – 532 Max – 532	Adm – 176 Min – 176 Max – 279	Yes	No	Enoxaparin 0.5 mg/kg/d	5	No	No	Discharged
5	79	M	Atrial fibrillation	Adm – 4 Max – 4	Adm – 587 Max – 685	Adm – 540 Max – 626	Adm – 104 Min – 101 Max – 560	Yes	Yes	Enoxaparin 1 mg/kg/d	14	No	No	Discharged
6	66	M	Obesity Hypertension COPD	Adm – 4 Max – 7	Adm – 687 Max – 7519	Adm – 502 Max – 902	Adm – 142 Min – 129 Max – 340	Yes	Yes	IV Heparin	57	No	No	Discharged
7	39	M	None	Adm – 3 Max – 3	Adm – 339 Max – 1228	Adm – 498 Max – 700	Adm – 172 Min – 172 Max – 749	Yes	No	Enoxaparin 1 mg/kg/d	10	No	No	Discharged
8	96	F	COPD Parkinson	Adm – 3 Max – 4	Adm – 525 Max – 1853	Adm – 605 Max – 609	Adm – 204 Min – 167 Max – 254	Yes	Yes	Enoxaparin 1 mg/kg/d	9	No	No	Discharged
9	63	M	Diabetes	Adm – 4 Max – 7	Adm – 644 Max – 6899	Adm – 731 Max – 810	Adm – 155 Min – 155 Max – 474	Yes	Yes	IV Heparin	76	No	No	Discharged
10	76	M	Coronary Heart Disease	Adm – 4 Max – 7	Adm – 1583 Max – 6522	Adm – 805 Max – 805	Adm – 133 Min – 111 Max – 580	Yes	Yes	IV Heparin	142	No	No	Still in hospital
11	68	F	Hypertension Hyperthyroidism	Adm – 3 Max – 7	Adm – 316 Max – 954	Adm – 436 Max – 648	Adm – 225 Min – 167 Max – 404	Yes	Yes	IV Heparin	24	No	No	Discharged
12	76	F	Atrial fibrillation	Adm – 3 Max – 7	Adm – 1968 Max – > 10000	Adm – 660 Max – 1045	Adm – 158 Min – 144 Max – 419	Yes	Yes	IV Heparin	104	No	VTE	Discharged
13	64	M	Hypertension Diabetes	Adm – 3 Max – 7	Adm – 342 Max – 3014	Adm – 508 Max – 518	Adm – 190 Min – 190 Max – 550	Yes	Yes	IV Heparin	30	No	No	Discharged

(Continued)

TABLE 1 | Continued

Subject	Age (years)	Gender	Comorbidities	WHO score	D-dimer (ng/mL FEU)	LDH (U/L)	Platelet count (*10^3/mm^3)	Antibiotics	Corticosteroids	Anticoagulation	In-hospital days	Bleeding	Macrothrombosis	Outcome
14	55	M	Hypertension	Adm – 5 Max – 6	Adm – 679 Max – 3599	Adm – 705 Max – 705	Adm – 295 Min – 295 Max – 460	Yes	Yes	IV Heparin	16	No	No	Discharged
15	66	M	Hypertension	Adm – 3 Max – 3	Adm – 441 Max – 441	Adm – 566 Max – 566	Adm – 224 Min – 224 Max – 285	Yes	No	Enoxaparin 0.5 mg/kg/d	3	No	No	Discharged
16	45	F	None	Adm – 3 Max – 3	Adm – 510 Max – 843	Adm – 461 Max – 461	Adm – 279 Min – 279 Max – 334	Yes	No	Enoxaparin 0.5 mg/kg/d	5	No	No	Discharged
17	53	F	Hypertension	Adm – 3 Max – 4	Adm – 395 Max – 406	Adm – 743 Max – 1034	Adm – 249 Min – 249 Max – 706	Yes	Yes	Enoxaparin 1 mg/kg/d	8	No	No	Discharged
18	35	M	Obesity Asthma	Adm – 3 Max – 3	Adm – 438 Max – 438	Adm – 520 Max – 599	Adm – 235 Min – 205 Max – 266	Yes	No	Enoxaparin 0.5 mg/kg/d	3	No	No	Discharged
19	52	M	Tobacco dependence	Adm – 3 Max – 4	Adm – 505 Max – 912	Adm – 368 Max – 830	Adm – 167 Min – 140 Max – 423	Yes	No	Enoxaparin 0.5 mg/kg/d	15	No	No	Discharged
20	67	F	Treated lymphoma	Adm – 3 Max – 4	Adm – 823 Max – 1234	Adm – 636 Max – 888	Adm – 125 Min – 105 Max – 325	Yes	Yes	Enoxaparin 1 mg/kg/d	12	No	No	Discharged
21	32	M	None	Adm – 3 Max – 4	Adm – 416 Max – 416	Adm – 408 Max – 797	Adm – 278 Min – 274 Max – 515	Yes	Yes	Enoxaparin 2 mg/kg/d	13	No	No	Discharged
22	66	M	Hypertension	Adm – 3 Max – 7	Adm – 720 Max – 4833	Adm – 611 Max – 618	Adm – 271 Min – 256 Max – 498	Yes	Yes	IV Heparin	22	No	No	Discharged
23	22	M	None	Adm – 3 Max – 3	Adm – 245 Max – 362	Adm – 520 Max – 970	Adm – 249 Min – 249 Max – 290	Yes	No	Enoxaparin 1 mg/kg/d	3	No	No	Discharged
24	42	F	None	Adm – 4 Max – 4	Adm – 644 Max – 1242	Adm – 571 Max – 716	Adm – 263 Min – 263 Max – 408	Yes	Yes	Enoxaparin 1 mg/kg/d	6	No	No	Discharged
25	65	M	Obesity	Adm – 4 Max – 4	Adm – 569 Max – 1346	Adm – 571 Max – 571	Adm – 186 Min – 179 Max – 265	Yes	No	Enoxaparin 1 mg/kg/d		No	No	Transferred
26	79	M	Cerebrovascular disease	Adm – 3 Max – 4	Adm – 1449 Max – 1686	Adm – 535 Max – 678	Adm – 267 Min – 217 Max – 318	Yes	Yes	Enoxaparin 1 mg/kg/d	12	No	No	Discharged
27	35	M	None	Adm – 4 Max – 5	Adm – 555 Max – 605	Adm – 630 Max – 709	Adm – 134 Min – 134 Max – 331	Yes	Yes	Enoxaparin 2 mg/kg/d	9	No	No	Discharged

Six patients received only the prophylactic dosage of heparin or enoxaparin; three patients started already with enoxaparin 0.5 mg/Kg twice and were kept on this dosage and in 18 patient's dosages were escalated to either full EV heparin or enoxaparin 1 mg/kg twice a day.

As of August 11th, of the 27 consecutive patients, 25(92%) were discharged from hospital after an median of 11 days. One patient was transferred to another hospital on the 4th day and lost follow-up. Nine patients (33%) were admitted to ICU, 8 (89%) of which have already been discharged to the ward after a median time of 44 days. Eight patients (30%) required intubation, and seven patients have already been successfully weaned after a median time of 11,5 days of mechanical ventilation. One patient is still under mechanical ventilation and required a tracheostomy. This patient had an infected sacral pressure ulcer and required multiple surgical procedures.

Interestingly enough, rotational thromboelastometry (ROTEM) performed in four patients, showed an increase in α-angle, amplitude 10 min after clotting time (A10) and maximum clot firmness (MCF) pointing to a persistent hypercoagulability state, despite their ongoing heparin use.

Figure 1 depicts the gradual improvement in PaO_2/FiO_2 ratio along the first 72 h in relation to pre-anticoagulation values. Analysis was conducted for the whole series (A) and considering only patients with moderate to severe disease (B) according to the WHO score ($p < 0.02$ for both groups). For non-mechanically ventilated patients PaO_2/FiO_2 ratio was calculated according to mask or nasal catheter oxygen flow and oxymetry (Lobete et al., 2013).

We observed no deaths due to any cause or haemorrhagic complications due to anticoagulation during the study period. Moreover, after three months, all but one patient were discharged home without supplementary oxygen.

DISCUSSION

Our results suggest the important role of hypercoagulative state and microthrombosis as the main mechanisms of organ failure in COVID-19 and the potential response to early anticoagulation therapy.

The significant improvement in oxygen exchange and clinical symptoms observed in these COVID-19 patients, in response to the anticoagulation, points to a potential role for systematic use of heparin in the treatment of such patients. The high incidence of thrombotic events that has been reported in COVID-19 patients (Klok et al., 2020), confirmed more recently, even in the presence of usual heparin prophylaxis (Middeldorp et al., 2020), as well as the fact that similar observations were reported in the other recent coronavirus outbreaks (de Wit et al., 2016; Giannis et al., 2020), further corroborate with this line of reasoning. This is not surprising, as severe cases of COVID-19 meet the laboratory criteria of DIC (Tang et al., 2020; Wu et al., 2020) of thrombotic pattern, in which fibrinogen does not drop and prothrombotic phenomena override the haemorrhagic ones (Wada et al., 2014). Moreover, specifically in patients with respiratory insufficiency caused by COVID-19 under mechanical ventilation, increased d-dimer, abnormal thromboelastography and high levels of fibrinogen point to a hypercoagulative status (Ranucci et al., 2020; Wright et al., 2020). Markers of hypercoagulability has been shown to be independent predictors of increased oxygen requirements in patients with COVID-19 (Rauch et al., 2020).

Thromboelastography showing a pattern of hypercoagulability despite the use of heparin during the course of viral diseases has been previously reported (Wilson et al., 2016). In fact, many viruses known to induce a state of hypercoagulability (Subramaniam and Scharrer, 2018) have a similar pattern of disease, including the timeframe of

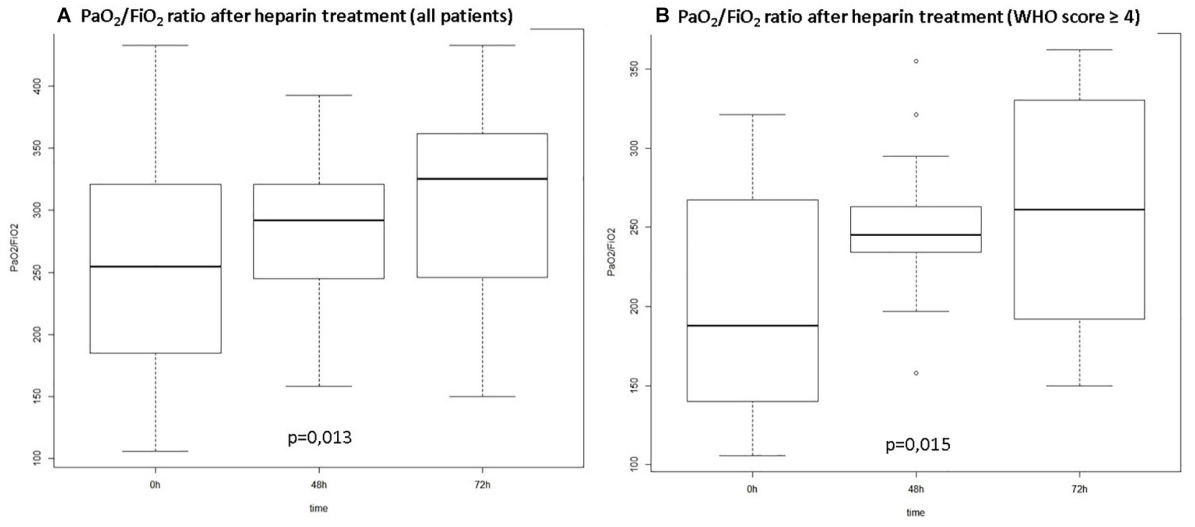


FIGURE 1 | PO_2/FiO_2 ratio over time from start of anticoagulation. **(A)** All patients included; **(B)** Patients with WHO score ≥4 at hospital admission (WHO Score: ordinal scale for clinical improvement proposed by the World Health Organization: 0. – no clinical or virological evidence of infection; 1. no limitation of activities; 2. limitations of activities; 3. hospitalized, no oxygen therapy; 4. oxygen by mask or nasal prongs; 5. non-invasive ventilation or high-flow oxygen; 6. intubation and mechanical ventilation; 7. ventilation plus additional organ support – pressors, renal replacement therapy, ECMO; 8. Death).

clinical manifestations (Gai et al., 2012), suggesting a common pattern of response.

Multiple phenomena are involved in the hypercoagulative status in COVID 19: the extensive denudation of epithelial and endothelial spaces causing a massive exposure of tissue factor, production of Von Willeband factor, platelet activation, netosis and pyroptosis, have been described as promoters of extensive microcirculation thrombosis in the severe cases of this disease (Wada et al., 2014; Teuwen et al., 2020). It has been shown that in patients with COVID-19, NETs increased with intubation or death as outcome and were inversely correlated with PaO2/FiO2 (Brinkmann et al., 2004; Middleton et al., 2020). Many autopsy findings confirm this pathophysiological rationale, showing a large amount of microthrombosis as well venous and arterial thrombosis in deceased patients (Dolhnikoff et al., 2020; Yao et al., 2020). Ultrastructural findings also corroborate the endothelial and epithelial destruction in multiple organs (Ackermann et al., 2020). More recently heparin treatment has been pointed to decrease mortality in severe COVID-19 (Ayerbe et al., 2020).

The PaO_2/FiO_2 ratio improvement observed in our patients after starting heparin is in agreement with the idea of a significant perfusion component explaining the mechanism of respiratory failure with the distinct pattern of marked hypoxia and preserved lung compliance that characterizes severe COVID-19 patients. It has been argued that this could be secondary to the loss of lung perfusion regulation and hypoxic vasoconstriction (Tian et al., 2020), but the clinical response to heparin rather suggests hypoxia due to extensive clogging of pulmonary microcirculation. HRCT studies have shown a consistent reduction in pulmonary blood volume in COVID-19 patients compared to healthy controls, particularly in vessels smaller than 5 mm, again pointing to microthrombi as a cause for hypoxia (Lins et al., 2020). Using electrical impedance tomography, it has been shown that dead space fraction was much more relevant than the shunt fraction as an explanation for the gas exchange derangement observed in the course of disease (Mauri et al., 2020). Moreover when a diagnosis of pulmonary embolism is made in patients with COVID-19, the phenotype is different than embolism in other patients, since it occurs only in areas already affected by the virus, with a lower thrombus load and lower prevalence of proximal embolism of main arteries (van Dam et al., 2020). Interestingly the use of tissue Plasminogen Activator (tPA) has been shown to promote a non-sustained elevation of PaO2/FiO2 ratio (Wang J. et al., 2020). In our opinion, given the marked hypercoagulability seen in these patients – and again in accordance with the autopsy findings – judicious tailoring of heparin doses is needed to prevent capillary reocclusion while avoiding the risks of bleeding complications.

The fact that this is a retrospective study without a control arm does not yet allow us to definitively conclude that heparin in tailored doses should be systematically employed in all COVID-19 patients. Nonetheless, our findings in this early group of patients certainly provide food for thought and perhaps a rationale to justify using a readily available and well-known drug such as heparin, even in larger doses than previously recommended to ameliorate the dim prognosis of such sick patients while we await the more solid data on this subject, as suggested recently (Iba et al., 2020).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Hospital Sirio-Libanes Institutional Review Board – Number of approval: 3.993.056. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors contributed equally on this manuscript.

ACKNOWLEDGMENTS

We would like to thank the Teaching Research Institute (IEP), Sirio-Libanes Hospital, São Paulo, Brazil. We also would like to acknowledge the hospital staff and their instrumental role in caring for all patients. This manuscript has been released as a pre-print at medRxiv (medRxiv 2020:2020.04.15.20067017) (Negri et al., 2020).

REFERENCES

Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al. (2020). Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in covid-19. *N. Engl. J. Med.* 383, 120–128. doi: 10.1056/nejmoa2015432

Ayerbe, L., Risco, C., and Ayis, S. (2020). The association between treatment with heparin and survival in patients with Covid-19. *J. Thromb.* 50, 298–301. doi: 10.1007/s11239-020-02162-z

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S., et al. (2004). Neutrophil extracellular traps kill bacteria. *Science* 303, 1532–1535. doi: 10.1126/science.1092385

Burzynski, L. C., Humphry, M., Pyrillou, K., Wiggins, K. A., Chan, J. N. E., Figg, N., et al. (2019). The coagulation and immune systems are directly linked through the activation of interleukin-1alpha by Thrombin. *Immunity* 50, 1033.e6-1042.e6.

Chen, G., Wu, D., Guo, W., Cao, Y., Huang, D., Wang, H., et al. (2020). Clinical and immunological features of severe and moderate coronavirus disease 2019. *J. Clin. Invest.* 130, 2620-2629.

Chen, T., Wu, D., Chen, H., Yan, W., Yang, D., Chen, G., et al. (2020). Clinical characteristics of. (113) deceased patients with coronavirus disease 2019: retrospective study. *BMJ* 368:m1091. doi: 10.1136/bmj.m1091

Cramer, C. L., Patterson, A., Alchakaki, A., and Soubani, A. O. (2017). Immunomodulatory indications of azithromycin in respiratory disease: a

concise review for the clinician. *Postgrad Med.* 129, 493–499. doi: 10.1080/00325481.2017.1285677

de Wit, E., van Doremalen, N., Falzarano, D., and Munster, V. J. (2016). SARS and MERS: recent insights into emerging coronaviruses. *Nat. Rev. Microbiol.* 14, 523–534. doi: 10.1038/nrmicro.2016.81

Dolhnikoff, M., Duarte-Neto, A. N., de Almeida Monteiro, R. A., Ferraz da Silva, L. F., Pierre de Oliveira, E., Nascimento Saldiva, P. H., et al. (2020). Pathological evidence of pulmonary thrombotic phenomena in severe COVID-19. *J. Thromb. Haemost.* 18, 1517–1519. doi: 10.1111/jth.14844

Gai, Z. T., Zhang, Y., Liang, M. F., Jin, C., Zhang, S., Zhu, C. B., et al. (2012). Clinical progress and risk factors for death in severe fever with thrombocytopenia syndrome patients. *J. Infect. Dis.* 206, 1095–1102.

Gattinoni, L., Coppola, S., Cressoni, M., Busana, M., Rossi, S., and Chiumello, D. (2020). Covid-19 does not lead to a "Typical" acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 201, 1299–1300. doi: 10.1164/rccm.202003-0817le

Giannis, D., Ziogas, I. A., and Gianni, P. (2020). Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. *J. Clin. Virol.* 127, 1386–1392.

Guo, W., Li, M., Dong, Y., Zhou, H., Zhang, Z., Tian, C., et al. (2020). Diabetes is a risk factor for the progression and prognosis of COVID-19. *Diabetes Metab. Res. Rev.* 31e:3319.

Han, H., Yang, L., Liu, R., Liu, F., Wu, K. L., Li, J., et al. (2020). Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. *Clin. Chem. Lab. Med.* 58, 1116-1120.

Iba, T., Levy, J. H., Levi, M., and Thachil, J. (2020). Coagulopathy in COVID-19. *J. Thromb. Haemost.* 18, 2103–2109.

Klok, F., Kruip, M., van der Meer, N., Arbous, M., Gommers, D., Kant, K., et al. (2020). Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb. Res.* 191, 145-147.

Lins, M., Vandevenne, J., Thillai, M., Lavon, B. R., Lanclus, M., Bonte, S., et al. (2020). Assessment of small pulmonary blood vessels in COVID-19 patients using HRCT. *Acad .Radiol.* 27, 1449-1455.

Lobete, C., Medina, A., Rey, C., Mayordomo-Colunga, J., Concha, A., and Menendez, S. (2013). Correlation of oxygen saturation as measured by pulse oximetry/fraction of inspired oxygen ratio with Pao2/fraction of inspired oxygen ratio in a heterogeneous sample of critically ill children. *J. Crit. Care.* 28, 538.e1–538.e7.

Mauri, T., Spinelli, E., Scotti, E., Colussi, G., Basile, M. C., Crotti, S., et al. (2020). Potential for lung recruitment and ventilation-perfusion mismatch in patients with the acute respiratory distress syndrome from coronavirus disease 2019. *Crit. Care Med.* 48, 1129–1134. doi: 10.1097/ccm.0000000000004386

Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., Manson, J. J., et al. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 395, 1033–1034. doi: 10.1016/s0140-6736(20)30628-0

Middeldorp, S., Coppens, M., van Haaps, T. F., Foppen, M., Vlaar, A. P., Muller, M. C. A., et al. (2020). Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J. Thromb. Haemost.* 18, 1995–2002.

Middleton, E. A., He, X. Y., Denorme, F., Campbell, R. A., Ng, D., Salvatore, S. P., et al. (2020). Neutrophil Extracellular Traps (NETs) Contribute to Immunothrombosis in COVID-19 Acute Respiratory Distress Syndrome. *Blood* 136, 1169-1179.

Negri, E. M., Piloto, B., Morinaga, L. K., Jardim, C. V. P., Lamy, S. A. E.-D., Ferreira, M. A., et al. (2020). Heparin therapy improving hypoxia in COVID-19 patients - a case series. *medRxiv[Preprint]*

Ranucci, M., Ballotta, A., Di Dedda, U., Bayshnikova, E., Dei Poli, M., Resta, M., et al. (2020). The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. *J. Thromb. Haemost.* 18, 1747–1751. doi: 10.1111/jth.14854

Rauch, A., Labreuche, J., Lassalle, F., Goutay, J., Caplan, M., Charbonnier, L., et al. (2020). Coagulation biomarkers are independent predictors of increased oxygen requirements in COVID-19. *J. Thromb. Haemost.* [Epub ahead of print].

Subramaniam, S., and Scharrer, I. (2018). Procoagulant activity during viral infections. *Front. Biosci.* 23:1060–1081. doi: 10.2741/4633

Tang, N., Bai, H., Chen, X., Gong, J., Li, D., and Sun, Z. (2020). Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J. Thromb. Haemost* 18, 1094-1099.

Teuwen, L. A., Geldhof, V., Pasut, A., and Carmeliet, P. (2020). COVID-19: the vasculature unleashed. *Nat Rev Immunol.* 20, 389–391. doi: 10.1038/s41577-020-0343-0

Tian, S., Hu, W., Niu, L., Liu, H., Xu, H., and Xiao, S. Y. (2020). Pulmonary pathology of early-phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. *J. Thorac. Oncol.* 15, 700-704.

van Dam, L. F., Kroft, L. J. M., van der Wal, L. I., Cannegieter, S. C., Eikenboom, J., de Jonge, E., et al. (2020). Clinical and computed tomography characteristics of COVID-19 associated acute pulmonary embolism: a different phenotype of thrombotic disease? *Thromb. Res.* 193, 86–89. doi: 10.1016/j.thromres.2020.06.010

Wada, H., Matsumoto, T., Yamashita, Y., and Hatada, T. (2014). Disseminated intravascular coagulation: testing and diagnosis. *Clin. Chim. Acta.* 436, 130–134. doi: 10.1016/j.cca.2014.04.020

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. (2020). Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 323, 1061-1069.

Wang, J., Hajizadeh, N., Moore, E. E., McIntyre, R. C., Moore, P. K., Veress, L. A., et al. (2020). Tissue plasminogen activator (tPA) treatment for COVID-19 associated acute respiratory distress syndrome (ARDS): a Case Series. *J. Thromb. Haemost* 18, 1752-1755.

Wilson, A. J., Martin, D. S., Maddox, V., Rattenbury, S., Bland, D., Bhagani, S., et al. (2016). Thromboelastography in the Management of Coagulopathy Associated With Ebola Virus Disease. *Clin Infect Dis.* 62, 610–612. doi: 10.1093/cid/civ977

World Health Organization [WHO] (2020). *Coronavirus disease (COVID-2019) R&D*. Geneva: WHO.

Wright, F. L., Vogler, T. O., Moore, E. E., Moore, H. B., Wohlauer, M. V., Urban, S., et al. (2020). Fibrinolysis Shutdown Correlation with Thromboembolic Events in Severe COVID-19 Infection. *J. Am. Coll. Surg.* 231, 193.e1-203.e1.

Wu, C., Chen, X., Cai, Y., Xia, J., Zhou, X., Xu, S., et al. (2020). Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 Pneumonia in Wuhan, China. *JAMA Intern. Med.* 180, 934-943.

Yao, X. H., Li, T. Y., He, Z. C., Ping, Y. F., Liu, H. W., Yu, S. C., et al. (2020). A pathological report of three COVID-19 cases by minimally invasive autopsies. *Zhonghua Bing Li Xue Za Zhi.* 49:E009.

Ye, Z., Zhang, Y., Wang, Y., Huang, Z., and Song, B. (2020). Chest CT manifestations of new coronavirus disease 2019 (COVID-19): a pictorial review. *Eur. Radiol.* 30, 4381-4389.

Yin, S., Huang, M., Li, D., and Tang, N. (2020). Difference of coagulation features between severe pneumonia induced by SARS-CoV2 and non-SARS-CoV2. *J. Thromb. Thromb.* 3, 1-4.

Zhang, J. J., Dong, X., Cao, Y. Y., Yuan, Y. D., Yang, Y. B., Yan, Y. Q., et al. (2020). Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 75, 1730-1741.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* 395, 1054–1062. doi: 10.1016/s0140-6736(20)30566-3

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

PERSPECTIVE
published: 06 November 2020
doi: 10.3389/fphys.2020.571367

SARS-CoV-2 Aiming for the Heart: A Multicenter Italian Perspective About Cardiovascular Issues in COVID-19

Matteo Briguglio[1], Mauro Porta[2], Francesca Zuffada[3], Alberto R. Bona[4], Tiziano Crespi[5], Fabio Pino[5], Paolo Perazzo[5], Marco Mazzocchi[5], Riccardo Giorgino[6], Giuseppe De Angelis[7], Alfonso Ielasi[8], Giuseppe De Blasio[9] and Maurizio Turiel[9]*

[1]IRCCS Orthopedic Institute Galeazzi, Scientific Direction, Milan, Italy, [2]IRCCS Orthopedic Institute Galeazzi, Neurology Unit, Milan, Italy, [3]ICCS Istituto Clinico Città Studi, Cardiology Unit, Milan, Italy, [4]ICCS Istituto Clinico Città Studi, Neurosurgery Unit, Milan, Italy, [5]IRCCS Orthopedic Institute Galeazzi, Intensive Care Unit, Milan, Italy, [6]University of Milan, Residency Program in Orthopedics and Traumatology, Milan, Italy, [7]ASST Rhodense, Cardiology Unit, Rho, Italy, [8]Istituto Clinico Sant'Ambrogio, Cardiology Unit, Milan, Italy, [9]IRCCS Orthopedic Institute Galeazzi, Cardiology Unit, Milan, Italy

OPEN ACCESS

Edited by:
Emmanuelle Vidal-Petiot, Assistance Publique Hopitaux De Paris, France

Reviewed by:
Pasquale Pagliaro, University of Turin, Italy
Maurizio Pesce, Centro Cardiologico Monzino (IRCCS), Italy

***Correspondence:**
Matteo Briguglio
matteo.briguglio@grupposandonato.it

Specialty section:
This article was submitted to Clinical and Translational Physiology, a section of the journal Frontiers in Physiology

Received: *10 June 2020*
Accepted: *30 September 2020*
Published: *06 November 2020*

Citation:
Briguglio M, Porta M, Zuffada F, Bona AR, Crespi T, Pino F, Perazzo P, Mazzocchi M, Giorgino R, De Angelis G, Ielasi A, De Blasio G and Turiel M (2020) SARS-CoV-2 Aiming for the Heart: A Multicenter Italian Perspective About Cardiovascular Issues in COVID-19. Front. Physiol. 11:571367. doi: 10.3389/fphys.2020.571367

The rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the high fatality rate of coronavirus disease 2019 (COVID-19) have been putting a strain on the world since December 2019. Infected individuals exhibit unpredictable symptoms that tend to worsen if age is advanced, a state of malnutrition persists, or if cardiovascular comorbidities are present. Once transmitted, the virus affects the lungs and in predisposed individuals can elicit a sequela of fatal cardiovascular consequences. We aim to present the pathophysiology of COVID-19, emphasizing the major cellular and clinical manifestations from a cardiological perspective. As a roaming viral particle or more likely *via* the Trojan horse route, SARS-CoV-2 can access different parts of the body. Cardiovascular features of COVID-19 can count myocardial injuries, vasculitis-like syndromes, and atherothrombotic manifestations. Deviations in the normal electrocardiogram pattern could hide pericardial effusion or cardiac inflammation, and dispersed microthrombi can cause ischemic damages, stroke, or even medullary reflex dysfunctions. Tailored treatment for reduced ejection fraction, arrhythmias, coronary syndromes, macrothrombosis and microthrombosis, and autonomic dysfunctions is mandatory. Confidently, evidence-based therapies for this multifaceted nevertheless purely cardiological COVID-19 will emerge after the global assessment of different approaches.

Keywords: cardiovascular system, coronavirus, SARS-CoV-2, COVID-19, infections, virulence, host-pathogen interactions, quality of health care

THE JOURNEY OF SARS-CoV-2

The little understanding of the natural diversity of the severe acute respiratory syndrome-related coronaviruses (SARS-CoVs) restricts the opportunities to control their zoonotic spillovers (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). Humans are therefore increasingly affected by outbreaks that put millions of people at risk. After the plagues of severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) in 2003 and of Middle East respiratory syndrome-related coronavirus

(MERS-CoV) in 2012, a familial coronavirus (SARS-CoV-2) was discovered after the first documented virus-related pneumonia in China at the end of December 2019. This new strain is primarily transmitted through respiratory droplets and is able to survive in the airway mucosa despite the presence of cleaning epithelial cells, protective lymphoid tissues, and immunocompetent nerve endings (Briguglio et al., 2020a). The optimized genomic feature to bind to the angiotensin-converting enzyme 2 (ACE2), which derives from either millions of random natural mutations during unnoticed human-to-human transmission (Xu et al., 2020a) or artificial laboratory manipulations (Andersen et al., 2020), is the major determinant for the highest viral replication (Hoffmann et al., 2020) and for the consequent respiratory (Harapan et al., 2020) and cardiovascular implications (Wu et al., 2020b). After acquiring a sufficiently high viral load in the upper cavity (Zou et al., 2020), SARS-CoV-2 infects the goblet and ciliated cells in charge of sputum expectoration (Sungnak et al., 2020). The diffusion through the mucous layer allows the ease of infection of alveolar epithelial type II cells and systemic organs that express ACE2 (Briguglio et al., 2020a). The resulting illness, named coronavirus disease 2019 (COVID-19), is multifaceted and unpredictable and can manifest with early smell disorders in over 80% of cases or result in the most severe conditions like sepsis-like shock or respiratory failure in 14% of cases (Remy et al., 2020; Wu and McGoogan, 2020). Globally, it has been observed that 1 in 16 patients has encountered fatal consequences (WHO situation report 132, May–June 2020), and several infected patients were old and malnourished (Briguglio et al., 2020d; Sattar et al., 2020). Importantly, epidemiological data have been shown that preexisting cardiovascular conditions could be another central virulence factor for disease progression. In addition, clinical findings showed that not a few numbers of COVID-19 patients encounter cardiac symptoms (Mehra et al., 2020). Since each structure and function of the cardiovascular system shows severe implications, it is crucial to discuss from a cardiological perspective the relationship between SARS-CoV-2 infection and the cardiovascular system in order to shed some light on the mechanisms that can lead to cardiac symptoms or fatal consequences in COVID-19 patients.

VIRUS-ASSOCIATED DAMAGE, PHASES OF DISEASE, AND PATIENT CLASSIFICATION

It is necessary to differentiate the types of SARS-CoV-2-associated damages, the various stages of the disease, and the classification of infected patients. The virus-associated damage is of two types:

- Type I damage (i.e., cytotoxicity), which is directly associated with the infiltration of the virus in those cells expressing ACE2 (pneumocytes, endothelial cells, cardiomyocytes, neuronal cells). This may lead to acute injuries in the lungs, the vasculature, the myocardium, and the brain (Kabbani and Olds, 2020; Mason, 2020).
- Type II damage, which occurs during the disease progression. It derives from hypoxemia, inflammation, and microthrombosis. In particular, pneumonia and acute respiratory distress syndrome are likely to lead to a mismatch between oxygen supply and demand (hypoxic damage). Moreover, the late increase in circulating cytokines is known to cause nonischemic multiple organ injuries (e.g., stress-cardiomyopathy, myocarditis, vasculitis-like syndromes), and the systemic inflammation or catecholamine rush are associated with plaque rupture or blood hypercoagulability (i.e., thrombi-derived ischemic damage; Basu-Ray et al., 2020; Matsushita et al., 2020; Xiong et al., 2020; Zheng et al., 2020).

Considering the disease progression, three distinct phases have been recognized, covering the early infection mechanisms, the body's response to the viral proliferation, and the late systemic phase of the illness.

- The incubation/proliferative phase: mild-to-moderate symptoms with fever, dry cough, headache, pharyngodynia, asthenia. This phase is biochemically characterized by mild lymphopenia and variations in some coagulation parameters, such as the D-dimer, thrombocytes, and international normalized ratio (INR). Lactate dehydrogenase as well as inflammatory markers like C-reactive protein and interleukin-6 may increase (Shi et al., 2020). Therapies to boost the immune response are certainly worth considering since early B lymphocyte reduction affects antibody production (Siddiqi and Mehra, 2020). This phase usually lasts a few days (Briguglio et al., 2020a).
- The respiratory phase: moderate-to-severe respiratory symptoms like shortness of breath and measurable hypoxemia. If a dysfunctional immune system was present, SARS-CoV-2 could proliferate quickly and lead to massive impairments of infiltrated tissues. This phase is characterized by increasing circulating levels of cytokines and chemokines, such as tumor necrosis factor-α, interleukins, interferon-γ, and chemoattractant proteins (Rokni et al., 2020). As long as the disease worsens, structural consequences include multiple patchy shadows in the lungs in mildly affected individuals or pleural fluid in the most severe cases (Yang et al., 2020). This phase normally starts to aggravate around 7–14 days after onset (Briguglio et al., 2020a).
- The systemic phase: moderate-to-severe systemic implications comprising acute distress respiratory syndrome, heart failure, and multisystem organ dysfunction. Troponin I and brain natriuretic peptide may be elevated in infected patients with cardiac involvement. The coagulopathy manifests with increased D-dimer and other fibrin degradation products, low platelet counts, and increased INR and prothrombin time (Lippi et al., 2020; Thachil et al., 2020). Severe lymphopenia, kidney injury, as well as elevated liver enzymes and cytokines may be found (Shi et al., 2020). Of note, lymphocyte attachment to the activated endothelium, together with their systemic redistribution and apoptosis, is supposed to be at the basis of low lymphocyte counts (Rokni et al., 2020). This phase might be conversely replaced by a recovery phase if the virus is effectively suppressed (Lin et al., 2020).

On the clinical bases, patients can be classified according to respiratory autonomy (Briguglio et al., 2020a):

- Level 0: asymptomatic, mostly home living.
- Level 1: mild symptoms, pharyngodynia, dry cough, mild fever; these individuals should not be hospitalized.
- Level 2: moderate symptoms, high fever, persistent cough, asthenia, dyspnea; these patients might require noninvasive oxygen therapy.
- Level 3: severe symptoms; these patients require invasive oxygen therapy and intensive care support. These patients were reported to meet the diagnostic criteria for sepsis, with the impaired liver, kidney, and lung functions presenting concomitantly with cold extremities, weak peripheral pulses, shock, and severe metabolic acidosis (Li et al., 2020b).

FROM LUNGS TO MYOCARDIUM INJURIES

The cardiovascular sequelae start with the viral binding to ACE2 in the lower airways, causing type I damage in pneumocytes (Leung et al., 2020). The altered diffusion of oxygen across the injured alveolar membrane is likely to ground hypoxic conditions that prevent proper tissue oxygenation. Locally, SARS-CoV-2 particles activate alveolar macrophages and T cells (Shi et al., 2020). The subsequent inflammation is known to stimulate hyaline membrane formation, wall thickening, and infiltration of circulating monocytes that differentiate into macrophages or fibroblast-like cells called fibrocytes that eventually favor fibrotic processes in the parenchyma (Pilling and Gomer, 2012). During the worsening of the respiratory phase, the overactive immunological response in the lungs alters the integrity of epithelial-endothelial barriers, with plasma components exuding in the alveolar cavity together with chemotactic monocytes and neutrophils (Li et al., 2013). In level 2 and level 3 patients, a cytokine storm might arise, being the main root for growing a worsening life-threatening systemic phase (Xu et al., 2020b). The recruitment of different leukocyte populations in the lungs could expose these cells to viral infiltration, ending up becoming Trojan horses (i.e., vectors for SARS-CoV-2, recall of the mythical subterfuge to enter the city of Troy). This mechanism was in fact shown for the familial predecessor SARS-COV-1 (Chen and Hsiao, 2004; Gu et al., 2005) and supposed for SARS-CoV-2 (Li et al., 2020b; Park, 2020), whose viral particles were found in blood samples and in the myocardium (Tavazzi et al., 2020; Wang et al., 2020b). If SARS-CoV-2 was able to infiltrate into the heart, it would be likely to elicit the secretion of cytokines from cardiac fibroblasts to subsequently increase the inflammatory milieu (van Nieuwenhoven and Turner, 2013) and to cause the recruitment of transendothelial monocytes (Lindner et al., 2014), neutrophils, and dendritic cells (Van der Borght and Lambrecht, 2018). Activated dendritic cells are known to trigger T cells (Eriksson et al., 2003), further promoting tissue damage. A plethora of immune cells, comprising macrophages and fibrocytes, may therefore populate these early myocardium lesions (Oudit et al., 2009; Pilling et al., 2009), each likely to have its own role in COVID-19-associated myocarditis and stress-cardiomyopathy (Xiong et al., 2020). Consequently, it would seem fair to assume that the myocardium of infected patients might be subjected not only to type I damage, as a consequence of direct myocardial cell injury, but also to type II damage mainly comprising the inflammation-derived grievance. Remarkably, even patients with mild respiratory symptoms can manifest early cardiovascular implications, such as acute myopericarditis (Inciardi et al., 2020), Takotsubo syndrome (Meyer et al., 2020), or acute myocardial infarction (Stefanini et al., 2020). Fulminant myocarditis was reported in level 2 patients (Hu et al., 2020; Zeng et al., 2020), and supraventricular tachycardia, decompensated heart failure, and cardiogenic shock were observed in aggravating level 3 patients (Fried et al., 2020). It is generally agreed that the lymphocytic count mirrors the nutritional status of the host (Briguglio et al., 2019), and it may be useful in predicting the patient's reservoirs against the infection since these cells decline as long as COVID-19 worsens (Peteranderl and Herold, 2017; Chan et al., 2020). This attenuated immune potential of the host increases the susceptibility to disease complications, and the coupling of severe pneumonia with myocardial injury is likely to lead to progressive cardiorespiratory deterioration. Severe patients were in fact reported to be 13-fold more exposed to cardiovascular complications than non-severe, with an increased troponin I and low-density epicardial adipose tissue possibly reflecting the extent of the damage to the myocardium (Hui Hui et al., 2020; Li et al., 2020a), ultimately known to be associated with a worse prognosis (Clerkin et al., 2020).

ENDOTHELIAL DYSFUNCTION AND ATHEROTHROMBOTIC MANIFESTATION

Endothelial dysfunction is a feature of COVID-19 that lingers from the proliferative to the systemic phase. If the viral load is high, probably boosted by an intense viral shedding in the blood flow (Chang et al., 2020), it is very likely that some particles directly affect the endothelium (Escher et al., 2020; Sardu et al., 2020). High levels of pro-inflammatory cytokines are associated with endothelial engrossment (Finkel et al., 1992; Cheng et al., 1999) that could progress to vasculitis-like syndromes in the vessels of the brain, the kidneys, or the gastrointestinal tract (Varga et al., 2020). In severe COVID-19 patients, the Kawasaki disease has been observed (Jones et al., 2020) together with cutaneous signs, such as the "COVID-19 toes" (Mazzotta et al., 2020) or the chilblain-like lesions (Papa et al., 2020). We can therefore assume that the endothelial dysfunctions in COVID-19 arise from both type I damage and the nonischemic type II damage. The dysfunctional endothelium elicits two events that are part of the "two-activation theory of the endothelium" (Chang, 2019): the release of inflammatory cytokines triggers the activation of inflammatory pathways, whereas the activation of the platelet and exocytosis of aberrant coagulation factors trigger the activation of microthrombotic pathways.

Viruses are known to directly affect hemostasis with their ability to agglutinate platelets, cause hemolysis, and lead to the formation of procoagulant complexes with antibodies (McKay and Margaretten, 1967; van Gorp et al., 1999). This latter mechanism may be advocated for SARS-CoV-2 by recent computational modeling that showed the possibility of the virus to cause hemoglobin derangements (Liu and Li, 2020). If this were the case, then the incorporation of the virus into Trojan horses would be plausible since white cells are known to commonly engulf hemoglobin in various tissues (Briguglio et al., 2020c). Aberrant coagulation is the underlying mechanism for ischemic heart disease, stroke, and venous thromboembolism, but it has been observed also in severe influenza pneumonia and SARS-CoV-1 (Chong et al., 2004; Yang and Tang, 2016). Similarly, the development of coagulopathy appears to be a noxious complication in severe level 2 and level 3 patients (Tang et al., 2020b). Clots can be found in kidney dialysis catheters, cause strokes, or leave portions of lungs bloodless. Spleen atrophy, hilar lymph node necrosis, and hepatomegaly were also observed (Li et al., 2020b). Thrombus formation was associated with increased mortality (Zhou et al., 2020), with most of level 3 patients meeting the criteria for the disseminated intravascular coagulation (i.e., consumptive of both platelets and clotting factors; Lillicrap, 2020). Once thrombi formed in capillary beds, the remodeling processes would be associated with leukocyte polarization and late recruitment of macrophages that are in charge of cell clearance and blood flow restoration through fibrinolytic processes (Pober and Sessa, 2014). This cascade of events (Virchow's triad) is nevertheless necessary for endothelial wall restoration (Mukhopadhyay et al., 2019). However, the immune derangements in COVID-19 are likely to alter the activation of both immune cells and the fibrinolytic system. For instance, neutrophil extracellular traps (NETs) are useful to entrap viruses in weblike structures, thus facilitating cleavage by macrophages. If neutrophils are abnormally activated, aggregated NETs and their associated antimicrobial factors may be key determinants in capillary destruction (Cicco et al., 2020), vessel obstruction (Leppkes et al., 2020), and lung injury (Wang et al., 2020a). Similarly, impaired activation of the fibrinolytic system activation can recirculate the material and thus increase the risk of distant thrombi-derived ischemic damages (i.e., disseminated intravascular microthrombosis). Although it is not known if a plaque rupture is as dangerous as the plaque before rupture (Schoenhagen et al., 2002), if circulating thrombi halt in the small coronary vessels, they can certainly contribute to myocardial injury (Hendren et al., 2020). Thromboembolic events can occur in the lungs of infected patients (Ai et al., 2020; Danzi et al., 2020), further impairing gas exchange. The pulmonary damage leads to poor perfusion in the coronary vessels, misbalance of oxygen supply/demand, reduced activity of the mitochondrial electron transport chain, acidosis, and oxidative damage from reactive oxygen species (ROS; Wu et al., 2020b), whose accumulation is also known to be elicited by the cytokine storm (Bhaskar et al., 2020). Importantly, tissue hypoxia is known to induce metabolic reprogramming in cardiomyocytes, thus being critical for the progression of numerous cardiovascular diseases (Abe et al., 2017, 2019).

ELECTRICAL DYSREGULATION, MEDULLARY REFLEX ALTERATION, AND AUTONOMIC DYSFUNCTION

Alike myocardial injuries, not all COVID-19 patients who manifest alterations in the cardiac electrophysiology, such as ST-segment or ST-T wave abnormalities, show concomitant chest tomographic opacities (Bangalore et al., 2020). It is therefore possible that in predisposed individuals, the cardiovascular system is affected before the respiratory system, with electrical dysregulations being caused by circulating levels of pro-inflammatory cytokines, stress hormones, electrolytic imbalances, or drug cardiotoxicity (Chung et al., 1990; Yokoyama et al., 1993; Hasan, 2013; Driggin et al., 2020), but SARS-CoV-2 might directly damage nerve fibers. The myocardium is innervated by sympathetic and vagal parasympathetic nerve fibers that intersect in local plexuses, ganglia, and pacemaker regions. The wide expression of ACE2 in nerve tissues and the neurotrophic nature of SARS-CoV-2 might render the cardiac nerve fibers a favorite prey (Briguglio et al., 2020a). Severe arrhythmias are nevertheless life-threatening conditions that may occur in over 30% of level 2 patients (Ferrari, 2020) and in higher rates in patients of level 3 (Huang et al., 2020). The prevalence and severity of electrocardiographic changes could reflect the progression of myocardial damage (Guo et al., 2020), but it is very likely that it is associated with disease progression. Defects of electrical impulses from the sinoatrial node to the ventricles might arise as drug-induced disorders, thereby requiring careful assessments before defining the pharmacological treatment of COVID-19 (Yogasundaram et al., 2014; Borba et al., 2020). Pulmonary stretch receptors, C-fibers in the alveolar wall, baroreceptors in the carotid sinuses, extra-carotid cardiopulmonary baroreceptors together with widespread metaboreceptors are critical for integrating breathing cycle, heart rate, and vascular resistance during ventilatory and arterial pressure changes (Sant'Ambrogio, 1982; Schelegle, 2003; Timmers et al., 2003; Kougias et al., 2010; Anand et al., 2014). Type II damages are likely to disrupt these nervous components, in turn compromising the responsiveness to local stimuli, the impulse activity in afferent glossopharyngeal and vagal fibers, and the reflexive outflow (Burki and Lee, 2010; van Gestel and Steier, 2010). The central processing would therefore receive vitiated information from the periphery, which grounds the lack of adaptation of intrapulmonary vessels of COVID-19 patients (Chu et al., 2020), with the outputs being equally artificial. For instance, it has been suggested that the state of "silent hypoxemia" (i.e., depressed dyspnea response) that was observed in a large number of COVID-19 patients could be associated with defects in the carotid body, which is known to express ACE2 (Tobin et al., 2020). The consequent poor regulation of blood displacement in the microcirculation to the lungs and the brain may therefore mirror a vitiated baroreceptor reflex and hemodynamics, as was indeed observed

in a COVID-19 patient (Ribas et al., 2020). Importantly, countless cardiovascular implications have been associated with the frequent renal involvement that was observed in level 2 and level 3 patients (Ronco et al., 2020). It is reasonable to believe that kidneys are subjected to both viral infiltration and several types of type II damages (Larsen et al., 2020; Su et al., 2020). Local polarization and subsequent activation of white blood cells easily disrupt the renin-angiotensin-aldosterone system (RAAS; Strutz and Zeisberg, 2006; Chen et al., 2016; Granot et al., 2017), in turn affecting the sympathetic noradrenergic and parasympathetic cholinergic neurotransmission (Miller and Arnold, 2019). Nevertheless, this intense extended autonomic system (EAS) activation was suggested to account for the multiple organ involvement of COVID-19 (Goldstein, 2020). Other than arrhythmias, level 3 patients were reported to be subjected to more frequent vasopressor support (Goyal et al., 2020). Some of these patients showed clinical involvement of the brainstem, especially of the respiratory center (Manganelli et al., 2020), which can imply a type I damage of SARS-Cov-2 *via* cerebrospinal fluid diffusion (Sun and Guan, 2020) or vagus nerve retrograde transport (Tassorelli et al., 2020). The autonomic center at the level of the lower medulla expresses ACE2 (Xia and Lazartigues, 2008), and it was shown to be highly infected by familial predecessors (Netland et al., 2008; Li et al., 2016). Non-epileptic seizures due to autonomic dysfunction were indeed reported in a COVID-19 patient (Logmin et al., 2020). The systemic inflammation, ischemic thrombotic/cardio-embolic injuries, or vasculitis at the level of capillary beds beneath the ependyma of the ventricle may similarly affect brainstem functions (Benghanem et al., 2020; Mirza and Das, 2020), being all hallmarks of the systemic phase of COVID-19. Endothelial damages in these critical areas are likely to affect afferent inputs from peripheral nerves, with subsequent lack of proper buffering of blood pressure fluctuations from the nucleus of the solitary tract (Cutsforth-Gregory and Benarroch, 2017). Clinically, the involvement of this medullary nucleus or of the dorsal motor nucleus of the vagus nerve might evoke nausea and vomiting frequently observed in COVID-19 patients (Goldstein, 2020). Electrical evaluation of both heart and brain activities, echocardiography, invasive hemodynamic monitoring, and serum brain natriuretic peptide can help clarify the cardiogenic component (Yufu et al., 2006; Mazeraud et al., 2016). Notably, any infection of central nervous tissues is accompanied by massive infiltration of leukocytes, such as dendritic cells from the perivascular region (Ludewig et al., 2016), that could serve as Trojan horses, further contributing to local affections.

PREEXISTING CARDIOVASCULAR CONDITIONS AS VIRULENCE FACTOR: IMPLICATIONS FOR DISEASE ONSET AND PROGRESSION

Although it is not possible to state whether the cardiovascular implications observed in COVID-19 derive from previous conditions or depend solely on the coronavirus-associated damages, it is reasonable to assume a causal link. From a molecular point of view, the upregulation of ACE2 in some cardiovascular diseases, such as ischemic heart disease or diabetes mellitus (Zisman et al., 2003), may certainly expose the sick individuals who contract the coronavirus to poorer prognosis (Wu et al., 2020b). The subsequent binding and downregulation of ACE2 expression by SARS-CoV-2 further prevent the conversion of angiotensin II, thus worsening pulmonary and cardiovascular outcomes (Datta et al., 2020). Accordingly, a higher ACE/ACE2 ratio might be a predisposing cause of worse outcomes in COVID-19, having angiotensin II dire vasoconstriction and pro-oxidant and pro-inflammatory effects in contrast to angiotensin (1–7) that is a vasodilator, antioxidant, and anti-inflammatory (Pagliaro and Penna, 2020). Clinically, it has been proposed that the more disturbed was the hemodynamic homeostasis prior to SARS-CoV-2 infection, the more severe could be the symptoms during COVID-19 and the higher would be the risk of long-term cardiovascular consequences (Zheng et al., 2020). Concerning the Italian cohort of patients, 1 in 3 had preexisting ischemic cardiomyopathy or diabetes mellitus, 1 in 4 already suffered from atrial fibrillation, and 1 in 10 had a history of stroke (Onder et al., 2020). The preexisting myocardial metabolic imbalances or atherosclerotic lesion might have played a major role in myocardial oxygen imbalances and plaque instabilities upon the advent of the systemic phase of COVID-19 (Bonow et al., 2020). Numerous mechanical (e.g., repetitive deformations derived from the cardiac cycle) and biological forces (e.g., inflammation) are known to undermine the stability of subclinical plaques (Arroyo and Lee, 1999; Yao et al., 2019), and they all occur during infections (Madjid et al., 2007; Campbell and Rosenfeld, 2015). In addition, a preexisting poor cardiac functional reserve is more likely to lead to a sudden cardiac insufficiency in patients with COVID-19, giving also the drug-related heart damage deriving from COVID-19 treatment (Wu et al., 2020a; Zheng et al., 2020). In the past, patients with comorbid cardiovascular diseases, such as coronary artery disease or heart failure, have been already recognized to be at higher risk of contagion and exacerbation of symptoms during viral respiratory infections (Nguyen et al., 2016). Furthermore, long-term damage to the cardiovascular system has been documented in hospitalized patients recovering from pneumonia (Corrales-Medina et al., 2015), thus highlighting the cardiorespiratory deteriorations of COVID-19. It is therefore reasonable to say that any previous hypoxic/vascular condition, cardiac inflammation, or autonomic dysfunction has to be recognized as a risk factor for COVID-19 onset and cardiovascular disease progression in any individual infected with SARS-CoV-2. Notably, the highest case/fatality ratio in older adults might be due to the increasing prevalence of frailty and comorbid cardiovascular diseases in advanced age (Briguglio et al., 2020b; Moccia et al., 2020), which is known to be associated with increased ACE/ACE2 ratio (Wang et al., 2016). While it is still controversial whether RAAS inhibitors are to

be administered to COVID-19 patients (Shibata et al., 2020), it is certain that the therapy with ACE inhibitors and angiotensin receptor blockers (ARBs) should definitely not be discontinued in patients with preexisting cardiovascular diseases (de Abajo et al., 2020; Vaduganathan et al., 2020).

CONCLUSIVE REMARKS

COVID-19 is a multifaceted illness that comprises several implications of cardiological nature, including hypoxemia, sustained activation of the endothelium, nonischemic injuries, leukocyte polarization, thrombi-derived ischemic damages, dysrhythmias, and autonomic dysfunctions (**Figure 1**). Given these considerations, it is reasonable to conclude that the more severe autonomic dysfunctions of critically ill patients, the more complex would be the preservation of hemodynamic balances, thereby increasing the likelihood of fatal cardiovascular consequences in COVID-19 or chronic cardiovascular damages in those who survive. In these patients, long-term remote electrophysiological monitoring might be useful to provide care as necessary after discharge (Lakkireddy et al., 2020). Understanding these pathophysiological mechanisms in COVID-19 is crucial to promptly triage early

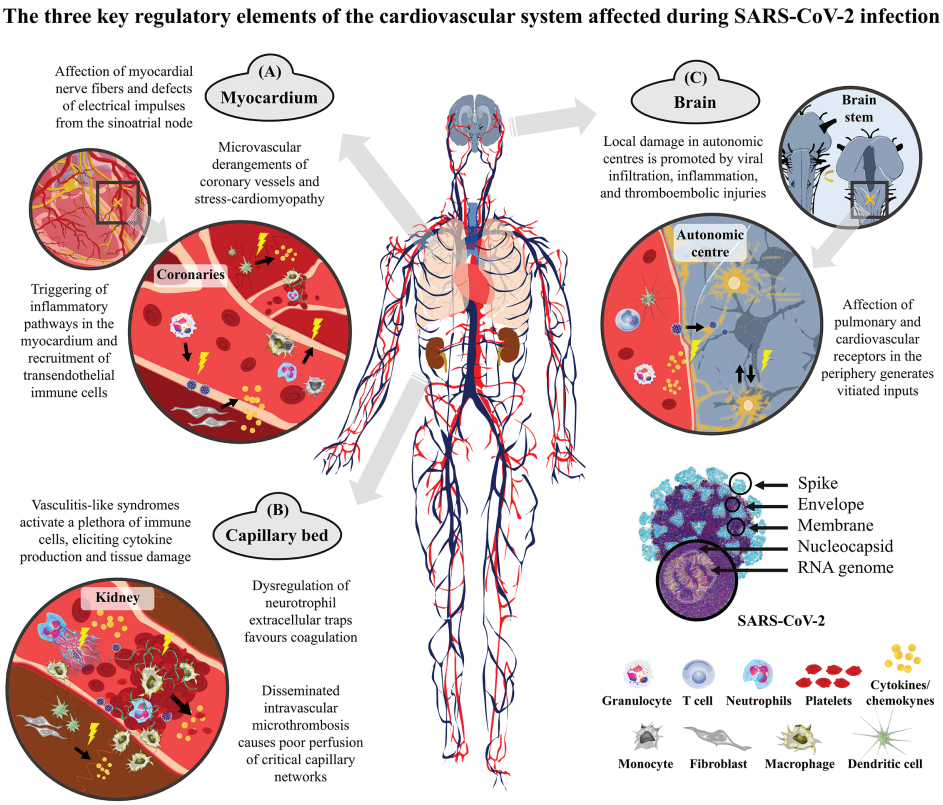


FIGURE 1 | Representation of the cardiovascular derangements of coronavirus disease 2019 (COVID-19) that are due to either direct cellular damage of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or indirect consequences of the exaggerated host's response. The severe acute respiratory syndrome coronavirus of 2019 (SARS-CoV-2) is a single-strand positive-sense RNA virus that spreads between humans mainly through the inhalation of respiratory droplets. Upon the collapse of the alveoli in the lungs, the virus can enter the bloodstream and distribute to systemic districts by cardiac pumping. It is likely that the transport in the blood is not as such, but carried by different types of white blood cells, such as T cells, granulocytes, and macrophages, which can therefore serve as vehicles. This Trojan route can guarantee the infiltration of the virus into normally inaccessible body districts. The systemic spread of the coronavirus elicits an exaggerated immune response in the most severe cases that strokes with hypoxic conditions. **(A)** In the myocardium, the excessive activation of the endothelial system upon viral damage and the enhanced inflammatory cell infiltration alter the coronary perfusion and the cardiac rhythm. Circulating monocytes and neutrophils infiltrate in the heart wall and parenchyma, with resident dendritic cells contributing to cytokine production and inflammatory/pro-fibrotic environment. **(B)** In the blood, the hyperactivation of both inflammatory and microthrombotic pathways leads to endothelium engrossment and coagulopathy. The activated vascular endothelium is targeted by neutrophils and monocytes, with thrombosis or bleedings being likely to occur because of the inflammation-derived imbalance between platelets, hypercoagulability, and altered fibrinolysis (fibrin in green). Thrombus-associated white blood cells produce inflammatory cytokines and proteases that contribute to local remodeling and fibroblast activation. Atherothrombotic manifestations may also be promoted by dysregulation of neutrophil extracellular traps (NETs). **(C)** The heart is innervated by vagal postganglionic fibers from the cardio-inhibitory center and by the cardiac postganglionic fibers from the spinal cord arising from the cardio-acceleratory center of the medulla. Sympatho-inhibitory and cardio-inhibitory baroreflexes together with arterial metaboreflexes encompass inputs to neurons located in the dorsolateral nucleus of the solitary tract that integrate the vasomotor tone and the automatism of the sinus node. Damages to these reflexes disturb these central pathways and ultimately disrupt the heart beat nuclei, eventually leading to irrepressible dysautonomia.

risk factors, tailor treatment according to the patient's severity and risk-benefit balance, and integrate evidence-based therapies depending on the disease phase (Carter et al., 2020; Mycroft-West et al., 2020). Drugs for COVID-19 have not been available yet (Kalil, 2020), but immunotherapies, extracorporeal membrane oxygenation, and low-molecular-weight heparin are being tested for effectiveness (Paranjpe et al., 2020; Perazzo et al., 2020; Ramanathan et al., 2020; Spyropoulos et al., 2020; Tang et al., 2020a; Thachil, 2020). In the meantime, cardiologists should stay up-to-date on recent and ongoing discoveries regarding COVID-19 and take a prominent role in the research studies or multidisciplinary teams.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MB formulated the hypothesis and wrote the first draft of the manuscript. MP, FZ, AB, TC, FP, PP, MM, RG, GA, AI, GB, and MT revised the first draft and contributed to manuscript sections. All authors contributed to manuscript revision and read and approved the submitted version.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ACKNOWLEDGMENTS

None that interests colleagues, institutions, or agencies in supporting this hypothesis. Yet all authors thank all the health workers and volunteers in Italy at the front lines of the fight against this SARS-CoV-2.

REFERENCES

Abe, H., Semba, H., and Takeda, N. (2017). The roles of hypoxia signaling in the pathogenesis of cardiovascular diseases. *J. Atheroscler. Thromb.* 24, 884–894. doi: 10.5551/jat.RV17009

Abe, H., Takeda, N., Isagawa, T., Semba, H., Nishimura, S., Morioka, M. S., et al. (2019). Macrophage hypoxia signaling regulates cardiac fibrosis via Oncostatin M. *Nat. Commun.* 10:2824. doi: 10.1038/s41467-019-10859-w

Ai, T., Yang, Z., Hou, H., Zhan, C., Chen, C., Lv, W., et al. (2020). Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. *Radiology* 296, E32–E40. doi: 10.1148/radiol.2020200642

Anand, A., Srivastava, N., Barwad, P., Ramakrishnan, S., Roy, A., and Bhargava, B. (2014). Dyspnea in Eisenmenger syndrome and its amelioration by sildenafil: role of J receptors. *Int. J. Cardiol.* 174, 574–578. doi: 10.1016/j.ijcard.2014.04.131

Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C., and Garry, R. F. (2020). The proximal origin of SARS-CoV-2. *Nat. Med.* 26, 450–452. doi: 10.1038/s41591-020-0820-9

Arroyo, L. H., and Lee, R. T. (1999). Mechanisms of plaque rupture: mechanical and biologic interactions. *Cardiovasc. Res.* 41, 369–375. doi: 10.1016/s0008-6363(98)00308-3

Bangalore, S., Sharma, A., Slotwiner, A., Yatskar, L., Harari, R., Shah, B., et al. (2020). ST-segment elevation in patients with Covid-19 - a case series. *N. Engl. J. Med.* 382, 2478–2480. doi: 10.1056/NEJMc2009020

Basu-Ray, I. S., Almaddah, N. K., Adeboye, A., and Soos, M. P. (2020). *Cardiac manifestations of coronavirus (COVID-19) [online]*. Treasure Island (FL): StatPearls Publishing https://www.ncbi.nlm.nih.gov/books/NBK556152/ (Accessed June 3 2020).

Benghanem, S., Mazeraud, A., Azabou, E., Chhor, V., Shinotsuka, C. R., Claassen, J., et al. (2020). Brainstem dysfunction in critically ill patients. *Crit. Care* 24:5. doi: 10.1186/s13054-019-2718-9

Bhaskar, S., Sinha, A., Banach, M., Mittoo, S., Weissert, R., Kass, J. S., et al. (2020). Cytokine storm in COVID-19-Immunopathological mechanisms, clinical considerations, and therapeutic approaches: the REPROGRAM consortium position paper. *Front. Immunol.* 11:1648. doi: 10.3389/fimmu.2020.01648

Bonow, R. O., O'Gara, P. T., and Yancy, C. W. (2020). Cardiology and COVID-19. *JAMA* 324, 1131–1132. doi: 10.1001/jama.2020.15088

Borba, M. G. S., Val, F. F. A., Sampaio, V. S., Alexandre, M. A. A., Melo, G. C., Brito, M., et al. (2020). Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: a randomized clinical trial. *JAMA Netw. Open* 3:e208857. doi: 10.1001/jamanetworkopen.2020.8857

Briguglio, M., Bona, A. R., Porta, M., Dell'osso, B., Pregliasco, F. E., and Banfi, G. (2020a). Disentangling the hypothesis of host dysosmia and SARS-CoV-2: the bait symptom that hides neglected neurophysiological routes. *Front. Physiol.* 11:671. doi: 10.3389/fphys.2020.00671

Briguglio, M., Gianola, S., Aguirre, M. F. I., Sirtori, P., Perazzo, P., Pennestri, F., et al. (2019). Nutritional support for enhanced recovery programs in orthopedics: future perspectives for implementing clinical practice. *Nutr. Clin. Metab.* 33, 190–198. doi: 10.1016/j.nupar.2019.04.002

Briguglio, M., Giorgino, R., Dell'Osso, B., Cesari, M., Porta, M., Lattanzio, F., et al. (2020b). Consequences for the elderly after COVID-19 isolation: FEaR (frail elderly amid restrictions). *Front. Psychol.* 11:565052. doi: 10.3389/fpsyg.2020.565052

Briguglio, M., Hrelia, S., Malaguti, M., De Vecchi, E., Lombardi, G., Banfi, G., et al. (2020c). Oral supplementation with sucrosomial ferric pyrophosphate plus L-ascorbic acid to ameliorate the martial status: a randomized controlled trial. *Nutrients* 12:386. doi: 10.3390/nu12020386

Briguglio, M., Pregliasco, F. E., Lombardi, G., Perazzo, P., and Banfi, G. (2020d). The Malnutritional status of the host as a virulence factor for new coronavirus SARS-CoV-2. *Front. Med.* 7:146. doi: 10.3389/fmed.2020.00146

Burki, N. K., and Lee, L. Y. (2010). Mechanisms of dyspnea. *Chest* 138, 1196–1201. doi: 10.1378/chest.10-0534

Campbell, L. A., and Rosenfeld, M. E. (2015). Infection and atherosclerosis development. *Arch. Med. Res.* 46, 339–350. doi: 10.1016/j.arcmed.2015.05.006

Carter, P., Anderson, M., and Mossialos, E. (2020). Health system, public health, and economic implications of managing COVID-19 from a cardiovascular perspective. *Eur. Heart J.* 41, 2516–2518. doi: 10.1093/eurheartj/ehaa342

Chan, K. W., Wong, V. T., and Tang, S. C. W. (2020). COVID-19: an update on the epidemiological, clinical, preventive and therapeutic evidence and guidelines of integrative Chinese-Western medicine for the management of 2019 novel coronavirus disease. *Am. J. Chin. Med.* 48, 737–762. doi: 10.1142/S0192415X20500378

Chang, J. C. (2019). Sepsis and septic shock: endothelial molecular pathogenesis associated with vascular microthrombotic disease. *Thromb. J.* 17:10. doi: 10.1186/s12959-019-0198-4

Chang, L., Yan, Y., and Wang, L. (2020). Coronavirus disease 2019: coronaviruses and blood safety. *Transfus. Med. Rev.* 34, 75–80. doi: 10.1016/j.tmrv.2020.02.003

Chen, P. C., and Hsiao, C. H. (2004). Re: to KF, Tong JH, Chan PK, et al. tissue and cellular tropism of the coronavirus associated with severe acute respiratory syndrome: an in-situ hybridization study of fatal cases. J Pathol 2004; 202: 157-163. *J. Pathol.* 203, 729–730. doi: 10.1002/path.1575

Chen, K., Wang, J. M., Yuan, R., Yi, X., Li, L., Gong, W., et al. (2016). Tissue-resident dendritic cells and diseases involving dendritic cell malfunction. *Int. Immunopharmacol.* 34, 1–15. doi: 10.1016/j.intimp.2016.02.007

Cheng, X. S., Shimokawa, H., Momii, H., Oyama, J., Fukuyama, N., Egashira, K., et al. (1999). Role of superoxide anion in the pathogenesis of cytokine-induced myocardial dysfunction in dogs in vivo. *Cardiovasc. Res.* 42, 651–659. doi: 10.1016/S0008-6363(98)00317-4

Chong, P. Y., Chui, P., Ling, A. E., Franks, T. J., Tai, D. Y., Leo, Y. S., et al. (2004). Analysis of deaths during the severe acute respiratory syndrome (SARS) epidemic in Singapore: challenges in determining a SARS diagnosis. *Arch. Pathol. Lab. Med.* 128, 195–204. doi: 10.1043/1543-2165(2004)128<195:AODDTS>2.0.CO;2

Chu, H., Chan, J. F., Wang, Y., Yuen, T. T., Chai, Y., Hou, Y., et al. (2020). Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: an ex vivo study with implications for the pathogenesis of COVID-19. *Clin. Infect. Dis.* 71, 1400–1409. doi: 10.1093/cid/ciaa410

Chung, M. K., Gulick, T. S., Rotondo, R. E., Schreiner, G. F., and Lange, L. G. (1990). Mechanism of cytokine inhibition of beta-adrenergic agonist stimulation of cyclic AMP in rat cardiac myocytes. Impairment of signal transduction. *Circ. Res.* 67, 753–763. doi: 10.1161/01.res.67.3.753

Cicco, S., Cicco, G., Racanelli, V., and Vacca, A. (2020). Neutrophil extracellular traps (NETs) and damage-associated molecular patterns (DAMPs): two potential targets for COVID-19 treatment. *Mediat. Inflamm.* 2020:7527953. doi: 10.1155/2020/7527953

Clerkin, K. J., Fried, J. A., Raikhelkar, J., Sayer, G., Griffin, J. M., Masoumi, A., et al. (2020). COVID-19 and cardiovascular disease. *Circulation* 141, 1648–1655. doi: 10.1161/CIRCULATIONAHA.120.046941

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses (2020). The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat. Microbiol.* 5, 536–544. doi: 10.1038/s41564-020-0695-z

Corrales-Medina, V. F., Alvarez, K. N., Weissfeld, L. A., Angus, D. C., Chirinos, J. A., Chang, C. C., et al. (2015). Association between hospitalization for pneumonia and subsequent risk of cardiovascular disease. *JAMA* 313, 264–274. doi: 10.1001/jama.2014.18229

Cutsforth-Gregory, J. K., and Benarroch, E. E. (2017). Nucleus of the solitary tract, medullary reflexes, and clinical implications. *Neurology* 88, 1187–1196. doi: 10.1212/WNL.0000000000003751

Danzi, G. B., Loffi, M., Galeazzi, G., and Gherbesi, E. (2020). Acute pulmonary embolism and COVID-19 pneumonia: a random association? *Eur. Heart J.* 41:1858. doi: 10.1093/eurheartj/ehaa254

Datta, P. K., Liu, F., Fischer, T., Rappaport, J., and Qin, X. (2020). SARS-CoV-2 pandemic and research gaps: understanding SARS-CoV-2 interaction with the ACE2 receptor and implications for therapy. *Theranostics* 10, 7448–7464. doi: 10.7150/thno.48076

de Abajo, F. J., Rodriguez-Martin, S., Lerma, V., Mejia-Abril, G., Aguilar, M., Garcia-Luque, A., et al. (2020). Use of renin-angiotensin-aldosterone system inhibitors and risk of COVID-19 requiring admission to hospital: a case-population study. *Lancet* 395, 1705–1714. doi: 10.1016/S0140-6736(20)31030-8

Driggin, E., Madhavan, M. V., Bikdeli, B., Chuich, T., Laracy, J., Biondi-Zoccai, G., et al. (2020). Cardiovascular considerations for patients, health care workers, and health systems during the COVID-19 pandemic. *J. Am. Coll. Cardiol.* 75, 2352–2371. doi: 10.1016/j.jacc.2020.03.031

Eriksson, U., Ricci, R., Hunziker, L., Kurrer, M. O., Oudit, G. Y., Watts, T. H., et al. (2003). Dendritic cell-induced autoimmune heart failure requires cooperation between adaptive and innate immunity. *Nat. Med.* 9, 1484–1490. doi: 10.1038/nm960

Escher, R., Breakey, N., and Lammle, B. (2020). Severe COVID-19 infection associated with endothelial activation. *Thromb. Res.* 190:62. doi: 10.1016/j.thromres.2020.04.014

Ferrari, F. (2020). COVID-19: updated data and its relation to the cardiovascular system. *Arq. Bras. Cardiol.* 114, 823–826. doi: 10.36660/abc.20200215

Finkel, M. S., Oddis, C. V., Jacob, T. D., Watkins, S. C., Hattler, B. G., and Simmons, R. L. (1992). Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* 257, 387–389. doi: 10.1126/science.1631560

Fried, J. A., Ramasubbu, K., Bhatt, R., Topkara, V. K., Clerkin, K. J., Horn, E., et al. (2020). The variety of cardiovascular presentations of COVID-19. *Circulation* 141, 1930–1936. doi: 10.1161/CIRCULATIONAHA.120.047164

Goldstein, D. S. (2020). The extended autonomic system, dyshomeostasis, and COVID-19. *Clin. Auton. Res.* 30, 299–315. doi: 10.1007/s10286-020-00714-0

Goyal, P., Choi, J. J., Pinheiro, L. C., Schenck, E. J., Chen, R., Jabri, A., et al. (2020). Clinical characteristics of Covid-19 in New York City. *N. Engl. J. Med.* 382, 2372–2374. doi: 10.1056/NEJMc2010419

Granot, T., Senda, T., Carpenter, D. J., Matsuoka, N., Weiner, J., Gordon, C. L., et al. (2017). Dendritic cells display subset and tissue-specific maturation dynamics over human life. *Immunity* 46, 504–515. doi: 10.1016/j.immuni.2017.02.019

Gu, J., Gong, E., Zhang, B., Zheng, J., Gao, Z., Zhong, Y., et al. (2005). Multiple organ infection and the pathogenesis of SARS. *J. Exp. Med.* 202, 415–424. doi: 10.1084/jem.20050828

Guo, T., Fan, Y., Chen, M., Wu, X., Zhang, L., He, T., et al. (2020). Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 5, 811–818. doi: 10.1001/jamacardio.2020.1017

Harapan, H., Itoh, N., Yufika, A., Winardi, W., Keam, S., Te, H., et al. (2020). Coronavirus disease 2019 (COVID-19): a literature review. *J. Infect. Public Health* 13, 667–673. doi: 10.1016/j.jiph.2020.03.019

Hasan, W. (2013). Autonomic cardiac innervation: development and adult plasticity. *Organogenesis* 9, 176–193. doi: 10.4161/org.24892

Hendren, N. S., Drazner, M. H., Bozkurt, B., and Cooper, L. T. Jr. (2020). Description and proposed Management of the Acute COVID-19 cardiovascular syndrome. *Circulation* 141, 1903–1914. doi: 10.1161/CIRCULATIONAHA.120.047349

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271–280.e8. doi: 10.1016/j.cell.2020.02.052

Hu, H., Ma, F., Wei, X., and Fang, Y. (2020). Coronavirus fulminant myocarditis saved with glucocorticoid and human immunoglobulin. *Eur. Heart J.* doi: 10.1093/eurheartj/ehaa190 [Epub ahead of print]

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Hui Hui, Y. Z., Yang, X., Wang, X., He, B., Li, L., Li, H., et al. (2020). Clinical and radiographic features of cardiac injury in patients with 2019 novel coronavirus pneumonia. medRxiv [Preprint]. doi: 10.1101/2020.02.24.20027052.

Inciardi, R. M., Lupi, L., Zaccone, G., Italia, L., Raffo, M., Tomasoni, D., et al. (2020). Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 5, 819–824. doi: 10.1001/jamacardio.2020.1096

Jones, V. G., Mills, M., Suarez, D., Hogan, C. A., Yeh, D., Bradley Segal, J., et al. (2020). COVID-19 and Kawasaki disease: novel virus and novel case. *Hosp. Pediatr.* 10, 537–540. doi: 10.1542/hpeds.2020-0123

Kabbani, N., and Olds, J. L. (2020). Does COVID19 infect the brain? If so, smokers might be at a higher risk. *Mol. Pharmacol.* 97, 351–353. doi: 10.1124/molpharm.120.000014

Kalil, A. C. (2020). Treating COVID-19-off-label drug use, compassionate use, and randomized clinical trials during pandemics. *JAMA* 323, 1897–1898. doi: 10.1001/jama.2020.4742

Kougias, P., Weakley, S. M., Yao, Q., Lin, P. H., and Chen, C. (2010). Arterial baroreceptors in the management of systemic hypertension. *Med. Sci. Monit.* 16, RA1–RA8.

Lakkireddy, D. R., Chung, M. K., Gopinathannair, R., Patton, K. K., Gluckman, T. J., Turagam, M., et al. (2020). Guidance for cardiac electrophysiology during the COVID-19 pandemic from the Heart Rhythm Society COVID-19 task force; electrophysiology section of the American College of Cardiology; and the electrocardiography and arrhythmias Committee of the Council on clinical cardiology, American Heart Association. *Heart Rhythm.* 17, e233–e241. doi: 10.1016/j.hrthm.2020.03.028

Larsen, C. P., Bourne, T. D., Wilson, J. D., Saqqa, O., and Sharshir, M. A. (2020). Collapsing glomerulopathy in a patient with coronavirus disease 2019 (COVID-19). *Kidney Int. Rep.* 5, 935–939. doi: 10.1016/j.ekir.2020.04.002

Leppkes, M., Knopf, J., Naschberger, E., Lindemann, A., Singh, J., Herrmann, I., et al. (2020). Vascular occlusion by neutrophil extracellular traps in COVID-19. *EBioMedicine* 58:102925. doi: 10.1016/j.ebiom.2020.102925

Leung, J. M., Yang, C. X., Tam, A., Shaipanich, T., Hackett, T. L., Singhera, G. K., et al. (2020). ACE-2 expression in the small airway epithelia of smokers and COPD patients: implications for COVID-19. *Eur. Respir. J.* 55:2000688. doi: 10.1183/13993003.00688-2020

Li, H., Liu, L., Zhang, D., Xu, J., Dai, H., Tang, N., et al. (2020b). SARS-CoV-2 and viral sepsis: observations and hypotheses. *Lancet* 395, 1517–1520. doi: 10.1016/S0140-6736(20)30920-X

Li, G., Liu, Y., Zhu, Y., Liu, A., Xu, Y., Li, X., et al. (2013). ACE2 activation confers endothelial protection and attenuates neointimal lesions in prevention of severe pulmonary arterial hypertension in rats. *Lung* 191, 327–336. doi: 10.1007/s00408-013-9470-8

Li, K., Wohlford-Lenane, C., Perlman, S., Zhao, J., Jewell, A. K., Reznikov, L. R., et al. (2016). Middle East respiratory syndrome coronavirus causes multiple organ damage and lethal disease in mice transgenic for human Dipeptidyl peptidase 4. *J. Infect. Dis.* 213, 712–722. doi: 10.1093/infdis/jiv499

Li, B., Yang, J., Zhao, F., Zhi, L., Wang, X., Liu, L., et al. (2020a). Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clin. Res. Cardiol.* 109, 531–538. doi: 10.1007/s00392-020-01626-9

Lillicrap, D. (2020). Disseminated intravascular coagulation in patients with 2019-nCoV pneumonia. *J. Thromb. Haemost.* 18, 786–787. doi: 10.1111/jth.14781

Lin, L., Lu, L., Cao, W., and Li, T. (2020). Hypothesis for potential pathogenesis of SARS-CoV-2 infection-a review of immune changes in patients with viral pneumonia. *Emerg. Microbes Infect.* 9, 727–732. doi: 10.1080/22221751.2020.1746199

Lindner, D., Zietsch, C., Tank, J., Sossalla, S., Fluschnik, N., Hinrichs, S., et al. (2014). Cardiac fibroblasts support cardiac inflammation in heart failure. *Basic Res. Cardiol.* 109:428. doi: 10.1007/s00395-014-0428-7

Lippi, G., Plebani, M., and Henry, B. M. (2020). Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis. *Clin. Chim. Acta* 506, 145–148. doi: 10.1016/j.cca.2020.03.022

Liu, W., and Li, H. (2020). COVID-19: Attacks the 1-Beta Chain of Hemoglobin and Captures the Porphyrin to Inhibit Human Heme Metabolism. ChemRxiv [Preprint]. doi: 10.26434/chemrxiv.11938173.v9.

Logmin, K., Karam, M., Schichel, T., Harmel, J., and Wojtecki, L. (2020). Non-epileptic seizures in autonomic dysfunction as the initial symptom of COVID-19. *J. Neurol.* 267, 2490–2491. doi: 10.1007/s00415-020-09904-2

Ludewig, P., Gallizioli, M., Urra, X., Behr, S., Brait, V. H., Gelderblom, M., et al. (2016). Dendritic cells in brain diseases. *Biochim. Biophys. Acta* 1862, 352–367. doi: 10.1016/j.bbadis.2015.11.003

Madjid, M., Vela, D., Khalili-Tabrizi, H., Casscells, S. W., and Litovsky, S. (2007). Systemic infections cause exaggerated local inflammation in atherosclerotic coronary arteries: clues to the triggering effect of acute infections on acute coronary syndromes. *Tex. Heart Inst. J.* 34, 11–18.

Manganelli, F., Vargas, M., Iovino, A., Iacovazzo, C., Santoro, L., and Servillo, G. (2020). Brainstem involvement and respiratory failure in COVID-19. *Neurol. Sci.* 41, 1663–1665. doi: 10.1007/s10072-020-04487-2

Mason, R. J. (2020). Pathogenesis of COVID-19 from a cell biologic perspective. *Eur. Respir. J.* 55:2000607. doi: 10.1183/13993003.00607-2020

Matsushita, K., Marchandot, B., Jesel, L., Ohlmann, P., and Morel, O. (2020). Impact of COVID-19 on the cardiovascular system: a review. *J. Clin. Med.* 9:1407. doi: 10.3390/jcm9051407

Mazeraud, A., Pascal, Q., Verdonk, F., Heming, N., Chretien, F., and Sharshar, T. (2016). Neuroanatomy and physiology of brain dysfunction in sepsis. *Clin. Chest Med.* 37, 333–345. doi: 10.1016/j.ccm.2016.01.013

Mazzotta, F., Troccoli, T., and Bonifazi, E. (2020). A new vasculitis at the time of COVID-19. *Eur. J. Pediat. Dermatol.* 30, 75–78. doi: 10.26326/2281-9649.30.2.2103

McKay, D. G., and Margaretten, W. (1967). Disseminated intravascular coagulation in virus diseases. *Arch. Intern. Med.* 120, 129–152. doi: 10.1001/archinte.1967.00300020001001

Mehra, M. R., Desai, S. S., Kuy, S., Henry, T. D., and Patel, A. N. (2020). Cardiovascular disease, drug therapy, and mortality in Covid-19. *N. Engl. J. Med.* 382:e102. doi: 10.1056/NEJMoa2007621

Meyer, P., Degrauwe, S., Van Delden, C., Ghadri, J. R., and Templin, C. (2020). Typical takotsubo syndrome triggered by SARS-CoV-2 infection. *Eur. Heart J.* 41:1860. doi: 10.1093/eurheartj/ehaa306

Miller, A. J., and Arnold, A. C. (2019). The renin-angiotensin system in cardiovascular autonomic control: recent developments and clinical implications. *Clin. Auton. Res.* 29, 231–243. doi: 10.1007/s10286-018-0572-5

Mirza, M., and Das, J.M. (2020). *Neuroanatomy, Area Postrema* [Online]. Treasure Island (FL): StatPearls Publishing: StatPearls [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK544249/ [Accessed 2020 April 29 2020].

Moccia, F., Gerbino, A., Lionetti, V., Miragoli, M., Munaron, L. M., Pagliaro, P., et al. (2020). COVID-19-associated cardiovascular morbidity in older adults: a position paper from the Italian society of cardiovascular researches. *Geroscience* 42, 1021–1049. doi: 10.1007/s11357-020-00198-w

Mukhopadhyay, S., Johnson, T. A., Duru, N., Buzza, M. S., Pawar, N. R., Sarkar, R., et al. (2019). Fibrinolysis and inflammation in venous thrombus resolution. *Front. Immunol.* 10:1348. doi: 10.3389/fimmu.2019.01348

Mycroft-West, C., Su, D., Elli, S., Guimond, S., Miller, G., Turnbull, J., et al. (2020). The 2019 coronavirus (SARS-CoV-2) surface protein (Spike) S1 Receptor Binding Domain undergoes conformational change upon heparin binding. bioRxiv [Preprint]. doi: 10.1101/2020.02.29.971093.

Netland, J., Meyerholz, D. K., Moore, S., Cassell, M., and Perlman, S. (2008). Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. *J. Virol.* 82, 7264–7275. doi: 10.1128/JVI.00737-08

Nguyen, J. L., Yang, W., Ito, K., Matte, T. D., Shaman, J., and Kinney, P. L. (2016). Seasonal influenza infections and cardiovascular disease mortality. *JAMA Cardiol.* 1, 274–281. doi: 10.1001/jamacardio.2016.0433

Onder, G., Rezza, G., and Brusaferro, S. (2020). Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. *JAMA* 323, 1775–1776. doi: 10.1001/jama.2020.4683

Oudit, G. Y., Kassiri, Z., Jiang, C., Liu, P. P., Poutanen, S. M., Penninger, J. M., et al. (2009). SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur. J. Clin. Investig.* 39, 618–625. doi: 10.1111/j.1365-2362.2009.02153.x

Pagliaro, P., and Penna, C. (2020). ACE/ACE2 ratio: a key also in 2019 coronavirus disease (Covid-19)? *Front. Med.* 7:335. doi: 10.3389/fmed.2020.00335

Papa, A., Salzano, A. M., Di Dato, M. T., and Varrassi, G. (2020). Images in practice: painful cutaneous Vasculitis in a SARS-Cov-2 IgG-positive child. *Pain Ther.* 21, 1–3. doi: 10.1007/s40122-020-00174-4

Paranjpe, I., Fuster, V., Lala, A., Russak, A., Glicksberg, B. S., Levin, M. A., et al. (2020). Association of treatment dose anticoagulation with in-hospital survival among hospitalized patients with COVID-19. *J. Am. Coll. Cardiol.* 76, 122–124. doi: 10.1016/j.jacc.2020.05.001

Park, M. D. (2020). Macrophages: a Trojan horse in COVID-19? *Nat. Rev. Immunol.* 20:351. doi: 10.1038/s41577-020-0317-2

Perazzo, P., Giorgino, R., Briguglio, M., Zuffada, M., Accetta, R., Mangiavini, L., et al. (2020). From standard to escalated anticoagulant prophylaxis in fractured older adults with SARS-CoV-2 undergoing accelerated orthopedic surgery. *Front. Med.* doi: 10.3389/fmed.2020.566770 [Epub ahead of print]

Peteranderl, C., and Herold, S. (2017). The impact of the interferon/TNF-related apoptosis-inducing ligand signaling axis on disease progression in respiratory viral infection and beyond. *Front. Immunol.* 8:313. doi: 10.3389/fimmu.2017.00313

Pilling, D., Fan, T., Huang, D., Kaul, B., and Gomer, R. H. (2009). Identification of markers that distinguish monocyte-derived fibrocytes from monocytes, macrophages, and fibroblasts. *PLoS One* 4:e7475. doi: 10.1371/journal.pone.0007475

Pilling, D., and Gomer, R. H. (2012). Differentiation of circulating monocytes into fibroblast-like cells. *Methods Mol. Biol.* 904, 191–206. doi: 10.1007/978-1-61779-943-3_16

Pober, J. S., and Sessa, W. C. (2014). Inflammation and the blood microvascular system. *Cold Spring Harb. Perspect. Biol.* 7:a016345. doi: 10.1101/cshperspect.a016345

Ramanathan, K., Antognini, D., Combes, A., Paden, M., Zakhary, B., Ogino, M., et al. (2020). Planning and provision of ECMO services for severe ARDS during the COVID-19 pandemic and other outbreaks of emerging infectious diseases. *Lancet Respir. Med.* 8, 518–526. doi: 10.1016/S2213-2600(20)30121-1

Remy, K. E., Brakenridge, S. C., Francois, B., Daix, T., Deutschman, C. S., Monneret, G., et al. (2020). Immunotherapies for COVID-19: lessons learned from sepsis. *Lancet Respir. Med.* 8, 946–949. doi: 10.1016/S2213-2600(20)30217-4

Ribas, V. R., Ribas, R. M. G., Barros, M. G. S., Ribas, K., Neto, N. A., Barros, M. G. S., et al. (2020). Hemodynamics, baroreflex index and blood biomarkers of a patient who died after being affected by COVID-19: case report. *Hematol. Transfus Cell Ther.* 42, 206–211. doi: 10.1016/j.htct.2020.06.004

Rokni, M., Ghasemi, V., and Tavakoli, Z. (2020). Immune responses and pathogenesis of SARS-CoV-2 during an outbreak in Iran: comparison with SARS and MERS. *Rev. Med. Virol.* 30:e2107. doi: 10.1002/rmv.2107

Ronco, C., Reis, T., and Husain-Syed, F. (2020). Management of acute kidney injury in patients with COVID-19. *Lancet Respir. Med.* 8, P738–P742. doi: 10.1016/S2213-2600(20)30229-0

Sant'Ambrogio, G. (1982). Information arising from the tracheobronchial tree of mammals. *Physiol. Rev.* 62, 531–569. doi: 10.1152/physrev.1982.62.2.531

Sardu, C., Gambardella, J., Morelli, M. B., Wang, X., Marfella, R., and Santulli, G. (2020). Hypertension, thrombosis, kidney failure, and diabetes: is COVID-19 an endothelial disease? A comprehensive evaluation of clinical and basic evidence. *J. Clin. Med.* 9:1417. doi: 10.3390/jcm9051417

Sattar, N., McInnes, I. B., and McMurray, J. J. V. (2020). Obesity a risk factor for severe COVID-19 infection: multiple potential mechanisms. *Circulation* 142, 4–6. doi: 10.1161/CIRCULATIONAHA.120.047659

Schelegle, E. S. (2003). Functional morphology and physiology of slowly adapting pulmonary stretch receptors. *Anat. Rec. A Discov. Mol. Cell Evol. Biol.* 270, 11–16. doi: 10.1002/ar.a.10004

Schoenhagen, P., Tuzcu, E. M., and Ellis, S. G. (2002). Plaque vulnerability, plaque rupture, and acute coronary syndromes: (multi)-focal manifestation of a systemic disease process. *Circulation* 106, 760–762. doi: 10.1161/01.cir.0000025708.36290.05

Shi, Y., Wang, Y., Shao, C., Huang, J., Gan, J., Huang, X., et al. (2020). COVID-19 infection: the perspectives on immune responses. *Cell Death Differ.* 27, 1451–1454. doi: 10.1038/s41418-020-0530-3

Shibata, S., Arima, H., Asayama, K., Hoshide, S., Ichihara, A., Ishimitsu, T., et al. (2020). Hypertension and related diseases in the era of COVID-19: a report from the Japanese Society of Hypertension Task Force on COVID-19. *Hypertens. Res.* 43, 1028–1046. doi: 10.1038/s41440-020-0515-0

Siddiqi, H. K., and Mehra, M. R. (2020). COVID-19 illness in native and immunosuppressed states: a clinical-therapeutic staging proposal. *J. Heart Lung Transplant.* 39, 405–407. doi: 10.1016/j.healun.2020.03.012

Spyropoulos, A. C., Ageno, W., and Barnathan, E. S. (2020). Hospital-based use of thromboprophylaxis in patients with COVID-19. *Lancet* 395:e75. doi: 10.1016/S0140-6736(20)30926-0

Stefanini, G. G., Montorfano, M., Trabattoni, D., Andreini, D., Ferrante, G., Ancona, M., et al. (2020). ST-elevation myocardial infarction in patients with COVID-19: clinical and angiographic outcomes. *Circulation* 141, 2113–2116. doi: 10.1161/CIRCULATIONAHA.120.047525

Strutz, F., and Zeisberg, M. (2006). Renal fibroblasts and myofibroblasts in chronic kidney disease. *J. Am. Soc. Nephrol.* 17, 2992–2998. doi: 10.1681/ASN.2006050420

Su, H., Yang, M., Wan, C., Yi, L. X., Tang, F., Zhu, H. Y., et al. (2020). Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int.* 98, 219–227. doi: 10.1016/j.kint.2020.04.003

Sun, T., and Guan, J. (2020). Novel coronavirus and central nervous system. *Eur. J. Neurol.* doi: 10.1111/ene.14227 [Epub ahead of print]

Sungnak, W., Huang, N., Becavin, C., Berg, M., Queen, R., Litvinukova, M., et al. (2020). SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat. Med.* 26, 681–687. doi: 10.1038/s41591-020-0868-6

Tang, N., Bai, H., Chen, X., Gong, J., Li, D., and Sun, Z. (2020a). Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J. Thromb. Haemost.* 18, 1094–1099. doi: 10.1111/jth.14817

Tang, N., Li, D., Wang, X., and Sun, Z. (2020b). Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J. Thromb. Haemost.* 18, 844–847. doi: 10.1111/jth.14768

Tassorelli, C., Mojoli, F., Baldanti, F., Bruno, R., and Benazzo, M. (2020). COVID-19: what if the brain had a role in causing the deaths? *Eur. J. Neurol.* 27, e41–e42. doi: 10.1111/ene.14275

Tavazzi, G., Pellegrini, C., Maurelli, M., Belliato, M., Sciutti, F., Bottazzi, A., et al. (2020). Myocardial localization of coronavirus in COVID-19 cardiogenic shock. *Eur. J. Heart Fail.* 22, 911–915. doi: 10.1002/ejhf.1828

Thachil, J. (2020). The versatile heparin in COVID-19. *J. Thromb. Haemost.* 18, 1020–1022. doi: 10.1111/jth.14821

Thachil, J., Tang, N., Gando, S., Falanga, A., Cattaneo, M., Levi, M., et al. (2020). ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J. Thromb. Haemost.* 18, 1023–1026. doi: 10.1111/jth.14810

Timmers, H. J., Wieling, W., Karemaker, J. M., and Lenders, J. W. (2003). Denervation of carotid baro- and chemoreceptors in humans. *J. Physiol.* 553, 3–11. doi: 10.1113/jphysiol.2003.052415

Tobin, M. J., Laghi, F., and Jubran, A. (2020). Why COVID-19 silent hypoxemia is baffling to physicians. *Am. J. Respir. Crit. Care Med.* 202, 356–360. doi: 10.1164/rccm.202006-2157CP

Vaduganathan, M., Vardeny, O., Michel, T., McMurray, J. J. V., Pfeffer, M. A., and Solomon, S. D. (2020). Renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. *N. Engl. J. Med.* 382, 1653–1659. doi: 10.1056/NEJMsr2005760

Van der Borght, K., and Lambrecht, B. N. (2018). Heart macrophages and dendritic cells in sickness and in health: a tale of a complicated marriage. *Cell. Immunol.* 330, 105–113. doi: 10.1016/j.cellimm.2018.03.011

van Gestel, A. J., and Steier, J. (2010). Autonomic dysfunction in patients with chronic obstructive pulmonary disease (COPD). *J. Thorac. Dis.* 2, 215–222. doi: 10.3978/j.issn.2072-1439.2010.02.04.5

van Gorp, E. C., Suharti, C., ten Cate, H., Dolmans, W. M., van der Meer, J. W., ten Cate, J. W., et al. (1999). Review: infectious diseases and coagulation disorders. *J. Infect. Dis.* 180, 176–186. doi: 10.1086/314829

van Nieuwenhoven, F. A., and Turner, N. A. (2013). The role of cardiac fibroblasts in the transition from inflammation to fibrosis following myocardial infarction. *Vasc. Pharmacol.* 58, 182–188. doi: 10.1016/j.vph.2012.07.003

Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., et al. (2020). Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 395, 1417–1418. doi: 10.1016/S0140-6736(20)30937-5

Wang, X. L., Iwanami, J., Min, L. J., Tsukuda, K., Nakaoka, H., Bai, H. Y., et al. (2016). Deficiency of angiotensin-converting enzyme 2 causes deterioration of cognitive function. *NPJ Aging Mech. Dis.* 2:16024. doi: 10.1038/npjamd.2016.24

Wang, J., Li, Q., Yin, Y., Zhang, Y., Cao, Y., Lin, X., et al. (2020a). Excessive neutrophils and neutrophil extracellular traps in COVID-19. *Front. Immunol.* 11:2063. doi: 10.3389/fimmu.2020.02063

Wang, W., Xu, Y., Gao, R., Lu, R., Han, K., Wu, G., et al. (2020b). Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*. 323, 1843–1844. doi: 10.1001/jama.2020.3786

Wu, Z., and McGoogan, J. M. (2020). Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. *JAMA* 323, 1239–1242. doi: 10.1001/jama.2020.2648

Wu, L., O'Kane, A. M., Peng, H., Bi, Y., Motriuk-Smith, D., and Ren, J. (2020b). SARS-CoV-2 and cardiovascular complications: from molecular mechanisms to pharmaceutical management. *Biochem. Pharmacol.* 178:114114. doi: 10.1016/j.bcp.2020.114114

Wu, C. I., Postema, P. G., Arbelo, E., Behr, E. R., Bezzina, C. R., Napolitano, C., et al. (2020a). SARS-CoV-2, COVID-19, and inherited arrhythmia syndromes. *Heart Rhythm.* 17, 1456–1462. doi: 10.1016/j.hrthm.2020.03.024

Xia, H., and Lazartigues, E. (2008). Angiotensin-converting enzyme 2 in the brain: properties and future directions. *J. Neurochem.* 107, 1482–1494. doi: 10.1111/j.1471-4159.2008.05723.x

Xiong, T. Y., Redwood, S., Prendergast, B., and Chen, M. (2020). Coronaviruses and the cardiovascular system: acute and long-term implications. *Eur. Heart J.* 41, 1798–1800. doi: 10.1093/eurheartj/ehaa231

Xu, J., Li, Y., Gan, F., Du, Y., and Yao, Y. (2020a). Salivary glands: potential reservoirs for COVID-19 asymptomatic infection. *J. Dent. Res.* 99:989. doi: 10.1177/0022034520918518

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., et al. (2020b). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med.* 8, 420–422. doi: 10.1016/S2213-2600(20)30076-X

Yang, W., Sirajuddin, A., Zhang, X., Liu, G., Teng, Z., Zhao, S., et al. (2020). The role of imaging in 2019 novel coronavirus pneumonia (COVID-19). *Eur. Radiol.* 30, 4874–4882. doi: 10.1007/s00330-020-06827-4

Yang, Y., and Tang, H. (2016). Aberrant coagulation causes a hyper-inflammatory response in severe influenza pneumonia. *Cell. Mol. Immunol.* 13, 432–442. doi: 10.1038/cmi.2016.1

Yao, B. C., Meng, L. B., Hao, M. L., Zhang, Y. M., Gong, T., and Guo, Z. G. (2019). Chronic stress: a critical risk factor for atherosclerosis. *J. Int. Med. Res.* 47, 1429–1440. doi: 10.1177/0300060519826820

Yogasundaram, H., Putko, B. N., Tien, J., Paterson, D. I., Cujec, B., Ringrose, J., et al. (2014). Hydroxychloroquine-induced cardiomyopathy: case report, pathophysiology, diagnosis, and treatment. *Can. J. Cardiol.* 30, 1706–1715. doi: 10.1016/j.cjca.2014.08.016

Yokoyama, T., Vaca, L., Rossen, R. D., Durante, W., Hazarika, P., and Mann, D. L. (1993). Cellular basis for the negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian heart. *J. Clin. Invest.* 92, 2303–2312. doi: 10.1172/JCI116834

Yufu, K., Takahashi, N., Nakagawa, M., Hara, M., Saikawa, T., and Yoshimatsu, H. (2006). Brain natriuretic peptide and cardiac autonomic function in type 2

diabetic patients. *Diabetes Res. Clin. Pract.* 72, 12–19. doi: 10.1016/j.diabres.2005.08.011

Zeng, J. H., Liu, Y. X., Yuan, J., Wang, F. X., Wu, W. B., Li, J. X., et al. (2020). First case of COVID-19 complicated with fulminant myocarditis: a case report and insights. *Infection* 48, 773–777. doi: 10.1007/s15010-020-01424-5

Zheng, Y. Y., Ma, Y. T., Zhang, J. Y., and Xie, X. (2020). COVID-19 and the cardiovascular system. *Nat. Rev. Cardiol.* 17, 259–260. doi: 10.1038/s41569-020-0360-5

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395, 1054–1062. doi: 10.1016/S0140-6736(20)30566-3

Zisman, L. S., Keller, R. S., Weaver, B., Lin, Q., Speth, R., Bristow, M. R., et al. (2003). Increased angiotensin-(1-7)-forming activity in failing human heart ventricles: evidence for upregulation of the angiotensin-converting enzyme homologue ACE2. *Circulation* 108, 1707–1712. doi: 10.1161/01.CIR.0000094734.67990.99

Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., et al. (2020). SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N. Engl. J. Med.* 382, 1177–1179. doi: 10.1056/NEJMc2001737

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 19 November 2020
doi: 10.3389/fphys.2020.599729

Biological Context Linking Hypertension and Higher Risk for COVID-19 Severity

*Caio A. M. Tavares[1], Matthew A. Bailey[2] and Adriana C. C. Girardi[3]**

[1] Geriatric Cardiology Unit, Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil, [2] Centre for Cardiovascular Science, The Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, United Kingdom, [3] Laboratory of Genetics and Molecular Cardiology, Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil

The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), represents a public health crisis of major proportions. Advanced age, male gender, and the presence of comorbidities have emerged as risk factors for severe illness or death from COVID-19 in observation studies. Hypertension is one of the most common comorbidities in patients with COVID-19. Indeed, hypertension has been shown to be associated with increased risk for mortality, acute respiratory distress syndrome, need for intensive care unit admission, and disease progression in COVID-19 patients. However, up to the present time, the precise mechanisms of how hypertension may lead to the more severe manifestations of disease in patients with COVID-19 remains unknown. This review aims to present the biological plausibility linking hypertension and higher risk for COVID-19 severity. Emphasis is given to the role of the renin-angiotensin system and its inhibitors, given the crucial role that this system plays in both viral transmissibility and the pathophysiology of arterial hypertension. We also describe the importance of the immune system, which is dysregulated in hypertension and SARS-CoV-2 infection, and the potential involvement of the multifunctional enzyme dipeptidyl peptidase 4 (DPP4), that, in addition to the angiotensin-converting enzyme 2 (ACE2), may contribute to the SARS-CoV-2 entrance into target cells. The role of hemodynamic changes in hypertension that might aggravate myocardial injury in the setting of COVID-19, including endothelial dysfunction, arterial stiffness, and left ventricle hypertrophy, are also discussed.

Keywords: COVID, hypertension, renin-angiotensin system, hemodynamic factors, inflammation, dipeptidyl peptidase 4

OPEN ACCESS

Edited by:
George Grant, University of Aberdeen, United Kingdom

Reviewed by:
Mark Chappell, Wake Forest School of Medicine, United States
Christoph Sinning, University Heart & Vascular Center Hamburg (UHZ), Germany

***Correspondence:**
Adriana C. C. Girardi
adriana.girardi@incor.usp.br

Specialty section:
This article was submitted to Clinical and Translational Physiology, a section of the journal Frontiers in Physiology

Received: *02 September 2020*
Accepted: *28 October 2020*
Published: *19 November 2020*

Citation:
Tavares CAM, Bailey MA and Girardi ACC (2020) Biological Context Linking Hypertension and Higher Risk for COVID-19 Severity. Front. Physiol. 11:599729. doi: 10.3389/fphys.2020.599729

INTRODUCTION

The severe acute respiratory coronavirus 2 (SARS-CoV-2) infection, named coronavirus disease 2019 (COVID-19), was initially described as a series of cases of atypical pneumonia arising in Wuhan, China, in December 2019 (Zhu et al., 2020). The rapid spread of COVID-19 in many countries worldwide has given rise to a global public health crisis of unprecedented proportions in the modern era. As of October 9, 2020, the SARS-CoV-2 has infected 36,669,238 individuals, with 1,063,863 deaths globally (Dong et al., 2020).

The clinical spectrum of COVID-19 ranges from asymptomatic infection to mild or moderate respiratory and associated symptoms (cough, sore throat, nasal congestion, myalgia, arthralgia, headache, shortness of breath) (Guan et al., 2020b) to severe pneumonia accompanied by multiorgan failure which may result in death. Accumulated evidence from the first months of the COVID-19 pandemic has also linked several risk factors with the development of severe morbidity and mortality, such as advanced age, male gender, and the coexistence of underlying chronic diseases. Indeed, the presence of comorbidities, especially hypertension, have been consistently reported as more common among patients with COVID-19 in severe conditions, admitted to the intensive care unit (ICI), who received mechanical ventilation or died, than among patients with mild symptoms (Guan et al., 2020b; Wang et al., 2020; Wu et al., 2020; Zhou et al., 2020a).

Hypertension represents one of the most prevalent comorbidities in patients with COVID-19. Since the first observational data available from China, in early March, hypertension has emerged as a potential risk factor for COVID-19 severity and mortality in different cohorts (Guan et al., 2020b; Zhang et al., 2020; Zhou et al., 2020a). With the pandemic progression worldwide, the association of hypertension and unfavorable outcomes was also seen in other countries such as Italy (Grasselli et al., 2020; Mancia et al., 2020) and the United States (Garg et al., 2020). However, at present, the precise impact of hypertension *per se* on COVID-19 severity is yet to be defined. This review aims to present the biological plausibility linking hypertension and higher risk for COVID-19 severity. To this end, we discuss how cellular, molecular, and functional alterations that underlie the pathophysiology of hypertension can impact the severity of the SARS-CoV-2 infection, thereby predisposing hypertensive patients to more complicated clinical outcomes.

ROLE OF THE RENIN-ANGIOTENSIN SYSTEM (RAS)

The RAS is a key player both in the SARS-CoV-2 transmissibility and in the pathophysiology of hypertension. It consists of a complex network of precursors, enzymes, effector peptides, and receptors that exerts a vital role in blood pressure control, extracellular volume homeostasis, and cardiac function, among several other physiological processes. Abnormal activation of RAS components, ultimately leading to the upregulation of angiotensin II (Ang II) and activation of its angiotensin II type 1 receptor (AT1R), contribute to the development and progression of hypertension (Carson et al., 2001; Dahlof et al., 2002; Crowley et al., 2005, 2006; Gurley et al., 2011).

Angiotensin-converting enzyme 2 (ACE2), a type I integral membrane protein, is a homolog of angiotensin-converting enzyme (ACE), the central enzyme of classical RAS (Tipnis et al., 2000). ACE2 is expressed in organs that are important for blood pressure control such as kidneys, vessels, brain, and heart, where it hydrolyzes Ang II (Donoghue et al., 2000; Tipnis et al., 2000). ACE2 is also found in the lungs, small intestine, ovaries, and testicles (Tipnis et al., 2000). Additionally, ACE2 has been identified as a functional receptor for the SARS-CoV-2 host cell entry (Hoffmann et al., 2020) as well as for its predecessor SARS-CoV (Kuba et al., 2005). Binding of the viral spike (S) protein of the SARS-CoV-2 to the extracellular domain of ACE2 triggers conformational changes that destabilize the membrane allowing the internalization of the SARS-CoV-2 along with ACE2, leading to ACE2 cell surface expression downregulation, viral replication and cell-to-cell transmission (Heurich et al., 2014; Hoffmann et al., 2020). During this process, the cleavage of the S protein by host cell proteases, including the transmembrane serine protease 2 (TMPRSS2), is essential for viral infectivity (Iwata-Yoshikawa et al., 2019). As such, TMPRSS2 constitutes a potential target for the treatment of SARS-CoV-2 infected patients.

As a bioactive component of the RAS, ACE2 functions as a counterregulatory enzyme, converting Ang II to Ang-(1-7). This heptapeptide binds to the Mas receptor (MasR), modestly reducing blood pressure, promoting vasodilation, increasing excretion of sodium and water by the kidneys, and exerting anti-inflammatory and antioxidant effects (Santos et al., 2018). These actions are directly opposed to those induced by the activation of the ACE/Ang II/AT1R axis. ACE converts Ang I to Ang II, which in turn acts on the AT1R, increasing blood pressure, inducing vasoconstriction, increasing renal tubular salt and water reabsorption, and increasing the production of reactive oxygen species (ROS) that promote inflammation and fibrosis (Benigni et al., 2010). The ACE/Ang II/AT1R and ACE2/Ang-(1-7)/MasR pathways are co-expressed in most tissues and act in an autocrine and paracrine manner. Thus, the balance between these pathways determines, at least in part, whether or not tissue damage will occur in response to pathological stimuli.

The kidney is a target for end-organ damage in hypertension, plays an active role in the pathogenesis of hypertension, and it is one of the sites of the highest levels of expression of ACE2 (Gembardt et al., 2005). Several studies have found that the protein and mRNA abundance, as well as the activity of ACE2, are reduced in the kidneys of experimental models of hypertension, including spontaneously hypertensive rats, renin transgenic hypertensive rats, aldosterone/NaCl-induced hypertension and the model of 2 kidneys 1 clip (2K1C) hypertensive rats (Soler et al., 2013). In mice on the C57BL/6 genetic background, (Gurley et al., 2006) have found that ACE2 deficiency was associated with a significant increase in blood pressure of ∼7 mmHg and that the absence of ACE2 considerably enhanced the severity of Ang II-dependent hypertension. Moreover, ACE2-deficient mice chronically treated with Ang II infusion displayed a more than 5-fold higher renal Ang II concentration than Ang II-treated wild-type animals, thereby suggesting that the more severe hypertension in ACE2-deficient mice may be attributed to an impaired metabolism of Ang II in the kidney (Gurley et al., 2006). Notably, Ang II upregulates ACE and downregulates ACE2 expression in human proximal tubule cells via an AT1R-mediated mechanism (Koka et al., 2008), thereby suggesting that ACE and ACE2 may be regulated in a balanced manner, which can be mediated via the local Ang II concentration. This synergistic regulation is observed in renal biopsies from humans, in which the ACE to ACE2 ratio is significantly higher in

subjects with hypertension than in subjects without hypertension (Wakahara et al., 2007). The human kidney is a target for the SARS-CoV-2 infection (Braun et al., 2020; Puelles et al., 2020). Acute kidney injury (AKI) has been observed in COVID-19 patients, and it is considered a marker of COVID-19 severity and an adverse prognostic factor for survival (Cheng et al., 2020). Renal Ang II overactivity in the setting of hypertension and potentiated by SARS-CoV-2 induced ACE2 internalization, may contribute to the pathogenesis of AKI in severely ill patients with COVID-19. Favoring this hypothesis are the findings of a prospective cohort study of 701 patients with COVID-19 conducted by Cheng et al. (2020). These authors investigated the association between inpatient use of medications and the development of AKI among patients with COVID-19. It was observed that none of the patients who were taking RAS inhibitors on admission or during hospitalization for COVID-19 developed AKI (Guan et al., 2020a).

Angiotensin-converting enzyme 2 expression is relatively abundant in the heart, where it can be found in cardiomyocytes endothelial cells, and fibroblasts (Santos et al., 2018). Crackower et al. (2002) have found that ACE2 knockout mice display increased heart content of Ang II and cardiac dysfunction characterized by a decrease in fractional shortening with slight ventricular dilation. Moreover, these authors have observed that cardiac dysfunction of ACE2 knockout mice progressed with age, and it was more pronounced in males than in females. The fact that cardiac phenotype and increased Ang II levels were completely reversible by deleting the ACE gene in ACE2 knockout mice strengths the notion that cardiac function is modulated by the balance between ACE and ACE2, and that the increase in local cardiac Ang II was involved in cardiac impairment (Crackower et al., 2002). The cardiac effects of ACE2 remain under debate since ACE2 deletion mediated-cardiac dysfunction was not observed by Gurley and colleagues. On the other hand, it is well accepted that increased cardiac Ang II, generated by cardiac ACE, drives left ventricular hypertrophy (LVH) in multiple settings, including hypertension (Sadoshima and Izumo, 1993; Crowley et al., 2006; Ainscough et al., 2009). Therefore, patients with hypertension are particularly susceptible to the imbalance between the ACE/Ang II/AT1R, and ACE2/Ang-(1-7)/MasR, further intensified by myocardial SARS-CoV-2-mediated ACE2 internalization (Huentelman et al., 2005). Indeed, loss of surface ACE2 in cardiac cells may be one of the underlying causes of acute, and perhaps long-term, exacerbation of cardiovascular disease in hypertensive patients infected with SARS-CoV-2.

Extrapolating data from SARS-CoV to SARS-CoV-2, one may postulate that the imbalance in the signaling and actions of products of ACE/ACE2, generated by the loss of ACE2 cell surface expression due to SARS-CoV-2 infection, may lead to severe acute respiratory failure in COVID-19 (Kuba et al., 2005). The existence of a causal relationship between the imbalanced ACE/ACE2 axis and the acute respiratory distress syndrome has been established through the use of genetically modified animals (Imai et al., 2005; Kuba et al., 2005). Imai et al. (2005) have found that acute lung injury induced by acid aspiration results in decreased expression of ACE2 and increased lung content of Ang II in wild-type mice. Additionally, ACE2 knockout mice with severe acute lung injury induced by acid aspiration or sepsis displayed a higher rate of mortality and lung failure than wild type mice with severe acute lung injury (Imai et al., 2005). Conversely, the genetic deletion of ACE in ACE2 knockout mice significantly attenuated these outcomes, demonstrating that ACE/Ang II drive severe lung, whereas ACE2 protects against it. The levels of ACE2 gene expression appear to be upregulated in the lung of patients with pulmonary hypertension when compared to controls (Pinto et al., 2020), however, to our knowledge, the modulation of lung ACE to ACE2 ratio in essential arterial hypertension remains elusive.

ROLE OF RAS INHIBITORS

During the early beginning of the COVID-19 pandemic, concerns emerged that RAS inhibitors, cornerstone treatment of several cardiovascular diseases, including hypertension, could promote viral interaction with host cells, leading to increased cell entry, viral replication and thereby COVID-19 exacerbation (Diaz, 2020; Esler and Esler, 2020). These concerns were primarily based on findings that ACE inhibitors (ACEi) or angiotensin II type 1 receptor (ARB) upregulate the expression and activity of ACE2, the SARS-CoV-2 receptor, in the kidneys and heart of experimental models of hypertension (Ferrario et al., 2005; Jessup et al., 2006; Wang et al., 2016).

As the pandemic evolved, several observational studies indicated that ACEi/ARBs use are not a risk factor for disease severity and may actually be related to milder disease and better outcomes (**Supplementary Table S1**) possibly by attenuating the imbalance between ACE/Ang II/AT1R and ACE2/Ang-(1-7)/MasR, reducing pathogenic inflammation and multiorgan injury. Also, evidence from population studies suggests that RAS inhibitors neither increase the risk of SARS-CoV-2 infection in patients with hypertension nor negatively impact the disease severity in those who are infected, establishing its safety and reinforcing that they should not be switched/stopped during the pandemic (Mancia et al., 2020; Mehta et al., 2020; Reynolds et al., 2020).

Ongoing clinical trials will add crucial information on the impact of RAS on COVID-19 severity. Currently, several studies are registered in the clinicaltrials.gov platform aiming to investigate the effects of ACEi/ARBs replacement or withdrawn on patients with COVID-19 (**Table 1**), the impact of ACEi/ARBs initiation in patients without hypertension on the risk of COVID-19 infection and severity (**Table 2**) and whether modulation of RAS by other agents with antihypertensive actions [AT1R biased agonist, Ang-(1-7) analogs, DPP4 inhibitors or recombinant ACE2] can impact COVID-19 outcomes (**Table 3**).

INFLAMMATION

There is strong evidence from human and experimental studies to show that chronic hypertension accrues sustained, low-grade inflammation, stimulating the adaptive immune

TABLE 1 | Ongoing randomized trials comparing ACEi/ARBs replacement or withdrawal in patients with COVID-19.

Category	NCT number	Study design	Acronym	Intervention arm	Study population	Target enrollment	Primary outcome measure
ACEi ARB replacement or withdraw	NCT04330300	Randomized, open-label	CORONACION	Switch RAAS inhibitor to alternative medication	Ambulatory hypertensive patients without COVID-19	2414	Composite: death, mechanical ventilation, ICU hospitalization or hospitalization for NIV
	NCT04351581	Randomized, single-blind (outcomes assessor)	RASCOVID-19	Discontinue RAAS inhibitor and start other medication as needed	Hospitalized patients with COVID-19 and use of RAAS inhibitors	215	Composite: death and days out of hospital within 14 days of recruitment
	NCT04353596	Randomized, single-blind (outcomes assessor)	ACEI-COVID	Stoping or replacing ACEi/ARB	COVID-19 infection ≤5 days	208	(1) SOFA/Death (2) ICU/MV/Death
	NCT04364893	Randomized, open-label	BRACE-CORONA	Temporally discontinuation of ACEi/ARB for 30 days	Hospitalized patients with COVID-19	700	Composite: days alive and out of hospital at 30 days
	NCT04329195	Randomized, open-label	ACORES-2	Discontinuation of RAS blocker	Hospitalized patients with COVID-19	554	Time to clinical improvement on a seven-category ordinal scale
	NCT04338009	Randomized, single-blind (participant)	REPLACECOVID	Discontinuation of ACEi/ARB	Hospitalized patients with COVID-19	152	Hierarchial/Composite: (1) time to death; (2) days at ECMO/MV;(3) days supported by RRT/VAD; (4) modified SOFA

BP, blood pressure ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; NIV, non-invasive ventilation; MV, mechanical ventilation; RRT, renal replacement therapy SOFA, Sequential Organ Failure Assessment; VAD, vasoactive drugs. For up-to-date information search the clinicaltrials.gov platform.

system. This may reflect tissue damage as a consequence of sustained high blood pressure, but experimental evidence also points to the role of the immune system in the generation of hypertension. Indeed, cells of the immune system, which contribute importantly to normal blood pressure homeostasis, may operate pathogenically in hypertension, contributing to pressure-dependent and independent organ damage (Mattson, 2019). Despite intense research, a unifying, mechanistic understanding of the interaction in health and disease has yet to emerge. The innate immune system has some "protective" roles: macrophages, for example, regulate extracellular fluid volume by buffering the release of salt from the skin for renal excretion (Machnik et al., 2009). Monocytes/macrophages can also scavenge reactive oxygen species (Rosenblat et al., 2013) and have a role in clearing vasoactive peptides such as endothelin-1 (Czopek et al., 2019), influencing local vasomotor tone and blood pressure. Depletion of monocytes/macrophages, or impairing their ability to clear endothelin-1, increases blood pressure over a few days in humans and mice (Guzik et al., 2007; Abais-Battad et al., 2018), particularly in the setting of a pre-existing challenge to blood pressure such as high salt or Ang II infusion. Conversely, T-cells and B-cells depletion are protective, reducing hypertension and vascular free-radical production in experimental models (Guzik et al., 2007; Abais-Battad et al., 2018). Thus, these cells of the adaptive immune system appear to be "pro-hypertensive" and experimentally, re-population of the T-cell pool restores the full hypertensive response to chronic Ang II infusion (Fehrenbach et al., 2020). Mechanistically, high blood pressure promotes T-cells activation, increasing their ability to invade organs such as the kidney that are susceptible to barotrauma (Itani et al., 2016). This invasive aspect appears to be directly related to pressure, rather than hormonal aspects such as RAS activation that may contribute to hypertension: preventing the pressure rise significantly reduces T-cell and B-cell infiltration (Shimada et al., 2020).

However, the picture is undoubtedly much more complex. For example, in the long-term, reducing the ability of macrophages to clear vasoactive endothelin-1 does not aggravate hypertensive injury, but unexpectedly protects against end-organ damage. This, in part, reflects the repolarizing of cells to an anti-inflammatory phenotype (Guyonnet et al., 2020). The role of the adaptive immune system is similarly, nuanced, and non-genomic modifiers may influence the "pro-hypertensive" phenotype of the T-cell (Seniuk et al., 2020).

Given the prevalence of hypertension in the general population, it is not surprising that this is a common comorbidity in patients hospitalized with coronavirus (Richardson et al., 2020). However, pre-existing hypertension increases the risk of developing severe disease and also of death (Zuin et al., 2020). How hypertension causes poor clinical outcomes in COVID-19 is not understood, but the intersection of blood pressure homeostasis and the immune system may be important. Certainly, SARS-CoV-2 infection features systemic inflammation and accumulation of inflammatory cytokines, the extent of which is strongly implicated in patient outcome (Huang et al., 2020). Viral interaction with ACE2 provides a further pivot point for

TABLE 2 | Ongoing randomized trials comparing ACEi/ARBs initiation to mitigate COVID-19 severity in patients with COVID-19.

Category	NCT number	Study design	Acronym	Intervention arm	Study population	Target enrollment	Primary outcome measure
ACEi or ARB initiation ACEi/ ARB initiation	NCT04345406	Randomized, open-label	N/A	ACE inhibitors	Patients with COVID-19 without contra-indication to ACE inhibitors	60	Number of patients with virological cure
	NCT04366050	Randomized, double-blind, placebo-controlled	RAMIC	Ramipril 2.5 mg for 14 days	Hospitalized patients or in a emergency department with COVID-19	560	Composite: death, need for ICU admission or MV
	NCT04355429	Randomized, open-label	CAPTOCOVID	Captopril 25 mg by nebulization	Hospitalized patients with COVID-19 needing oxygen	230	Ventilator free survival at 14 days
	NCT04360551	Randomized, double-blind, placebo controlled	N/A	Telmisartan 40 mg	Outpatients with COVID-19	40	Maximal clinical severity on a seven-category ordinal scale
	NCT04335786	Randomized, double-blind, placebo-controlled	PRAETORIAN-COVID	Valsartan 80 to 160 mg titrated by blood pressure	Hospitalized patients with COVID-19	651	Composite: death, mechanical ventilation or ICU admission
	NCT04394117	Randomized, single-blind (outcomes assessor)	CLARITY	Initiation of an ARB or switching from non-RAAS inhibitor to ARB	Confirmed COVID-9	605	Improvement on a seven-category ordinal scale
	NCT04340557	Randomized, open-label	N/A	Losartan 12.5 mg up titrated according to BP	Hospitalized patients with COVID-19 and mild to moderate hypoxia	200	Need for MV
	NCT04312009	Randomized, double-blind, placebo-controlled	N/A	Losartan 50 mg daily	Hospitalized patients with COVID-19 requiring oxygen therapy	200	The difference in P/F ratio at 7 days
	NCT04343001	Randomized, factorial design (2 × 2 × 2), open-label	CRASH-19	Losartan 100 mg daily Other interventions: Aspirin, Simvastatin	Hospitalized patients with COVID-19	10000	Mortality up to 28 days

(Continued)

TABLE 2 | Continued

Category	NCT number	Study design	Acronym	Intervention arm	Study population	Target enrollment	Primary outcome measure
	NCT04328012	Randomized, double-blind, placebo-controlled, 4 groups (parallel)	COVIDMED	Losartan 25 mg daily Other interventions: Lopinavir/Ritonavir, Hydroxychloroquine	Hospitalized patients with COVID-19	4000	The difference in the ordinal scale of disease severity
	NCT04359953	Randomized, open-label, 4 groups (parallel)	COVID-Aging	Telmisartan 40 mg twice daily Other interventions: Azithromycin, Hydroxychloroquine	Hospitalized patients with COVID-19 and age ≥75 years or ≥60 years if dementia	1600	Mortality up to 14 days
	NCT04351724	Randomized, open-label, adaptative trial	ACOVACT	RAS Blockade substudy Candesartan 4 mg daily, uptitrated Other interventions: Chloroquine, Lopinavir/Ritonavir, Rivaroxaban and Clazakizumab	Hospitalized patients with COVID-19 and blood pressure ≥120/80 mmHg	500	Sustained clinical improvement on a seven-category ordinal scale
	NCT04356495	Randomized, open-label, multi-arm multi-stage trial	COVERAGE	Telmisartan 20 mg daily Other interventions: Hydroxychloroquine, Imatinib, and Faviparavir	Outpatients with COVID-19	1057	Primary outcomes: 1- Mortality up to 14 days 2- Need for hospitalization up to 14 days
	NCT04447235	Randomized, double-blind, placebo-controlled	TITAN	Ivermectin plus Losartan 50 mg daily	Cancer patients with COVID-19	176	Composite: mortality, need for MV or ICU admission up to 28 days
	NCT04311177	Randomized, double-blind, placebo controlled	N/A	Losartan 25 mg	Symptomatic COVID-19 infection	516	Hospital admission up to 15 days
	NCT04355936	Randomized, open-label	N/A	Telmisartan 80 mg twice daily	COVID-19 infection	400	CRP at days 1.8 and 15
	NCT04428268	Randomized, double-blind	N/A	Losartan 25 mg twice daily Chloroquine vs. Chloroquine/Losartan	Hospitalized patients with COVID-19	20	Mortality up to 28 days

TABLE 3 | Ongoing clinical trials testing the hypothesis that modulation of RAS components can impact COVID-19 severity.

Category	NCT number	Study design	Acronym	Intervention arm	Study population	Target enrollment	Primary outcome measure
Recombinant ACE2	NCT04382950	Randomized, open-label	N/A	Recombinant ACE2 infusion plus aerosolized isotretinoin	Hospitalized patients with COVID-19 and respiratory failure	24	Fever
	NCT04375046	Randomized, open-label	Bacterial ACE2	Recombinant ACE2 infusion	Hospitalized patients with COVID-19	24	(1) Fever (2) Viral load
	NCT04335136	Randomized, double-blind, placebo-controlled	APN01-COVID-19	Recombinant ACE2 infusion	Hospitalized patients with COVID-19	200	Composite: death or mechanical ventilation up to 28 days or hospital discharge
Biased agonist of AT1R	NCT04419610	Randomized, double-blind, placebo-controlled	N/A	TRV027 at 12 mg/hour until discharge or 7 days	Hospitalized patients with COVID-19	60	Mean change from baseline D-dimer at day 8
Ang 1-7 analogs	NCT04332666	Randomized, double-blind, placebo controlled	ATCO	Angiotensin-(1-7) infusion (venous) of 0.2 mcg/Kg/h for 48h	Hospitalized patients with COVID-19 respiratory failure and MV	60	Composite: mortality and MV-free days
	NCT04375124	Non-randomized, open label	N/A	angiotensin peptide (1-7) derived plasma	Hospitalized patients with COVID-19	20	Mortality up to 4 months
	NCT04401423	Randomized, double-blind, placebo-controlled	TXA COVID-19 Clinical Trial	TXA127 0.5 mg/kg per day	Hospitalized patients with COVID-19 requiring oxygen therapy	100	1-Acute kidney injury up to 7 days 2-Need for VM up to 7 days
DPP4 inhibitors	NCT04341935	Randomized, open-label	N/A	Linagliptin 5 mg daily	Hospitalized patients with COVID-19	20	Changes in glucose levels
	NCT04371978	Randomized, open-label	N/A	Linagliptin 5 mg daily	Hospitalized patients with COVID-19	100	Time to clinical improvement (WHO scale of COVID-19)

Ang-1-7, Angiotensin-1-7; AT1R, Angiotensin II type 1 Receptor; DPP4, Dipeptidyl peptidase 4.

local inflammation since ACE2 converts pro-inflammatory Ang II to Ang-(1-7), which has anti-inflammatory roles. It is evident that the inflammation response to COVID-19 is amplified in hypertensives compared to normotensive controls (Yang et al., 2020). In humans, high levels of systemic inflammation driven by infection induce a short-lived, extensive endothelial dysfunction (Hingorani et al., 2000) that would be anticipated to transiently increase cardiovascular risk. It is not difficult to imagine that this risk would be exaggerated for individuals with a vulnerable cardiovascular system, such as those with hypertension. Of importance, in patients receiving antihypertensive medicines, those on ACEi or ARBs had reduced levels of inflammatory biomarkers (C-reactive protein and procalcitonin) and better outcomes than those on other antihypertensive medication (Yang et al., 2020). These outcomes from a retrospective, single-center cohort (Wuhan, China) study give some insight suggesting that immune system/blood pressure interactions are important for COVID-19 severity, and also that ACEi, may have beneficial cardiovascular effects in this setting beyond blood pressure control. Indeed, targeting excessive inflammation is an attractive strategy to improve the health of the arterial cardiovascular system (Zanoli et al., 2020) and may be particularly relevant in understanding cardiovascular risk in COVID-19.

HEMODYNAMIC FACTORS

Endothelial Dysfunction

Endothelial cells play a vital role in cardiovascular homeostasis by controlling vasomotor tone, maintaining vascular integrity, exerting barrier protecting effects, and preventing platelet and leukocyte adhesion and aggregation (Deanfield et al., 2007). It also regulates fibrinolysis and the coagulation cascade, provides antiproliferative and anti-inflammatory actions, and protects against oxidative stress (Deanfield et al., 2007). In turn, abnormalities of the vascular endothelium significantly contribute to a plethora of cardiovascular disorders.

A large body of evidence demonstrates the presence of endothelial dysfunction in patients with hypertension (Watson et al., 2008). Endothelial dysfunction is characterized by imbalanced vasodilation and vasoconstriction, elevated ROS and pro-inflammatory mediators, as well as reduced bioavailability of nitric oxide (NO) (Deanfield et al., 2007; Watson et al., 2008). As aforementioned, Ang II, via AT1R, is a potent activator of oxidative and inflammatory cascades, the primary mediators of endothelial dysfunction. Under physiological conditions, however, the ACE2/Ang-(1-7)/MasR axis stimulates the activity of the endothelial NO synthase, increasing NO production. Adding up to this effect, Ang-(1-7) decreases the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase stimulated by Ang II, directly modulating the generation of reactive ROS (Sampaio et al., 2007).

Many severe COVID-19 patients show signs of a cytokine storm that could be aggravated due to overactivation of Ang II, increased production of ROS, and a preexistent pro-inflammatory state, features of hypertension associated-chronic endothelial dysfunction. In fact, endotheliitis and an increase in D-dimer (a marker of activation of coagulation and fibrinolysis) have been described in the pathological findings of patients with COVID-19 (Tang et al., 2020; Varga et al., 2020). Therefore, it is plausible to postulate that circulating SARS-CoV-2 may interact with endothelial cells of hypertensive patients culminating both in direct viral injury and in a dysfunctional response to infection, amplificating chemokine release, inflammatory cell adhesion and migration through the endothelial barrier, ultimately leading to a procoagulant state and tissue damage (i.e., myocardial injury, acute respiratory distress syndrome) (Ackermann et al., 2020; Bermejo-Martin et al., 2020).

Arterial Stiffness

Arterial stiffness describes the reduced capability of an artery to expand and contract in response to pressure changes. It is measured by carotid-femoral pulse wave velocity and is an independent predictor of cardiovascular (CV) events and mortality in patients with hypertension (Laurent et al., 2001).

Potentially, high arterial stiffness could have deleterious effects in patients with SARS-CoV-2 infection thorough different putative mechanisms: (i) chronically, arterial stiffness can increase the energy penetration of the increased pulsatile flow from the larger arteries, damaging target organs (brain, kidney, heart) and aggravating the infection by mitigating the functional reserves of different systems; (ii) the COVID-19 cytokine-storm may cause ventricular-arterial decoupling in the setting of low systemic vascular resistance and elevated heart rate. In this scenario, patients with high arterial stiffness could be more prone to ventricular-arterial decoupling by increasing pulsatile components of the total arterial load to the left ventricle (LV): proximal aortic impedance, wave reflections and arterial tree compliance (Chirinos et al., 2014, 2019; Ikonomidis et al., 2019) which ultimately leads to increased myocardial oxygen demand, CV inefficiency, and left ventricle (LV) energetic failure (Chemla et al., 2003; Guarracino et al., 2014); and (iii) increased arterial stiffness is associated with reduced coronary flow reserve in hypertensive patients (Ikonomidis et al., 2008) and lower diastolic blood pressure (coronary perfusion pressure), rendering these patients more vulnerable to myocardial injury and ischemia - a known complication of COVID-19 (Hendren et al., 2020).

Another intriguing aspect warranting exploration is whether survivors of COVID-19 will develop long term vascular sequelae of the infection, such as increased arterial stiffness and accelerated vascular aging. The Artery Society recently launched a collaborative, multicenter, research project to evaluate the vascular impact of the infection[1] and will periodically test different biomarkers of aging in patients that had COVID-19.

Left Ventricle Hypertrophy

Prolonged systemic hypertension results in hypertensive target-organ damage, and the most common manifestation of this is left ventricular hypertrophy (LVH). LVH - due to cellular hypertrophy and expansion of extracellular matrix - is defined as an increase in the mass of the left ventricle secondary to chronically elevated afterload and neurohormonal stimuli

[1] http://www.arterysociety.org/our-activities/cartesian-2/

(Diez and Frohlich, 2010). Arterial stiffness plays a major role in LVH as it accelerates pulse wave velocity, causing premature arrival of wave reflections to the central aorta and by producing amplification of the mid-to-late systolic pressure, chronically stressing the LV (Chirinos et al., 2019). Allied to neurohormonal stimuli, both processes generate a series of molecular, cellular, and structural adaptations leading to cardiac remodeling (Nwabuo and Vasan, 2020). LVH is not only a marker of hypertension-related target organ damage but also an independent risk factor for CV complications (Haider et al., 1998; Sundstrom et al., 2001; Narayanan et al., 2014; Bang et al., 2017; Afify et al., 2018) that can occur after weaning of the initial compensatory mechanism due to contractile, electrical, structural or metabolic abnormalities (Pitoulis and Terracciano, 2020).

Myocardial injury among patients hospitalized with COVID-19 has been described since the early reports of the disease (Zhou et al., 2020a). Although being an important prognostic factor in severe cohorts (Guo et al., 2020; Lala et al., 2020; Shi et al., 2020a,b), the exact mechanism of myocardial injury is not fully understood as multiple plausible mechanisms often coexist in a single patient including multiorgan failure, types 1 and 2 myocardial infarction, disseminated intravascular coagulation, endothelial cell dysfunction, pre-existing chronic injury, pulmonary hypertension, among others (Jaffe et al., 2020). It is also unknown if there is a causal relationship between myocardial injury and disease severity or if it is solely a marker of pre-existing cardiovascular disease. LVH-related changes in the myocardial tissue and extracellular matrix might be related to both an increased risk of myocardial injury due to several pathophysiological pathways (increased cardiac Ang II, endothelial dysfunction, chronic inflammation, upregulated cardiac DPP4 expression) and an abolished cardiovascular response to the stress related to the infection.

Left ventricular hypertrophy imposed changes of cardiac structure function can complicate further the management of these patients in the intensive care unit (ICU), as LV compliance and diastolic function are impaired: the rise in the end-diastolic pressure narrows the optimal volume status for hemodynamic stability without pulmonary congestion (Sanfilippo et al., 2018); sinus tachycardia or supraventricular arrhythmias with rapid ventricular response can trigger hemodynamic collapse by reduced LV filling time-related to increase in the heart rate (Borlaug et al., 2011); increased LV filling pressure is also an independent risk factor for weaning failure from mechanical ventilation (Papanikolaou et al., 2011; Konomi et al., 2016; Liu et al., 2016) and the use high positive end-expiratory pressure during mechanical ventilation can lead to additional impairment in LV relaxation (Chin et al., 2013; Juhl-Olsen et al., 2013).

Also, the electrical remodeling due to LVH in hypertensive patients might be related to an increased risk of malignant ventricular arrhythmias and sudden cardiac death (Aro and Chugh, 2016), as they are being treated in the ICU with other aggravating factors such as mechanical ventilation, vasoactive drugs, medications that prolong the QT interval, and electrolyte disturbances. This overlap of hemodynamics and electrical disorders reflecting cellular and molecular remodeling due to LVH can indeed be the cause of poorer outcomes in patients with COVID-19 being treated in the ICU and poises a great challenge for clinicians that need to untangle the complexity of a serious illness aggravated by pre-existing conditions.

ROLE OF DIPEPTIDYL PEPTIDASE 4 (DPP4)

Bioinformatic approaches based on protein crystal structure predicted that the middle east respiratory syndrome coronavirus (MERS-CoV) receptor DPP4 displays a high affinity with the SARS-CoV-2 spike protein (Li et al., 2020). This thereby suggests that SARS-CoV-2 may utilize DPP4 as a coreceptor, in addition to ACE2, to gain entry into the host cell. Nevertheless, the results of free energy calculation revealed that SARS-CoV-2 spike protein binds ACE2 with higher affinity than that of DPP4 (Li et al., 2020). Moreover, it was shown that only Hela and baby kidney hamster (BHK2) cells transfected with human ACE2, but not with human DPP4, were capable of being infected with SARS-CoV-2 (Hoffmann et al., 2020; Letko et al., 2020; Zhou et al., 2020b). However, further research is necessary to defined whether or not DPP4 may mediate the SARS-CoV-2 entry into permissive cells.

Dipeptidyl peptidase is a serine peptidase expressed on the surface of several cell types, including epithelial and endothelial cells and lymphocytes (Kenny et al., 1976; Marguet et al., 2000; Lambeir et al., 2003). It also exists as a soluble circulating form in plasma and other body fluids (Lambeir et al., 2003). Through its enzymatic function, DPP4 modulates the biological activity of several circulating hormones, neuropeptides, cytokines, and chemokines. In addition to its peptidase, activity DPP4 interacts with several proteins, including the renal proximal tubule Na^+/H^+ exchanger isoform 3 (NHE3) (Girardi et al., 2001), fibronectin and collagen, adenosine deaminase (ADA), C-X-C chemokine receptor type 4, underscoring the potential role of DPP4 in sodium retention, fibrosis, and inflammation. The importance of DPP4 for the scientific and medical community has considerably raised since the approval of inhibitors of DPP4 activity, known as gliptins, for the treatment of type 2 diabetes (T2D).

The gliptins do not bind to the putative receptor binding site of SARS-CoV-2 (Li et al., 2020). However, it does not exclude the possibility that DPP4 inhibition may indirectly attenuate the severity of COVID-19, due to the role that DPP4 plays in the pathophysiology of common comorbidities in patients with COVID-19 (Ryskjaer et al., 2006; Dos Santos et al., 2013; Zhong et al., 2013), including hypertension. Indeed, successive clinical studies have demonstrated that gliptins confer renal and cardiovascular benefits in patients with hypertension with or without T2D (Nistala and Savin, 2017). Intriguingly, although known primarily for its role as competitive inhibitors, gliptins are also capable of reducing DPP4 protein and mRNA abundance in the heart, kidneys, and endothelial cells of experimental animals of cardiovascular and metabolic diseases (Dos Santos et al., 2013; Kanasaki et al., 2014; Arruda-Junior et al., 2016; Beraldo et al., 2019). Whether altered DPP4 expression in the setting of hypertension, as well as of other comorbidities,

contributes to SARS-CoV-2 infectivity, and COVID-19 severity is currently undetermined.

Recent evidence suggests the existence of an interplay between DPP4 and tissue RAS (Aroor et al., 2016; Beraldo et al., 2019). In renal proximal tubule cells, Ang II, through AT1R, enhances DPP4 activity, whereas inhibition of DPP4 mitigates Ang II-mediated activation of AT1R signaling and its downstream effects (Aroor et al., 2016). In rats with chronic kidney disease (CKD) and hypertension, the administration of the DPP4 inhibitor sitagliptin ameliorated hypertension, kidney function and restored the cardiac ratio of Ang II to Ang-(1-7) concentrations in the heart by reducing the levels of Ang II and increasing the content of Ang-(1-7) (Beraldo et al., 2019). Interestingly, sitagliptin was capable of upregulating ACE2 expression in the heart of rats with CKD and as well as in control animals (Beraldo et al., 2019). In line with these findings, Zhang et al. (2015) found that the DPP4 inhibitor linagliptin lowered the expression of the AT1R and upregulated the activity of ACE2 in the heart in rats with Ang II-induced hypertension. Collectively, these studies support the hypothesis that increased DPP4 activity and expression can favor an imbalance between ACE/Ang II/AT1R and ACE2/Ang-(1-7)/MasR.

The vascular activity and expression of DPP4 are increased in hypertensive rats (Linardi et al., 2004; Savignano et al., 2017), suggesting that this peptidase may contribute to impaired vascular function associated with high blood pressure. Accordingly, extensive studies have shown that DPP4 inhibitors play a protective effect against hypertension-related vascular events, such as endothelial dysfunction and increased arterial stiffness (Kishimoto et al., 2019; Zhang et al., 2019). The vasoprotective effects of DPP4 inhibition are mediated through multiple mechanisms, including improved NO bioavailability by upregulation of endothelial NOS, and thus, endothelium-dependent relaxation; reduction of ROS generation, and cyclooxygenase-2 expression; as well as by suppression of inflammatory responses (Zhang et al., 2019; Liu et al., 2020).

Several studies have demonstrated that DPP4 inhibitors ameliorate LVH (Dos Santos et al., 2013; Arruda-Junior et al., 2016; Beraldo et al., 2019; Nakajima et al., 2019; Nam et al., 2019; Okabe et al., 2020), whereas upregulated activity and expression of heart DPP4 is associated with cardiac remodeling and dysfunction (Dos Santos et al., 2013; Arruda-Junior et al., 2016; Beraldo et al., 2019). The antihypertrophic effects of the DPP4 inhibitor teneligliptin have been recently unraveled in an experimental model of Ang II-induced hypertension (Okabe et al., 2020). The authors found that the administration of teneligliptin to C57BL/6J mice suppressed Ang II-induced NADPH oxidase 4 mRNA overexpression, ROS production, and attenuated LVH without affecting blood pressure (Okabe et al., 2020). DPP4 inhibition has also mitigated LV remodeling and dysfunction in other experimental models of hypertension, such as spontaneously hypertensive rats and Dahl salt-sensitive rats (Nakajima et al., 2019; Nam et al., 2019). However, in the

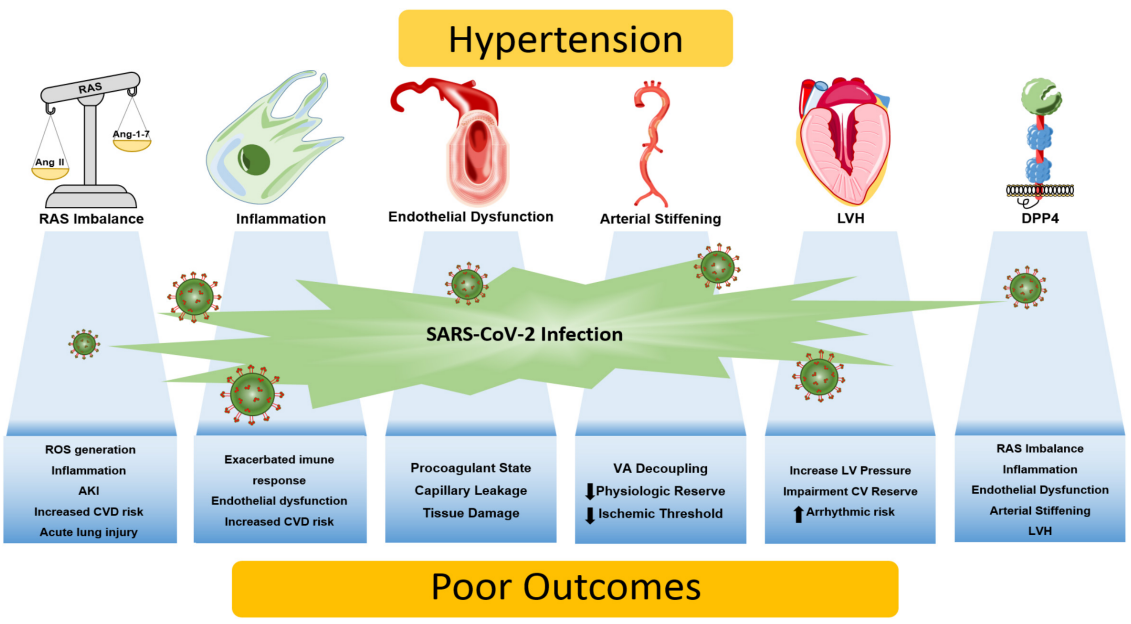


FIGURE 1 | Putative mechanisms linking hypertension and COVID-19 severity. Patients with hypertension are more prone to a vicious interplay between RAS imbalance, chronic low-grade inflammation, and elevated DPP4 activity and expression. Dysregulation of these biological processes may be aggravated by the SARS-CoV-2 infection, giving rise to an exacerbated immune response that culminates in tissue damage/dysfunction. Also, end-organ damage caused by chronic hypertension diminishes cardiovascular reserve, as arterial stiffening, endothelial dysfunction and left ventricular hypertrophy emerges, leading to synergic processes that increase the susceptibility to know complications of COVID-19 including myocardial injury and ischemia, acute lung injury, thrombosis, acute kidney injury, ventricular arrhythmias and potentially death. AKI, acute kidney injury; CV, cardiovascular; CVD, cardiovascular disease; DPP4, dipeptidyl peptidase 4; LV, left ventricle; LVH, left ventricular hypertrophy; RAS, renin-angiotensin system; ROS, reactive oxygen species; VA, ventricle-atrial.

latter two studies, the gliptin-induced amelioration of cardiac remodeling and dysfunction was accompanied by blood pressure lowering effects.

CONCLUDING REMARKS

The relationship between hypertension, SARS-CoV-2 infection, and tissue injury is complex and multifactorial. Untangling the importance of several pathophysiological mechanisms in COVID-19 severity is still a work in progress, as scientific and clinical knowledge is continually being updated during the current pandemic. Through putative cellular, molecular and functional mechanisms, we provide a conceptual framework on how these biological processes may interact and lead to COVID-19 severity in patients with pre-existing hypertension: the role of the RAS, inflammation, endothelial dysfunction, arterial stiffness, left ventricular hypertrophy and DPP4 are summarized in **Figure 1**. In brief, patients with hypertension can be more prone to RAS imbalance, which in turn lead to vasoconstriction/inflammation due to unopposed Ang II effect, aggravated by increased DPP4 vascular activity/expression and by chronic low-grade inflammation. This dysregulated response, allied with diminished physiologic cardiovascular reserve induced by hypertension - arterial stiffening, left ventricular hypertrophy and endothelial dysfunction – creates the perfect milieu for both COVID-19 related tissue injury and worsening of cardiac, renal and vascular function.

Targeting these biological processes might attenuate the inflammatory response, reduce tissue injury, and ultimately lead to better outcomes in hypertensive patients with SARS-CoV-2 infection. Also, understanding the pathophysiology of hypertension in cardiovascular hemodynamics and how it might lead to poor outcomes in COVID-19 patients can aid the clinician in making decisions at the bedside. Finally, the role of RAS inhibitors needs to be further investigated, but, to the best of our knowledge, there is no known harmful impact of these medications neither on the risk of infection or disease severity. Noteworthy, preliminary data suggest that these antihypertensive agents may, in fact, confer a protective effect.

AUTHOR CONTRIBUTIONS

All authors conceived, wrote the manuscript and contributed to the article and approved the submitted version.

FUNDING

AG was supported by the São Paulo State Research Foundation (FAPESP) Grants 2016/22140-7 and 2020/05338-3. MB has funding from the Medical Research Council (MR/S01053X/1), British Heart Foundation (PG/16/98/32568), Kidney Research UK (IN001/20170302), and Diabetes UK (17/0005685).

ACKNOWLEDGMENTS

We thank Acaris Benetti for the preparation of **Figure 1** and João Carlos Ribeiro da Silva for their helpful comments and discussion on the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.599729/full#supplementary-material

REFERENCES

Abais-Battad, J. M., Lund, H., Fehrenbach, D. J., Dasinger, J. H., and Mattson, D. L. (2018). Rag1-null Dahl SS rats reveal that adaptive immune mechanisms exacerbate high protein-induced hypertension and renal injury. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 315, R28–R35.

Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al. (2020). Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N. Engl. J. Med.* 383, 120–128. doi: 10.1056/nejmoa2015432

Afify, H. M. A., Waits, G. S., Ghoneum, A. D., Cao, X., Li, Y., and Soliman, E. Z. (2018). Peguero electrocardiographic left ventricular hypertrophy criteria and risk of mortality. *Front. Cardiovasc. Med.* 5:75. doi: 10.3389/fcvm.2018.00075

Ainscough, J. F., Drinkhill, M. J., Sedo, A., Turner, N. A., Brooke, D. A., Balmforth, A. J., et al. (2009). Angiotensin II type-1 receptor activation in the adult heart causes blood pressure-independent hypertrophy and cardiac dysfunction. *Cardiovasc. Res.* 81, 592–600. doi: 10.1093/cvr/cvn230

Aro, A. L., and Chugh, S. S. (2016). Clinical diagnosis of electrical versus anatomic left ventricular hypertrophy: prognostic and therapeutic implications. *Circ. Arrhythm. Electrophysiol.* 9:e003629.

Aroor, A., Zuberek, M., Duta, C., Meuth, A., Sowers, J. R., Whaley-Connell, A., et al. (2016). Angiotensin II stimulation of DPP4 activity regulates megalin in the proximal tubules. *Int. J. Mol. Sci.* 17:780. doi: 10.3390/ijms17050780

Arruda-Junior, D. F., Martins, F. L., Dariolli, R., Jensen, L., Antonio, E. L., Dos Santos, L., et al. (2016). Dipeptidyl peptidase IV inhibition exerts renoprotective effects in rats with established heart failure. *Front. Physiol.* 7:293. doi: 10.3389/fphys.2016.00293

Bang, C. N., Soliman, E. Z., Simpson, L. M., Davis, B. R., Devereux, R. B., Okin, P. M., et al. (2017). Electrocardiographic left ventricular hypertrophy predicts cardiovascular morbidity and mortality in hypertensive patients: the ALLHAT study. *Am. J. Hypertens.* 30, 914–922. doi: 10.1093/ajh/hpx067

Benigni, A., Cassis, P., and Remuzzi, G. (2010). Angiotensin II revisited: new roles in inflammation, immunology and aging. *EMBO Mol. Med.* 2, 247–257. doi: 10.1002/emmm.201000080

Beraldo, J. I., Benetti, A., Borges-Júnior, F. A., Arruda-Junior, D. F., Martins, F. L., Jensen, L., et al. (2019). Cardioprotection conferred by sitagliptin is associated with reduced cardiac angiotensin II/Angiotensin-(1-7) balance in experimental chronic kidney disease. *Int. J. Mol. Sci.* 20:1940. doi: 10.3390/ijms20081940

Bermejo-Martin, J. F., Almansa, R., Torres, A., Gonzalez-Rivera, M., and Kelvin, D. J. (2020). COVID-19 as a cardiovascular disease: the potential role of chronic endothelial dysfunction. *Cardiovasc. Res.* 116, e132–e133.

Borlaug, B. A., Jaber, W. A., Ommen, S. R., Lam, C. S., Redfield, M. M., and Nishimura, R. A. (2011). Diastolic relaxation and compliance reserve during dynamic exercise in heart failure with preserved ejection fraction. *Heart* 97, 964–969. doi: 10.1136/hrt.2010.212787

Braun, F., Lütgehetmann, M., Pfefferle, S., Wong, M. N., Carsten, A., Lindenmeyer, M. T., et al. (2020). SARS-CoV-2 renal tropism associates with acute kidney injury. *Lancet* 396, 597–598. doi: 10.1016/s0140-6736(20)31759-1

Carson, P., Giles, T., Higginbotham, M., Hollenberg, N., Kannel, W., and Siragy, H. M. (2001). Angiotensin receptor blockers: evidence for preserving target organs. *Clin. Cardiol.* 24, 183–190. doi: 10.1002/clc.4960240303

Chemla, D., Antony, I., Lecarpentier, Y., and Nitenberg, A. (2003). Contribution of systemic vascular resistance and total arterial compliance to effective arterial elastance in humans. *Am. J. Physiol. Heart Circ. Physiol.* 285, H614–H620.

Cheng, Y., Luo, R., Wang, K., Zhang, M., Wang, Z., Dong, L., et al. (2020). Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int.* 97, 829–838. doi: 10.1016/j.kint.2020.03.005

Chin, J. H., Lee, E. H., Kim, W. J., Choi, D. K., Hahm, K. D., Sim, J. Y., et al. (2013). Positive end-expiratory pressure aggravates left ventricular diastolic relaxation further in patients with pre-existing relaxation abnormality. *Br. J. Anaesth.* 111, 368–373. doi: 10.1093/bja/aet061

Chirinos, J. A., Rietzschel, E. R., Shiva-Kumar, P., De Buyzere, M. L., Zamani, P., Claessens, T., et al. (2014). Effective arterial elastance is insensitive to pulsatile arterial load. *Hypertension* 64, 1022–1031. doi: 10.1161/hypertensionaha.114.03696

Chirinos, J. A., Segers, P., Hughes, T., and Townsend, R. (2019). Large-artery stiffness in health and disease: JACC state-of-the-art review. *J. Am. Coll. Cardiol.* 74, 1237–1263. doi: 10.1016/j.jacc.2019.07.012

Crackower, M. A., Sarao, R., Oudit, G. Y., Yagil, C., Kozieradzki, I., Scanga, S. E., et al. (2002). Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 417, 822–828.

Crowley, S. D., Gurley, S. B., Herrera, M. J., Ruiz, P., Griffiths, R., Kumar, A. P., et al. (2006). Angiotensin II causes hypertension and cardiac hypertrophy through its receptors in the kidney. *Proc. Natl. Acad. Sci. U.S.A.* 103, 17985–17990. doi: 10.1073/pnas.0605545103

Crowley, S. D., Gurley, S. B., Oliverio, M. I., Pazmino, A. K., Griffiths, R., Flannery, P. J., et al. (2005). Distinct roles for the kidney and systemic tissues in blood pressure regulation by the renin-angiotensin system. *J. Clin. Invest.* 115, 1092–1099. doi: 10.1172/jci23378

Czopek, A., Moorhouse, R., Guyonnet, L., Farrah, T., Lenoir, O., Owen, E., et al. (2019). A novel role for myeloid endothelin-B receptors in hypertension. *Eur. Heart J.* 40, 768–784. doi: 10.1093/eurheartj/ehy881

Dahlof, B., Devereux, R. B., Kjeldsen, S. E., Julius, S., Beevers, G., De Faire, U., et al. (2002). Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet* 359, 995–1003. doi: 10.1016/s0140-6736(02)08089-3

Deanfield, J. E., Halcox, J. P., and Rabelink, T. J. (2007). Endothelial function and dysfunction: testing and clinical relevance. *Circulation* 115, 1285–1295. doi: 10.1161/circulationaha.106.652859

Diaz, J. H. (2020). Hypothesis: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the risk of severe COVID-19. *J. Travel Med.* 27:taaa041.

Diez, J., and Frohlich, E. D. (2010). A translational approach to hypertensive heart disease. *Hypertension* 55, 1–8. doi: 10.1161/hypertensionaha.109.141887

Dong, E., Du, H., and Gardner, L. (2020). An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect. Dis.* 20, 533–534. doi: 10.1016/s1473-3099(20)30120-1

Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., et al. (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ. Res.* 87, E1–E9.

Dos Santos, L., Salles, T. A., Arruda-Junior, D. F., Campos, L. C. G., Pereira, A. C., Barreto, A. L. T., et al. (2013). Circulating dipeptidyl peptidase IV activity correlates with cardiac dysfunction in human and experimental heart failure. *Circulation* 6, 1029–1038. doi: 10.1161/circheartfailure.112.000057

Esler, M., and Esler, D. (2020). Can angiotensin receptor-blocking drugs perhaps be harmful in the COVID-19 pandemic? *J. Hypertens* 38, 781–782. doi: 10.1097/hjh.0000000000002450

Fehrenbach, D. J., Dasinger, J. H., Lund, H., Zemaj, J., and Mattson, D. L. (2020). Splenocyte transfer exacerbates salt-sensitive hypertension in rats. *Exp. Physiol.* 105, 864–875. doi: 10.1113/ep088340

Ferrario, C. M., Jessup, J., Chappell, M. C., Averill, D. B., Brosnihan, K. B., Tallant, E. A., et al. (2005). Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation* 111, 2605–2610. doi: 10.1161/circulationaha.104.510461

Garg, S., Kim, L., Whitaker, M., O'halloran, A., Cummings, C., Holstein, R., et al. (2020). Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed coronavirus disease 2019 – COVID-NET, 14 States, March 1-30, 2020. *MMWR Morb. Mortal. Wkly. Rep.* 69, 458–464. doi: 10.15585/mmwr.mm6915e3

Gembardt, F., Sterner-Kock, A., Imboden, H., Spalteholz, M., Reibitz, F., Schultheiss, H. P., et al. (2005). Organ-specific distribution of ACE2 mRNA and correlating peptidase activity in rodents. *Peptides* 26, 1270–1277. doi: 10.1016/j.peptides.2005.01.009

Girardi, A. C. C., Degray, B. C., Nagy, T., Biemesderfer, D., and Aronson, P. S. (2001). Association of Na+-H+ exchanger isoform NHE3 and dipeptidyl peptidase IV in the renal proximal tubule. *J. Biol. Chem.* 276, 46671–46677. doi: 10.1074/jbc.m106897200

Grasselli, G., Zangrillo, A., Zanella, A., Antonelli, M., Cabrini, L., Castelli, A., et al. (2020). Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. *JAMA* 323, 1574–1581. doi: 10.1001/jama.2020.5394

Guan, W. J., Liang, W. H., Zhao, Y., Liang, H. R., Chen, Z. S., Li, Y. M., et al. (2020a). Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. *Eur. Respir. J.* 55:2000547.

Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W. H., Ou, C. Q., He, J. X., et al. (2020b). Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* 382, 1708–1720.

Guarracino, F., Ferro, B., Morelli, A., Bertini, P., Baldassarri, R., and Pinsky, M. R. (2014). Ventriculoarterial decoupling in human septic shock. *Crit. Care* 18, R80.

Guo, T., Fan, Y., Chen, M., Wu, X., Zhang, L., He, T., et al. (2020). Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 5, 811–818. doi: 10.1001/jamacardio.2020.1017

Gurley, S. B., Allred, A., Le, T. H., Griffiths, R., Mao, L., Philip, N., et al. (2006). Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice. *J. Clin. Invest.* 116, 2218–2225. doi: 10.1172/jci16980

Gurley, S. B., Riquier-Brison, A. D. M., Schnermann, J., Sparks, M. A., Allen, A. M., Haase, V. H., et al. (2011). AT1A angiotensin receptors in the renal proximal tubule regulate blood pressure. *Cell Metab.* 13, 469–475. doi: 10.1016/j.cmet.2011.03.001

Guyonnet, L., Czopek, A., Farrah, T. E., Baudrie, V., Bonnin, P., Chipont, A., et al. (2020). Deletion of the myeloid endothelin-B receptor confers long-term protection from angiotensin II-mediated kidney, eye and vessel injury. *Kidney Int.* 98, 1193–1209. doi: 10.1016/j.kint.2020.05.042

Guzik, T. J., Hoch, N. E., Brown, K. A., Mccann, L. A., Rahman, A., Dikalov, S., et al. (2007). Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J. Exp. Med.* 204, 2449–2460. doi: 10.1084/jem.20070657

Haider, A. W., Larson, M. G., Benjamin, E. J., and Levy, D. (1998). Increased left ventricular mass and hypertrophy are associated with increased risk for sudden death. *J. Am. Coll. Cardiol.* 32, 1454–1459. doi: 10.1016/s0735-1097(98)00407-0

Hendren, N. S., Drazner, M. H., Bozkurt, B., and Cooper, L. T. Jr. (2020). Description and proposed management of the acute COVID-19 cardiovascular syndrome. *Circulation* 141, 1903–1914. doi: 10.1161/circulationaha.120.047349

Heurich, A., Hofmann-Winkler, H., Gierer, S., Liepold, T., Jahn, O., and Pohlmann, S. (2014). TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. *J. Virol.* 88, 1293–1307. doi: 10.1128/jvi.02202-13

Hingorani, A. D., Cross, J., Kharbanda, R. K., Mullen, M. J., Bhagat, K., Taylor, M., et al. (2000). Acute systemic inflammation impairs endothelium-dependent dilatation in humans. *Circulation* 102, 994–999. doi: 10.1161/01.cir.102.9.994

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271–280.e8.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506.

Huentelman, M. J., Grobe, J. L., Vazquez, J., Stewart, J. M., Mecca, A. P., Katovich, M. J., et al. (2005). Protection from angiotensin II-induced cardiac hypertrophy and fibrosis by systemic lentiviral delivery of ACE2 in rats. *Exp. Physiol.* 90, 783–790. doi: 10.1113/expphysiol.2005.031096

Ikonomidis, I., Aboyans, V., Blacher, J., Brodmann, M., Brutsaert, D. L., Chirinos, J. A., et al. (2019). The role of ventricular-arterial coupling in cardiac disease and heart failure: assessment, clinical implications and therapeutic interventions. A consensus document of the European society of cardiology working group on aorta & peripheral vascular diseases, European association of cardiovascular imaging, and heart failure association. *Eur. J. Heart Fail.* 21, 402–424. doi: 10.1002/ejhf.1436

Ikonomidis, I., Lekakis, J., Papadopoulos, C., Triantafyllidi, H., Paraskevaidis, I., Georgoula, G., et al. (2008). Incremental value of pulse wave velocity in the determination of coronary microcirculatory dysfunction in never-treated patients with essential hypertension. *Am. J. Hypertens.* 21, 806–813. doi: 10.1038/ajh.2008.172

Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., et al. (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 436, 112–116. doi: 10.1038/nature03712

Itani, H. A., Mcmaster, W. G., Saleh, M. A., Nazarewicz, R. R., Mikolajczyk, T. P., Kaszuba, A. M., et al. (2016). Activation of human T cells in hypertension: studies of humanized mice and hypertensive humans. *Hypertension* 68, 123–132. doi: 10.1161/hypertensionaha.116.07237

Iwata-Yoshikawa, N., Okamura, T., Shimizu, Y., Hasegawa, H., Takeda, M., and Nagata, N. (2019). TMPRSS2 contributes to virus spread and immunopathology in the airways of murine models after coronavirus infection. *J. Virol.* 93, e01815-18. doi: 10.1128/JVI.01815-18

Jaffe, A. S., Cleland, J. G. F., and Katus, H. A. (2020). Myocardial injury in severe COVID-19 infection. *Eur. Heart J.* 41, 2080–2082. doi: 10.1093/eurheartj/ehaa447

Jessup, J. A., Gallagher, P. E., Averill, D. B., Brosnihan, K. B., Tallant, E. A., Chappell, M. C., et al. (2006). Effect of angiotensin II blockade on a new congenic model of hypertension derived from transgenic Ren-2 rats. *Am. J. Physiol. Heart Circ. Physiol.* 291, H2166–H2172.

Juhl-Olsen, P., Hermansen, J. F., Frederiksen, C. A., Rasmussen, L. A., Jakobsen, C. J., and Sloth, E. (2013). Positive end-expiratory pressure influences echocardiographic measures of diastolic function: a randomized, crossover study in cardiac surgery patients. *Anesthesiology* 119, 1078–1086. doi: 10.1097/aln.0b013e3182a10b40

Kanasaki, K., Shi, S., Kanasaki, M., He, J., Nagai, T., Nakamura, Y., et al. (2014). Linagliptin-mediated DPP-4 inhibition ameliorates kidney fibrosis in streptozotocin-induced diabetic mice by inhibiting endothelial-to-mesenchymal transition in a therapeutic regimen. *Diabetes Metab. Res. Rev* 63, 2120–2131. doi: 10.2337/db13-1029

Kenny, A. J., Booth, A. G., George, S. G., Ingram, J., Kershaw, D., Wood, E. J., et al. (1976). Dipeptidyl peptidase IV, a kidney brush-border serine peptidase. *Biochem. J.* 157, 169–182. doi: 10.1042/bj1570169

Kishimoto, S., Kinoshita, Y., Matsumoto, T., Maruhashi, T., Kajikawa, M., Matsui, S., et al. (2019). Effects of the dipeptidyl peptidase 4 inhibitor alogliptin on blood pressure in hypertensive patients with type 2 diabetes mellitus. *Am. J. Hypertens.* 32, 695–702. doi: 10.1093/ajh/hpz065

Koka, V., Huang, X. R., Chung, A. C., Wang, W., Truong, L. D., and Lan, H. Y. (2008). Angiotensin II up-regulates angiotensin I-converting enzyme (ACE), but down-regulates ACE2 via the AT1-ERK/p38 MAP kinase pathway. *Am. J. Pathol.* 172, 1174–1183. doi: 10.2353/ajpath.2008.070762

Konomi, I., Tasoulis, A., Kaltsi, I., Karatzanos, E., Vasileiadis, I., Temperikidis, P., et al. (2016). Left ventricular diastolic dysfunction–an independent risk factor for weaning failure from mechanical ventilation. *Anaesth. Intensive Care* 44, 466–473. doi: 10.1177/0310057x1604400408

Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., et al. (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.* 11, 875–879. doi: 10.1038/nm1267

Lala, A., Johnson, K. W., Januzzi, J. L., Russak, A. J., Paranjpe, I., Richter, F., et al. (2020). Prevalence and impact of myocardial injury in patients hospitalized with COVID-19 infection. *J. Am. Coll. Cardiol.* 76, 533–546.

Lambeir, A. M., Durinx, C., Scharpé, S., and De Meester, I. (2003). Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. *Crit. Rev. Clin. Lab. Sci* 40, 209–294. doi: 10.1080/713609354

Laurent, S., Boutouyrie, P., Asmar, R., Gautier, I., Laloux, B., Guize, L., et al. (2001). Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 37, 1236–1241. doi: 10.1161/01.hyp.37.5.1236

Letko, M., Marzi, A., and Munster, V. (2020). Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat. Microbiol.* 5, 562–569. doi: 10.1038/s41564-020-0688-y

Li, Y., Zhang, Z., Yang, L., Lian, X., Xie, Y., Li, S., et al. (2020). The MERS-CoV receptor DPP4 as a candidate binding target of the SARS-CoV-2 Spike. *iScience* 23:101160. doi: 10.1016/j.isci.2020.101160

Linardi, A., Panunto, P. C., Ferro, E. S., and Hyslop, S. (2004). Peptidase activities in rats treated chronically with N(omega)-nitro-L-arginine methyl ester (L-NAME). *Biochem. Pharmacol.* 68, 205–214. doi: 10.1016/j.bcp.2004.03.016

Liu, H., Guo, L., Xing, J., Li, P., Sang, H., Hu, X., et al. (2020). The protective role of DPP4 inhibitors in atherosclerosis. *Eur. J. Pharmacol.* 875:173037. doi: 10.1016/j.ejphar.2020.173037

Liu, J., Shen, F., Teboul, J. L., Anguel, N., Beurton, A., Bezaz, N., et al. (2016). Cardiac dysfunction induced by weaning from mechanical ventilation: incidence, risk factors, and effects of fluid removal. *Crit. Care* 20:369.

Machnik, A., Neuhofer, W., Jantsch, J., Dahlmann, A., Tammela, T., Machura, K., et al. (2009). Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat. Med.* 15, 545–552. doi: 10.1038/nm.1960

Mancia, G., Rea, F., Ludergnani, M., Apolone, G., and Corrao, G. (2020). Renin-angiotensin-aldosterone system blockers and the risk of Covid-19. *N. Engl. J. Med.* 382, 2431–2440. doi: 10.1056/nejmoa2006923

Marguet, D., Baggio, L., Kobayashi, T., Bernard, A. M., Pierres, M., Nielsen, P. F., et al. (2000). Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc. Natl. Acad. Sci. U.S.A.* 97, 6874–6879. doi: 10.1073/pnas.120069197

Mattson, D. L. (2019). Immune mechanisms of salt-sensitive hypertension and renal end-organ damage. *Nat. Rev. Nephrol.* 15, 290–300. doi: 10.1038/s41581-019-0121-z

Mehta, N., Kalra, A., Nowacki, A. S., Anjewierden, S., Han, Z., Bhat, P., et al. (2020). Association of use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers with testing positive for coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 5, 1020–1026. doi: 10.1001/jamacardio.2020.1855

Nakajima, Y., Ito, S., Asakura, M., Min, K. D., Fu, H. Y., Imazu, M., et al. (2019). A dipeptidyl peptidase-IV inhibitor improves diastolic dysfunction in Dahl salt-sensitive rats. *J. Mol. Cell Cardiol.* 129, 257–265. doi: 10.1016/j.yjmcc.2019.03.009

Nam, D. H., Park, J., Park, S. H., Kim, K. S., and Baek, E. B. (2019). Effect of gemigliptin on cardiac ischemia/reperfusion and spontaneous hypertensive rat models. *Korean J. Physiol. Pharmacol.* 23, 329–334.

Narayanan, K., Reinier, K., Teodorescu, C., Uy-Evanado, A., Chugh, H., Gunson, K., et al. (2014). Electrocardiographic versus echocardiographic left ventricular hypertrophy and sudden cardiac arrest in the community. *Heart Rhythm* 11, 1040–1046. doi: 10.1016/j.hrthm.2014.03.023

Nistala, R., and Savin, V. (2017). Diabetes, hypertension, and chronic kidney disease progression: role of DPP4. *Am. J. Physiol. Renal Physiol.* 312, F661–F670.

Nwabuo, C. C., and Vasan, R. S. (2020). Pathophysiology of hypertensive heart disease: beyond left ventricular hypertrophy. *Curr. Hypertens. Rep* 22:11.

Okabe, K., Matsushima, S., Ikeda, S., Ikeda, M., Ishikita, A., Tadokoro, T., et al. (2020). DPP (Dipeptidyl Peptidase)-4 inhibitor attenuates Ang II (Angiotensin II)-induced cardiac hypertrophy via GLP (Glucagon-Like Peptide)-1-Dependent Suppression of Nox (Nicotinamide Adenine Dinucleotide Phosphate Oxidase) 4-HDAC (Histone Deacetylase) 4 Pathway. *Hypertension* 75, 991–1001. doi: 10.1161/hypertensionaha.119.14400

Papanikolaou, J., Makris, D., Saranteas, T., Karakitsos, D., Zintzaras, E., Karabinis, A., et al. (2011). New insights into weaning from mechanical ventilation: left ventricular diastolic dysfunction is a key player. *Intensive Care Med.* 37, 1976–1985. doi: 10.1007/s00134-011-2368-0

Pinto, B. G. G., Oliveira, A. E. R., Singh, Y., Jimenez, L., Gonçalves, A. N. A., Ogava, R. L. T., et al. (2020). ACE2 expression is increased in the lungs of patients with comorbidities associated with severe COVID-19. *J. Infect. Dis.* 222, 556–563. doi: 10.1093/infdis/jiaa332

Pitoulis, F. G., and Terracciano, C. M. (2020). Heart plasticity in response to pressure- and volume-overload: a review of findings in compensated and

decompensated phenotypes. *Front. Physiol.* 11:92. doi: 10.3389/fphys.2020.00092

Puelles, V. G., Lütgehetmann, M., Lindenmeyer, M. T., Sperhake, J. P., Wong, M. N., Allweiss, L., et al. (2020). Multiorgan and Renal Tropism of SARS-CoV-2. *N. Engl. J. Med.* 383, 590–592. doi: 10.1056/nejmc2011400

Reynolds, H. R., Adhikari, S., Pulgarin, C., Troxel, A. B., Iturrate, E., Johnson, S. B., et al. (2020). Renin-Angiotensin-Aldosterone System Inhibitors and Risk of Covid-19. *N. Engl. J. Med.* 382, 2441–2448.

Richardson, S., Hirsch, J. S., Narasimhan, M., Crawford, J. M., Mcginn, T., Davidson, K. W., et al. (2020). Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. *JAMA* 323, 2052–2059.

Rosenblat, M., Elias, A., Volkova, N., and Aviram, M. (2013). Monocyte-macrophage membrane possesses free radicals scavenging activity: stimulation by polyphenols or by paraoxonase 1 (PON1). *Free Radic. Res.* 47, 257–267. doi: 10.3109/10715762.2013.765562

Ryskjaer, J., Deacon, C. F., Carr, R. D., Krarup, T., Madsbad, S., Holst, J., et al. (2006). Plasma dipeptidyl peptidase-IV activity in patients with type-2 diabetes mellitus correlates positively with HbAlc levels, but is not acutely affected by food intake. *Eur. J. Endocrinol.* 155, 485–493. doi: 10.1530/eje.1.02221

Sadoshima, J., and Izumo, S. (1993). Molecular characterization of angiotensin II–induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. *Critical role of the AT*1 receptor subtype. *Circ. Res.* 73, 413–423. doi: 10.1161/01.res.73.3.413

Sampaio, W. O., Henrique De Castro, C., Santos, R. A., Schiffrin, E. L., and Touyz, R. M. (2007). Angiotensin-(1-7) counterregulates angiotensin II signaling in human endothelial cells. *Hypertension* 50, 1093–1098. doi: 10.1161/hypertensionaha.106.084848

Sanfilippo, F., Scolletta, S., Morelli, A., and Vieillard-Baron, A. (2018). Practical approach to diastolic dysfunction in light of the new guidelines and clinical applications in the operating room and in the intensive care. *Ann. Intensive Care* 8, 100.

Santos, R. A. S., Sampaio, W. O., Alzamora, A. C., Motta-Santos, D., Alenina, N., Bader, M., et al. (2018). The ACE2/Angiotensin-(1-7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1-7). *Physiol. Rev.* 98, 505–553. doi: 10.1152/physrev.00023.2016

Savignano, F. A., Crajoinas, R. O., Pacheco, B. P. M., Campos, L. C. G., Shimizu, M. H. M., Seguro, A. C., et al. (2017). Attenuated diuresis and natriuresis in response to glucagon-like peptide-1 in hypertensive rats are associated with lower expression of the glucagon-like peptide-1 receptor in the renal vasculature. *Eur. J. Pharmacol* 811, 38–47. doi: 10.1016/j.ejphar.2017.05.054

Seniuk, A., Thiele, J. L., Stubbe, A., Oser, P., Rosendahl, A., Bode, M., et al. (2020). B6.R*ag*1 knockout mice generated at the Jackson Laboratory in 2009 show a robust wild-type hypertensive phenotype in response to Ang II (Angiotensin II). *Hypertension* 75, 1110–1116. doi: 10.1161/hypertensionaha.119.13773

Shi, S., Qin, M., Cai, Y., Liu, T., Shen, B., Yang, F., et al. (2020a). Characteristics and clinical significance of myocardial injury in patients with severe coronavirus disease 2019. *Eur. Heart J.* 41, 2070–2079. doi: 10.1093/eurheartj/ehaa408

Shi, S., Qin, M., Shen, B., Cai, Y., Liu, T., Yang, F., et al. (2020b). Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* 5, 802–810. doi: 10.1001/jamacardio.2020.0950

Shimada, S., Abais-Battad, J. M., Alsheikh, A. J., Yang, C., Stumpf, M., Kurth, T., et al. (2020). Renal perfusion pressure determines infiltration of leukocytes in the kidney of rats with angiotensin II-induced hypertension. *Hypertension* 76, 849–858. doi: 10.1161/hypertensionaha.120.15295

Soler, M. J., Wysocki, J., and Batlle, D. (2013). ACE2 alterations in kidney disease. *Nephrol. Dial. Transplant.* 28, 2687–2697. doi: 10.1093/ndt/gft320

Sundstrom, J., Lind, L., Arnlov, J., Zethelius, B., Andren, B., and Lithell, H. O. (2001). Echocardiographic and electrocardiographic diagnoses of left ventricular hypertrophy predict mortality independently of each other in a population of elderly men. *Circulation* 103, 2346–2351. doi: 10.1161/01.cir.103.19.2346

Tang, N., Li, D., Wang, X., and Sun, Z. (2020). Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J. Thromb. Haemost.* 18, 844–847. doi: 10.1111/jth.14768

Tipnis, S. R., Hooper, N. M., Hyde, R., Karran, E., Christie, G., and Turner, A. J. (2000). A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* 275, 33238–33243. doi: 10.1074/jbc.m002615200

Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., et al. (2020). Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 395, 1417–1418.

Wakahara, S., Konoshita, T., Mizuno, S., Motomura, M., Aoyama, C., Makino, Y., et al. (2007). Synergistic expression of angiotensin-converting enzyme (ACE) and ACE2 in human renal tissue and confounding effects of hypertension on the ACE to ACE2 ratio. *Endocrinology* 148, 2453–2457.

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 323, 1061–1069.

Wang, X., Ye, Y., Gong, H., Wu, J., Yuan, J., Wang, S., et al. (2016). The effects of different angiotensin II type 1 receptor blockers on the regulation of the ACE-AngII-AT1 and ACE2-Ang(1-7)-Mas axes in pressure overload-induced cardiac remodeling in male mice. *J. Mol. Cell Cardiol.* 97, 180–190.

Watson, T., Goon, P. K., and Lip, G. Y. (2008). Endothelial progenitor cells, endothelial dysfunction, inflammation, and oxidative stress in hypertension. *Antioxid. Redox. Signal.* 10, 1079–1088.

Wu, C., Chen, X., Cai, Y., Xia, J., Zhou, X., Xu, S., et al. (2020). Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med.* 180, 934–943.

Yang, G., Tan, Z., Zhou, L., Yang, M., Peng, L., Liu, J., et al. (2020). Effects of angiotensin II receptor blockers and ACE (Angiotensin-Converting Enzyme) inhibitors on virus infection, inflammatory status, and clinical outcomes in patients with COVID-19 and hypertension: a single-center retrospective study. *Hypertension* 76, 51–58.

Zanoli, L., Briet, M., Empana, J. P., Cunha, P. G., Mäki-Petäjä, K. M., Protogerou, A. D., et al. (2020). Vascular consequences of inflammation: a position statement from the ESH Working Group on Vascular Structure and Function and the ARTERY Society. *J. Hypertens.* 38, 1682–1698.

Zhang, J., Chen, Q., Zhong, J., Liu, C., Zheng, B., and Gong, Q. (2019). DPP-4 inhibitors as potential candidates for antihypertensive therapy: improving vascular inflammation and assisting the action of traditional antihypertensive drugs. *Front. Immunol.* 10:1050. doi: 10.3389/fimmu.2019.01050

Zhang, J. J., Dong, X., Cao, Y. Y., Yuan, Y. D., Yang, Y. B., Yan, Y. Q., et al. (2020). Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 75, 1730–1741.

Zhang, L. H., Pang, X. F., Bai, F., Wang, N. P., Shah, A. I., Mckallip, R. J., et al. (2015). Preservation of glucagon-like peptide-1 level attenuates angiotensin II-induced tissue fibrosis by altering AT1/AT 2 receptor expression and angiotensin-converting enzyme 2 activity in rat heart. *Cardiovasc. Drugs. Ther.* 29, 243–255.

Zhong, J., Rao, X., Deiuliis, J., Braunstein, Z., Narula, V., Hazey, J., et al. (2013). A potential role for dendritic cell/macrophage-expressing DPP4 in obesity-induced visceral inflammation. *Diabetes Metab. Res. Rev.* 62, 149–157.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020a). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395, 1054–1062.

Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., et al. (2020b). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., et al. (2020). A novel coronavirus from patients with Pneumonia in China, 2019. *N. Engl. J. Med.* 382, 727–733.

Zuin, M., Rigatelli, G., Zuliani, G., Rigatelli, A., Mazza, A., and Roncon, L. (2020). Arterial hypertension and risk of death in patients with COVID-19 infection: systematic review and meta-analysis. *J. Infect.* 81, e84–e86.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Public Health

ORIGINAL RESEARCH
published: 19 November 2020
doi: 10.3389/fpubh.2020.604339

Covid-19 Mortality: A Matter of Vulnerability Among Nations Facing Limited Margins of Adaptation

Quentin De Larochelambert[1], *Andy Marc*[1], *Juliana Antero*[1], *Eric Le Bourg*[2] and *Jean-François Toussaint*[1,3]*

[1] EA7329, Institute for Research in bioMedicine and Epidemiology of Sport (IRMES), Paris, France, [2] Centre de Recherche sur la Cognition Animale (CRCA), Centre de Biologie Intégrative (CBI Toulouse), Université de Toulouse, CNRS, UPS, Toulouse, France, [3] CIMS, Hôtel-Dieu, Assistance Publique—Hôpitaux de Paris, Paris, France

Context: The human development territories have been severely constrained under the Covid-19 pandemic. A common dynamics has been observed, but its propagation has not been homogeneous over each continent. We aimed at characterizing the non-viral parameters that were most associated with death rate.

Methods: We tested major indices from five domains (demography, public health, economy, politics, environment) and their potential associations with Covid-19 mortality during the first 8 months of 2020, through a Principal Component Analysis and a correlation matrix with a Pearson correlation test. Data of all countries, or states in federal countries, showing at least 10 fatality cases, were retrieved from official public sites. For countries that have not yet finished the first epidemic phase, a prospective model has been computed to provide options of death rates evolution.

Results: Higher Covid death rates are observed in the [25/65°] latitude and in the [−35/−125°] longitude ranges. The national criteria most associated with death rate are life expectancy and its slowdown, public health context (metabolic and non-communicable diseases (NCD) burden vs. infectious diseases prevalence), economy (growth national product, financial support), and environment (temperature, ultra-violet index). Stringency of the measures settled to fight pandemia, including lockdown, did not appear to be linked with death rate.

Conclusion: Countries that already experienced a stagnation or regression of life expectancy, with high income and NCD rates, had the highest price to pay. This burden was not alleviated by more stringent public decisions. Inherent factors have predetermined the Covid-19 mortality: understanding them may improve prevention strategies by increasing population resilience through better physical fitness and immunity.

Keywords: COVID-19, demography, environment, public health, lockdown, niche adaptation

OPEN ACCESS

Edited by:
Georges Leftheriotis, Université Côte d'Azur, France

Reviewed by:
Martin Dlouhy, University of Economics, Prague, Czechia
Gregoire P. Millet, University of Lausanne, Switzerland

***Correspondence:**
Jean-François Toussaint
jean-francois.toussaint@aphp.fr

Specialty section:
This article was submitted to Health Economics, a section of the journal Frontiers in Public Health

Received: *15 September 2020*
Accepted: *26 October 2020*
Published: *19 November 2020*

Citation:
De Larochelambert Q, Marc A, Antero J, Le Bourg E and Toussaint J-F (2020) Covid-19 Mortality: A Matter of Vulnerability Among Nations Facing Limited Margins of Adaptation. Front. Public Health 8:604339. doi: 10.3389/fpubh.2020.604339

INTRODUCTION

Out of the many environmental options, human populations have concentrated in the most favorable development niche, characterized by a local mean annual temperature around 11–15°C (1), corresponding to a narrow latitude strip. In the plains of that strip, the highest life expectancies have been experienced by the populations and most of the human longevity maxima have been recorded (2), showing that the niche coincides with and allows for the highest capacities of the human physiological development (1) and wealth creation, associated with elevated gross domestic product (GDP) (3, 4).

Experiencing a recent phase of stagnation, nations encounter intrinsic and extrinsic limits: plateauing has been demonstrated in the progression of life expectancy (5–7), adult height (8), or physiological maxima (9, 10). As a consequence, societies seem to face reduced margins of adaptability (2, 10, and become more susceptible to new constraints. In fact, individuals have a limited organism shaped by physical (11) and evolutionary constraints (12), and modulated by their interactions with the environment, resulting in an age-related decline in performances (10) with a potential maximal longevity (7). Hence, global threats may put the human development niche at higher risks. Demographical, social, economic, and health parameters may underline population vulnerabilities following the recent development phase.

Countries with the highest life expectancy have demographically transitioned to greater proportions of older and frailer populations, susceptible to increased mortality rates when facing physical or biological aggressors, such as temperature elevations (13) or infections (14). Concomitantly, the causes of death in these nations have transitioned from infectious to chronic diseases: mainly cardio-vascular diseases (CVD), metabolic (diabetes, high blood pressure), and neuro-degenerative diseases or cancers. In addition, metabolic and CVD risk factors associated with high death rates, such as sedentary lifestyle, poor nutrition quality, or obesity, have a large prevalence in high income countries (15, 16) and rise in developing ones (17–19). Such comorbidities were early associated with a higher risk of death from Covid-19 (20).

The balance between the prior demographic, environmental, economic, health, or social factors in each nation may partially determine Covid-19 mortality rates, as well as the efforts made by governments to contain the pandemic. We hypothesized that nations characterized by limited progression of life expectancies, with high chronic disease rates, metabolic comorbidities, and high GDP produced higher vulnerabilities to Covid-19 and were associated with higher mortality rates during the first 8 months phase of the pandemic.

Hence, this study aimed to investigate the power of associations between Covid-19 death rates and demographic (e.g., life expectancy and its progression), health (e.g., major risk factors and lifestyles), environmental (temperature, humidity), and economic parameters (e.g., GDP and development index) as well as indices characterizing the governments' responses (e.g., stringency and containment measures) in every country affected by the pandemic.

METHODS

Studied Countries

From the 188 countries that have declared at least one case, only those counting a minimum of 10 deaths due to Covid-19 up to the study end point (31th August 2020) were included. China and US were also analyzed by states or regions, when each of them reached the 10 deaths threshold.

Variables of Interest

The studied outcome was the death rate due to Covid-19. Its association was tested with environmental [temperature, humidity, ultra-violet (UV) index]; demographic [life expectancy (LE), progression of LE]; health (CVD death rate, cancer death rate, infectious diseases death rate, obesity rate, sedentary, or inactive lifestyle); GDP and with each government response (containment and health index, stringency index, and economic support index).

The mortality rate due to Covid-19 was calculated as the ratio between the total number of deaths and the population size of each country, state, or region. It can be displayed as the number of deaths per 100,000 inhabitants and/or transformed into its decimal logarithm.

To test the optimal development niche effect, the Covid-19 mortality rates were analyzed according to the latitude and longitude of each country. Both were characterized by the barycenter of the country (GPS coordinates). Likewise, each state in the USA and each region in China was analyzed with its own latitude and longitude [as reported on the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU)] for the environmental analysis.

Data Collection

Daily data on the number of cases and deaths due to Covid-19 were collected up to the study end point via the Johns Hopkins University data source (https://github.com/CSSEGISandData/COVID-19). The latest population sizes available, used to calculate the mortality rate were extracted from the UNdata website (http://data.un.org/Data.aspx?d=POP&f=tableCode%3A22). The same data source was used to obtain the GDP for each country, the last year with available data being used.

Daily environmental data (temperature, humidity, and UV index) were collected via the Darsky website (https://darksky.net/). They were recorded from the beginning of the pandemic (defined as the day when the country reached a total of 10 deaths due to Covid-19) until the peak of the pandemic. To calculate the pandemic peak (PP), the number of cumulative deaths was theorized with a non-symmetrical logistic regression:

$$Y(t) = c + \frac{d - c}{\left(1 + \exp\left(b(\log(t) - \log(e))\right)\right)^f}$$

where Y: logarithm of the number of deaths per 100,000 population

t : time in days

b, c, d, e, f : model parameters

The parameter d controls the height of the asymptote of the curve. The parameters b and f jointly control the magnitude, which represents the speed of transition between the two asymptotes. The parameter e controls the position of the slope and the parameter c the left asymptote of the curve.

The maximum of the derivative of this function was used to determine PP in each country. We calculated the mean of each of the three variables (temperature, humidity, and UV) in each country for a period starting at the beginning of the epidemic and ending the day of the PP.

Several countries (e.g., India, Argentina, etc.) have not yet reached the peak of the epidemic first wave. In order to take this parameter into account, the analyzes were also done with a death number that was estimated at the 99% time point (i.e., when the epidemic reaches 99% of the total death toll from the first epidemic wave) according to the logistic regression above. The estimated number of deaths for each country as well as all analyzes with such simulated data are presented in the Supplementary Material (**Supplementary Table 1**, **Supplementary Figures 1–3**), where the actual number of deaths for each country at the last known date is compared to the theoretical number according to the model.

The geographical coordinates corresponding to the barycenter of each country were retrieved thanks to the package *rgeos* in the R software. Latitude is expressed negatively in the southern hemisphere, positively in the northern one. Relative to the Greenwich meridian, longitude is expressed negatively for the western countries, positively for the eastern ones.

The obesity rate was calculated as the percent of the country total population considered to be obese, according to the last year this prevalence data was publicly available. Adult obesity is defined through a Body Mass Index (BMI) greater to or equal to 30 kg/m^2. Data were collected from *The World Factbook* from the US intelligence agency (https://www.cia.gov/library/publications/the-world-factbook/fields/367.html).

The inactive lifestyle was characterized by the prevalence (percentage of the population) of adults performing <150 min of moderate-intensity physical activity per week, or <75 min of vigorous-intensity physical activity per week, or equivalent. Data were retrieved from the website of the World Health Organization (https://apps.who.int/gho/data/node.main.A893?lang=en). This prevalence is based on self-reported physical activity captured using the GPAQ (Global Physical Activity Questionnaire), the IPAQ (International Physical Activity Questionnaire), or a similar questionnaire covering activity at work/in the household, for transport, and during leisure time.

The current life expectancies were collected from the World Bank, based on the last year these data were available (https://data.worldbank.org/indicator/SP.DYN.LE00.IN). To calculate the progression of LE, we used data from 2010 up to now. The α coefficient of the linear regression between current LE and the 2010 one was determined to estimate the progression trend. The greater the index, the greater the life expectancy gains in the last decade.

The burden resulting from major chronic diseases (CVD, metabolic diseases, cancer) and from infectious diseases in the previous population death rates was estimated by the proportion of the mortality rates associated with these major causes compared to the all-cause mortality rates. Both sexes and all age classes were taken into account. Data were retrieved from the IHME "GDP results tools" (http://ghdx.healthdata.org/gbd-results-tool) up to the last year the mortality rate was available. They appear as "Neoplasms death rate," "CV and MD death rate," and "Infectious diseases death rate."

We used the Oxford university data source to characterize the state responses, regarding the containment and health index, the stringency index, and the economic support index (https://www.bsg.ox.ac.uk/research/research-projects/coronavirus-government-response-tracker) including public health measures taken by each country at short term. The Oxford COVID-19 Government Response Tracker (OxCGRT) systematically collects information on several different common policy responses that governments have taken to respond to the pandemic on 17 indicators. The data from the 17 indicators were aggregated into a set of three common indices, reporting a number between 1 and 100 to reflect the level of government's action on each topic: (1) the containment and health index combines lockdown restrictions and closures with measures such as testing policies and contact tracing, short term investment in healthcare, as well as investments in vaccines; (2) the economic support index records measures such as income support and debt relief; (3) the original stringency index records the strictness of lockdown and policies that primarily aimed at restricting population mobility.

Statistical Analysis

To study the relationship between environmental variables and the Covid-19 mortality rate, we carried out a linear ($y = \alpha.x + \beta$) and a two-degree polynomial ($y = \alpha_1.\ x + \ \alpha_2.x^2 + \beta$) analysis, taking into account the notion of physiological optima (21) through an optimized link between thermodynamics and physiology/pathology (parameters of air-borne diseases such as influenza also show a maximal transmission rate for a specific range of ambient temperatures–20). For each of the three environmental variables, we kept the best of the two models based on the adjusted coefficient of determination, taking into account the complexity of the model.

To test potential associations between the studied parameters, a Principal Component Analysis (PCA) was computed. Pearson correlation coefficients and tests for association were computed to measure the correlation between each pair of parameters. The results are presented in a correlation matrix. For these analyzes, we used the absolute value of latitude, representing the deviation from latitude 0. Finally, when a polynomial regression was determined for environmental variables, the deviation from the maximal value was used to test the association with Covid mortality.

Results are considered significant at $p < 0.05$. All statistical analyses were performed with R (version 3.6.1; The R Foundation for Statistical Computing, Vienna, Austria).

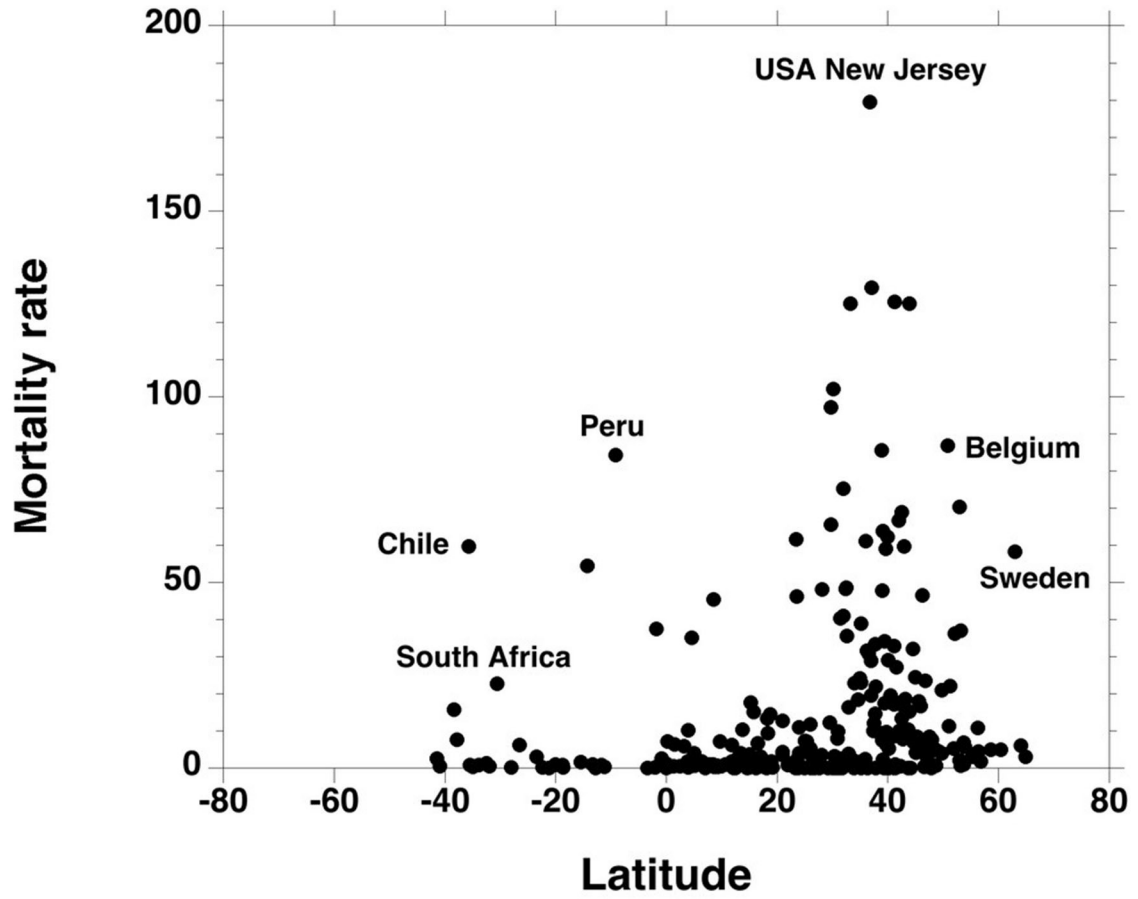


FIGURE 1 | Each point represents the Covid-19 mortality rate of a country or a state, according to its latitude. Greater mortality rates were mostly observed in the [25 /65] latitude interval.

RESULTS

One hundred and sixty countries were included in the study (**Supplementary Table 1**), accounting for a total of 846,395 deaths due to Covid-19 up to the study end point (31th August 2020).

Covid-19 Mortality and the Global Niche

The geographical relation between Covid-19 mortality rate and latitude shows that higher mortality rates were observed in the 25/65° northern parallels (**Figure 1**, **Supplementary Figure 4**). The [25–65°] latitude intervals (North and South) delimited an area where 78% of all Covid-19 deaths were recorded (in the European continent, this area includes Spain and Italy up to the southern part of Sweden; in the Americas, it covers the state of Texas up to the Hudson Bay; the southern part of Brazil and the states under it; in the African continent: the Maghreb states and South Africa). This area includes the states with the highest recorded death rates (New-Jersey in the Americas, Belgium in Europe).

Negative longitudes (American Countries) were also associated with higher death rates (**Figure 2**). The [−35/−125°] longitude interval (West) delimited an area where 57% of all Covid-19 deaths were recorded.

Covid-19 Mortality and the Environment

Polynomial regression was used for the relationship between the number of deaths per 100,000 inhabitants according to temperature ($R^2 = 0.21$) and humidity ($R^2 = 0.05$) (**Figure 3**). A linear relationship was preferred for the UV index ($R^2 = 0.11$). Maximal death rates are obtained for a temperature T_{max} of 10.1°C, a humidity H_{max} of 55%, and a zero UV index. Deviations from T_{max} and H_{max} were used for the multifactorial analysis of death rates with temperature and humidity.

Principal Component Analysis

Combining the studied parameters, the first and second factorial planes of the PCA represent 60.27% of the information (**Figure 2**). The first axis concentrates 37.55% of total inertia and axis 2 represents 13.56% of it. The third factorial axis represents

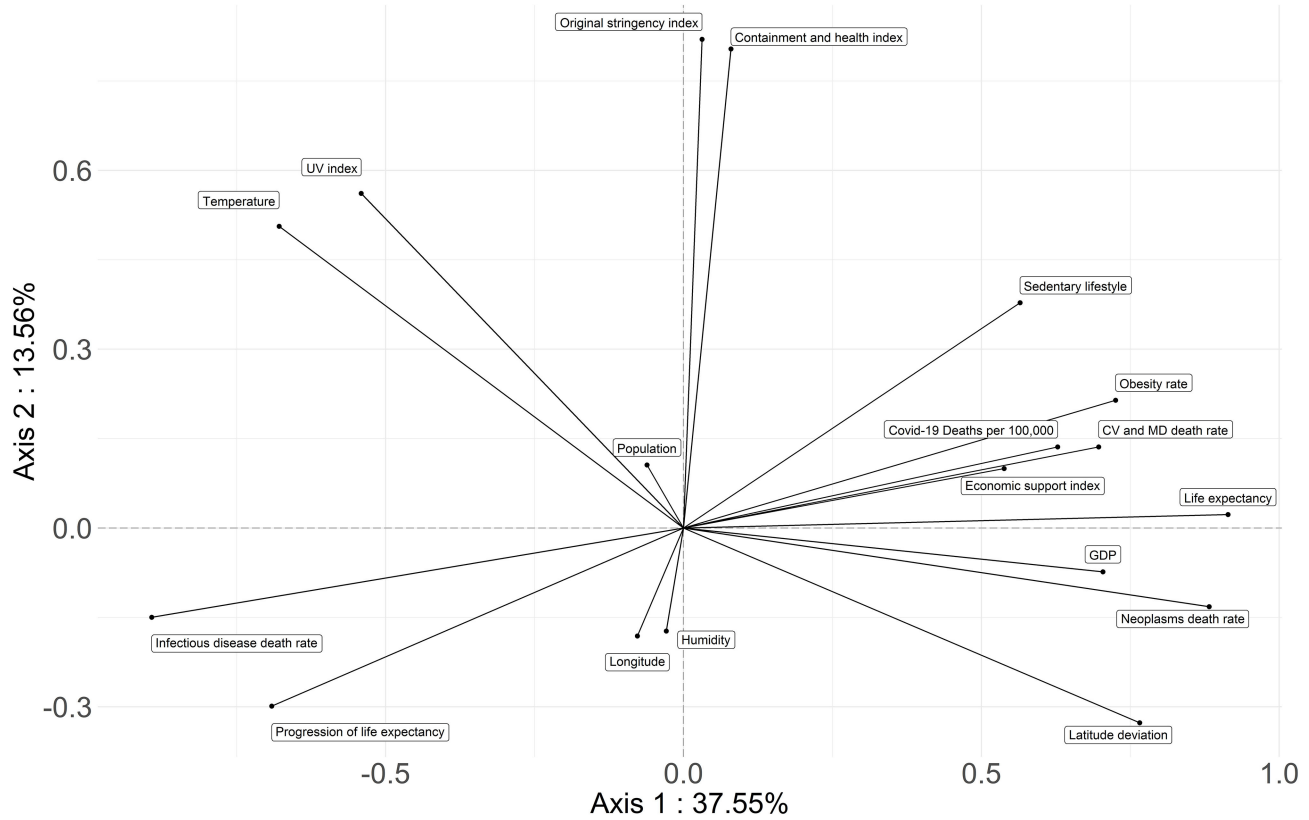


FIGURE 2 | PCA first factorial plane: axis 1 is horizontal; axis 2 is vertical. The more distant a variable is from the center, the more it is correlated with the first or the second factorial axis. The studied parameters regrouped the countries associated with Covid-19 highest death rates on the right of the horizontal axis. These are the high income countries with a high LE but a low progression of it, high sedentarity, obesity, high deviation from latitude 0 and low longitude (Asian countries have a high longitude, while it is negative in the Americas). Countries associated with low Covid-19 death rates have a low GDP, a low LE but a great margin of progression for it, a high prevalence of infectious diseases, a greater deviation from optimum temperature and UV index; they occupy the left part of the axis. Lockdown stringency, containment index and ambiant humidity are not correlated with Covid-19 mortality, as they are linked to the second axis. The cloud of individuals on the first factorial plane is presented in the **Supplementary Figure 5**.

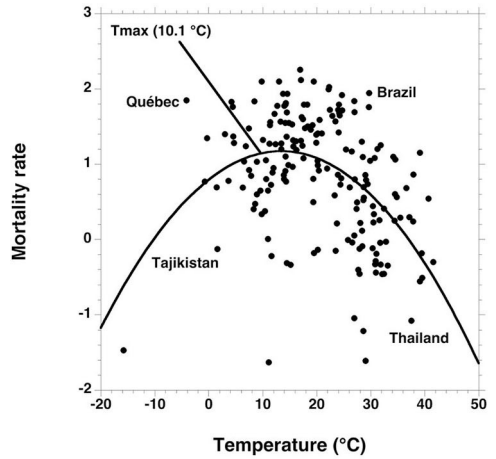
9.16% of the information. The cloud of individuals on the first factorial plane is presented in **Supplementary Figure 5**.

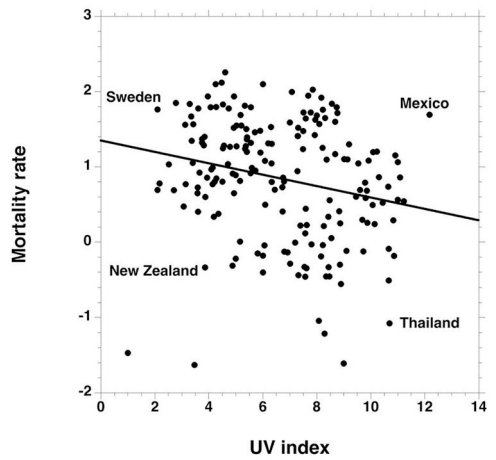
The first axis of PCA opposes two groups of countries (**Figures 2**, **4**, **5**). High income northern countries are positively correlated to this axis: they provide high economic support, have higher LE but lower progression of LE, more frequent sedentary lifestyle, larger obesity rates, and higher mortality from CVD and cancer. Occupying the left part of the axis are countries with a low GDP, lower life expectancy but greater progression of LE, higher death rate from infectious diseases, greater deviation from optimum temperature, and UV index. Covid-19 death rate is higher in countries strongly and positively correlated with the first factorial axis on the right.

The government's responses (i.e., the severity index and the containment and health index) are strongly correlated with the second factorial axis (**Figures 2**, **4**). The death rate from Covid-19 is not correlated with this axis. Therefore, the death rate appears not to be linked with the responses of governments.

The third axis shows a relationship between Covid-19 mortality and longitude as well as obesity and sedentarity (**Figures 4**, **5**). American countries have a higher obesity rate and a higher Covid-19 mortality rate; Asian countries have lower obesity rates and lower Covid-19 mortality rates.

The correlation matrix (**Figure 6**) shows that the Covid-19 mortality rate is positively correlated to a group of variables composed of the inactive lifestyle ($r = 0.46$, $p < 10^{-6}$), obesity rate ($r = 0.55$, $p < 10^{-11}$), GDP ($r = 0.40$, $p < 10^{-7}$), economic support index ($r = 0.31$, $p < 10^{-3}$), life expectancy ($r = 0.50$, $p < 10^{-11}$), burden of mortality due to CVD ($r = 0.33$, $p < 10^{-3}$), cancer ($r = 0.47$, $p < 10^{-9}$), and deviation from latitude 0 ($r = 0.41$, $p < 10^{-3}$). The mortality rate due to Covid-19 is negatively correlated to another group of variables composed of the mortality rate from infectious diseases ($r = -0.50$, $p < 10^{-9}$), the progression of life expectancy ($r = -0.37$, $p < 10^{-4}$), longitude ($r = -0.36$, $p < 10^{-3}$), the deviation from optimum temperature ($r = -0.39$, $p < 10^{-5}$), UV index ($r = -0.37$, $p < 10^{-43}$). There is no significant correlation with the deviation





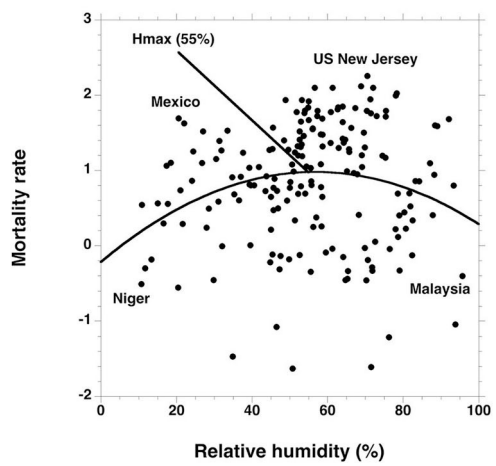


FIGURE 3 | Polynomial or linear regression between the logarithm of the mortality rates due to Covid-19 and the UV index, humidity, and average temperature from the local beginning of the pandemic up to the peak.

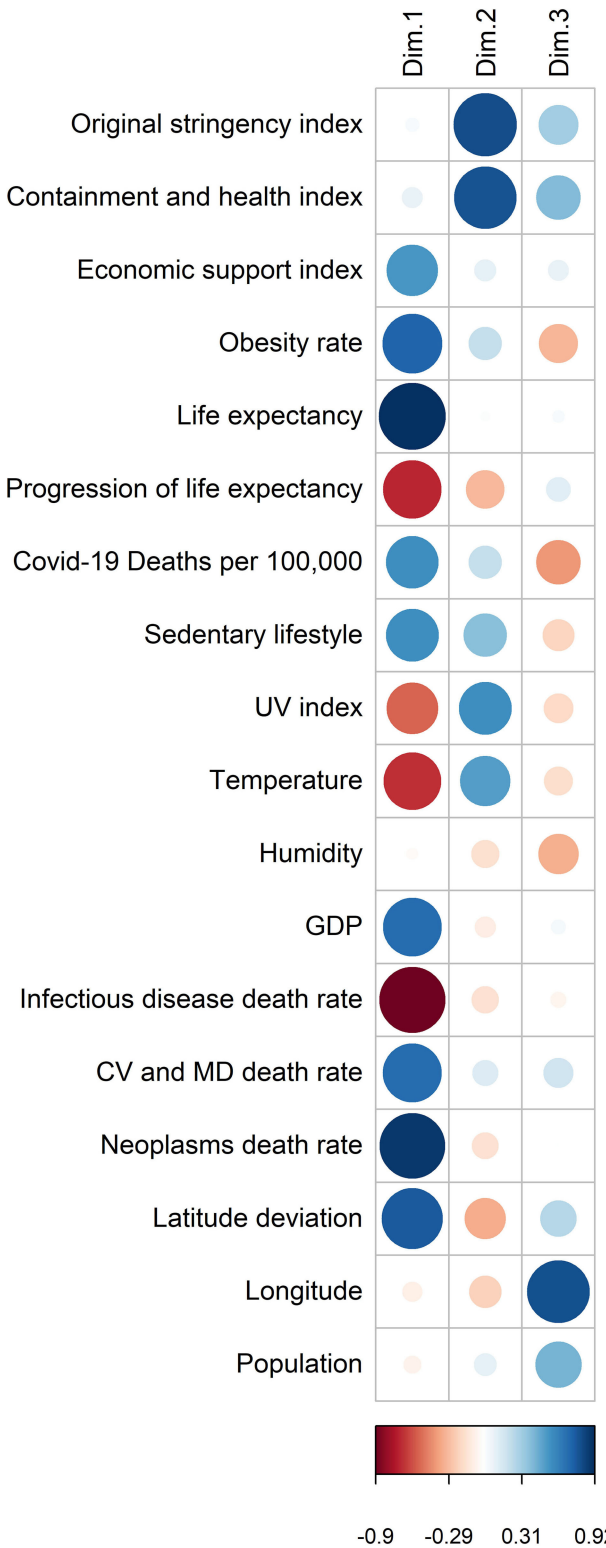


FIGURE 4 | Coordinates of the variables on the first 3 factorial axes. The larger the circle, the more the variable is correlated with the axis. A blue circle indicates that the variable is negatively correlated, a red circle indicates that the variable is positively correlated. The scale shows the coordinates of the variables on the axis.

from optimum humidity ($r = 0.03$, $p = 0.52$), the containment and health index ($r = 0.07$, $p = 0.51$), the original stringency index ($r = 0.07$, $p = 0.36$), and population size ($r = -0.05$, $p = 0.35$). A negative correlation also relates obesity and longitude ($r = -0.33$, $p < 10^{-4}$).

The principal component analysis as well as the correlation matrix with the estimated data are presented in **Supplementary Figures 1–3**. The analyzes with the estimated death number at the end of the first epidemic wave do not change the conclusions of the analyzes on the real data. The direction of the correlations as well as their significance in the correlation matrix are unchanged as well.

DISCUSSION

Main Findings

This analysis shows that higher Covid-19 mortality rates are mostly found in countries experiencing higher life expectancies and showing a recent slowdown of this progression. Most of these developed and aging societies are latitudinally located over the 25° parallel. They also have higher GDP and chronic diseases levels (e.g., CVD and cancer) associated with major metabolic risk factors (e.g., inactive lifestyle, sedentarity, and obesity). High temperature and UV levels are associated with low death rates such that northern and western countries pay the most severe toll to Covid-19.

In the PCA, the first axis shows a strong correlation between Covid-19 death rates and countries inside the [25/65°] latitudinal strip, while the third axis reveals two correlations with Covid-19 death rates: one with longitude, a second one with obesity. This suggests that states in the Americas plagued with frequent inactive lifestyle and higher obesity rates than Asian countries experienced a higher death toll.

This is consistent with the hypothesis of an optimal human development niche, that has aggregated favorable health, demographic, environment, and economic parameters (1). However, though previously positive, they now expose populations to higher vulnerabilities to both infectious (Covid-19) or physical constraints (heat waves). Regarding government's actions (i.e., containment and stringency index), no association was found with the outcome, suggesting that the other studied factors were more important in the Covid-19 mortality than political measures implemented to fight the virus, except for the economic support index. It may however be important to decipher this positive relation in a plausible chronological order: it does not seem that a higher economic support would induce a higher Covid-19 mortality, but rather that a higher death toll rate

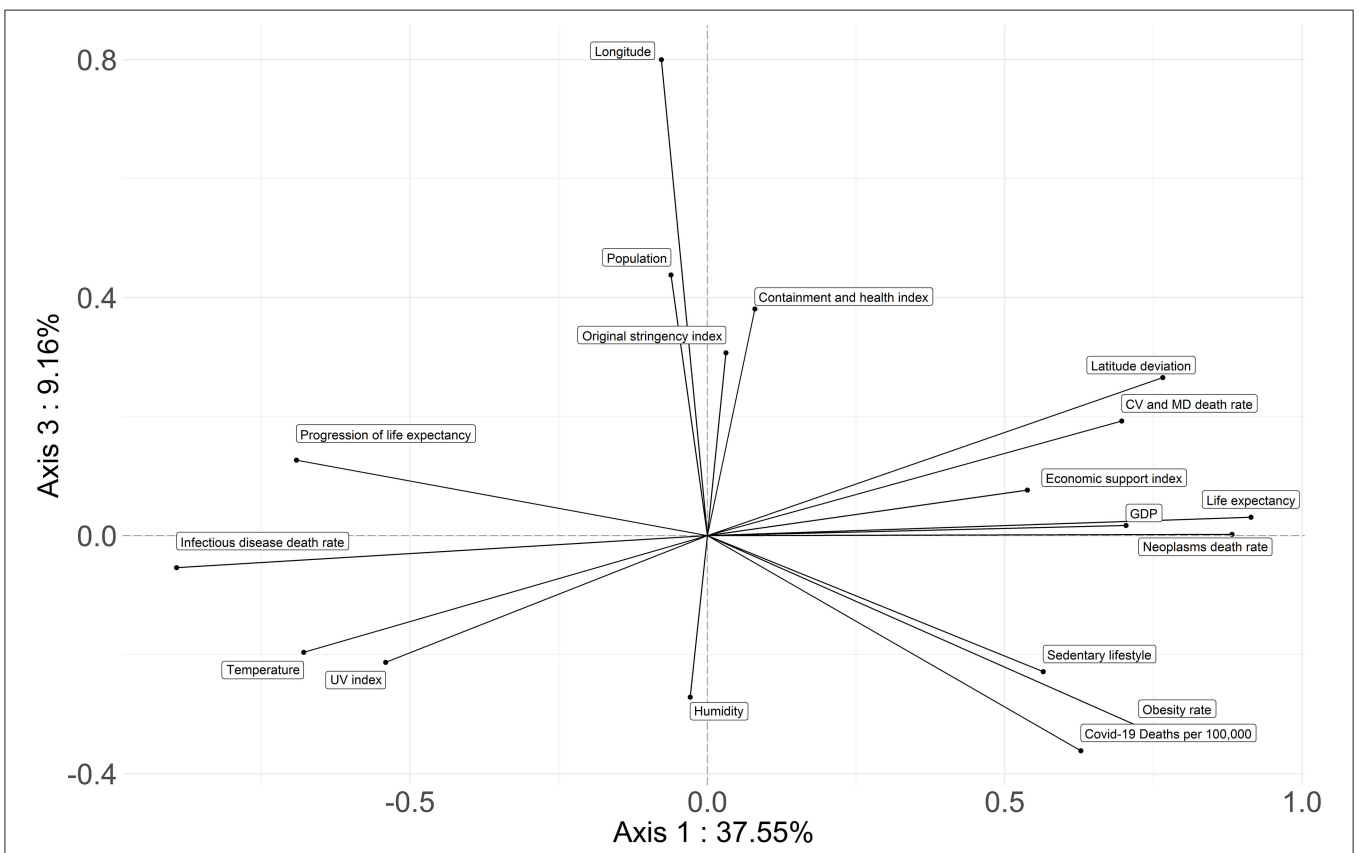


FIGURE 5 | PCA third factorial plane: axis 1 is horizontal; axis 3 is vertical. The studied parameters regrouped the countries associated with Covid-19 highest death rates on the right of the horizontal axis. Longitude and obesity rates are related to the third axis.

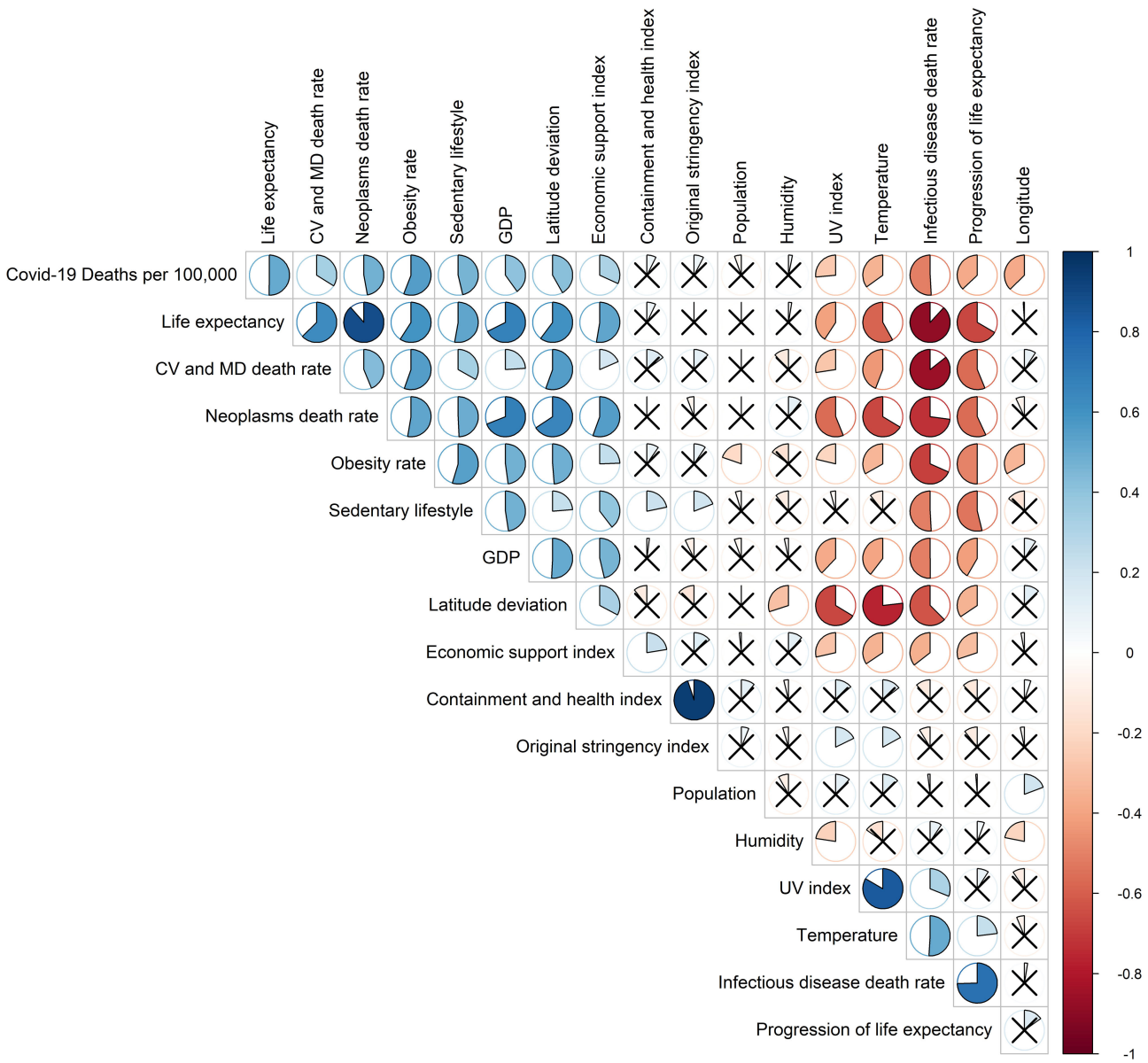


FIGURE 6 | Correlation matrix: a larger area in the circles indicates a stronger correlation between the row and the column variables. A blue circle indicates a positive correlation coefficient; a red circle indicates a negative one; a full circle corresponds to $r = 1$ or -1; an empty circle corresponds to $r = 0$. If the Pearson correlation test was not significant, a cross on the circle was added.

provoked a larger societal reaction, including a higher amount of economic measures, when available.

The design of this study aimed to draw a global description of the Covid-19 mortality and its associations with several major parameters. It is out of scope to speculate on any cause-effect relation. Nevertheless, some explanatory hypothesis may be proposed: countries displaying greater susceptibility (determined by a more fragile balance between health, demographic, environment, and economic parameters) seem to have narrower margins of adaptation and be therefore more vulnerable to main aggressors.

The crucial link between a hazard—or external threat—and a disaster is illustrated by the notion of vulnerable populations (22). Vulnerability is the result of complex interactions of distinct risks, exposure to the threat, and the lack of defenses or resources to deal with it. During a pandemic situation, the foremost indicator of countries health fragility may be seen in the proportion of older people (who were the SARS-CoV-2 major target), given the ineluctable diminished performances and resilience with age (23). Resulting from both biological and social processes, the decline in health and physical strength and the increasing disabilities particularly affect

old people, bringing them closer to vulnerability thresholds. The highest proportions of elderly people are observed in countries with higher life expectancy (24, 25). Such nations may suffer from higher mortality levels when new aggressors appear.

Previous studies have illustrated the relation between frailty and mortality (26). For instance, the 2003 heatwave killed 30,000 to 50,000 people in Europe and 15,000 in France (13, 27), 80% of them being elderly people. Among centenarians, who are more likely to decline suddenly, mortality due to infections increases (e.g., pneumonia) (14). Accordingly, the Covid-19 mortality was the highest among the elderly worldwide (28). Moving toward higher life expectancy will therefore expose greater proportions of people to high mortality rates, especially when facing mass threats or when environment conditions largely evolves.

Concomitantly to a high life expectancy, the development afforded by an elevated GDP usually favors inactive lifestyles, sedentary behaviors, and obesity (15, 29), increasing the risk for hypertension, diabetes, and CVD, the most frequent comorbidities associated with Covid-19 mortality (30–32). With an epidemiological transition toward more prevalent chronic diseases, countries with high life expectancy have also increased concurrent risks, restraining their adaptability margins.

The associations found among two opposed groups of countries suggest important inherent factors, predetermining the consequences of global threats. Properly understanding the relations between those parameters may help to provide new prevention strategies. Covid-19 has prompted a wide range of responses from governments around the world, yet the contagion and mortality curves are very homologous among countries (33). This is reinforced by our findings regarding the lack of any association with the government's actions taken during the pandemic. In that sense, the determining demographic, health, development, and environment factors seem much more important to anticipate the lethal consequences of the Covid-19 than government's actions, especially when such actions are led by political goals more than by sanitary ones. This last result however cannot predict that other types of measure would not reduce the pandemia death load.

This study highlights the great difficulties of adaptation that most countries will face (34, 35). Climate change for instance will disturb the optimal niche by forcing the ideal development temperature toward north. Infection balance and human resilience supported by local species equilibrium may be impaired as a result. Understanding where the risks and weaknesses are in each country is an important starting point when preparing to face new threats. In the Covid-19 case, an advisable strategy may be to increase populations immunity and resilience (36) and prevent sedentary behaviors through higher physical activity and better physical fitness. Hence, political strategies restricting physical activity (e.g., closing sport facilities) may refrain the enhancement of population immunity in response to present and future viral aggressors.

The first limitation of this study is the uncertainty and reliability of the recorded national data on Covid-19 deaths, given the diverse counting methods in the different countries. We also acknowledge the limit of the reliability of the input data, since it refers to worldwide data collections. However, these are the least uncertain and the most reliable sources. Furthermore, the large size of the datasets compensates for the internal variability.

Another limitation is that the pandemic is not over, with American countries displaying a kinetics partially diverging from the European ones. While a clear mortality peak was observed in Europe with a quick decrease after it, it is not the case in several American countries: Mexico, Peru, and Brazil show a lasting plateau for the time being and the USA experienced a spring peak in the eastern states and a summer peak in southern ones. If finally, Covid-linked mortality would be higher in countries of Latin America than in richer countries, it would be necessary to understand the peculiar features, absent of our analysis, explaining such a result. A large dependence to seasonal parameters may also modify some conclusions at the end of the pandemic (e.g., if mortality does not decrease for months in these countries). But it may not change the conclusions about the first phase we deal with in that study. Indeed, countries with the highest death toll could still be in the Americas as USA have already experienced a first regression of life expectancy, whereas Mexico also shows one of the highest obesity rates.

This study has focused on the explosive sub-exponential phase of Covid-19 epidemic in each country. However, a previous period of propagation has probably started in the summer or fall of 2019. It is difficult to account for it, but such a diffusion phase of SARS-CoV-2 may be investigated through both International Air Transport Association (IATA) databases and modeling of airplane transport (37). The situation in islands such as Taiwan, New Zealand, or Iceland, that quickly imposed restrictive measures on air transport, shows that the virus has not become endemic in these first 8 months. After a rapid propagation phase, only the re-importation of subjects contaminated outside the island provoked new local cases. Finally, we did not account for the various viral sub-types, that could modify the relations shown here, as they may theoretically have a different impact on death rate. The main recorded variants however did not appear to produce such a difference on mortality (38).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

QD, AM, JA, ELB, and J-FT conceived, designed, performed, and analyzed the research. QD and AM conceived, designed, and collected data from website. QD, ELB, and J-FT carried out the

statistical analyzes. QD, JA, ELB, and J-FT wrote the manuscript. All authors read, review and approved the final manuscript.

ACKNOWLEDGMENTS

We thank Bernard Swynghedauw and Gilles Boeuf for manuscript reading and fruitful discussion.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2020.604339/full#supplementary-material

Supplementary Table 1 | Number of deaths as of August 31th, 2020, number of theoretical deaths according to our model as of August 31th, 2020 and number of deaths estimated at the end of the first epidemic wave according to our model for each country included in the analysis. The estimated death number for each country was obtained from the logistic regression (see section Methods—Data collection) when the first epidemic wave was expected to reach 99% of the total death toll. The correlation matrix as well as the principal component analysis were performed with data to assess whether the results may change at the end of the first epidemic wave.

Supplementary Figure 1 | First factorial plan of the principal component analysis with the estimated data (see Section Methods—Data collection). Results are the same with estimated data and actual data.

Supplementary Figure 2 | Coordinates of the variables of the principal component analysis with the estimated data (see Section Methods—Data collection). Results are the same with estimated data and actual data.

Supplementary Figure 3 | Correlation matrix with the estimated death numbers obtained from the logistic equation (see Section Methods—Data collection). Results are the same with estimated data and actual data.

Supplementary Figure 4 | Covid-19 mortality is expressed in numbers in the top figures and in rates in the bottom figures. It is expressed in raw data on the left and in decimal logarithm on the right.

Supplementary Figure 5 | The position of the countries on the graph represents their correlation according to the variables of the first factorial plane. For example, countries positively correlated to axis 1 (right), will be positively correlated to variables to the right of axis 1.

REFERENCES

1. Xu C, Kohler TA, Lenton TM, Svenning J-C, Scheffer M. Future of the human climate niche. *Proc Natl Acad Sci U S A*. (2020) 117:11350–5. doi: 10.1073/pnas.1910114117
2. Olshansky SJ, Carnes BA. The future of human longevity. In: P. Uhlenberg, editor. *International Handbook of Population Aging International Handbooks of Population* (Springer Netherlands) (2009). p. 731–45. Available online at: http://link.springer.com/chapter/10.1007/978-1-4020-8356-3_33 (accessed August 8, 2013).
3. Preston SH. The Changing relation between mortality and level of economic development. *Popul Stud*. (1975) 29:231–48. doi: 10.1080/00324728.1975.10410201
4. Global Burden of Disease Health Financing Collaborator Network. Trends in future health financing and coverage: future health spending and universal health coverage in 188 countries, 2016-40. *Lancet*. (2020) 391:1783–98. doi: 10.1016/ S0140-6736(18)30697-4
5. Olshansky SJ, Carnes BA. Inconvenient truths about human longevity. *J Gerontol Ser A*. (2019) 74:S7–S12. doi: 10.1093/gerona/glz098
6. Dong X, Milholland B, Vijg J. Evidence for a limit to human lifespan. *Nature*. (2016) 538:257–9. doi: 10.1038/nature19793
7. Antero-Jacquemin JS, Berthelot G, Marck A, Noirez P, Latouche A, Toussaint J-F, et al. Learning from leaders: life-span trends in olympians and supercentenarians. *J Gerontol A Biol Sci Med. Sci*. (2015) 70:944–9. doi: 10.1093/gerona/glu130
8. NCD Risk Factor Collaboration (NCD-RisC). A century of trends in adult human height. *eLife*. (2016) 5:e13410. doi: 10.7554/eLife.13410
9. Berthelot G, Sedeaud A, Marck A, Antero-Jacquemin J, Schipman J, Saulière G, et al. Has athletic performance reached its peak? *Sports Med Auckl NZ*. (2015) 45:1263–71. doi: 10.1007/s40279-015-0347-2
10. Marck A, Antero J, Berthelot G, Saulière G, Jancovici J-M, Masson-Delmotte V, et al. Are we reaching the limits of Homo sapiens? *Front Physiol*. (2017) 8:812. doi: 10.3389/fphys.2017.00812
11. West GB, Brown JH. The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. *J Exp Biol*. (2005) 208:1575–92. doi: 10.1242/jeb.01589
12. Dhar PK, Giuliani A. Laws of biology: why so few? *Syst Synth Biol*. (2010) 4:7–13. doi: 10.1007/s11693-009-9049-0
13. Valleron A-J, Boumendil A. Epidemiology and heat waves: analysis of the 2003 episode in France. *C R Biol*. (2004) 327:1125–41. doi: 10.1016/j.crvi.2004.09.009
14. Evans CJ, Ho Y, Daveson BA, Hall S, Higginson IJ, Gao W, et al. Place and cause of death in centenarians: a population-based observational study in England, 2001 to 2010. *PLoS Med*. (2014) 11:e1001653. doi: 10.1371/journal.pmed.1001653
15. Olshansky SJ, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, et al. A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med*. (2005) 352:1138–45. doi: 10.1056/NEJMsr043743
16. Daar AS, Singer PA, Leah Persad D, Pramming SK, Matthews DR, Beaglehole R, et al. Grand challenges in chronic non-communicable diseases. *Nature*. (2007) 450:494–6. doi: 10.1038/450494a
17. Abegunde DO, Mathers CD, Adam T, Ortegon M, Strong K. The burden and costs of chronic diseases in low-income and middle-income countries. *Lancet*. (2007) 370:1929–38. doi: 10.1016/S0140-6736(07)61696-1
18. Beaglehole R, Epping-Jordan J, Patel V, Chopra M, Ebrahim S, Kidd M, et al. Improving the prevention and management of chronic disease in low-income and middle-income countries: a priority for primary health care. *Lancet*. (2008) 372:940–9. doi: 10.1016/S0140-6736(08)61404-X
19. Oni T, McGrath N, BeLue R, Roderick P, Colagiuri S, May CR, et al. Chronic diseases and multi-morbidity - a conceptual modification to the WHO ICCC model for countries in health transition. *BMC Public Health*. (2014) 14:575. doi: 10.1186/1471-2458-14-575
20. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. (2020) 323:1061–9. doi: 10.1001/jama.2020.1585
21. El Helou N, Tafflet M, Berthelot G, Tolaini J, Marc A, Guillaume M, et al. Impact of environmental parameters on marathon running performance. *PLoS One*. (2012) 7:e037407. doi: 10.1371/journal.pone.0037407
22. Schröder-Butterfill E, Marianti R. A framework for understanding old-age vulnerabilities. *Ageing Soc*. (2006) 26:9–35. doi: 10.1017/S0144686X05004423
23. Berthelot G, Bar-Hen A, Marck A, Foulonneau V, Douady S, Noirez P, et al. An integrative modeling approach to the age-performance relationship in mammals at the cellular scale. *Sci Rep*. (2019) 9:418. doi: 10.1038/s41598-018-36707-3
24. Waite LJ. The demographic faces of the elderly. *Popul Dev Rev*. (2004) 30:3–16. Available online at: www.jstor.org/stable/3401460 (accessed November 01, 2020).
25. Le Bourg, E. Is life expectancy of french women going to plateau and oscillate? *Gerontology*. (2019) 65:288–93 doi: 10.1159/000497179
26. Buchman AS, Wilson RS, Bienias JL, Bennett DA. Change in frailty and risk of death in older persons. *Exp Aging Res*. (2009) 35:61–82. doi: 10.1080/03610730802545051

27. Trigo RM, García-Herrera R, Díaz J, Trigo IF, Valente MA. How exceptional was the early August 2003 heatwave in France? *Geophys. Res. Lett*. (2005) 32:1–4. doi: 10.1029/2005GL022410
28. Baqui P, Bica I, Marra V, Ercole A, van der Schaar M. Ethnic and regional variations in hospital mortality from COVID-19 in Brazil: a cross-sectional observational study. *Lancet Glob Health*. (2020) 8:e1018–e26. doi: 10.1016/S2214-109X(20)30285-0
29. Chaix B, Chauvin P. Tobacco and alcohol consumption, sedentary lifestyle and overweightness in france: a multilevel analysis of individual and area-level determinants. *Eur J Epidemiol*. (2003) 18:531–8. doi: 10.1023/A:1024642301343
30. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. *JAMA*. (2020) 323:2052–9. doi: 10.1001/jama.2020.6775
31. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. *Int J Infect Dis*. (2020) 94:91–5. doi: 10.1016/j.ijid.2020.03.017
32. Fakhry AbdelMassih A, Ghaly R, Amin A, Gaballah A, Kamel A, Heikal B, et al. Obese communities among the best predictors of COVID-19-related deaths. *Cardiovasc Endocrinol Metab*. (2020) 9:102–7. doi: 10.1097/XCE.0000000000000218
33. *Coronavirus Government Response Tracker*. (2020). Available online at: https://www.bsg.ox.ac.uk/research/research-projects/coronavirus-government-response-tracker (accessed August 10, 2020).
34. Toussaint JF, Swynghedauw B, Boeuf G. *L'Homme Peut-il S'adapter à Lui-Même?* Paris: QUAE Publisher (2012). Available online at: https://www.quae.com/produit/1135/9782759219452/l-homme-peut-il-s-adapter-a-lui-meme.
35. Fauci AS, Moren DJ. The perpetual challenge of infectious diseases. *N Engl J Med*. (2012) 366:454–61. doi: 10.1056/NEJMra1108296
36. Campbell JP, Turner JE. Debunking the myth of exercise-induced immune suppression: redefining the impact of exercise on immunological health across the lifespan. *Front Immunol*. (2018) 9:648. doi: 10.3389/fimmu.2018.00648
37. Colizza V, Barrat A, Barthelemy M, Valleron AJ, Vespignani A. Modeling the worldwide spread of pandemic influenza: baseline case and containment interventions. *PLoS Med*. (2007) 4:e13. doi: 10.1371/journal.pmed.0040013
38. Grubaugh ND, Hanage WP, Rasmussen AL. Making sense of mutation: what D614G means for the COVID-19 pandemic remains unclear. *Cell*. (2020) 182:794–5. doi: 10.1016/j.cell.2020.06.040

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 19 November 2020
doi: 10.3389/fphys.2020.595005

Study of Clinical and Biological Characteristics of Moroccan Covid-19 Patients With and Without Olfactory and/or Gustatory Dysfunction

Hasnae Benkirane[1], Jaafar Heikel[2], Fatima Zahra Laamiri[3], Amina Bouziani[1], Houria Lahmam[1], Ayoub Al-Jawaldeh[4], Noureddine El Haloui[1], Khalid Ennibi[5], Naim Akhtar-Khan[6], El Mostafa El Fahime[7], Majdouline Obtel[8], Amina Barkat[9] and Hassan Aguenaou[1]*

[1] CNESTEN, Joint Research Unit in Nutrition and Food, Regional Designated Center of Nutrition (AFRA/IAEA), Ibn Tofail University, Kenitra, Morocco, [2] Mohammed VI University of Health Sciences, Casablanca, Morocco, [3] Laboratory of Health Sciences and Technology, Higher Institute of Health Sciences of settat, Hassan First University of Settat, Settat, Morocco, [4] Nutrition, Department of NonCommunicable Diseases and Mental Health World Health Organization (WHO),Regional Office for the Eastern Mediterranean (EMRO), Abdul Razzak Al-Sanhouri, Cairo, Egypt, [5] Center of Virology, Infectious and Tropical Diseases, Mohammed V Military Hospital, Rabat, Morocco, [6] Inserm Research Center, U1231 INSERM/UB/AgroSup, Team-Physiology of Nutrition & Toxicology, Faculty of Life Sciences, University Bourgogne Franche-Comté (UBFC), Dijon, France, [7] Functional Genomic Platform, UATRS-CNRST, Rabat, Morocco, [8] Laboratory of Community Health, Preventive Medicine and Hygiene, Laboratory of Epidemiology and Clinical Research, Department of Public Heath, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco, [9] Health and Nutrition Research Team of the Mother-Child Couple, Faculty of Medicine, Mohammed 5th University, Rabat, Morocco

OPEN ACCESS

Edited by:
Denis Noble,
University of Oxford, United Kingdom

Reviewed by:
Hamid Reza Marateb,
Universitat Politecnica de Catalunya, Spain
Ramon Bossardi Ramos,
Albany Medical College, United States

***Correspondence:**
Amina Bouziani
amina.bouziani@uit.ac.ma

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: 14 August 2020
Accepted: 20 October 2020
Published: 19 November 2020

Citation:
Benkirane H, Heikel J, Laamiri FZ, Bouziani A, Lahmam H, Al-Jawaldeh A, El Haloui N, Ennibi K, Akhtar-Khan N, El Fahime EM, Obtel M, Barkat A and Aguenaou H (2020) Study of Clinical and Biological Characteristics of Moroccan Covid-19 Patients With and Without Olfactory and/or Gustatory Dysfunction. Front. Physiol. 11:595005. doi: 10.3389/fphys.2020.595005

Background: The epidemic of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), presents a significant and urgent threat to global health. This alarming viral infection, declared as pandemic by the WHO in February 2020, has resulted millions of infected patients and thousands of deaths around the world. In Morocco, despite the efforts made by the authorities, the SARS-CoV-2 continues to spread and constitutes a burden of morbidity and mortality. The objective of this study is to describe clinical characteristics of COVID-19 Moroccan patients and to establish the relationship between specific clinical symptoms, namely ageusia and/or anosmia, with these characteristics.

Methods: We performed a descriptive, non-interventional cross-sectional study analyzing data from 108 patients admitted to the VINCI clinic, Casablanca (Morocco). The database includes 39 parameters including epidemiological characteristics, anthropometric measurements and biological analyzes.

Results: The average of age of the patients was 43.80 ± 15.75 years with a sex ratio of 1:1. The mean body mass index of the patients was 25.54 ± 4.63 Kg/m^2. The majority of patients had, at least, one comorbidity and among 75% symptomatic patients, about 50% had, at least, three symptoms namely, fever (40.7%), cough (39.8%), myalgia (28.7%), and anosmia and/or ageusia (20.4%). From biological analyzes, we noticed lymphopenia and an elevated protein C reactive and lactate dehydrogenases levels in 24.1, 36.1, and 35.2% of patients, respectively. A disturbance in liver function markers

was observed in 15.7% of cases. For the other hemostasis parameters, high levels of prothrombin and platelets were reported in 14.6 and 14.8% of patients, respectively. Comparisons related to the presence of anosmia and/or ageusia did not show any difference for demographic and anthropometric characteristics, while a possibility of a significant difference was revealed for certain biological parameters, particularly the levels of lymphocytes, D-dimer and troponin.

Conclusion: This study provides significant findings that will be used not only to supplement previous studies carried out in Morocco in order to resume the epidemiological situation in comparison with other countries, but also to improve the quality of the diagnosis of COVID-19 patients by identifying all the symptoms of the disease and better understanding its clinical outcomes.

Keywords: ageusia, anosmia, biological, epidemiological, demographic characteristics, Moroccan patients, COVID-19

INTRODUCTION

The world is currently experiencing an alarming epidemic called coronavirus disease 2019 (COVID-19), caused by an infectious new viral strain belonging to the coronavirus family, i.e., severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), first detected in the Wuhan district of eastern China (Lu and Stratton, 2020). Infection of this virus quickly reached all corners of the world and saturated even the most resilient health systems (Organisation mondiale de la santé (OMS), 2020). Globally, 20% of infected subjects developed a severe or critical form of the disease, with a fatality rate currently above 3%, with higher rates in the older people and in those with chronic diseases (World Health Organization, 2020a).

Common signs of SARS-CoV-2 infection are respiratory symptoms, fever, cough, myalgia, shortness of breath, sore throat, and dyspnea. In more severe cases, the infection can cause pneumonia, severe acute respiratory syndrome and kidney failure (Hussain et al., 2020). However, some people, although infected, have only very mild symptoms or remain asymptomatic (Hussain et al., 2020).

Recent reports have also demonstrated the appearance of a new symptom in COVID-19 patients, i.e., sudden loss of the sense of smell and/or taste with the absence of the common clinical viral symptoms (Lechien et al., 2020a). It is a specific sign of COVID-19 infection that was recently added, by the WHO, to the list of other symptoms (World Health Organization, 2020a). The presence of anosmia and ageusia may appear in the early stages of COVID-19 (Zhou et al., 2020a) and represent an important diagnostic tool (Lee et al., 2020). Furthermore, the loss of taste and smell varies well according to sex and age. Interestingly, these sensorial alterations were generally more prevalent in women than men and young patients compared to adults (Giacomelli et al., 2020; Lechien et al., 2020b).

In parallel to these apparent symptoms, other immunological, biochemical and biological markers have been highlighted in COVID-19 patients (Yang et al., 2020c), they generally cover inflammatory and obesity indicators (Yang et al., 2020c; Arthur et al., 2020). Therefore, old age, chronic metabolic diseases and male sex make a favorable environment for SARS-CoV-2 infection that, in turn, can trigger acute and fatal hyperinflammation, called "cytokine storm" (Xu et al., 2020b). Several hypotheses have been proposed, suggesting that the smell and/or taste dysfunction could be due to inflammation of the nasal or oral neurological tissues. However, the physiopathological mechanism of this phenomenon remains unknown (Baig et al., 2020).

Additionally, obesity which is a major risk factor of several chronic diseases, including cardiovascular complications, diabetes, cancer, kidney dysfunction, etc., is also associated with a higher risk of respiratory tract infections, and hence the virus installation. Actually, an increase in adiposity has been shown to modify the integrity of the respiratory epithelium, which could lead to dysfunction of the respiratory tract (Honce and Schultz-Cherry, 2019) with an inflammatory response resulting in immunosuppression that could promote viral infections (Khan, 2006). In term of oro-sensory perception, olfaction or taste disorders can lead to weight gain or to an aggravation of the infection by distorting the feeling of food satiation (Tomassini et al., 2017). Furthermore, body mass index (BMI) affects olfactory function (Skrandies and Zschieschang, 2015). It has been suggested that obese subjects are at high risk of SARS-CoV-2 infection because they already have a low olfactory and gustatory capacity due to obesity which will mask the decrease in taste and odor induced by SARS-CoV-2 infection (Khan et al., 2020).

Most importantly, it has been increasingly evidenced that COVID-19 patients, before showing any clinical sign related to the viral infection, exhibit a loss of chemosensory perception of smell and taste. As mentioned above, some obese subjects suffer from the loss of gustatory perception, which may be explained by the association of obesity with "low grade" inflammation, marked with high concentrations of pro-inflammatory cytokines. COVID-19 is also marked with a "inflammatory cytokine storm." So, we decided to categorize our COVID-19 patient population as with or without this oro-naso-sensory perception to better shed light at this aspect in relation with inflammation (Khan et al., 2020).

Besides, in Morocco, the data available on coronavirus mainly focused on generalities (description of the virus, its mode of spread and diagnosis, the adopted treatments, the number of cases, etc.) (Bourhanbour and Bakkouri, 2020; Traore et al., 2020; Zoukal et al., 2020). However, further studies testing more characteristics of COVID 19 patients remain limited. Therefore, the present study aims to describe anthropometric and clinical characteristics of Moroccan patients with COVID-19 and to define differences for each parameter according to the olfactory or gustatory dysfunction. This study will also allow us to specify the part of the population most at risk (males or females, young or old population, etc.) to assess the condition of the patients studied and to study the disease's severity in order to guide treatment and therapeutic management of patients.

MATERIALS AND METHODS

Study Design and Recruitment Site

This is a descriptive, non-interventional cross-sectional study. It was carried out among COVID-19 patients admitted to the VINCI clinic in Casablanca (Morocco) from 20 March to 04 June 2020.

The study, including patient monitoring and all performed analyzes, was carried out in partnership with the VINCI clinic in Casablanca, Mother and Child Health & Nutrition Research team, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Ministry of Health as well as National Center for Scientific and Technical Research, Rabat (Morocco).

A trained medical staff of the VINCI clinic realized data collection, using a validated questionnaire by all parties involved in this study and presented as separated sections. Thus, the identity of each patient and its contact details were recorded. Likewise, measurements of anthropometric and biological parameters were carried out.

In addition, data on olfactory or gustatory dysfunction (taste and/or smell) were also collected from questions reported in a separate section and which are scored based on questionnaires already used by researchers (Mattos et al., 2019; Lechien et al., 2020a).

Description of the Participants

The data-set covers patients admitted for COVID-19 at the VINCI Clinic in Casablanca. Indeed, only information on adult patients (men and women) confirmed by a positive COVID-19 (using PCR test and CT chest) and aged 18 years or older was noted. Thus, a total of 108 patients were enrolled in this study. This represents the total number of patients who, were hospitalized at the Vinci clinic at the time of writing this article. With the lifting of confinement, the cases' number is currently increasing. The Vinci clinic is again involved in hospitalizations.

Details of Data Collection and Measurements

Among patients meeting the inclusion criteria, the following steps, measures and interventions have been performed by the clinical staff in-charge:

Infection Confirmation

Infection confirmation was enrolled based on PCR test and radiological examination.

PCR's details

PCR was carried out at Pasteur's institute in Casablanca. Indeed, extraction of viral RNA from nasopharyngeal and oropharyngeal swabs was adopted according to the "Berlin protocol" which was developed and made available worldwide in mid-January 2020 by Professor Christian Drosten, Director of the Institute of Virology at the Charité Hospital in Berlin. This test targets the SARS-CoV-2 E and RdRp gene (Corman et al., 2020).

Radiological examination

This examination was realized using a United UCT 528 multi-barrette scanner with use of the CORADS score and determination, by specific software, of the percentage of the reached territory.

Demographic Data

For each participant, data was collected on age, sex and alcohol or tobacco use. Studied patients were classified into different age groups, i.e., 18–34, 35–44, 45–54, 55–64, and ≥65 years.

Anthropometric Measurements

The anthropometric parameters were measured following the WHO measurement standards (World Health Organization, 1995). These parameters included body weight and height. The BMI was calculated as the body weight in kilogram divided by the height squared in meter. According to obtained BMI values, the participants were classified into different groups, based on the WHO reference values (World Health Organization, 1995), as follows: underweight, BMI < 18.5 Kg/m^2; normal, $18.5 \leq$ BMI < 25 Kg/m^2; overweight, $25 \leq$ BMI < 30 Kg/m^2; obesity class 1, $30 \leq$ BMI < 35 Kg/m^2; obesity class 2, $35 \leq$ BMI < 40 Kg/m^2, and obesity class 3: BMI ≥ 40 Kg/m^2.

Clinical Survey

During the consultation, a clinical survey was prospectively realized for each patient. Data on common symptoms associated with COVID-19 infection principally fever, cough, myalgia, headache, asthenia, pharyngitis, digestive disorders, breathing difficulties and anosmia and/or ageusia were noted. Also, the presence of any other conditions and specifically severe non-communicable diseases, such as diabetes, cardiovascular disease, dyslipidemia, high blood pressure, etc., was recorded.

Olfactory and Gustatory Survey

Using a validated questionnaire, the anosmia and/or ageusia was/were evaluated in a specific section. This part was attributed to define the olfactory and/or gustatory dysfunction.

Biological Analysis

In the Institute Pasteur Laboratory in Casablanca, a number of biological parameters were analyzed in blood samples. On the first day of consultation and after 5 days of hospitalization, a set of biological parameter such as glycemia, hemoglobin (Hb), white blood cells (WBC), lymphocyte, prothrombin time

(PT), reactive protein C (CRP), ferritin, D-dimers, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), troponin, creatine phosphokinase (CPK), lactate dehydrogenase (LDH) were measured for each recruited patient.

The biological analysis of these parameters was made on the basis of paraclinical examinations recommended by the national scientific commission of COVID-19. In addition to the etiological diagnosis and epidemiological surveillance, these laboratory examinations are used to monitor patients according to their cases and their comorbidities, to guide treatment and therapeutic management, and also to avoid a double bacterial infection.

Therewith, for each studied parameter, a defined diagnostic technique was used:

- **Hemogram (Hb, WBC, lymphocyte)**: Sample: whole blood EDTA- SYSMEX flow cytometry- Reference values: adult annals clinical biology 2014-Pediatrics RFL 2009;
- **Prothrombin:** Sample: citrated plasma-SIEMENS Reagent (Human Thromboplastin) Sysmex CA 620 Automate
- **CRP:** ROCHE immunoturbidimetric technique;
- **Ferritin:** ROCHE Electrochemiluminescence technique;
- **D-Dimers**: ELFA bioMérieux automated technique;
- **ASAT, ALAT, and GGT transaminases**: IFCC technique 37°C ROCHE;
- **Troponin:** Electrochemiluminescence technique on COBAS ROCHE;
- **C.P.K and LDH**: Technique UV 37 ROCHE.

Statistical Analysis

Statistical analysis was performed using SPSS software-version 23.0 (IBM SPSS Statistics 23.0.0.0, New York, NY, United States). Baseline characteristics and clinical features of all patients with SARS-CoV-2 infection were described, as numbers and percentages by adding confidence interval estimates for better precision. Biological parameters were also analyzed and presented as mean ± standard deviation or as medians (quartiles), and each variable was categorized into different groups (Low, normal, and high), according to the biological variations, as follows:

- Hb (Man) categorized into 2 groups: <13.4 and >13.4 g/dl;
- Hb (Woman) categorized into 2 groups: <11.5 and >13.4 g/dl;
- WBC categorized into 3 groups: <4550/mm^3, 4550–11000 mm^3, and 30–155 mg/l;
- CRP categorized into 3 groups: <5, 5–30, and 30–155 mg/l;
- CPK categorized into 2 groups: <190 and >190 UI/l;
- LDH categorized into 2 groups: <225 and >225 UI/l;
- Procalcitonin categorized into 2 groups: >0,5 and >0,5 ng/ml;
- AST categorized into 2 groups: <40 and >40 UI/l;
- ALT categorized into 2 groups: <41 and >41 UI/l;
- Prothrombin time categorized into 3 groups: <70, 70–130 and >130%.

The normality of the distribution was evaluated using the Kolmogorov-Smirnov (KS) test.

Between the group of patients with olfactory (or gustatory) dysfunction and those without such dysfunction, comparisons have been made according to demographic, anthropometric and biological data employing different statistical tests depending on the studied variable. The comparison of quantitative variables with normal distribution was carried out by the student *t*-test and when the parametric hypotheses were not satisfactory, the Mann-Whitney test was performed. Association between qualitative variables was made using the Chi-square test or Fisher's exact test. A value of $p < 0.05$ was considered for all statistical analyzes.

NB: Variables with missing data < 1% (GGT gamma-glutamyltranspeptidase, procalcitonin, and troponinemia) or <5% (BMI: Body mass index) were processed after exclusion of missing data. While for CPK and D-dimer, the missing data is greater than 10%, therefore statistical comparison tests were applied on valid data.

Ethics

This study is a part of an overall project on the COVID-19 pandemic which has obtained the approval from the Ethical Committee and Biomedical Research (CEBR) of the College of Medicine and Pharmacy of Mohammed V University (Rabat, Morocco). The Committee approved the inclusion of the patients' data, provided by the VINCI clinic, in order to analyze all the data and to make the different possible correlations (Approval number: 17/20, delivered on 12/06/2020). Written informed consent was obtained from each participating patient or from their family member in the case of severe conditions.

RESULTS

General Characteristics of the Study Population

A total of 108 patients with laboratory-confirmed COVID-19 were included in this study. The general characteristics of the population are shown in **Table 1**. The age of our patients ranged from 18 to 82 years with an average of 43.80 ± 15.75 years. Almost one third of the patients (27.77%) were over 55 years old and 32.4% were young patients under 35 years old. The distribution by sex was statistically similar. The mean BMI of the patients was 25.54 ± 4.63 years with extremes of 18.90 and 42.90 Kg/m^2. Almost half of the patients (42.59%) were either overweight (28.8%; 95% CI: 20.2–37.5) or obese (15.4%; 95% CI: 8.7–22.1). The majority of patients neither consumed tobacco, nor alcohol. Analysis of the comorbidity profile revealed that 28.7% had, at least, one comorbidity (95% CI: 20.4–37). Diabetes and arterial hypertension were the most common comorbidities.

Clinical Characteristics of the Patients

Analysis of **Table 2**, representing the distribution of patients on admission according to clinical signs, showed that 25% (95% CI: 15.7–33.3) had no clinical signs. Infection was symptomatic in three quarters of the study population (75%; 95% CI: 66.7–84.3).

Among the 81 symptomatic patients, we found that more than half (59.25%) had more than three clinical signs

TABLE 1 | Characteristics of COVID-19 patients.

Characteristics	*n* = 108 (%)	95% confidence interval (CI)
Age groups (years)		
[18–35]	35 (32.4)	24.1–40.7
[35–45]	17 (15.7)	9.3–23.1
[45–55]	26 (24.1)	15.7–32.4
[55–65]	20 (18.5)	11.1–25.9
≥65	10 (9.3)	3.7–14.8
Sex		
Male	54 (50)	40.7–59.3
Female	54 (50)	40.7–59.3
BMI categories (*n* = 104)		
Normal weight	58 (55.8)	46.2–66.3
Overweight	30 (28.8)	20.2–37.5
Obesity class 1	9 (8.7)	3.8–14.4
Obesity class 2	6 (5.8)	1.9–10.6
Obesity class 3	1 (1)	0–2.9
Tabaco consumption		
No	97 (89.8)	83.3–95.4
Yes	11 (10.2)	4.6–16.7
Alcohol consumption		
No	89 (82.4)	75–88.9
Yes	19 (17.6)	11.1–25
Diabetes		
No	95 (88)	81.5–93.5
Yes	13 (12)	6.5–18.5
Arterial hypertension		
No	92 (85.2)	77.8–91.7
Yes	16 (14.8)	8.3–22.2
Dyslipidemia		
No	103 (95.4)	91.7–99.1
Yes	5 (4.6)	0.9–8.3
Chronic respiratory disease		
No	101 (93.5)	88.9–98.1
Yes	7 (6.5)	1.9–11.1
Cardiovascular disease		
No	106 (98.1)	95.4–100
Yes	2 (1.9)	0–4.6

Values are expressed in count and percentage. BMI (body mass index): Underweight: BMI < 18.5 Kg/m^2; Normal weight: 18.5 ≤ BMI < 25 Kg/m^2; Overweight: 25 ≤ BMI < 30 Kg/m^2; Obesity class 1: 30 ≤ BMI < 35 Kg/m^2; Obesity class 2: 35 ≤ IMC < 40 Kg/m^2; Obesity class 3: IMC ≥ à 40 Kg/m^2.

TABLE 2 | Clinical symptoms of COVID-19 patients.

Clinical signs	*n* = 108 (%)	95% confidence interval (CI)
Fever and chills		
No	64 (59.3)	50–68. 5
Yes	44 (40.7)	31.5–50
Cough/dyspnea		
No	65 (60.2)	50.9–68.5
Yes	43 (39.8)	31.5–49.1
Myalgia		
No	77 (71.3)	63–79.6
Yes	31 (28.7)	20.4–37
Ageusia/anosmia		
No	86 (79.6)	72.2–87
Yes	22 (20.4)	13–27.8
Headache		
No	91 (84.3)	77.8–90.7
Yes	17 (15.7)	9.3–22.2
Asthenia/tiredness		
No	81 (75)	66.7–82.4
Yes	27 (25)	17.6–33.3
Pharyngitis		
No	95 (88)	81.5–94.4
Yes	13 (12)	5.6–18.5
Digestive disorders		
No	93 (86.1)	79.6–91.7
Yes	15 (13.9)	8.3–20.4
Fever or chills		
No	104 (96.3)	92.6–99.1
Yes	4 (3.7)	0.9–7.4
Polypnoea		
No	104 (96.3)	92.6–99.1
Yes	4 (3.7)	0.9–7.4
Oxygen desaturation		
No	97 (89.8)	84.3–94.4
Yes	11 (10.2)	5.6–15.7
High blood pressure		
No	94 (87)	80.6–93.5
Yes	14 (13)	6.5–19.4

Values are expressed in count and (percentage).

with a preponderance of the following signs: fever, cough, myalgia, asthenia, ageusia and anosmia. The distribution of patients according clinical signs and comorbidity was statistically similar ($p = 0.177$). In fact, the number of symptomatic patients was statistically higher as compared to asymptomatic patients both in the group with comorbidity (83.9 vs. 16.9%) and in the group without comorbidity (71.4 vs. 28.6%).

A minority of the patients suffered from cardiovascular disorders other than high blood pressure or chronic respiratory conditions, while nephropathy was almost absent in all of the patients.

Distribution of Covid-19 Patients by Age Groups According to General Characteristics and Clinical Symptoms

The distribution of Covid-19 patients according to their general parameters, comorbidities and clinical signs in relation to age groups, is described in **Table 3**.

Overall, the statistical analysis revealed a statistically significant difference between the age groups concerning the following variables: Alcohol ($p = 0.041$), diabetes ($p < 0.001$), dyslipidemia ($p = 0.017$), oxygen desaturation ($p = 0.011$) and high blood pressure ($p = 0.003$).

The distribution of patients for these main parameters was marked by high numbers, particularly for the group of participants aged between 55 and 65 years and for those aged ≥65 years.

TABLE 3 | Distribution of Covid-19 patients by age groups according to general characteristics and clinical symptoms.

Characteristics	Age groups (years) $N = 108$					P
	[18–35[	[35–45[	[45–55[	[55–65[	≥65	
Sex						0.686*
Male	19 (35.2)	9 (16.7)	12 (22.2)	11 (20.4)	3 (5.6)	
Female	16 (29.6)	8 (14.8)	14 (25.9)	9 (16.7)	7 (13)	
BMI categories ($n = 104$)						0.540**
Normal weight	23 (39.7)	9 (15.5)	13 (22.4)	11 (19)	2 (3.4)	
Overweight	8 (26.7)	6 (20)	6 (20)	5 (16.7)	5 (16.7)	
Obesity	4 (25)	2 (12.5)	4 (25)	4 (25)	2 (12.5)	
Tabaco consumption (yes)	3 (27.3)	5 (45.5)	2 (18.2)	1 (9.1)	0 (0)	0.127**
Alcohol consumption (yes)	6 (31.6)	3 (15.8)	2 (10.5)	8 (42.1)	0 (0)	0.041**
Diabetes (yes)	0 (0)	1 (7.7)	2 (15.4)	4 (30.8)	6 (46.6)	**<0.001****
Arterial hypertension (yes)	0 (0)	0 (0)	3 (18.8)	4 (43.8)	6 (37.5)	**<0.001****
Dyslipidemia (yes)	0 (0)	1 (20)	0 (0)	2 (40)	2 (40)	**0.017****
Chronic respiratory disease (yes)	4 (57.1)	2 (28.6)	0 (0)	1 (14.3)	0 (0)	0.305**
Cardiovascular disease (yes)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	0.094**
Fever and chills (yes)	11 (25.0)	8 (18.2)	10 (22.7)	11 (25.0)	4 (9.1)	0.510**
Cough/dyspnea (yes)	11 (25.6)	8 (18.6)	8 (18.6)	9 (20.9)	7 (16.3)	0.184**
Myalgia (yes)	9 (29.0)	3 (9.7)	7 (22.6)	7 (22.6)	5 (16.1)	0.443**
Ageusia/anosmia (yes)	10 (45.5)	1 (4.5)	5 (22.7)	4 (18.2)	2 (9.1)	0.455**
Headache (yes)	3 (17.6)	2 (11.8)	4 (23.5)	6 (35.3)	2 (11.8)	0.309**
Asthenia/tiredness (yes)	9 (33.3)	3 (11.1)	3 (11.1)	7 (25.9)	5 (18.5)	0.117**
Pharyngitis (yes)	4 (30.8)	2 (15.4)	2 (15.4)	4 (30.8)	1 (7.7)	0.800**
Digestive disorders (yes)	8 (53.3)	1 (6.7)	4 (26.7)	2 (13.3)	0 (0)	0.364**
Fever or chills (yes)	0 (0)	1 (25.0)	1 (25.0)	2 (50.0)	0 (0)	0.261**
Polypnoea (yes)	0 (0)	1 (25.0)	0 (0)	2 (50.0)	1 (25.0)	0.082**
Oxygen desaturation	1 (9.1)	1 (9.1)	1 (9.1)	5 (45.5)	3 (27.3)	**0.011****
High blood pressure	1 (7.1)	1 (7.1)	2 (14.3)	6 (42.9)	4 (28.6)	**0.003****

*Values are expressed as number and percent. * Pearson chi-square test. **: exact test of Fisher. A value of $p < 0.05$ is considered significant.*

Biological Characteristics of the Study Population

Table 4 summarizes the main laboratory parameters of patients on admission. Analysis of the results showed hyperglycemia in 25.2% (95% CI: 17.8–34.6). WBC count were low (<4050/mm^3) in 12% of cases (95% CI: 6.5–18.5), normal (4050–11000/mm^3) in 81.5% of cases (95% CI: 74.1–88) and high (>11000/mm^3) in only 6.5% (95% CI: 1.9–12) of cases. The lymphocyte count was below 1241/mm^3 in 24.1% (95% CI: 16.7–32.4), normal in 75% (95% CI: 66.7–82.4) and above 3919/mm^3 in only 0.9% (95% CI: 0–2.87) of cases. The prevalence of anemia was 11.1% (95% CI: 2.8–13.1) in men and 14.8% in women (95% CI: 5.6–25.9). Exploration of hemostasis parameters showed a disturbance of PT whose levels exceeded 100% in 14.6% (95% CI: 7.8–22.3), as well as platelets whose number was less than 161 × 10^3/mm3 in 14.8% (95% CI: 8.3–21.3) of cases. Our results also revealed an increase in inflammation markers, in particular CRP, which exceeded 5 mg/l, in 36.1% of cases (95% CI: 26.9–45.4), ferritin which was above 400 ng/ml in 13.9% of cases (95% CI: 7.4–20.4) and D-dimers whose increase (>500 ng/ml) was noticed in 13.2% (95% CI: 5.9–22.1) of patients. A disturbance of biological markers of hepatic function was also marked by an increase in aspartate aminotransferase (AST > 40 UI/l), alanine aminotransferase (ALT > 41 UI/l) and gamma-glutamyl transpeptidase (GGT > 60 UI/l) in 15.7% (95% CI: 9.3–23.1), 19.4% (95% CI: 12–26.9) and in 18.9% (95% CI: 11.3–26.4) of patients, respectively. In addition, our results reported a slight alteration in blood troponin and creatinine phosphokinase (CPK) which were, respectively, higher than 14 ng/l in 7.5% (95% CI: 2.8–13.1) and 190 IU/l in 7.8% (95% CI: 2.2–13.3). Furthermore, our results revealed an increase in lactate dehydrogenase (LDH) and procalcitonin (PCT) which exceeded, respectively, 225 UI/l in 35.2% (95% CI: 26.9–44.4) and 0.5 mg/ml in 2.8% (95% CI: 0–6.5) of patients.

Comparison of COVID-19 Patients With Alterations in Oro-Naso-Sensory Parameters

In order to explore the demographic and anthropometric profile in the group with olfactory and/or taste dysfunction, we compared these parameters (**Table 5**). The comparison of the biological parameters made it possible to highlight a statistically significant difference which concerned only the number of lymphocytes, the levels of troponin and D-dimer in the blood. Indeed, we observed in the group with taste and or olfactory dysfunction compared to the group without any alteration in this function, a significantly high increase in the percentage of patients with lymphopenia (29.1% vs. 4.5%; $p = 0.019$), D-dimer

TABLE 4 | Biological characteristics of COVID-19 patients.

Biological parameters	Number of patient *n*	Result
Hb (g/dl)[a]	108	14.02 ± 1.44
Hb < 13.4 g/dl[b] (men)		6 (11.1)
Hb < 13.5 g/dl[b] (women)		8 (14.8)
Platelet count ($\times 10^3$/mm^3)[a]	108	235.34 ± 77.15
Platelet count (<161 × 103/mm^3)[b]		16 (14.8)
WBC count/ (mm^3)[c]	108	6390 [4895–7830]
WBC < 4050/ (mm^3)[b]		13 (12)
Lymphocyte count (/mm^3)[a]	108	1787.91 ± 762.67
Lymphocyte < 1241/ (mm^3)[b]		26 (24.1)
Blood glucose (g/l)[c]	107	1.10 [0.94–1.26]
Blood glucose > 1,26 (g/l)[b]		27 (25.2)
CRP (mg/l)[c]	108	2.25 [0.96–10.17]
CRP > 5 (mg/l)[b]		39 (36.1)
Ferritin (ng/ml)[c]	108	136.70 [52.75–253.40]
Férritin > 400 (ng/ml)[b]		15 (13.9)
CPK (IU/L)[c]	90	101 [64–148]
CPK > 190 (IU/l)[b]		7 (7.8)
LDH (IU/L)[c]	108	202.50 [170.5–263.25]
LDH > 225 (UI/l)[b]		38 (35.2)
Troponin (ng/l)[c]	107	4.30 [3.7–6.4]
Troponin > 14 (ng/l)[b]		8 (7.5)
D-dimer (ng/ml)[c]	67	203.50 [117–389.75]
D-dimer > 500 (ng/ml)[b]		9 (13.2)
PCT (ng/ml)[c]	107	0.05 [0.05–0.05]
PCT > 0.5 (mg/ml)[b]		3 (2.8)
AST (IU/L)[c]	108	23.50 [19.25–31.75]
AST > 40 (UI/l)[b]		17 (15,7)
ALT (IU/L)[c]	108	22.50 [15–38.75]
AST > 41 (UI/l)[b]		21 (19.4)
GGT (IU/L)[c]	107	21 [15–49.25]
GGT > 60 (UI/l)[b]		20 (18.9)
PT (%)[a]	106	91.49 ± 12.11
PT > 100 (%)[b]		15 (14.6)

Values are expressed as mean ± standard deviation (a), as number and percent (b) or median and quartile (c).
Hb, hemoglobin; WBC, White blood cell count; CRP, protein C reactive; CPK, creatinine phosphokinase; LDH, lactate dehydrogenases; PCT, procalcitonin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltranspeptidase; PT, Prothrombin time.
Reference intervals for normal subject: Hb: 13.4–17 g/dl (men), 11.5–15.5 g/dl (women); Platelet count: 161–398 × 10^3/mm^3; WBC: 4050–11000/mm^3; Lymphocyte count: 1241–3919/mm^3; Blood glucose: 0.74–1.26 g/l; CRP: <5 mg/l; Ferritin: 30–400 ng/ml; CPK: <190 UI/l; LDH: <225 UI/l; Troponin: <14 ng/l; D-dimer: <500 ng/ml PCT: <0,5 ng/ml; AST: <40 UI/l; ALT: <401 UI/l; GGT: <60 UI/l; PT: 70–130%

TABLE 5 | Olfactory and gustatory dysfunctions in COVID-19 patients.

Characteristics	olfactory or gustatory dysfunction		*p*
	No *n* = 86 (%)	**Yes *n* = 22 (%)**	
Age (years)a	44.34 ± 15.62	41.68 ± 16.44	0.483$^{\alpha}$
Age group^{s}			0.455$^{\gamma}$
[18–35]	25 (71.4)	10 (28.6)	
[35–45]	16 (94.1)	1 (5.9)	
[45–55]	21 (80.8)	5 (19.2)	
[55–65]	16 (80.0)	4 (20)	
≥65	8 (80)	2 (20)	
BMI (Kg/m^2)[a]	25.59 ± 4.78	25.26 ± 4.20	0.771$^{\alpha}$
BMI *categories* ($n = 104$)[b]			0.942$^{\gamma}$
Normal weight	47 (81)	11 (19)	
Overweight	23 (76.7)	7 (23.3)	
Obesity	13 (81.3)	3 (18.8)	
Sex			0.633$^{\mu}$
Male	44 (81.5)	10 (18.5)	
Female	42 (77.8)	12 (22.2)	
Hb (g/dl)[a]	13.91 ± 1.42	14.43 ± 1.49	0.135$^{\alpha}$
WBC count (/mm^3)[c]	6520 [4905–8055]	5820 [4307.5–7395]	0.282$^{\beta}$
Platelet count (/mm^3)[a]	238.37 ± 76	223.5 ± 82.24	0.422$^{\alpha}$
Blood glucose (g/l)[c]	1.09 [0.94–1.24]	1.1 [0.93–1.30]	0.761$^{\beta}$
C-reactive protein (mg/l)	2.5 [0.98–14.57]	1.79 [0.90–5.92]	0.387$^{\beta}$
Ferritin (ng/ml)[c]	135.7 [51.25–259.27]	140.6 [57.52–226.1]	0.749$^{\beta}$
CPK (IU/L)[c]	98 [62.50–147.50]	108 [70.50–162]	0.371$^{\beta}$
LDH (IU/L)[c]	204 [162.75–265.75]	190.5 [178.50-.]	0.900$^{\beta}$
Troponin (>14 ng/l)[b]	4 (4.7)	4 (18.2)	0.032$^{\mu}$
D-dimer (>500 ng/ml)[b]	7 (13.2)	9 (40.9)	0.008$^{\mu}$
AST (IU/L)[c]	23 [19.75–34]	24 [17.75–28.50]	0.921$^{\beta}$
ALT (IU/L)[c]	22.50 [15–39.25]	22 [16.75–37.25]	0.593$^{\beta}$
GGT (IU/L)[c]	21 [15–54.50]	21 [15–38]	0.583$^{\beta}$
PT (%)[a]	90.69 ± 12.38	94.54 ± 10.74	0.185$^{\alpha}$

Values are expressed as mean ± standard deviation (a), as number and percent (b) or median and quartile (c). α: Student's t test; ß: Mann-Whitney test; γ: exact test of Fisher; μ: Pearson chi-square test. A value of $p < 0.05$ is considered significant. Hb, hemoglobin; WBC, White blood cell count; CRP, protein C reactive; CPK, creatinine phosphokinase; LDH, lactate dehydrogenases; PCT, procalcitonin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltranspeptidase; PT, Prothrombin time.
Reference intervals for normal subject: Hb: 13.4–17 g/dl (men), 11.5–15.5 g/dl (women); Platelet count: 161–398 × 10^3/mm^3; WBC: 4050–11000/mm^3; Lymphocyte count: 1241–3919/mm^3; Blood glucose: 0.74–1.26 g/l; CRP: <5 mg/l; Ferritin: 30–400 ng/ml; CPK: <190 UI/l; LDH: <225 UI/l; Troponin: <14 ng/l; D-dimer: <500 ng/ml; AST: <40 UI/l; ALT: <401 UI/l; GGT: <60 UI/l; PT: 70–130%

>500 ng/ml (77.8 vs. 22.2% $p = 0.002$) and troponinemia >14 ng/l (18.2% vs. 4.1%; $p = 0.032$).

DISCUSSION

Our study aims to describe the demographic, anthropometric, clinical and biological characteristics of Moroccan COVID-19 patients. It presents a detailed analysis which complements previous Moroccan researches by analyzing different characteristics cited and possible correlations. In particular the differences of each parameter according to the olfactory or taste dysfunction were examined. In general, the results of our study show some points of similarity and others of differences with the previous studies for the different parameters. However, the present research work reports original results in relation to the symptoms like anosmia or ageusia wherein we did not observe any difference according to demographic and anthropometric characteristics. A possibility of a significant difference was noticed, however, for certain biological parameters, particularly the levels of lymphocytes, of the D-dimer and of the troponin.

We recruited 108 adult patients with a mean age of 43.80 ± 15.75 years and a sex ratio of 1:1. There was no difference in the proportion of men and women, which was inconsistent with the results of a study that have been conducted by Guan et al. (2020) who observed that men were more likely to be infected than women. The same result has been demonstrated by a Danish team (Kragholm et al., 2020).

In terms of the analysis of BMI values, we observed that almost half of the patients (44.3%) were overweight or obese (28.8 and 15.4% respectively). A similar obesity prevalence (48.3%) among COVID patients has been published by Finer et al. (2020), while, other researchers noted a high prevalence of obesity among patients with SARS-CoV-2 infection (Arthur et al., 2020; Peng et al., 2020). Furthermore, our results suggest that overweight and obesity could be risk factors for severe infection with COVID-19 in line with emerging data published in other clinical studies (Hamer et al., 2020; Sattar et al., 2020). Indeed, potential mechanisms have been linked to immune hyperresponsiveness, altered metabolic responses and pulmonary dysfunction, including a reduction in forced expiratory volume and a forced biological capacity, to the problem of overweight or obesity (Khan et al., 2020; Sattar et al., 2020). Also, lipid peroxidation is a key factor giving rise to reactive lipid aldehydes which will affect the prognosis of patients infected with SARS-CoV-2 (Demetrios et al., 2020). Physiologically, it has been shown that angiotensin converting enzyme 2 (ACE2) is the assumed receptor for the entry of SARS-CoV-2 in host cells. Given the expression level of this receptor is very high in the tissues, the possibility of risk in obese patients infected by this virus increases greatly (Zhou et al., 2020c).

In relation to comorbidities, this survey pointed out that almost a third of the population had, at least, one comorbidity with a slight dominance for diabetes and hypertension compared to the other studied comorbidities (dyslipidemia, chronic respiratory disease and cardiovascular disease). Similar results were found in a cohort of 85 patients in Wuhan, China, with first-degree diabetes followed by high blood pressure in patients presenting 68% for comorbidities (Du et al., 2020).

For non-communicable diseases, lifestyle risk factors have been consistently associated with morbidity, mortality, and loss of disease-free life years (Colpani et al., 2018; Nyberg et al., 2020; Schlesinger et al., 2020). For example, physical inactivity and smoking appear to be independently associated with a higher risk of community-acquired pneumonia and pneumonia mortality (Baik et al., 2000; Wang et al., 2014; Paulsen et al., 2017). However, the evidence from alcohol consumption and diet on the risk of respiratory infection is less clear (Paulsen et al., 2017; Hamer et al., 2019). Fortunately, most of the participants in this study were non-smokers (about 89.8%) and were not alcohol dependent (82.4%).

On the other hand, data on the characteristics of clinical complications revealed that a quarter of patients did not present any symptoms. In the symptomatic population, the predominant clinical signs were fever (40.7%), cough (39.8%), myalgia (28.7%), fatigue (25%), and anosmia and/or ageusia (20.4%). Nevertheless, high blood pressure, cardiovascular disorders and respiratory conditions were present in a limited number of our patients and nephropathy was almost absent. Such results have been reported by numerous researches studying COVID-19 patients (Wang et al., 2020; Yu et al., 2020; Zhang et al., 2020b). However, other studies observed an association between the SARS-CoV-2 infection and the elevated risk of developing diseases such as chronic kidney disease, heart disorders, diabetes, etc. (Arentz et al., 2020; Gorbalenya et al., 2020).

Regarding biological parameters, our results showed that hyperleukocytosis and lymphopenia, which represent a key indicator of infection, were noticed in some patients. In this context, numerous studies have reported that more than 80% of infected patients with SARS-CoV-2 presented these symptoms particularly lymphopenia (Mostafa et al., 2020; Yang et al., 2020c). Indeed, many researchers have revealed that in patients who died from lymphopenia, a trace of severe SARS-CoV-2 infection was confirmed. This can be explained by the death of endothelial cells due to endothelial dysfunction in certain chronic diseases which then causes excessive leakage of WBC and a disruption of the blood tissue barrier that may reflect the lymphocytes decrease found in patients with severe COVID infection (Bermejo-Martin et al., 2020). More studies have also reported that a significant increase in WBC presents a clinical worsening sign that has been shown to be significantly elevated in dead subjects (Henry et al., 2020). However, another study has found normal WBC values with lymphocyte decrease in diagnosed COVID-19 patients (Golnaz et al., 2020).

Concerning inflammatory markers, our results also detected a particular increase in CRP in 36.1% of patients with a general median value of 2.25 mg/l. Such an increase might be due to viral inflammation (Sproston and Ashworth, 2018). In our study, CRP reflected the COVID-19 pathogenesis presenting an immune response to this viral infection (Mostafa et al., 2020). In clinical laboratories, the test of this marker is currently widely used for the assessment of SARS-CoV-2 infection (Zhang et al., 2020b). For D-dimer, ferritin and LDH, as other parameters of inflammation, we also noted an increase in 13.2, 13.9, and 35.2% of cases, respectively. This finding is in line with results of previous researches which showed that blood levels of D-dimers, presenting also a sign of coagulation, were higher in severe SARS-CoV-2 infected cases (Wang et al., 2020; Yang et al., 2020b), while a study by Sun Ziyong of Huazhong University of Science and Technology in Wuhan, China, has shown that D-dimer is linked to a poor prognosis for COVID-19 patients (Tang et al., 2020b). Similarly, elevated ferritin levels could be interpreted as a sign of infection with SARS-CoV-2. In agreement with this observation, Zhou and his collaborators have also noted high ferritin levels in 200 adult patients (Zhou et al., 2020b). Indeed (Wenzhong and Hualan, 2020), based on an *in silico* analysis (not yet validated by peers) of the SARS-CoV-2 genome, reported the sequences encoding non-structural proteins that attack hemoglobin, in particular one of the beta chains, from which they would extract the iron atom, leading to ferritin increase in blood of COVID-19 patients.

Besides D-dimer, prothrombin and platelets are other important coagulation and thrombotic indicators commonly used in clinical laboratories for the early diagnosis of infection. Several reports have pointed out that high level of prothrombin is generally linked with the severity of the infection, of which hypercoagulation is the main result of this increase (Ling et al.,

2020; Zhou et al., 2020a). Interestingly, the number of platelets has been shown to be negatively correlated with the risk of mortality in SARS-CoV-2 infected patients (Tang et al., 2020a). However, the precise mechanism by which the SARS-CoV-2 virus acts on platelet function remains unclear (Manne et al., 2020).

Additionally, the present study demonstrated a disruption of biological markers of liver function with an increase in AST, ALT and GGT transaminases, in accordance with previous studies that have shown an increase in transaminases in 25 to 35% of COVID-19 patients (Fan et al., 2020; Xu et al., 2020a; Zhang et al., 2020a; Zhou et al., 2020a).

As for the comparison of patients with and without anosmia and/or ageusia according to their demographic and anthropometric, we found no statistically significant difference between the two groups. Nevertheless, the comparison of the biological parameters revealed a significant difference between two groups with remarkable lymphopenia, a high level of D-dimer and troponemia in patients with anosmia and/or ageusia. This is in the opposite direction with the study of Hornuss et al. (2020) who reported similar clinical and laboratory test outcomes in patients with and without anosmia or hyposmia. Likewise, another study COVID-19 patients showed no difference in lymphocyte and D-dimer levels between those with peripheral nervous system disorders with loss of taste and smell as the main symptom and those without these disorders and, therefore, without anosmia or ageusia (Ling et al., 2020). These noticeable alterations in biological parameters including inflammatory (D-dimer) and immune (lymphocytes) indicators in subjects suffering from anosmia and/or ageusia can be attributed to the severity of the clinical symptoms which strongly activate the immune system, leading to a severe inflammatory (Trotier et al., 2007; Khan et al., 2020; Ling et al., 2020).

There are several limitations, linked to the current study. First, the processed data relate to a single hospital center, which does not allow conclusions to be drawn on all Moroccan patients. Secondly, with regard to the symptom of dysfunction of the senses, details on the type (olfactory or gustatory or both) and on the specificity of the total or partial loss have not been made. Thirdly, no specific test on the loss of taste and smell was performed, and these observations are derived from self-reported questionnaire. Fourth, although PCR tests can usually determine whether a person is currently infected with the Sars-Cov-2 virus, they are still not 100% accurate. Indeed, false negatives can occur with a frequency of around 30% because, within 2 weeks of exposure. Thus, this test does not determine whether people who have been exposed to SARS-CoV-2 will develop Covid-19 or not (Longokolo et al., 2020) and it is not recommended by the WHO for clinical use (Haute Autorité de Santé (HAS), 2020).

CONCLUSION

This study is the first of its kind to be conducted among Moroccan patients with SARS-CoV-2 infection. It provides important information on the demographic, anthropometric, clinical and biological characteristics of these patients. The majority of cases present the common coronavirus symptoms with remarkable disturbances in inflammatory and other biological markers as an immune response to defend against the viral infection. Thus, this is an essential work that presents the part of the Moroccan Health Department's efforts to improve the quality of the diagnosis of COVID-19 based on the identification of all the disease symptoms. It also presents an important baseline study for future studies focusing on the possible correlations between the SARS-CoV-2 epidemic and its various symptoms, especially the olfactory and gustatory dysfunctions, considered to be as an important sign for the diagnosis of patients, especially for asymptomatic cases.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation after terminating its exploitation for future publications.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee and Biomedical Research (CEBR) of the College of Medicine and Pharmacy of Mohammed V University (Rabat, Morocco) (Approval umber: 17/20, delivered on 12/06/2020). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HB, HA, and NE designed, coordinated and drafted the manuscript for publication. HA had oversight responsibility over the project. JH, EE, and KE collected the data and provided laboratory analyzes. HB and HL wrote the manuscript. ABO translated the manuscript. ABA, FL, and MO analyzed the results and performed statistical analysis. HA and ABA reviewed the manuscript. AE performed the scientific review of the manuscript. NA-K contributed to the critical advice for editing the manuscript. All authors read and approved the final version of the manuscript.

FUNDING

The study was entirely carried out using the database provided by the VINCI clinic, which fully covered hospitalization costs of the 108 COVID patients included in this study.

ACKNOWLEDGMENTS

We would to kindly thank all participants in the present study for their patience, time and consent. We also thank to health workers, medical staff of the VINCI clinic, partners, local authorities and all persons who successfully aided in the realization of this work research.

REFERENCES

Arentz, M., Yim, E., Klaff, L., et al. (2020). Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington Statejama. *JAMA* 323, 1612–1614. doi: 10.1001/jama.4326

Arthur, S., Chetboun, M., Poissy, J., Raverdy, V., Noulette, J., Duhame, A., et al. (2020). High Prevalence of Obesity in Severe Acute RespiratorySyndrome Coronavirus-2 (SARS-CoV-2) Requiring Invasive Mechanical Ventilation. *Obesity* 28, 1195–1199. doi: 10.1002/oby.22831

Baig, A. M., Khaleeq, A., Ali, U., and Syeda, H. (2020). Evidence of the COVID-19 Virus Targeting the CNS: Tissue Distribution, Host-Virus Interaction, and Proposed Neurotropic Mechanisms. *ACS Chem. Neurosci.* 11, 995–998. doi: 10.1021/acschemneuro.0c00122

Baik, I., Curhan, G. C., Rimm, E. B., Bendich, A., Willett, W. C., and Fawzi, W. W. (2000). A prospective study of age and lifestyle factors in relation to community-acquired pneumonia in US men and women Arch. *Intern. Med.* 160, 3082–3308. doi: 10.1001/archinte.160.20.3082

Bermejo-Martin, J. F., Almansa, R., Menéndez, R., Mendez, R., Kelvin, D. J., and Torres, A. (2020). Lymphopenic community acquired pneumonia as signature of severe COVID-19 infection: Lymphopenia in severe COVID-19 infection. *J. Infect.* 80:e23–e24. doi: 10.1016/j.jinf.2020.02.029

Bourhanbour, A. D., and Bakkouri, J. E. (2020). Connaissances actuelles de l'immunopathologie du COVID-19. *Revue marocaine de santepublique* 7:10.

Colpani, V., Baena, C. P., Jaspers, L., vanDijk, G. M., and Farajzadegan, Z. (2018). Lifestyle factors, cardiovascular disease and all-cause mortality in middle-aged and elderly women: a systematic review and meta-analysis Eur. *J. Epidemiol.* 33, 831–845. doi: 10.1007/s10654-018-0374-z

Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K. W., et al. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance* 25:23. doi: 10.2807/1560-7917.ES.2020.25.3.2000045

Demetrios, P., Margină, D., Tsarouhas, K., Tekos, F., Stan, M., Nikitovic, D., et al. (2020). Obesity - a risk factor for increased COVID-19 prevalence, severity and lethality. *Mol. Med. Rep.* 22, 9–19. doi: 10.3892/mmr.2020.11127

Du, Y., Tu, L., Zhu, P., Mu, M., Wang, R., Yang, P., et al. (2020). Clinical Features of 85 Fatal Cases of COVID-19 from Wuhan: A Retrospective Observational Study. *Am. J. Respir. Crit. Care Med.* 201, 1372–1379. doi: 10.1164/rccm.202003-0543oc

Fan, Z., Chen, L., Li, J., Cheng, X., Jingmao Yang, Tian, C., et al. (2020). Clinical Features of COVID-19-Related Liver Damage. *Clin. Gastroenterol. Hepatol.* 18, 1561–1566. doi: 10.1016/j.cgh.2020.04.002

Finer, N., Garnett, S., and Bruun, J. (2020). COVID−19 and obesity. *Clin. Obesity* 10:e12365. doi: 10.1111/cob.12365

Giacomelli, A., Pezzati, L., Conti, F., et al. (2020). Self-reported olfactory and taste disorders in sars-cov-2 patients: A cross-sectional study. *Clin. Infect. Dis.* doi: 10.1093/cid/ciaa330

Golnaz, V., Mansourian, M., Karimi, R., Ghahdarijani, K. H., Rouhi, P., Shariati, M., et al. (2020). Inflammatory markers in Covid-19 Patients: a systematic review and meta-analysis. Preprint. doi: 10.1101/2020.04.29.20084863

Gorbalenya, A. E., Baker, S. C., Baric, R. S., et al. (2020). Severe acute respiratory syndrome-related coronavirus: the species and its viruses—a statement of the Coronavirus Study Group. *bioRxiv* 11, Preprint. doi: 10.1101/2020.02.07.937862 (preprint).

Guan, W., Ni, Z., Hu, Y., Liang, W., Ou, C., He, J., et al. (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* 382, 1708–1720. doi: 10.1056/NEJMoa2002032

Hamer, M., Kivimäki, M., Gale, C., and Batty, G. (2020). Lifestyle risk factors, inflammatory mechanisms, and COVID-19 hospitalization: A community-based cohort study of 387,109 adults in UK. *Brain Behav. Immun.* 87, 184–187. doi: 10.1016/j.bbi.2020.05.059

Hamer, M., O'Donovan, G., and Stamatakis, E. (2019). Lifestyle risk factors, obesity and infectious disease mortality in the general population: linkage study of 97,844 adults from England and Scotland. *Prev. Med.* 123, 65–70. doi: 10.1016/j.ypmed.2019.03.002

Haute Autorité de Santé (HAS) (2020). *Cahier des charges définissant les modalités d'évaluation des performances des tests sérologiques détectant les anticorps dirigés contre le SARS-CoV-2*. France: Haute Autorité de Santé.

Henry, B. M., de Oliveira, M. H. S., Benoit, S., Plebania, M., and Lippi, G. (2020). Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin. Chem. Lab. Med.* 58, 1021–1028. doi: 10.1515/cclm-2020-0369

Honce, R., and Schultz-Cherry, S. (2019). Impact of obesity on influenza a virus pathogenesis, immune response, and evolution. *Front. Immunol.* 10:1071. doi: 10.3389/fimmu.2019.01071

Hornuss, D., Lange, B., Schroter, N., Rieg, S., Kern, W. V., and Wagner, D. (2020). Anosmia in COVID-19 patients. *Clin. Microbiol. Infect.* 26, 1426–1427. doi: 10.1016/j.cmi.2020.05.017

Hussain, A., Kaler, J., Tabrez, E., Tabrez, S., and Tabrez, S. (2020). Novel COVID-19: A ComprehensiveReview of Transmission, Manifestation, and Pathogenesis. *Cureus* 12:e8184. doi: 10.7759/cureus.8184

Khan, A. S., Hichami, A., and Khan, N. A. (2020). Obesity and COVID-19: Oro-Naso-Sensory Perception. *J. Clin. Med.* 9:2158. doi: 10.3390/jcm9072158

Khan, N. A. (2006). Inflammation & Immunity: Implications in obesity and type-2 diabetes. Oilseeds Fats Crop. *Lipids* 13, 343–351. doi: 10.1051/ocl.2006.0047

Kragholm, K., Andersen, M., Gerds, T., Butt, J., Østergaard, L., Polcwiartek, C., et al. (2020). Association between male sex and outcomes of Coronavirus Disease 2019 (Covid-19) – a Danish nationwide, register-based study. *Clin. Infect. Dis.* 2020:ciaa924. doi: 10.1093/cid/ciaa924

Lechien, J. R., Chiesa-Estomba, C. M., De Siati Daniele, R., Horoi Mihaela, Le Bon, Serge, D., et al. (2020a). Olfactory and gustatory dysfonctions as a clinical presentation of mild to moderate forms of the coronavirus disease (COVID-19): a multicenter european study. *Eur. Arch. OtoRhinoLaryngol.* 277, 2251–2261. doi: 10.1007/s00405-020-05965-1

Lechien, J. R., Chiesa-Estomba, C. M., Place, S., Van Laethem, Y., Cabaraux, P., Mat, Q., et al. (2020b). Clinical and Epidemiological Characteristics of 1,420 European Patients with mild-to-moderate Coronavirus Disease 2019. *J. Intern. Med.* 288:335–344. doi: 10.1111/joim.13089

Lee, Y., Min, P., Lee, S., and Kim, S.-W. (2020). Prevalence and Duration of Acute Loss of Smell or Taste in COVID-19 Patients. *J. Kor. Med. Sci.* 35:e174. doi: 10.3346/jkms.2020.35.e174

Ling, M., Jin, H., Wang, M., Hu, Y., Chen, S., He, Q., et al. (2020). Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 inWuhan. China. *JAMA Neurol.* 77, 683–690. doi: 10.1001/jamaneurol.2020.1127

Longokolo, M.-M., Murhula, K. I., Kamwiziku, G., Ndona, M. M., Situakibanza, N. T. H., Bompeka, L. F., et al. (2020). Tests diagnostiques de l'infection à Coronavirus (COVID-19) : des atouts et des limites. *Ann. Afr. Méd.* 13, 3720–3726.

Lu, H., and Stratton, C. W. (2020). Outbreak of pneumonia of unknownetiology in Wuhan, China: The mystery and the miracle. *J. Med. Virol.* 92, 401–402. doi: 10.1002/jmv.25678

Manne, B. K., Denorme, F., Middleton, E., Portier, I., Rowley, J., Stubben, C., et al. (2020). Platelet Gene Expression and Function in COVID-19 Patients. *Blood* 136, 1317–1329. doi: 10.1182/blood.2020007214/1745894/blood

Mattos, J. L., Edwards, C., Schlosser, R. J., Hyer, M., Mace, J. C., Smith, T. L., et al. (2019). A brief version of the questionnaire of olfactory disorders in patients with chronic rhinosinusitis. *Int. Forum Allergy Rhinol.* 9, 1144–1150. doi: 10.1002/alr.22392

Mostafa, J., Bayani, M., Shokri, M., Sadeghi-Haddad-Zavareh, M., Babazadeh, A., Yeganeh, B., et al. (2020). Clinical and laboratory findings from patients with COVID-19 pneumonia in Babol North of Iran: a retrospective cohort study. *Intern. Med.* 0, 1–15.

Nyberg, S. T., Singh-Manoux, A., Pentti, J., Madsen, I. E. H., Sabia, S., Alfredsson, L., et al. (2020). Association of healthy lifestyle with years lived without major chronic diseases. *JAMA Int. Med.* 180, 760–768. doi: 10.1001/jamainternmed.2020.0618

Organisation mondiale de la santé (OMS) (2020). *Maladie à coronavirus 2019 (COVID-19) : questions-réponses*. Geneva: Organisation mondiale de la santé.

Paulsen, J., Askim, A., Mohus, R. M., et al. (2017). Associations of obesity and lifestyle with the risk and mortality of bloodstream infection in a general population: a 15-year follow-up of 64 027 individuals in

the HUNT Study. *Int. J. Epidemiol.* 46, 1573–1581. doi: 10.1093/ije/dyx091

Peng, Y. D., Meng, K., Guan, H. Q., et al. (2020). Clinical characteristics and outcomesof 112 cardiovascular disease patients infected by 2019-nCoV. *Zhonghua Xin Xue Guan Bing Za Zhi* 48:E004.

Sattar, N. I, McInnes, B., and McMurray, J. J. V. (2020). Obesity Is a Risk Factor for Severe COVID-19 Infection. Multiple Potential Mechanisms. *Circulation* 142, 4–6. doi: 10.1161/CIRCULATIONAHA.120.047659

Schlesinger, S., Neuenschwander, M., Ballon, A., Nöthlings, U., and Barbaresko, J. (2020). Adherence to healthy lifestyles and incidence of diabetes and mortality among individuals with diabetes: a systematic review and meta-analysis of prospective studies J. *Epidemiol. Community Health* 74, 481–487. doi: 10.1136/jech-2019-213415

Skrandies, W., and Zschieschang, R. (2015). Olfactory and gustatory functions and its relation to body weight. *Physiol. Behav.* 142, 1–4. doi: 10.1016/j.physbeh.2015.01.024

Sproston, N. R., and Ashworth, J. J. (2018). Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front. Immunol.* 9:754. doi: 10.3389/fimmu.2018.00754

Tang, N., Bai, H., Chen, X., Gong, J., Li, D., and Sun, Z. (2020a). Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J. Thromb. Haemost.* 18, 1094–1099. doi: 10.1111/jth.14817

Tang, N., Li, D., Wang, X., and Sun, Z. (2020b). Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J. Thromb. Haemost.* 18, 844–847. doi: 10.1111/jth.14768

Tomassini, B. I., Ozdener, M. H., Melis, M., Love-Gregory, L., Mitreva, M., Abumrad, N. A., et al. (2017). Variant in a common odorant-binding protein geneis associatedwith bitter sensitivity in people. *Behav. Brain Res.* 329, 200–204. doi: 10.1016/j.bbr.2017.05.015

Traore, B., Gladys, T. B., Samira, N., and Samira, H. (2020). COVID-19: Priseen charge therapeutique. *Revue marocaine de santepublique* 7:10. doi: 10.1016/s0992-5945(08)70274-5

Trotier, D., Bensimon, J. L., Herman, P., Tran, Ba, Huy, P., et al. (2007). Inflammatory obstruction of the olfactory clefts and olfactory loss in humans: a new syndrome? *Chem. Senses* 32, 285–292. doi: 10.1093/chemse/bjl057

Wang, D., Hu, B., Hu, C., et al. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 323, 1061–1069. doi: 10.1001/jama.1585 published online Feb 7.

Wang, H. E., Baddley, J., Griffin, R. L., et al. (2014). Physical inactivity and long-term rates of community-acquired sepsis. *Prev. Med.* 65, 58–64. doi: 10.1016/j.ypmed.2014.04.017

Wenzhong, L., and Hualan, L. (2020). *COVID-19: Attacks the 1-Beta Chain of Hemoglobin and Captures the Porphyrin to Inhibit Heme Metabolism*. Preprint.doi: 10.26434/chemrxiv.11938173.v5

World Health Organization (1995). *Physical Status: The Use and Interpretation of Anthropometry*. Geneva: WHO.

World Health Organization (2020a). *Coronavirus disease (COVID-19) pandemic*. Geneva:World Health Organization. Available Online at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019

World Health Organization (2020b). *March 2020*. Geneva:World Health Organization. Available Online at: https://www.who.int/health-topics/coronavirus.2020

World Health Organization (2020c):*mise à jour de la Stratégie covid-19*. Geneva:World Health Organization. Available Online at: https://www.who.int/docs/default-source/coronaviruse/strategy-update-french.pdf?sfvrsn=b1cfe48a_2

Xu, L., Liu, J., Lu, M., Yang, D., and Zheng, X. (2020a). Liver injury during highly pathogenic human coronavirus infections. *Liver Int.* 40, 998–1004. doi: 10.1111/liv.14435

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., et al. (2020b). Pathological findingsof COVID-19 associated with acute respiratorydistress syndrome. *Lancet Respir. Med.* 8, 420–422. doi: 10.1016/S2213-2600(20)30076-X

Yang, A.-P., Liu, J.-P., Tao, W.-Q., and Li, H.-M. (2020a). The diagnostic and predictiverole of NLR, d-NLR and PLR in COVID-19 patients. *Int. Immunopharmacol.* 84:106504. doi: 10.1016/j.intimp.106504

Yang, W., Cao, Q., Qin, L., Wang, X., Cheng, Z., Pan, A., et al. (2020b). Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19): A multicenter study in Wenzhou city, Zhejiang, China. *J. Infect.* 80, 388–393. doi: 10.1016/j.jinf.2020.02.016

Yang, X., Yu, Y., Xu, J., SHu, H., Liu, H., Wu, Y., et al. (2020c). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir. Med.* 8, 475–481. doi: 10.1016/s2213-2600(20)30079-5

Yu, P., Zhu, J., Zhang, Z., Han, Y., and Huang, L. (2020). A familial cluster of infection associated with the 2019 novel coronavirus indicating potential person-to-person transmission during the incubation period. *J. Infect. Dis.* 221, 1757–1761. doi: 10.1093/infdis/jiaa077

Zhang, J., Zhou, L., Yang, Y., Peng, W., Wang, W., and Chen, X. (2020b). Therapeutic and triagestrategies for 2019 novel coronavirus disease in fever clinics. *Lancet Respir. Med.* 8, 11–12e. doi: 10.1016/S2213-2600(20)30071-0

Zhang, J.-J., Dong, X., Cao, Y.-Y., Yuan, Y.-D., Yang, Y.-B., Yan, Y.-Q., et al. (2020a). Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Epidemiol. Genet.* 75, 1730–1741. doi: 10.1111/all.14238

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020a). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan. China: a retrospective cohort study. *Lancet* 395, 1054–1062. doi: 10.1016/s0140-6736(20)30566-3

Zhou, M., Zhang, X., and Qu, J. (2020b). Coronavirus disease 2019 (covid-19): A clinical update. *Front. Med.* 14, 126–135. doi: 10.1007/s11684-020-0767-8

Zhou, P., Yang, X., Wang, X., et al. (2020c). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273. doi: 10.1038/s41586-020-2012-7

Zoukal, S., Nani, S., and Hassoune, S. (2020). Place des tests antigéniques et sérologiquesdans le diagnostic de l'infection a COVID19: Revue systématique. *Revue Marocaine de Sante Publique* 7:10.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 10 December 2020
doi: 10.3389/fphys.2020.587013

Pathophysiology of SARS-CoV-2 in Lung of Diabetic Patients

Tales Lyra Oliveira[1,2†], Igor Santana Melo[3†], Léia Cardoso-Sousa[4], Igor Andrade Santos[5], Mohamad Bassim El Zoghbi[2], Caroline Gusson Shimoura[6], Renata Pereira Georjutti[6], Olagide Wagner Castro[3], Luiz Ricardo Goulart[7,8], Ana Carolina Gomes Jardim[5], Thúlio Marquez Cunha[9] and Robinson Sabino-Silva[4]**

[1] Heart Institute, Faculty of Medicine, University of São Paulo, São Paulo, Brazil, [2] Medical School, Municipal University of São Caetano do Sul, São Paulo, Brazil, [3] Institute of Biological Sciences and Health, Federal University of Alagoas, Alagoas, Brazil, [4] Department of Physiology, Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, Brazil, [5] Laboratory of Virology, Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, Brazil, [6] Department of Physiology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX, United States, [7] Institute of Biotechnology, Federal University of Uberlândia, Uberlândia, Brazil, [8] Department of Medical Microbiology and Immunology, University of California, Davis, Davis, CA, United States, [9] Department of Pulmonology, School of Medicine, Federal University of Uberlândia, Uberlândia, Brazil

OPEN ACCESS

Edited by:
Matthew A. Bailey, University of Edinburgh, United Kingdom

Reviewed by:
Gabriele Valli, Azienda Ospedaliera San Giovanni Addolorata, Italy
Hari H. Subramanian, Boston Scientific, United States

***Correspondence:**
Robinson Sabino-Silva
robinsonsabino@gmail.com
Thúlio Marquez Cunha
thcunha@yahoo.com.br

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Respiratory Physiology, a section of the journal Frontiers in Physiology

Received: *24 July 2020*
Accepted: *27 October 2020*
Published: *10 December 2020*

Citation:
Oliveira TL, Melo IS, Cardoso-Sousa L, Santos IA, El Zoghbi MB, Shimoura CG, Georjutti RP, Castro OW, Goulart LR, Jardim ACG, Cunha TM and Sabino-Silva R (2020) Pathophysiology of SARS-CoV-2 in Lung of Diabetic Patients. Front. Physiol. 11:587013. doi: 10.3389/fphys.2020.587013

Novel coronavirus disease (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Its impact on patients with comorbidities is clearly related to fatality cases, and diabetes has been linked to one of the most important causes of severity and mortality in SARS-CoV-2 infected patients. Substantial research progress has been made on COVID-19 therapeutics; however, effective treatments remain unsatisfactory. This unmet clinical need is robustly associated with the complexity of pathophysiological mechanisms described for COVID-19. Several key lung pathophysiological mechanisms promoted by SARS-CoV-2 have driven the response in normoglycemic and hyperglycemic subjects. There is sufficient evidence that glucose metabolism pathways in the lung are closely tied to bacterial proliferation, inflammation, oxidative stress, and pro-thrombotic responses, which lead to severe clinical outcomes. It is also likely that SARS-CoV-2 proliferation is affected by glucose metabolism of type I and type II cells. This review summarizes the current understanding of pathophysiology of SARS-CoV-2 in the lung of diabetic patients and highlights the changes in clinical outcomes of COVID-19 in normoglycemic and hyperglycemic conditions.

Keywords: betacoronavirus infection, angiotensin-converting enzyme, SGLT1, diabetes mellitus, pneumonia

PHYSIOLOGICAL REVIEWS SUMMARY

(1) The airway surface liquid (ASL) plays a pivotal role in lung defense. Diabetes is related with higher ASL glucose concentration, ASL volume accumulation in alveolar space, imbalance of reactive oxidative species (ROS), and inflammatory chemokine production.
(2) The COVID-19 infection promotes injuries in type I and type II pneumocytes and lung endothelial lesions, with subsequent additional secretion of protein-rich exudate in the alveolar space and intravascular coagulation in lung vessel, which leads to a reduction in surfactant and gas exchange.
(3) The association between diabetes and SARS-CoV-2 increases the glucose and protein concentration in ASL, leading to increase the risk of pneumonia.

(4) The prevalence and severity of hypoxemia and severe hyperinflammation is higher in COVID-19 diabetic patients.
(5) The harmful clinical outcomes and mortality rate of COVID-19 are higher in diabetic subjects.

BACKGROUND

COVID-19 Scenario

Currently, there are more than 38 million infected cases and more than 1,000,000 deaths confirmed worldwide due to the spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathological agent of coronavirus disease 2019 (COVID-19) (WHO, 2020). Transmission of SARS-CoV-2 occurs through respiratory droplets exhaled during coughing and sneezing by symptomatic and asymptomatic infected subjects (Cho et al., 2018). The SARS-CoV-2 infection occurs also through inhalation of oral droplets or by touching contaminated surfaces and then scrubbing nose, mouth, or eyes. Occasionally, the SARS-CoV-2 spread was documented by aerosols suspended in the air (Correia et al., 2020; Sabino-Silva et al., 2020). The SARS-CoV-2 incubation period ranges between 2 and 14 days (expected average around 5 days). The most frequent clinical outcomes include fever, cough, sore throat, headache, fatigue, myalgia, and breathlessness. Altogether, these initial symptoms are similar to other respiratory infections (Yang et al., 2020). The clinical features of COVID-19 ranges from asymptomatic state to death and also include self-limiting respiratory complications [acute respiratory distress syndrome (ARDS)] and severe progressive pneumonia (Cao et al., 2020; Chen et al., 2020; Huang et al., 2020).

Although the mortality rates of SARS-CoV-2 are debated, it is well established that elderly people (65 years and older) and subjects with other comorbidities such as cardiovascular diseases, hypertension, and diabetes mellitus (DM) are more susceptible to severe illness and subsequent mortality (Singh et al., 2020; Wang et al., 2020). Indeed, the prevalence of DM was the second most common comorbidity in several cohorts of COVID-19 patients (Stein, 2020; Stoian et al., 2020; Zhou F. et al., 2020). The DM prevalence is about 425 million worldwide, corresponding to 8.8% of adults between 20 and 79 years (Cho et al., 2018). It was suggested around 20% of a diabetic population without diagnostic in England and it is expected that this percentage is higher in lower-income countries (Clark et al., 2020). The association between DM and COVID-19 increases the risk of a more severe illness, ARDS, hospitalization, and death (Chan-Yeung and Xu, 2003). Multiple hypotheses have been proposed to support the association between DM and COVID-19 severity. Briefly, diabetic patients with inadequate metabolic control frequently present reduced anti-viral immune response, which is also associated with pathogen proliferation (Philips et al., 2003; Rothe et al., 2020). Moreover, DM is commonly accomplished by magnified reactive oxidative species (ROS) with no counter regulation by appropriate antioxidant defense, which can be further related with the exacerbated inflammatory response to SARS-CoV-2 and ARDS (Rothe et al., 2020).

Based on published evidence, we have focused on discussing the pathophysiological principles on infection and SARS-CoV-2 immune response of lung cells in normoglycemic and hyperglycemic conditions, and assessment findings to frame lung interactions between SARS-CoV-2 infection and DM, paving the way to better understand the unique characteristics of the SARS-CoV-2 infection in diabetic lungs. We also summarized current evidences of subcellular distribution of glucose transporters in lung and highlighted the effect of higher airway surface liquid (ASL) glucose concentration on SARS-CoV-2 proliferation, as well as its relationship with bacterial proliferation, inflammation, oxidative stress, and lung tissue injury.

LUNG PHYSIOLOGY

The distal lung alveolar epithelium is mainly composed of two pneumocytes. Type I pneumocytes coat around 92% of lung alveolar surface and their vital function is to promote O_2 and CO_2 exchange through the alveolar-capillary barrier (Stone et al., 1992; Mossel et al., 2008). Type II pneumocytes secrete surfactant into the alveolar space, which is a biofluid capable to reduce the surface tension at the air/liquid interface (Veldhuizen and Haagsman, 2000) and hence also contribute to keeping the alveoli open and facilitate gas exchange. As expected, type II pneumocytes injury frequently reduces the surfactant secretion to the ASL in alveolar space, which is accomplished by reduction in lung compliance and atelectasis (Mossel et al., 2008). The maintenance of an adequate level of ASL with optimal levels of surfactant and balanced oxidative/antioxidative condition are pivotal to perform adequate lung function (Mossel et al., 2008).

In physiological conditions, the pneumocytes and pericytes contribute to provide a compartment barrier and vascular integrity. This alveolar-capillary barrier restrains the physical interactions between pneumocytes and immune cells, which is paramount to avoid inflammation. Additionally, it prevents coagulation due to the physiological secretion of coagulation inhibitors, glycoproteins, and glycolipids promoting a protective coat with anti-coagulation activity. In physiological circumstances, the desquamated alveolar cells display immunomodulatory responses to induce cytokine production, leukocyte recruitment, and scavenger properties, specially related with MHC class II-mediated antigen processing, which reinforces the role in immune surveillance against viruses and bacteria in the lung (Teuwen et al., 2020).

Angiotensin-converting enzyme 2 (ACE2) is one of the key players of the renin angiotensin aldosterone system (Jia et al., 2009). ACE2 is a classical type 1 integral membrane glycoprotein expressed by lung epithelial cells (Fang et al., 2020; Pal and Bhansali, 2020). In more detail, ACE2 expression has been detected in type I and type II pneumocytes in humans and animal models (Hamming et al., 2004; van den Brand et al., 2008); however, the ACE2 expression in type II is higher than in type I pneumocytes (Hamming et al., 2004; van den Brand et al., 2008). ACE2 hydrolyzes angiotensin II into Ang (1-7), which plays important anti-inflammatory and antioxidant roles to protect lungs against ARDS (Tikellis and Thomas, 2012; Zou et al., 2014).

It is important to point out that ACE2 is also expressed in other lung immune cell as T and B lymphocytes, fibroblasts, natural killer (NK) cells, and monocytes (Cheng et al., 2020; Fu et al., 2020).

Glucose Fluxes and Subcellular Distribution of Glucose Transporters in Lung

The regulation of ASL composition plays a pivotal role in lung defense (Baker et al., 2006b). Due to a counterbalance of glucose efflux and influx in the alveolar epithelia, the glucose concentration in ASL is around 0.4 mM, which correspond about 12 times lower than the plasma and extracellular liquid (ECL) (Baker et al., 2007; Baker and Baines, 2018). The fenestrated capillaries of the lung warrant passive communication of glucose between the blood and ECL. Despite the presence of tight junctions in alveolar epithelial cells, glucose molecules are capable to move from the ECL to the ASL through a paracellular pathway (Saumon et al., 1996). Glucose can also access the ASL via transcellular pathway when the intracellular glucose concentration is higher than ASL (Pezzulo et al., 2011). Bearing in mind that glucose can be transported by two types of glucose transporters, the Na(+)/glucose cotransporters (SGLT) and the facilitative glucose transporters (GLUT) (Sabino-Silva et al., 2010), it established that glucose uptake is generated by GLUT2 in proximal airways and through the SGLT1 in distal alveolar cells, leading to lower glucose concentration in ASL (Garnett et al., 2012b; Oliveira et al., 2016).

The glucose transport by GLUT2 and GLUT10 appears to be related with glucose regulation in lung proximal airways (Kalsi et al., 2009; Pezzulo et al., 2011; Garnett et al., 2012a). On the other side, the SGLT1 protein has been detected in both type I and type II pneumocytes (Bodega et al., 2010; Oliveira et al., 2016). The SGLT1 cotransport 2 Na^+ ions, one molecule of glucose and 264 H_2O in luminal membrane of pneumocytes I, and this protein can transport glucose into the cytoplasm of pneumocytes against its concentration gradient (Sabino-Silva et al., 2010). In fact, we have previously demonstrated the functional role of SGLT1 in distal alveolar cells by instillation of an SGLT inhibitor, which promoted an increase of ASL glucose concentration in non-diabetic animals and reinforced the pivotal role of SGLT1 to maintain low ASL glucose concentration. The pharmacological blockage of SGLT1 was also suitable to increase the ASL volume (Oliveira et al., 2016). Furthermore, it is established that Aquaporins (AQPs) also promotes water reabsorption in alveolar cells, which is paramount to maintain an adequate volume of ASL in alveolar space (Schmidt et al., 2017; **Figure 1**).

PATHOPHYSIOLOGY OF DM IN LUNG

Regulation of ASL Glucose Concentration in DM

Glucose diffuses mainly from plasma to ASL through paracellular pathway due to the glucose concentration gradient. DM is characterized by classical pre-existing vascular dysfunction. In this context, moderate injury in this epithelial barrier can occur in non-controlled diabetic patients (Li et al., 2019; Teuwen et al., 2020). As described in a normoglycemic condition, the ASL glucose concentration is also correlated with glycemia in DM (Baker et al., 2006b; Oliveira et al., 2016). In a hyperglycemic scenario, the referred increase in glucose flux from plasma to ASL is not counterbalanced by a parallel adjustment by SGLT1-mediated re-uptake of glucose, which explain the higher ASL glucose concentration in distal lung (Oliveira et al., 2016). Bearing in mind the osmotic effect due to the high ASL glucose concentration, these changes could explain the higher total water content in lung during hyperglycemic condition, which can be related with higher volume of ASL, leading to reduced gas exchange rates of O_2 and CO_2 between alveolar space and lung capillaries. Frequently, the mild increase in ASL can be compensated by ventilatory response mechanisms (Oliveira et al., 2016). It is important to point out that the pathological reduction of O_2 and CO_2 exchange through the lumen of pneumocytes and blood occurs in poorly controlled diabetic patients mainly under bacterial, inflammatory, or oxidant disruption (Stone et al., 1992; Mossel et al., 2008).

Oxidative Stress and Inflammatory Outcomes of DM Related With ASL Glucose Regulation in Lung

Although ACE2 expression was described in type I and type II pneumocytes (Helenius and Aebi, 2001; Vrhovac et al., 2015), a distinguishing analysis on these cells was not shown in diabetic conditions. It revealed an increase in ACE2/ACE ratio in the lungs of long-term diabetics (Roca-Ho et al., 2017), indicating a shift to trigger inflammatory and ROS activities. The imbalance associated with higher ROS formation and reduced capability to detoxify the reactive intermediates promotes oxidative stress. It is currently accepted that hyperglycemia activates a metabolic signaling route, which culminates in higher levels of ROS formation (Volpe et al., 2018). Besides, several transcription factors related to ROS also trigger inflammation (Chatterjee, 2016). Additionally, inflammation leads immune cells to release cytokines to recruit additional immune cells to the oxidative stress region. Reflexively, higher levels of ROS delivery by immune cells also promotes tissue injury, triggering more inflammation (Chatterjee, 2016). We have also shown that oxidative stress dysregulation was parallelly associated with hyperglycemia and diabetic complications (Diniz Vilela et al., 2016) due to impairment of lipids and proteins in several tissues, such as lung (Baynes, 1991).

We also have previously demonstrated the increase of ASL glucose concentration promoted by SGLT1 inhibitors in the lungs of diabetic animals (Oliveira et al., 2016); however, the opposite effect on ASL glucose concentration was observed under the beta-adrenergic agonist application due to the SGLT1 translocation to plasma membrane of type I and type II pneumocytes. In this context, we also showed that SGLT1 inhibitors promote bronchial inflammation (interferon-γ and Interleucin-1β), reduction on antioxidant system, and atelectasis associated with significant decrease in survival rate in an ARDS promoted by cecum ligation and puncture

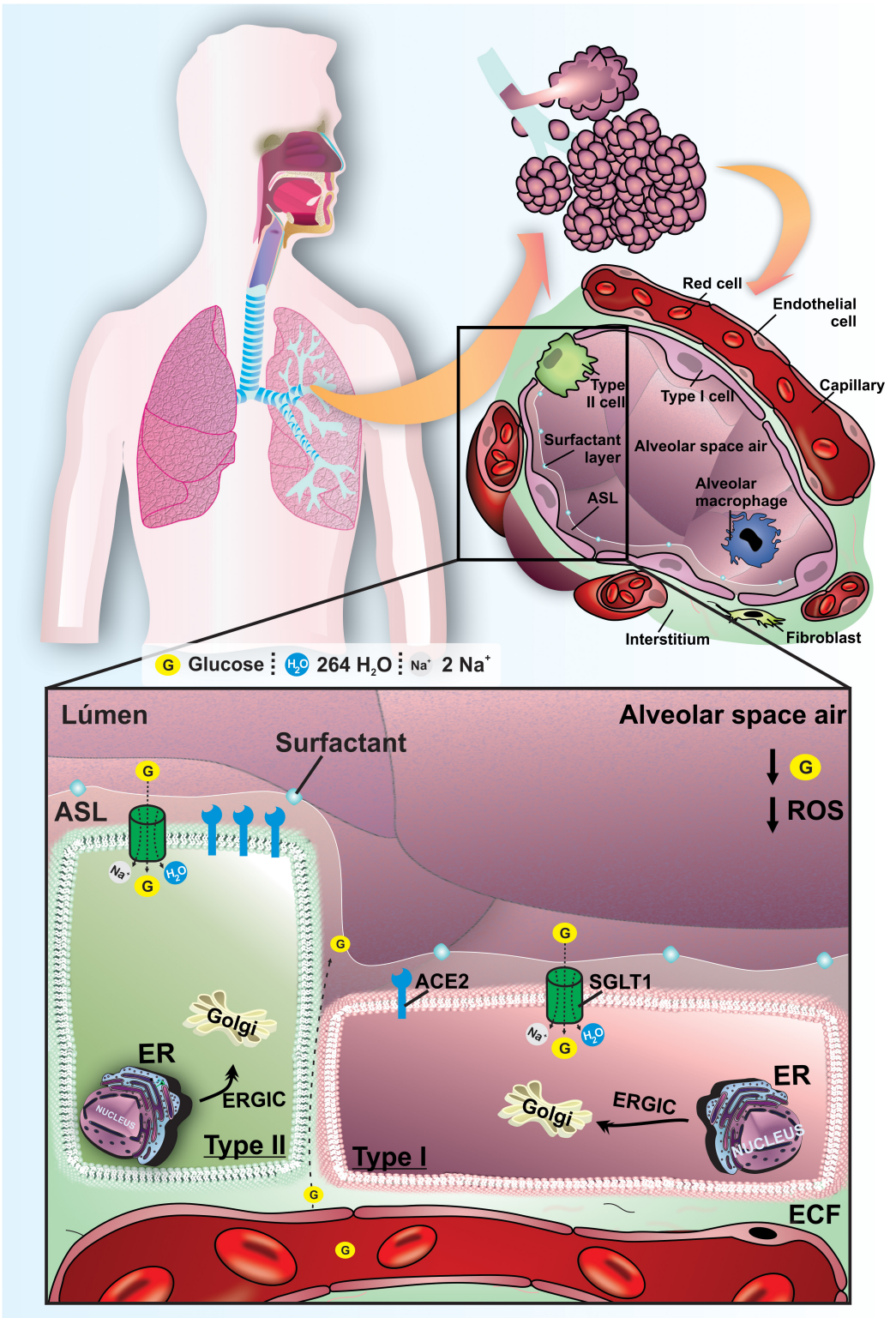


FIGURE 1 | Functional mechanisms to maintain lung homeostasis and to regulated ASL glucose concentration in ASL of healthy normoglycemic subjects. Schematic representation of the lungs of normoglycemic subjects. The low concentration of glucose and the reduced volume in the ASL are mainly maintained by SGLT1 in the apical membrane of type I and II pneumocytes. ACE is present in pneumocytes, with greater expression in type II pneumocytes. The oxidative profile is in balance with low ROS production. G, glucose; SGLT1, Type 1 Na^+/glucose/H_2O cotransporter; ECF, extracellular fluid; ER, endoplasmic reticulum; ERGIC, endoplasmic reticulum-Golgi intermediate compartment; ACE2, angiotensin-converting enzyme 2; ROS, reactive oxygen species. ECL, extracellular liquid.

(CLP)-induced sepsis animal model (Cardoso-Sousa et al., 2019). Otherwise, a specific β2-adrenergic agonist reduced bronchial inflammation (interleucin-1β), preserved higher levels of antioxidant system, and reduced pulmonary atelectasis associated with bronchodilation (Cardoso-Sousa et al., 2019). The SGLT1-triggered water transport is feasible directly together with classical Na^+ and glucose transport, and also indirectly as a powerful facilitator of passive water cotransport (Erokhova et al., 2016). Accordingly, we suggested that SGLT1 translocation to plasma membrane of type I and type II pneumocytes could decrease the ASL volume (Oliveira et al., 2016), facilitating the O_2 and CO_2 exchange, which indirectly could reduce lung inflammation. Altogether, changes in lung SGLT1 translocation were linked with ASL glucose concentration, ASL volume, bronchial inflammation, antioxidant system, and atelectasis in diabetic models.

Effect of DM on Lung Bacterial Proliferation Related With ASL Glucose Regulation

Despite consistent airway exposure to bacteria, ASL is usually sterile (Pezzulo et al., 2011). The proportion between bacterial growth and bacterial killing in ASL guide the outcome to infection or sterility. Several innate immune mechanisms are activated to remove bacteria as antimicrobials, cough, phagocytes, and mucociliary clearance (Pezzulo et al., 2011). It is well recognized that the reduced ASL glucose concentrations is paramount to airway defense against infection, which is capable of restricting bacterial growth by the reduction in nutrient availability (Baker and Baines, 2018) and reduced immune mechanism (Pezzulo et al., 2011). In fact, the hyperglycemia and higher ASL glucose concentration in diabetic patients has been strongly related with higher prevalence of respiratory complications (Pezzulo et al., 2011) and also predisposes to bacterial respiratory infection (Garnett et al., 2013; Oliveira et al., 2016). Multiple respiratory pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* are isolated more frequently from respiratory secretions of hyperglycemic patients, and it has been associated with increased glucose concentration in ASL (Philips et al., 2005; Baker et al., 2006a). Despite the direct effect of hyperglycemia on ASL glucose concentration, and consequently on bacterial proliferation (Baker et al., 2006b, 2007; Baker and Baines, 2018), we demonstrated the power of the SGLTs to modulate these effects in diabetic conditions (Oliveira et al., 2016; **Figure 2**). Indeed, we proved that the blockage of the SGLT1 transport in distal alveolar cells by instillation of phlorizin was also capable of promoting bacterial proliferation of MRSA and *P. aeruginosa* in both non-diabetic and diabetic condition (Oliveira et al., 2016), which support the relationship between high ASL glucose concentration and bacterial proliferation. Altogether, these *in vivo* studies appear to be in line with clinical outcomes in respiratory system of diabetic patients (Baker and Baines, 2018). The increased surveillance for an optimal glucose control may permit decreased numbers of hospitalizations and improve outcomes of diabetic patients (Shubrook et al., 2017).

Despite several clinical outcomes described in the respiratory system of diabetic subjects, the relationship of the currently most prominent endocrine disease and lung has been considered neglected (Khateeb et al., 2019). Dual SGLT1/SGLT2 inhibitors were considered in the DM treatment (Inagaki et al., 2013; Polidori et al., 2013; Powell et al., 2013); however, the definitive long-term adverse effects on respiratory tract especially in cases of associated bacterial and viral infections could be further studied. The component and volume regulations of ASL open perspectives to new treatments capable to reduce ASL glucose concentration associated with reduction of ASL volume. Additionally, it is important to address studies to evaluate the potential long-term effects of β-adrenoreceptor agonists in the lung due to the induced tolerance after prolonged administration (Raffay et al., 2014).

PATHOPHYSIOLOGY OF COVID-19 IN LUNG

The Replicative Cycle of SARS-CoV-2 in Alveolar Lung Cells

Severe acute respiratory syndrome coronavirus 2 is a positive single-stranded RNA (ssRNA [+]) virus, shielded by a nucleocapsid protein (N) shell and encompassed by a lipid bilayer conferring it a pleomorphic spherical shape. The envelope (E) and membrane (M) proteins and the spike (S) glycoproteins are anchored on its surface (Ding and Liang, 2020; Santos et al., 2020).

Briefly, the entry mechanism of SARS-CoV-2 into the host cells is similar to what occurs in the alveolar lung cells. The SARS-CoV-2 entry in alveolar cells includes receptor binding, proteolytic activation for membranes fusion, and viral internalization. The viral entry is promoted by interaction of S protein with the ACE2 in alveolar cells. The S protein is constituted by the S1 subunit (contain the receptor binding domain, RBD, which is responsible for interacting with host cell receptors) and the S2 subunit (triggers the fusion of viral and endosome membranes allowing viral entry into the host cells). The RBD/ACE2 interaction triggers the endocytosis of viral particle and endosome formation (Hoffmann et al., 2020a; Santos et al., 2020; Walls et al., 2020).

The host surface cell receptor ACE2 was recently recognized as a functional receptor of SARS-CoV-2 (Li et al., 2003). The ACE2 ectodomain possesses peptide motifs suitable to cleave several peptides. The activity of ACE2 can be managed by the attachment of ligand with ectodomain, receptor internalization, and transcription/translating interplay (Imai et al., 2005; Jia et al., 2009). In addition, since the entry of SARS-CoV-2 occurs connected with ACE2, it might decrease the functional ACE2 in the lung (Lukassen et al., 2020). Later, a cleavage of the S1/S2 subunits by host proteases, such as furin and transmembrane protease/serine (TMPRSS), exposes the S2 subunit enabling the fusion of SARS-CoV-2 and endosome membranes. These proteases are attached on the cellular surface, among them, the TMPRSS2 was

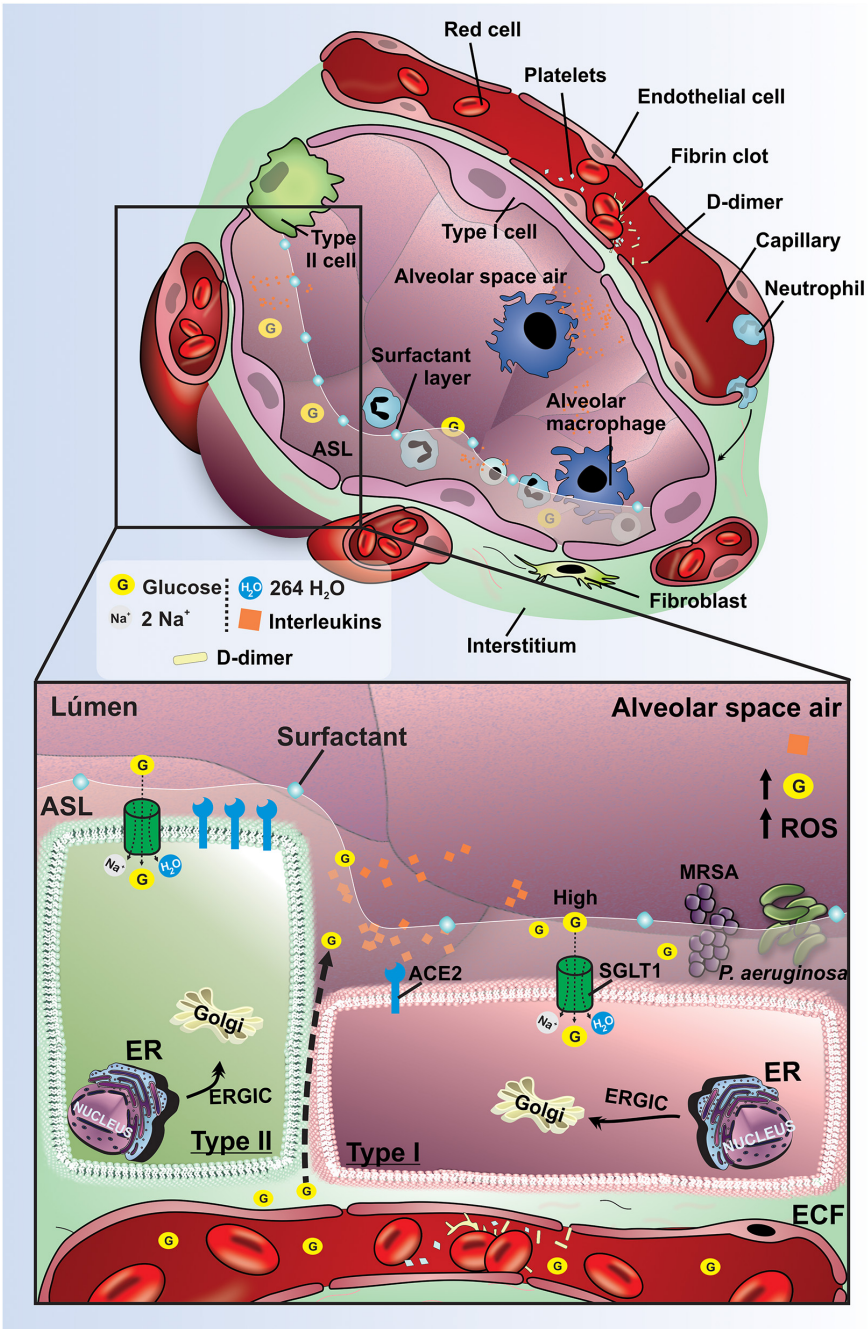


FIGURE 2 | Pathophysiological mechanisms to regulate lung function and ASL glucose concentration effects on diabetic hyperglycemic patients. Schematic representation of pathophysiological mechanisms related to DM in the lung. The high concentration of glucose and the increased volume in the ASL are maintained mainly by the low expression of SGLT1 in the apical membrane of type I and II pneumocytes in the lungs of diabetics. Hyperglycemia promotes increased paracellular glucose transport from blood to ASL. The evolution of DM promotes activation of the inflammatory cascade with the production of interleukins, increased ROS, and endothelial damage. These changes provide an increased risk for pneumonia due to the proliferation of bacteria *P. aeruginosa* and MRSA. G, glucose; SGLT1, Na^+/glucose/H_2O type 1 cotransporter; ECF, extracellular fluid; ER, endoplasmic reticulum; ERGIC, endoplasmic reticulum-Golgi intermediate compartment; ACE2, receptor angiotensin-converting enzyme 2; ROS, reactive oxygen species; *P. aeruginosa*, *pseudomonas aeruginosa*; MRSA, Methicillin-resistant *Staphylococcus aureus*; Orange rhombus: interleukins.

described as an important protease for SARS-CoV-2 entry in lung cells (Lukassen et al., 2020). To support the crucial role of molecules derived from glucose and carbohydrates in the attachment with receptors, the N-linked glycans adhered in S protein can also modulate the effect of activated proteases (Walls et al., 2016).

The N-terminal domain (NTD) on S1 subunit of coronaviruses is suitable to bind in carbohydrate moieties (sugar-binding galectin motifs and 9-O-acetylated neuraminic acid) (Peng et al., 2011), while the C-domain (CTD) is capable to connect to protein receptors as ACE2. The S1 domains are extensively associated with N-linked glycans, which are pivotal to virion attachment (Helenius and Aebi, 2001). Recently, it was suggested that carbohydrates derived from glucose molecules, as the 22 N-linked glycosylation sequons, and other oligosaccharides are attached into S protein to aid the interaction between SARS-CoV-2 and host cell receptors (Grant et al., 2020), suggesting a potential effect of a hyperglycemic milieu. A significant part of S protein is covered by glycans; however, the ACE2 binding domain in the S1 subunit is uncovered by glycans, which can be paramount to SARS-CoV-2 entry in host cells (Watanabe et al., 2020) and open perspectives to new therapeutic strategies.

After SARS-CoV-2 viral entry occurs, the fusion between the viral envelope with endosome membrane, resulting in the exposure of the nucleocapsid. Subsequently, the viral uncoating is triggered and the viral genome is released into the cytoplasm. The exposure of the RNA permits the viral replication, and genomic RNA is partially translated to produce non-structural proteins (nsps) from two open reading frames (ORFs), ORF1a and ORF1b. The ORF1a yield polyprotein 1a (pp1a) is subsequently cleaved into 11 nsps. The continued translation of ORF1b produces the polyprotein 1ab (pp1ab), which is cleaved into 15 nsps. Proteolytic cleavages are mediated by viral proteases nsp3 and nsp5 (Belouzard et al., 2009; Kim et al., 2020). Nsps will assemble to form a replicase-transcriptase complex (RTC), which is responsible for RNA synthesis, replication, and transcription of nine subgenomic RNAs (sgRNAs) (Fehr and Perlman, 2015). The viral genomic RNA is transcribed into negative-strand RNAs, which are intermediates of genome replication by serving as a template for the synthesis of positive-sense genomic RNAs and sgRNAs (Kim et al., 2020). The sgRNAs act as mRNAs for structural and accessory genes localized downstream of the replicase polyproteins (Sethna et al., 1991; Fehr and Perlman, 2015). SARS-CoV-2 is known to have 6 accessory proteins (3a, 6, 7a, 7b, 8, and 10) (Kim et al., 2020). The structural proteins S, E, and M are translated from short sgRNAs, inserted in the endoplasmic reticulum (ER), and directed to an intermediate compartment of ER with Golgi (ERGIC). Viral genomes are encapsulated by N protein and assembled with the structural proteins to form virus particles (Siu et al., 2008; Fehr and Perlman, 2015), where the M protein binds to E protein and later to the nucleocapsid. Finally, the S protein is incorporated into virions, completing the virion assembly, and then transported to the cell surface in vesicles, and released by exocytosis (Ye and Hogue, 2007; Kuo and Masters, 2013; Fehr and Perlman, 2015; Bojkova et al., 2020; Zhou Y. et al., 2020; **Figure 3**).

Clinical Course of COVID-19

It is important bear in mind the clinical characteristics of COVID-19 to understand specific phenotype factors and evaluate the immunomodulation response in normoglycemic and hyperglycemic conditions. In this context, a three phase clinical classification to COVID-19 was proposed: (1) viremia phase; (2) acute pulmonary phase; (3) severe hyperinflammation phase (Cao and Li, 2020; Siddiqi and Mehra, 2020).

(1) *Viremia phase*: The incubation period occurs in early stages of infection of SARS-CoV-2 and is characterized by the viral entry into host cells, replication and establishment of infection mainly in the respiratory system. After the incubation period, the viremia phase can be asymptomatic or related to mild symptoms (Cao and Li, 2020; Siddiqi and Mehra, 2020). In this phase, the diagnostic is frequently performed by RT-PCR using nasopharyngeal swabs samples or, more recently, saliva (Sabino-Silva et al., 2020). The probability of SARS-CoV-2 detection increases with multiplication of SARS-CoV-2 and the presence of lymphopenia and neutrophilia is suitable. It also reported changes in high resolution computer tomography (HRCT) of the chest and presence of IgM against SARS-CoV-2. The seroconversion can occur 4 days after symptoms onset; however, it occurs in high frequency after 14 days. It is expected that immunocompetent subjects with additional risk factors are capable of generating sufficient immune responses to strongly suppress the SARS-CoV-2 replication (Cao and Li, 2020; Sethuraman et al., 2020; Siddiqi and Mehra, 2020).

(2) *Acute pulmonary phase*: In this period, the presence of several symptoms is expected as cough, fever, and occasionally shortness of breath. It is characterized as mild symptoms in the absence of hypoxia ($PaO_2/FiO_2 > 300$ mmHg), and severe under hypoxia ($PaO_2/FiO_2 < 300$ mmHg). HRCT of the chest can indicate lung infiltrates and typical COVID-19-ground-glass opacities. The lymphopenia and systemic inflammatory biomarkers can be higher in blood tests. As expected, the changes in chest imaging and blood test parameters are in accordance with severity degree (Siddiqi and Mehra, 2020). Most patients in this phase are also capable of suppressing SARS-CoV-2 infection with no exacerbated immunomodulatory response.

(3) *Severe hyperinflammation phase*: A minority of SARS-CoV-2 infected patients develop the most severe phase of the disease, which is characterized as pulmonary and systemic hyperinflammation due to the elevation of several inflammatory markers as IL-2, IL-6, IL-7, D-dimer, C-reactive protein, tumor necrosis factor-α, macrophage inflammatory protein 1-α, and ferritin (Siddiqi and Mehra, 2020). The characteristics in this phase can be similar to ARDS, which determines the presence of non-cardiogenic pulmonary edema, hypoxia, and, frequently, mechanical ventilation demand (Matthay et al., 2018). To note, the hypoxic respiratory failure due to ARDS is the leading mortality cause in SARS-CoV-2 infected patients. Additionally, the ARDS has been related to damages in the integrity of the alveolar-capillary barrier, which mediate additional inflammatory cell infiltration and development of pro-coagulative markers.

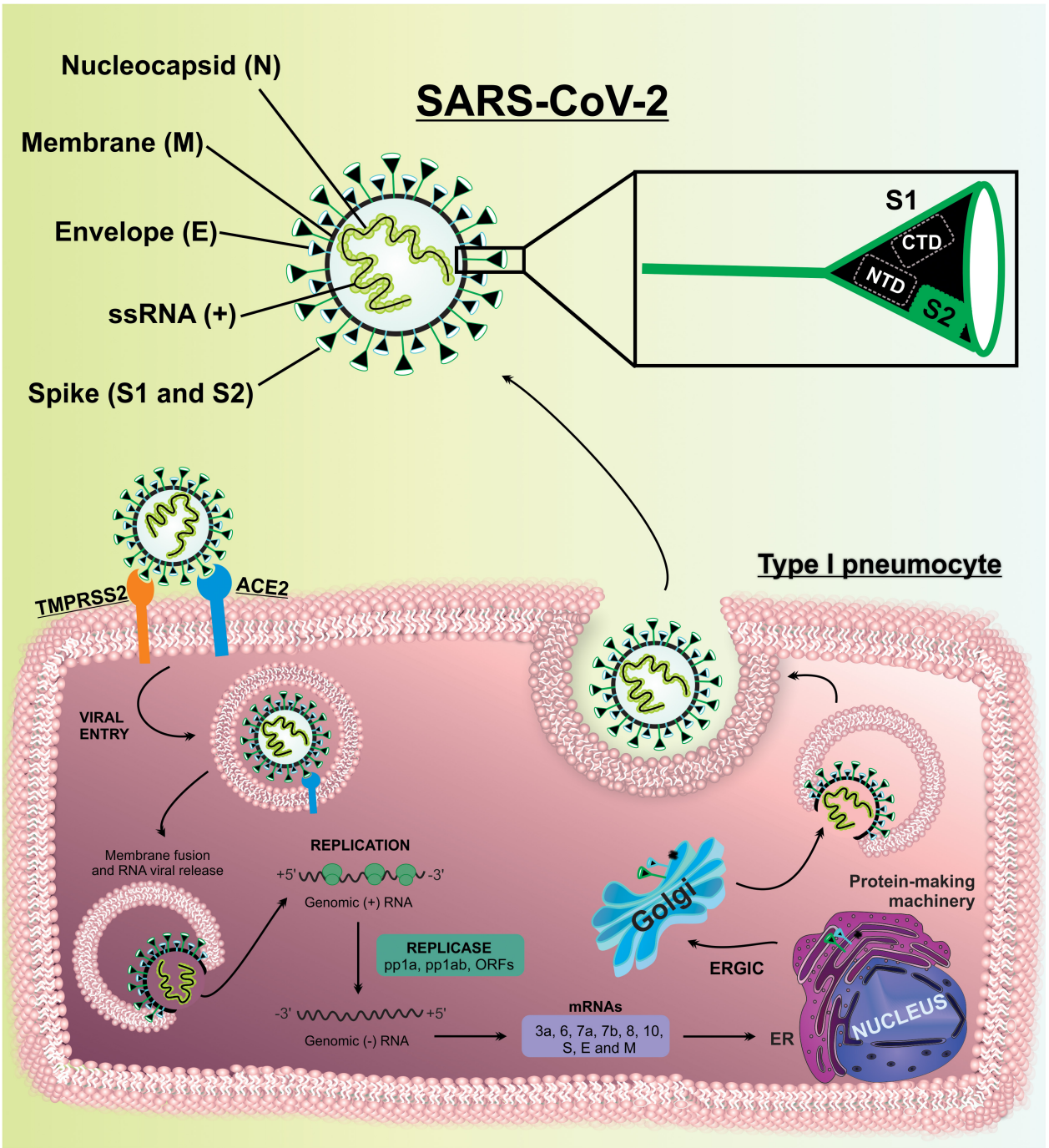


FIGURE 3 | SARS-CoV-2 structure and SARS-CoV-2 replication cycle in lung cells. The main structural proteins of SARS-CoV-2 are spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. The S, E, and M proteins are assembled in the viral membrane and the N protein merge in the viral RNA. SARS-CoV-2 interacts with ACE2, and subsequently with TMPRSS2, in lung cells. After SARS-CoV-2 entry occurs the release of viral genome and replication. Structural and non-structural proteins are assembly and packaging to SARS-CoV-2 release by exocytosis.

Usually, COVID-19 patients in severe hyperinflammation display lung vascular leakage and airway liquid accumulation (pulmonary edema) as a result of multiple mechanisms. Considering the ability of SARS-CoV-2 to gain entry in type I and type II pneumocytes and also in vasculature endothelial blood vessels cells by the expression of ACE2, it is expected to have a diffuse inflammatory profile in these epithelial cells that can lead to cellular lysis and apoptosis. The cell death in this region permits the fluxes of a protein-rich exudate biofluid derived from plasma and extracellular fluid to the alveolar space.

As expected, the presence of this rich-protein biofluid in alveolar space can reduce the O_2 and CO_2 diffusion due to the increase of liquid layer and presence of proteins, which lead to higher fluid density. In moderate and severe fluxes of this protein-rich exudate biofluid, the compensatory ventilatory response can be inefficient to maintain adequate exudate O_2 and CO_2 exchanges (Oliveira et al., 2016). Considering the resemblance between inflammatory response in the lung of patients with COVID-19 and sepsis (Tomar et al., 2020), it is expected that SGLT1 is also reduced in luminal membrane of pneumocytes (Cardoso-Sousa et al., 2019), which can resonate in ASL glucose concentration and ASL volume repercussion. Additional effort needs to be expended to raise this question.

The decrease in the functional ACE2 in the lung can activate the kallikrein–bradykinin system by an indirect pathway, promoting increase in vascular permeability. Besides, it is capable of activating neutrophils and secreting ROS. Furthermore, immunoinflammatory cytokines can promote additional inter-endothelial gaps. The acid hyaluronic pathway is also activated by inflammatory cells leading to fluid retention in alveolar space. Altogether, these several pathophysiological and immunological mechanisms stimulate vascular leakage and increased vascular permeability leading to pulmonary symptomatology in COVID-19 patients (**Figure 4**).

PATHOPHYSIOLOGY OF COVID-19 IN THE LUNG OF DIABETIC PATIENTS

Effects of SARS-CoV-2 on the Lung of Diabetic Patients

The general mechanism of SARS-CoV-2 infection in hyperglycemic diabetic subjects is similar to normoglycemic subjects; however, we point out the potential pathophysiological changes in the lung of diabetic patients suitable to changing the clinical course of COVID-19. In a genome-wide association, type I and type II DM were also related with higher ACE2 expression in lung study based on genotype expression of 5,515 subjects (Rao et al., 2020). Until now, the prevalence of a diabetic population with COVID-19 is similar with DM prevalence among general population, which indicates similar susceptibility to SARS-CoV-2 infection (Apicella et al., 2020; Jeong et al., 2020). In a pathophysiological context, it suggests similar ACE2 expression in nasal and oral mucosal cells in both normoglycemic and hyperglycemic populations. On the other hand, the increase of ACE2 in type I and type II pneumocytes (Roca-Ho et al., 2017) can be related with parallel changes in severity of COVID-19 in diabetic comparing to normoglycemic population, which is also reflected in higher mortality in diabetic subjects (Jeong et al., 2020). Thus, the higher ACE2 expression in pneumocytes could promote parallel entry and replication of SARS-CoV-2, which can be associated with the severity of COVID-19 in diabetics.

The higher risk to develop worse outcomes in diabetic population infected with SARS-CoV-2 (Apicella et al., 2020) also can be associated with the reduced ACE2/ACE ratio in diabetic condition, which could be critical to several pathophysiological mechanisms of COVID-19 (Roca-Ho et al., 2017). To counter regulate the opposite effects of ACE, it is important to emphasize that ACE2 expression is pivotal to promote anti-inflammatory, antioxidative stress and antifibrotic adjustments in the lung and to keep the vascular integrity of lung capillaries (Dalan et al., 2020). However, the reduced ACE2/ACE ratio is a classical characteristic that could be associated evaluating anti-inflammatory and antioxidant systems, which is reduced in the lung of diabetic mice (Roca-Ho et al., 2017).

In the second step, in the SARS-CoV-2 entry also occurs the internalization of ACE2, which can reduce the probability of additional virions entry in these infected cells. Dichotomously, it promotes additional pro-inflammatory and pro-fibrotic regulations in the lung, facilitating microvascular leakage in the respiratory system due to ACE-Ang-II-AT1R axis, since the decrease in ACE2 will decrease the Ang 1-7 formation, which has anti-inflammatory effects (Dalan et al., 2020). This evidence suggests a worst clinical scenario in DM including (i) higher ACE2 expression facilitating the SARS-CoV-2 infection, and (ii) a reduced ACE2/ACE ratio post SARS-CoV-2 infection triggering an heightened inflammatory and oxidative responses in diabetic patients increase the risk of a more severe form of COVID-19, especially in the acute pulmonary and severe hyperinflammation phase (Volpe et al., 2018).

Recently, it was described that glucose concentration could be directly related with higher levels of SARS-CoV-2 in monocytes from bronchoalveolar lavage (BAL), which was related with glycolysis to produce ATP and with a new metabolic and proteomic profile triggered by glucose (Codo et al., 2020). It is already established that beta-coronaviruses, as SARS-CoV-2, need the cellular machinery for viral replication (Walls et al., 2020). The inhibition of SARS-CoV-2 replication due to reduced glucose transport by 2-deoxy-D-glucose (2-DG) and the ATP synthase inhibition by oligomycin suggests that the glucose metabolism in lung cells can develop a pivotal role in COVID-19 (Codo et al., 2020). Besides, higher glucose levels were also associated with parallel expression of IL-1β and other proinflammatory cytokines as TNF-α, IL-6, and IFN-α, IFN-β, and IFN-λ (Codo et al., 2020). These cytokines play critical roles in the cytokine storm and lung injury of COVID-19 patients (Shi et al., 2020), because under high glucose conditions SARS-CoV-2 infected monocytes can promote pulmonary epithelial cell death (Codo et al., 2020). These changes in inflammatory profile in diabetic condition are identical to the profile described in severe COVID-19 patients with the worst clinical outcome (Lucas et al., 2020). It was demonstrated that an elevation in inflammatory cytokine levels in acute pulmonary phase was related with poor clinical outcomes. The inflammatory cytokines signature is similar in COVID-19 patients that develop moderate and severe outcomes during 10 days from symptom onset; subsequently, in moderate patients this inflammatory profile declined, and it remains maintained and elevated in severe patients (Lucas et al., 2020), suggesting parallel regulations in diabetes.

The higher viral replication in hyperglycemic condition (Codo et al., 2020) also produces rapid cell death by apoptosis, increasing the immune cells recruitment. Subsequently, an

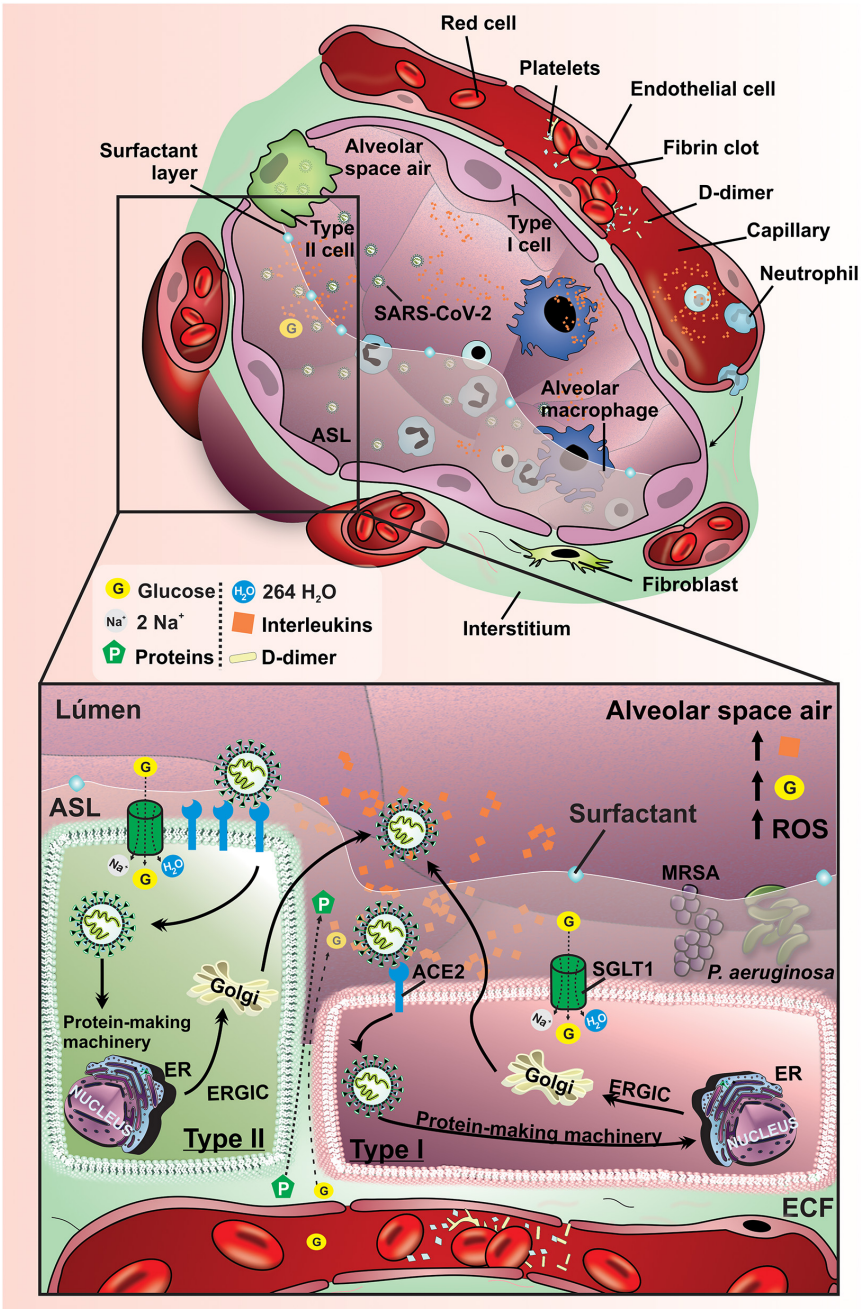


FIGURE 4 | Pathophysiological mechanisms affecting lung function and ASL glucose concentration COVID-19 patients. Schematic representation of pathophysiological mechanisms related to SARS-CoV-2 infection in the lung of non-diabetic subjects. Spike glycoproteins from the SARS-CoV-2 envelope binds to ACE2, allowing the inoculation of the viral genome into type I and II pneumocytes. The virus uses the host cells machinery to replicate and infect other cells, which can result in the activation of an inflammatory cascade as well as promoting damage to the vascular endothelium. These changes promote an increase of the production of interleukins, activation of ROS, damage to the alveolar epithelium and endothelium, which allows the leakage of liquid and glucose from the interstitium into the alveoli. These changes provide an increased risk for pneumonia due to the proliferation of bacteria *P. aeruginosa* and MRSA. G, glucose; SGLT1, Na^+/glucose/H_2O type 1 cotransporter; ECF, extracellular fluid; ER, endoplasmic reticulum; ERGIC, endoplasmic reticulum-Golgi intermediate compartment; ACE2r, receptor angiotensin-converting enzyme 2; ROS, reactive oxygen species; *P. aeruginosa*, *Pseudomonas aeruginosa*; MRSA, Methicillin-resistant *Staphylococcus aureus*; Orange rhombus: interleukins.

excessive alveolar exudative and interstitial inflammatory reaction might occur as outcome. In diabetes, the hypersecretion of the pro-inflammatory cytokines can provoke the "cytokine storm," resulting in lung tissue destruction by cytotoxic granules in patients with the worst clinical outcomes. The alveolar cell destruction leads to additional recruitment of immune cells with

an excessive alveolar exudative and interstitial inflammatory reaction (Mirastschijski et al., 2020).

Angiotensin-converting enzyme 2 expression in the lung is upregulated on SARS-CoV-2 infection, with type 2 pneumocytes potentially serving as a key cell type facilitating pulmonary inflammation (Drucker, 2020); notably, interleukin IL-6 may be further exaggerated in response to a stimulus as seen in diabetic patients with COVID-19 (Chan-Yeung and Xu, 2003). The additional expression of ACE2 in type II alveolar cells are capable to promote cell death after the SARS-CoV-2 entry (Hoffmann et al., 2020b; Zhou F. et al., 2020). In diabetes, the additional damage in type II alveolar cells infected by SARS-CoV-2 drastically reduces pulmonary surfactant production and secretion to the alveolar space causing atelectasis, reduced blood oxygenation, lung fibrosis, edema, impaired regeneration, and ultimately, leading to respiratory failure (Alcorn, 2017; Fang et al., 2020).

In this context, the SARS-CoV-2 infection in diabetic patients makes them more prone to develop severe stages of diseases, ARDS, and increased mortality (Wu and McGoogan, 2020). In summary, DM, promotes changes in immunity, increase glucose concentration, exudate, and fluid volume in the ASL, as well as endothelial lesion with formation of disseminated intravascular coagulation (Tang et al., 2020). Thus, SARS-CoV-2 infection in diabetic patients causes a potential reduction in gas exchange, hypoxemia, due to the damage in both ventilation and tissue perfusion. Moreover, the association between DM and SARS-CoV-2 increases the protein and glucose concentration in ASL, leading to higher risk of pulmonary infections (Gao et al., 2020).

Clinical Course of COVID-19 Diabetic Patients

The clinical course of COVID-19 in diabetic patients is similar to the clinical course described previously to normoglycemic patients. However, the prevalence of more severe phases as acute pulmonary and severe hyperinflammation phases is more frequent (Hussain et al., 2020; Pal and Bhansali, 2020; Singh et al., 2020). The classical pathophysiology of ARDS is focused on fibrin-rich exudates triggers by activation of the coagulation system and inhibition of fibrinolysis. A subgroup of patients with COVID-19-associated ARDS with higher mortality is characterized by low pulmonary compliance and high plasmatic D-dimer concentration. The endothelial dysfunction in the lung is critical to reduce gas exchange and to induce pro-thrombotic responses, which is associated with the worst prognosis (Grasselli et al., 2020). In this context, the endothelial dysfunction related to diabetes is associated with pro-inflammatory, pro-thrombotic, and significantly increased intracellular ROS (de Jongh et al., 2004; Schnabel et al., 2013; Khalaf et al., 2020). Furthermore, diabetic patients with COVID-19 exhibit disseminated intravascular coagulation and higher levels of D-dimer, leading to more severe endothelial injury and coagulation abnormalities, which has been associated with a higher mortality rate (Shi et al., 2020; Zhu et al., 2020). Other research groups also showed that D-dimer levels are higher in diabetic patients (Bhandari et al., 2020) and it is in accordance with higher levels of D-dimer in COVID-19 patient when glucose level is higher than 11 mmol/L, which was associated with parallel death rate (Li et al., 2020). D-dimer was also increased in uncontrolled glycemic subjects compared to optimal controlled diabetic subjects (Bhandari et al., 2020), reinforcing the importance of adequate surveillance in glycemic control to COVID-19 patients. Therefore, scientific evidence shows that optimal glucose control in diabetic patients with COVID-19 was associated with a significant reduction of inflammatory cytokines and D-dimer levels, decreasing the risk of COVID-19 complications. Poorly controlled DM has been linked to inhibited lymphocyte proliferative response to different kinds of stimuli, as well as impaired monocyte/macrophage and neutrophil functions (Hussain et al., 2020). A recent study with

TABLE 1 | Multifactorial mechanisms involved to the higher risk of worse outcomes in diabetic subjects associated with COVID-19.

Effectors in diabetic *compared* to normoglycemic condition	SARS-CoV-2 related mechanisms in diabetic compared to normoglycemic condition	Reference
↑ Expression of ACE2 in lung	↑ SARS-CoV-2 entry in respiratory cells	Roca-Ho et al., 2017; Rao et al., 2020
↓ ACE2/ACE ratio	↓ Anti-inflammatory, antifibrotic and antioxidant systems	Dalan et al., 2020
Internalization of ACE2 in the SARS-CoV-2 entry	↑ Pro-inflammatory, fibrotic and oxidant systems	Dalan et al., 2020
↑ Glucose concentration in bronchoalveolar lavage and ↑ Intracellular ATP production	↑ SARS-CoV-2 levels and ↑ levels of proinflammatory cytokines as IL-1β, TNF-α, IL-6, and IFN-α, IFN-β, and IFN-λ in lung	Codo et al., 2020
Inflammatory cytokines signature	Parallel inflammatory cytokine levels in COVID-19 patients with diabetes and COVID-19 patients with worst clinical outcomes	Codo et al., 2020; Lucas et al., 2020
↑ Viral replication in hyperglycemic condition	↑ Apoptosis, ↑ immune cells, ↑alveolar exudative and ↑interstitial inflammatory recruitment	Codo et al., 2020; Mirastschijski et al., 2020
Additional damages in type II alveolar ↑ viral replication in hyperglycemic condition	↓ Surfactant production causing atelectasis, reduced blood oxygenation, lung fibrosis and lung edema	Alcorn, 2017; Fang et al., 2020
↑ Endothelial dysfunction related to diabetes	Additional increase in ROS production, ↑ pro-inflammatory and ↑ Pro-thrombotic	de Jongh et al., 2004; Khalaf et al., 2020
↑ Protein and glucose concentration in ASL	↑ Risk of secondary infections	Oliveira et al., 2016; Gao et al., 2020;
↑ Levels of D-dimer	↑ Endothelial injury and coagulation abnormalities, which has been associated with a higher mortality rate	Shi et al., 2020; Zhu et al., 2020

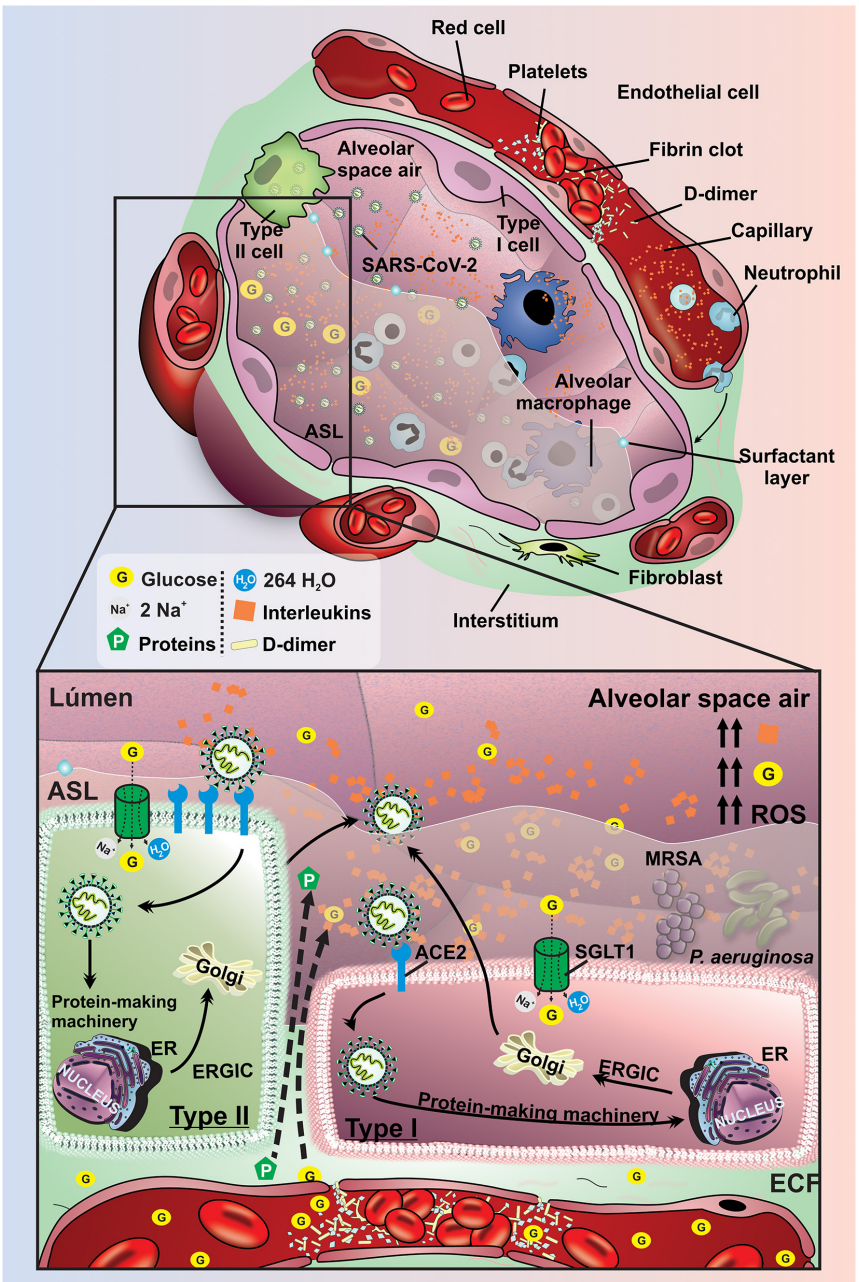


FIGURE 5 | Pathological manifestations of lung tissue in diabetic patient affected by SARS-CoV-2. Schematic representation of pathophysiological mechanisms related to SARS-CoV-2 infection in the lung of diabetic subjects. Spike glycoproteins from the SARS-CoV-2 envelope binds to ACE2 allowing the inoculation of the viral genome into type I and II pneumocytes. The virus uses the host cells machinery to replicate and infect other cells which can result in the activation of a disseminated inflammatory cascade. The high concentration of glucose and the increased volume in the ASL are maintained mainly by the low expression of SGLT1 in the apical membrane of type I and II pneumocytes in the condition of DM. These changes promote a potential increase in the production of interleukins, activation of ROS, damage to the alveolar epithelium and endothelium, which allows the leakage of liquid and glucose from the interstitial to the alveoli. In addition, the risk of developing pneumonia due to the proliferation of *P. aeruginosa* and MRSA bacteria is greatly increased in diabetic individuals with COVID-19. G, glucose; SGLT1, Na^+/glucose/H_2O type 1 cotransporter; ECF, extracellular fluid; ER, endoplasmic reticulum; ERGIC, endoplasmic reticulum-golgi intermediate compartment; ACE2, receptor angiotensin-converting enzyme 2; ROS, reactive oxygen species; *P. aeruginosa*, *Pseudomonas aeruginosa*; MRSA, Methicillin-resistant *Staphylococcus aureus*; Orange rhombus: interleukins.

595 consecutive hospitalized COVID-19 patients demonstrates that pneumonia and secondary infection were 2 times higher in diabetic than normoglycemic patients, which was parallel with changes in death rate (Akbariqomi et al., 2020). Thus, the adequate diabetes treatment maintaining optimal glucose levels may be an effective method for achieving glycemic targets

and reducing mortality in diabetic patients with COVID-19 (Sardu et al., 2020).

Taken together, it is pivotal to study the association between these synergic mechanisms to understand the effect of oxidative stress and inflammation in the lung of diabetic patients. Finally, studies have shown that patients with SARS-CoV-2 can develop secondary bacterial pneumonia and consequently worsen their clinical condition (Dong et al., 2020; Jin et al., 2020). SARS-CoV-2 can infect and kill alveolar pneumocytes and macrophages, leading to increased susceptibility to secondary bacterial pneumonia (Rothan and Byrareddy, 2020; Xu et al., 2020). Several pathophysiological mechanisms involved in worst clinical outcomes described in diabetes are summarized in **Table 1**. Thus, the risk of developing secondary bacterial pneumonia by dual SGLT inhibitors in diabetic patients with SARS-CoV-2 should be considered. On the other hand, the blocked SGLT1 in luminal membrane of type I and type II cells could be capable to reduce the intracellular glucose fluxes, which can reduce SARS-CoV-2 proliferation (**Figure 5**).

Diabetes mellitus are definitely at an increased risk of severe COVID-19 outcomes. Hence, it is advisable that community-dwelling residents having underlying DM take extra precautions to not contract the virus by adopting social distancing and strict hand and respiratory hygiene (Chan-Yeung and Xu, 2003).

FINAL REMARKS

Altogether, the latest advances in pathophysiology have challenged the understanding about the lung environment under normoglycemic and hyperglycemic conditions. The functional regulation of glucose/water transporters in luminal membrane of type I and type II pneumocytes and the pivotal role of ASL glucose concentration modulation broaden the horizons in lung pathogenesis of diabetic patients infected with SARS-CoV-2. The global spread of COVID-19 has led to an urgent effort to draw the complex pathophysiology of the SARS-CoV-2 infections in the lung of diabetic patients. In this context, we described the current view about the effects of higher ASL glucose concentration, intracellular glucose metabolism, and glycans in the bacterial proliferation, inflammation, oxidative stress, and pro-thrombotic responses in the lung, which frequently leads to harmful clinical outcomes in diabetic patients. Finally, this draw of the lung interaction between SARS-CoV-2 infection and DM paves the way to better understand unique characteristics of the SARS-CoV-2 in lung of diabetic patients, indicating that advances in intensive glycemic control may be an effective method for reduce mortality in diabetic patients with COVID-19. In summary, DM promotes an increase in ASL glucose concentration, ASL volume accumulation in alveolar space, imbalance of ROS, and inflammatory chemokine production. The COVID-19 infection triggers type I and type II pneumocytes damages and additional lung endothelial lesions, with subsequent additional secretion of protein-rich inflammatory fluid in the alveolar space and intravascular coagulation in lung vessel, which leads to a reduction in surfactant and gas exchange. As expected, the prevalence and severity of hypoxemia, severe hyperinflammation, and pro-thrombotic responses and pneumonia are higher in COVID-19 diabetic patients.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

The authors received financial support from the CAPES–Prevention and Combat of Outbreaks, Endemics, Epidemics and Pandemics (#23038.014934/2020-59), FAPEMIG (Minas Gerais Research Foundation APQ-00587-14-SICONV 793988/2013; APQ-02872-16 and APQ-03385-18), and Chiesi Foundation Onlus. The Brazilian funding agencies CNPq, CAPES (Coordination for the Improvement of Higher Education), and FAPEMIG provide financial support to the National Institute of Science and Technology in Theranostics and Nanobiotechnology-INCT-Teranano (CNPq-465669/2014-0). Sabino-Silva, R received a fellowship from PrInt CAPES/UFU.

REFERENCES

Akbariqomi, M., Hosseini, M. S., Rashidiani, J., Sedighian, H., Biganeh, H., Heidari, R., et al. (2020). Clinical characteristics and outcome of hospitalized COVID-19 patients with diabetes: a single-center, retrospective study in Iran. *Diabetes Res. Clin. Pract.* 169:108467. doi: 10.1016/j.diabres.2020.108467

Alcorn, J. L. (2017). "Pulmonary surfactant trafficking and homeostasis," in *Lung Epithelial Biology in the Pathogenesis of Pulmonary Disease*, eds. V. K. Sidhaye and M. Koval (Amsterdam: Elsevier), 59–75. doi: 10.1016/B978-0-12-803809-3.00004-X

Apicella, M., Campopiano, M. C., Mantuano, M., Mazoni, L., Coppelli, A., and Del Prato, S. (2020). COVID-19 in people with diabetes: understanding the reasons for worse outcomes. *Lancet. Diabetes Endocrinol.* 8, 782–792. doi: 10.1016/S2213-8587(20)30238-2

Baker, E. H., and Baines, D. L. (2018). Airway glucose homeostasis: a new target in the prevention and treatment of pulmonary infection. *Chest* 153, 507–514. doi: 10.1016/j.chest.2017.05.031

Baker, E. H., Clark, N., Brennan, A. L., Fisher, D. A., Gyi, K. M., Hodson, M. E., et al. (2007). Hyperglycemia and cystic fibrosis alter respiratory fluid glucose concentrations estimated by breath condensate analysis. *J. Appl. Physiol.* 102, 1969–1975. doi: 10.1152/japplphysiol.01425.2006

Baker, E. H., Janaway, C. H., Philips, B. J., Brennan, A. L., Baines, D. L., Wood, D. M., et al. (2006a). Hyperglycaemia is associated with poor outcomes in patients admitted to hospital with acute exacerbations of chronic obstructive pulmonary disease. *Thorax* 61, 284–289. doi: 10.1136/thx.2005.051029

Baker, E. H., Wood, D. M., Brennan, A. L., Clark, N., Baines, D. L., and Philips, B. J. (2006b). Hyperglycaemia and pulmonary infection. *Proc. Nutr. Soc.* 65, 227–235. doi: 10.1079/pns2006499

Baynes, J. W. (1991). Role of oxidative stress in development of complications in diabetes. *Diabetes* 40, 405–412. doi: 10.2337/diab.40.4.405

Belouzard, S., Chu, V. C., and Whittaker, G. R. (2009). Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *Proc. Natl. Acad. Sci. U.S.A.* 106, 5871–5876. doi: 10.1073/pnas.0809524106

Bhandari, S., Rankawat, G., Singh, A., Gupta, V., and Kakkar, S. (2020). Impact of glycemic control in diabetes mellitus on management of COVID-19 infection. *Int. J. Diabetes Dev. Ctries* 40, 340–345. doi: 10.1007/s13410-020-00868-7

Bodega, F., Sironi, C., Armilli, M., Porta, C., and Agostoni, E. (2010). Evidence for Na +-glucose cotransporter in type I alveolar epithelium. *Histochem. Cell Biol.* 134, 129–136. doi: 10.1007/s00418-010-0725-7

Bojkova, D., Klann, K., Koch, B., Widera, M., Krause, D., Ciesek, S., et al. (2020). Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. *Nature* 583, 469–472. doi: 10.1038/s41586-020-2332-7

Cao, B., Wang, Y., Wen, D., Liu, W., Wang, J., Fan, G., et al. (2020). A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. *N. Engl. J. Med.* 382, 1787–1799. doi: 10.1056/NEJMoa2001282

Cao, W., and Li, T. (2020). COVID-19: towards understanding of pathogenesis. *Cell Res.* 30, 367–369. doi: 10.1038/s41422-020-0327-4

Cardoso-Sousa, L., Aguiar, E. M. G., Caixeta, D. C., Vilela, D. D., da Costa, D. P., Silva, T. L., et al. (2019). Effects of salbutamol and phlorizin on acute pulmonary inflammation and disease severity in experimental sepsis. *PLoS One* 14:e0222575. doi: 10.1371/journal.pone.0222575

Chan-Yeung, M., and Xu, R. H. (2003). SARS: epidemiology. *Respirology* 8(Suppl. 1), S9–S14. doi: 10.1046/j.1440-1843.2003.00518.x

Chatterjee, S. (2016). "Oxidative stress, inflammation, and disease," in *Oxidative Stress and Biomaterials*, eds. T. Dziubla and D. A. Butterfield (Amsterdam: Elsevier), 35–58. doi: 10.1016/B978-0-12-803269-5.00002-4

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395, 507–513. doi: 10.1016/S0140-6736(20)30211-7

Cheng, H., Wang, Y., and Wang, G. Q. (2020). Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. *J. Med. Virol.* 92, 726–730. doi: 10.1002/jmv.25785

Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W., et al. (2018). IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res. Clin. Pract.* 138, 271–281. doi: 10.1016/j.diabres.2018.02.023

Clark, A., Jit, M., Warren-Gash, C., Guthrie, B., Wang, H. H. X., Mercer, S. W., et al. (2020). Global, regional, and national estimates of the population at increased risk of severe COVID-19 due to underlying health conditions in 2020: a modelling study. *Lancet. Glob. Heal* 8, E1003–E1017. doi: 10.1016/S2214-109X(20)30264-3

Codo, A. C., Davanzo, G. G., Monteiro, L. B., de Souza, G. F., Muraro, S. P., Virgilio-da-Silva, J. V., et al. (2020). Elevated glucose levels favor SARS-CoV-2 infection and monocyte response through a HIF-1α/Glycolysis-Dependent Axis. *Cell Metab.* 132, 437.e5–446.e5. doi: 10.1016/j.cmet.2020.07.007

Correia, G., Rodrigues, L., Gameiro da Silva, M., and Gonçalves, T. (2020). Airborne route and bad use of ventilation systems as non-negligible factors in SARS-CoV-2 transmission. *Med. Hypotheses* 141:109781. doi: 10.1016/j.mehy.2020.109781

Dalan, R., Bornstein, S. R., El-Armouche, A., Rodionov, R. N., Markov, A., Wielockx, B., et al. (2020). The ACE-2 in COVID-19: foe or friend? *Horm. Metab. Res.* 52, 257–263. doi: 10.1055/a-1155-0501

de Jongh, R. T., Serné, E. H., IJzerman, R. G., de Vries, G., and Stehouwer, C. D. A. (2004). Impaired microvascular function in obesity: implications for obesity-associated microangiopathy, hypertension, and insulin resistance. *Circulation* 109, 2529–2535. doi: 10.1161/01.CIR.0000129772.26647.6F

Ding, S., and Liang, T. J. (2020). Is SARS-CoV-2 also an enteric pathogen with potential fecal–oral transmission? A COVID-19 virological and clinical review. *Gastroenterology* 159, 53–61. doi: 10.1053/j.gastro.2020.04.052

Diniz Vilela, D., Gomes Peixoto, L., Teixeira, R. R., Belele Baptista, N., Carvalho Caixeta, D., Vieira de Souza, A., et al. (2016). The role of metformin in controlling oxidative stress in muscle of diabetic rats. *Oxid. Med. Cell. Longev.* 2016:6978625. doi: 10.1155/2016/6978625

Dong, X., Cao, Y. Y., Lu, X. X., Zhang, J. J., Du, H., Yan, Y. Q., et al. (2020). Eleven faces of coronavirus disease 2019. *Allergy Eur. J. Allergy Clin. Immunol* 75, 1699–1709. doi: 10.1111/all.14289

Drucker, D. J. (2020). Coronavirus infections and type 2 Diabetes—Shared pathways with therapeutic implications. *Endocr. Rev.* 41:bnaa011. doi: 10.1210/endrev/bnaa011

Erokhova, L., Horner, A., Ollinger, N., Siligan, C., and Pohl, P. (2016). The Sodium glucose cotransporter SGLT1 is an extremely efficient facilitator of passive water transport. *J. Biol. Chem.* 291, 9712–9720. doi: 10.1074/jbc.M115.706986

Fang, L., Karakiulakis, G., and Roth, M. (2020). Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir. Med.* 8:e21. doi: 10.1016/S2213-2600(20)30116-8

Fehr, A. R., and Perlman, S. (2015). "Coronaviruses: an overview of their replication and pathogenesis," in *Coronaviruses: Methods and Protocols*, eds H. J. Maier and E. Bickerton (New York, NY: Springer), 1–23. doi: 10.1007/978-1-4939-2438-7_1

Fu, J., Zhou, B., Zhang, L., Balaji, K. S., Wei, C., Liu, X., et al. (2020). Expressions and significances of the angiotensin-converting enzyme 2 gene, the receptor of SARS-CoV-2 for COVID-19. *Mol. Biol. Rep.* 47, 4383–4392. doi: 10.1007/s11033-020-05478-4

Gao, Y., Li, T., Han, M., Li, X., Wu, D., Xu, Y., et al. (2020). Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. *J. Med. Virol.* 92, 791–796. doi: 10.1002/jmv.25770

Garnett, J. P., Baker, E. H., and Baines, D. L. (2012a). Sweet talk: insights into the nature and importance of glucose transport in lung epithelium. *Eur. Respir. J.* 40, 1269–1276. doi: 10.1183/09031936.00052612

Garnett, J. P., Nguyen, T. T., Moffatt, J. D., Pelham, E. R., Kalsi, K. K., Baker, E. H., et al. (2012b). Proinflammatory mediators disrupt glucose homeostasis in airway surface liquid. *J. Immunol.* 189, 373–380. doi: 10.4049/jimmunol.1200718

Garnett, J. P., Gray, M. A., Tarran, R., Brodlie, M., Ward, C., Baker, E. H., et al. (2013). Elevated paracellular glucose flux across cystic fibrosis airway epithelial monolayers is an important factor for *Pseudomonas aeruginosa* Growth. *PLoS One* 8:e0076283. doi: 10.1371/journal.pone.0076283

Grant, O. C., Montgomery, D., Ito, K., and Woods, R. J. (2020). Analysis of the SARS-CoV-2 spike protein glycan shield: implications for immune recognition. *bioRxiv* [Preprint]. doi: 10.1101/2020.04.07.030445

Grasselli, G., Tonetti, T., Protti, A., Langer, T., Girardis, M., Bellani, G., et al. (2020). Pathophysiology of COVID-19-associated acute respiratory distress syndrome: a multicentre prospective observational study. *Lancet. Respir. Med.* doi: 10.1016/S2213-2600(20)30370-2

Hamming, I., Timens, W., Bulthuis, M. L. C., Lely, A. T., Navis, G. J., and van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203, 631–637. doi: 10.1002/path.1570

Helenius, A., and Aebi, M. (2001). Intracellular functions of N-linked glycans. *Science* 291, 2364–2369. doi: 10.1126/science.291.5512.2364

Hoffmann, M., Kleine-Weber, H., and Pöhlmann, S. (2020a). A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Mol. Cell* 78, 779.e5–784.e5. doi: 10.1016/j.molcel.2020.04.022

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020b). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271.e8–280.e8. doi: 10.1016/j.cell.2020.02.052

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan. *China. Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Hussain, A., Bhowmik, B., and do Vale Moreira, N. C. (2020). COVID-19 and diabetes: knowledge in progress. *Diabetes Res. Clin. Pract.* 162:108142. doi: 10.1016/j.diabres.2020.108142

Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., et al. (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 436, 112–116. doi: 10.1038/nature03712

Inagaki, N., Kondo, K., Yoshinari, T., Maruyama, N., Susuta, Y., and Kuki, H. (2013). Efficacy and safety of canagliflozin in Japanese patients with type 2 diabetes: a randomized, double-blind, placebo-controlled, 12-week study. *Diabetes. Obes. Metab.* 15, 1136–1145. doi: 10.1111/dom.12149

Jeong, I.-K., Ho Yoon, K., and Kyu Lee, M. (2020). Diabetes and COVID-19: global and regional perspectives. *Diabetes Res. Clin. Pract.* 166:108303. doi: 10.1016/j.diabres.2020.108303

Jia, H. P., Look, D. C., Tan, P., Shi, L., Hickey, M., Gakhar, L., et al. (2009). Ectodomain shedding of angiotensin converting enzyme 2 in human airway

epithelia. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 297, L84–L96. doi: 10.1152/ajplung.00071.2009

Jin, Y.-H., Cai, L., Cheng, Z.-S., Cheng, H., Deng, T., Fan, Y.-P., et al. (2020). A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Mil. Med. Res.* 7:4. doi: 10.1186/s40779-020-0233-6

Kalsi, K. K., Baker, E. H., Fraser, O., Chung, Y. L., MacE, O. J., Tarelli, E., et al. (2009). Glucose homeostasis across human airway epithelial cell monolayers: role of diffusion, transport and metabolism. *Pflugers Arch. Eur. J. Physiol.* 457, 1061–1070. doi: 10.1007/s00424-008-0576-4

Khalaf, M., Scott-Ward, T., Causer, A., Saynor, Z., Shepherd, A., Górecki, D., et al. (2020). Cystic fibrosis transmembrane conductance regulator (CFTR) in human lung microvascular endothelial cells controls oxidative stress, reactive oxygen-mediated cell signaling and inflammatory responses. *Front. Physiol.* 11:879. doi: 10.3389/fphys.2020.00879

Khateeb, J., Fuchs, E., and Khamaisi, M. (2019). Diabetes and lung disease: an underestimated relationship. *Rev. Diabet. Stud.* 15, 1–15. doi: 10.1900/RDS.2019.15.1

Kim, D., Lee, J. Y., Yang, J. S., Kim, J. W., Kim, V. N., and Chang, H. (2020). The Architecture of SARS-CoV-2 Transcriptome. *Cell* 181, 914.e10–921.e10. doi: 10.1016/j.cell.2020.04.011

Kuo, L., and Masters, P. S. (2013). Functional analysis of the murine coronavirus genomic RNA packaging signal. *J. Virol.* 87, 5182–5192. doi: 10.1128/jvi.00100-13

Li, W., Moore, M. J., Vasilieva, N., Sui, J., Wong, S. K., Berne, M. A., et al. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 426, 450–454. doi: 10.1038/nature02145

Li, X., Sun, X., and Carmeliet, P. (2019). Hallmarks of endothelial cell metabolism in health and disease. *Cell Metab.* 30, 414–433. doi: 10.1016/j.cmet.2019.08.011

Li, Y., Han, X., Alwalid, O., Cui, Y., Cao, Y., Liu, J., et al. (2020). Baseline characteristics and risk factors for short-term outcomes in 132 COVID-19 patients with diabetes in Wuhan China: a retrospective study. *Diabetes Res. Clin. Pract.* 166:108299. doi: 10.1016/j.diabres.2020.108299

Lucas, C., Wong, P., Klein, J., Castro, T. B. R., Silva, J., Sundaram, M., et al. (2020). Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature* 584, 463–469. doi: 10.1038/s41586-020-2588-y

Lukassen, S., Chua, R. L., Trefzer, T., Kahn, N. C., Schneider, M. A., Muley, T., et al. (2020). SARS-CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. *EMBO J.* 39:e105114. doi: 10.15252/embj.20105114

Matthay, M. A., Zemans, R. L., Zimmerman, G. A., Arabi, Y. M., Beitler, J. R., Mercat, A., et al. (2018). Acute respiratory distress syndrome. *Nat. Rev. Dis. Prim.* 5, 1–22. doi: 10.1038/s41572-019-0069-0

Mirastschijski, U., Dembinski, R., and Maedler, K. (2020). Lung surfactant for pulmonary barrier restoration in patients with COVID-19 pneumonia. *Front. Med.* 7:254. doi: 10.3389/fmed.2020.00254

Mossel, E. C., Wang, J., Jeffers, S., Edeen, K. E., Wang, S., Cosgrove, G. P., et al. (2008). SARS-CoV replicates in primary human alveolar type II cell cultures but not in type I-like cells. *Virology* 372, 127–135. doi: 10.1016/j.virol.2007.09.045

Oliveira, T. L., Candeia-Medeiros, N., Cavalcante-Araújo, P. M., Melo, I. S., Fávaro-Pípi, E., Fátima, L. A., et al. (2016). SGLT1 activity in lung alveolar cells of diabetic rats modulates airway surface liquid glucose concentration and bacterial proliferation. *Sci. Rep.* 6, 1–11. doi: 10.1038/srep21752

Pal, R., and Bhansali, A. (2020). COVID-19, diabetes mellitus and ACE2: The conundrum. *Diabetes Res. Clin. Pract.* 162:108132. doi: 10.1016/j.diabres.2020.108132

Peng, G., Sun, D., Rajashankar, K. R., Qian, Z., Holmes, K. V., and Li, F. (2011). Crystal structure of mouse coronavirus receptor-binding domain complexed with its murine receptor. *Proc. Natl. Acad. Sci. U.S.A.* 108, 10696–10701. doi: 10.1073/pnas.1104306108

Pezzulo, A. A., Gutiérrez, J., Duschner, K. S., McConnell, K. S., Taft, P. J., Ernst, S. E., et al. (2011). Glucose depletion in the airway surface liquid is essential for sterility of the airways. *PLoS One* 6:e0016166. doi: 10.1371/journal.pone.0016166

Philips, B. J., Meguer, J.-X., Redman, J., and Baker, E. H. (2003). Factors determining the appearance of glucose in upper and lower respiratory tract secretions. *Intensive Care Med.* 29, 2204–2210. doi: 10.1007/s00134-003-1961-2

Philips, B. J., Redman, J., Brennan, A., Wood, D., Holliman, R., Baines, D., et al. (2005). Glucose in bronchial aspirates increases the risk of respiratory MRSA in intubated patients. *Thorax* 60, 761–764. doi: 10.1136/thx.2004.035766

Polidori, D., Sha, S., Mudaliar, S., Ciaraldi, T. P., Ghosh, A., Vaccaro, N., et al. (2013). Canagliflozin lowers postprandial glucose and insulin by delaying intestinal glucose absorption in addition to increasing urinary glucose excretion: results of a randomized, placebo-controlled study. *Diabetes Care* 36, 2154–2161. doi: 10.2337/dc12-2391

Powell, D. R., Smith, M., Greer, J., Harris, A., Zhao, S., DaCosta, C., et al. (2013). LX4211 increases serum glucagon-like peptide 1 and peptide YY levels by reducing sodium/glucose cotransporter 1 (SGLT1)-mediated absorption of intestinal glucose. *J. Pharmacol. Exp. Ther.* 345, 250–259. doi: 10.1124/jpet.113.203364

Raffay, T., Kc, P., Reynolds, J., Di Fiore, J., MacFarlane, P., and Martin, R. J. (2014). Repeated β2-adrenergic receptor agonist therapy attenuates the response to rescue bronchodilation in a hyperoxic newborn mouse model. *Neonatology* 106, 126–132. doi: 10.1159/000362684

Rao, S., Lau, A., and So, H.-C. (2020). Exploring diseases/traits and blood proteins causally related to expression of ACE2, the putative receptor of SARS-CoV-2: a mendelian randomization analysis highlights tentative relevance of diabetes-related traits. *Diabetes Care* 43, 1416–1426. doi: 10.2337/dc20-0643

Roca-Ho, H., Riera, M., Palau, V., Pascual, J., and Soler, M. (2017). Characterization of ACE and ACE2 expression within different organs of the NOD mouse. *Int. J. Mol. Sci.* 18:563. doi: 10.3390/ijms18030563

Rothan, H. A., and Byrareddy, S. N. (2020). The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J. Autoimmun.* 109:102433. doi: 10.1016/j.jaut.2020.102433

Rothe, C., Schunk, M., Sothmann, P., Bretzel, G., Froeschl, G., Wallrauch, C., et al. (2020). Transmission of 2019-NCOV infection from an asymptomatic contact in Germany. *N. Engl. J. Med.* 382, 970–971. doi: 10.1056/NEJMc2001468

Sabino-Silva, R., Jardim, A. C. G., and Siqueira, W. L. (2020). Coronavirus COVID-19 impacts to dentistry and potential salivary diagnosis. *Clin. Oral Investig.* 24, 1619–1621. doi: 10.1007/s00784-020-03248-x

Sabino-Silva, R., Mori, R. C., David-Silva, A., Okamoto, M. M., Freitas, H. S., and Machado, U. F. (2010). The Na(+)/glucose cotransporters: from genes to therapy. *Brazilian J. Med. Biol. Res.* 43, 1019–1026. doi: 10.1590/s0100-879x2010007500115

Santos, I. D. A., Grosche, V. R., Sabino-Silva, R., and Jardim, A. C. G. (2020). Antivirals against Coronaviruses: candidate drugs for the SARS-CoV-2 treatment? *Front. Microbiol.* 11:1818. doi: 10.3389/FMICB.2020.01818

Sardu, C., D'Onofrio, N., Balestrieri, M. L., Barbieri, M., Rizzo, M. R., Messina, V., et al. (2020). Outcomes in patients with hyperglycemia affected by covid-19: can we do more on glycemic control? *Diabetes Care* 43, 1408–1415. doi: 10.2337/dc20-0723

Saumon, G., Martet, G., and Loiseau, P. (1996). Glucose transport and equilibrium across alveolar-airway barrier of rat. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 270(2 Pt 1), L183–L190. doi: 10.1152/ajplung.1996.270.2.l183

Schmidt, H., Michel, C., Braubach, P., Fauler, M., Neubauer, D., Thompson, K. E., et al. (2017). Water permeability adjusts resorption in lung epithelia to increased apical surface liquid volumes. *Am. J. Respir. Cell Mol. Biol.* 56, 372–382. doi: 10.1165/rcmb.2016-0161OC

Schnabel, R. B., Yin, X., Larson, M. G., Yamamoto, J. F., Fontes, J. D., Kathiresan, S., et al. (2013). Multiple inflammatory biomarkers in relation to cardiovascular events and mortality in the community. *Arterioscler. Thromb. Vasc. Biol.* 33, 1728–1733. doi: 10.1161/ATVBAHA.112.301174

Sethna, P. B., Hofmann, M. A., and Brian, D. A. (1991). Minus-strand copies of replicating coronavirus mRNAs contain antileaders. *J. Virol.* 65, 320–325. doi: 10.1128/jvi.65.1.320-325.1991

Sethuraman, N., Jeremiah, S. S., and Ryo, A. (2020). Interpreting diagnostic tests for SARS-CoV-2. *J. Am. Med. Assoc.* 323, 2249–2251. doi: 10.1001/jama.2020.8259

Shi, Y., Wang, Y., Shao, C., Huang, J., Gan, J., Huang, X., et al. (2020). COVID-19 infection: the perspectives on immune responses. *Cell Death Differ.* 27, 1451–1454. doi: 10.1038/s41418-020-0530-3

Shubrook, J., Butts, A., Chamberlain, J. J., Johnson, E. L., Leal, S., Rhinehart, A. S., et al. (2017). Standards of medical care in diabetes—2017 abridged for primary care providers. *Clin. Diabetes* 35, 5–26. doi: 10.2337/cd16-0067

Siddiqi, H. K., and Mehra, M. R. (2020). COVID-19 illness in native and immunosuppressed states: a clinical–therapeutic staging proposal. *J. Hear. Lung Transplant.* 39, 405–407. doi: 10.1016/j.healun.2020.03.012

Singh, A. K., Gupta, R., Ghosh, A., and Misra, A. (2020). Diabetes in COVID-19: Prevalence, pathophysiology, prognosis and practical considerations. *Diabetes Metab. Syndr.* 14, 303–310. doi: 10.1016/j.dsx.2020.04.004

Siu, Y. L., Teoh, K. T., Lo, J., Chan, C. M., Kien, F., Escriou, N., et al. (2008). The M, E, and N structural proteins of the severe acute respiratory syndrome coronavirus are required for efficient assembly, trafficking, and release of virus-like particles. *J. Virol.* 82, 11318–11330. doi: 10.1128/jvi.01052-08

Stein, R. A. (2020). COVID-19: Risk groups, mechanistic insights and challenges. *Int. J. Clin. Pract.* 74, e13512. doi: 10.1111/ijcp.13512

Stoian, A. P., Banerjee, Y., Rizvi, A. A., and Rizzo, M. (2020). Diabetes and the COVID-19 pandemic: how insights from recent experience might guide future management. *Metab. Syndr. Relat. Disord.* 18, 173–175. doi: 10.1089/met.2020.0037

Stone, K. C., Mercer, R. R., Freeman, B. A., Chang, L. Y., and Crapo, J. D. (1992). Distribution of lung cell numbers and volumes between alveolar and nonalveolar tissue. *Am. Rev. Respir. Dis.* 146, 454–456. doi: 10.1164/ajrccm/146.2.454

Tang, N., Bai, H., Chen, X., Gong, J., Li, D., and Sun, Z. (2020). Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J. Thromb. Haemost.* 18, 1094–1099. doi: 10.1111/jth.14817

Teuwen, L. A., Geldhof, V., Pasut, A., and Carmeliet, P. (2020). COVID-19: the vasculature unleashed. *Nat. Rev. Immunol.* 20, 389–391. doi: 10.1038/s41577-020-0343-0

Tikellis, C., and Thomas, M. C. (2012). Angiotensin-converting enzyme 2 (ACE2) is a key modulator of the renin angiotensin system in health and disease. *Int. J. Pept.* 2012, 1–8. doi: 10.1155/2012/256294

Tomar, B., Anders, H.-J., Desai, J., and Mulay, S. R. (2020). Neutrophils and Neutrophil Extracellular Traps Drive Necroinflammation in COVID-19. *Cells* 9:1383. doi: 10.3390/cells9061383

van den Brand, J. M. A., Haagmans, B. L., Leijten, L., van Riel, D., Martina, B. E. E., Osterhaus, A. D. M. E., et al. (2008). Pathology of experimental SARS coronavirus infection in cats and ferrets. *Vet. Pathol.* 45, 551–562. doi: 10.1354/vp.45-4-551

Veldhuizen, E. J., and Haagsman, H. P. (2000). Role of pulmonary surfactant components in surface film formation and dynamics. *Biochim. Biophys. Acta* 1467, 255–270. doi: 10.1016/s0005-2736(00)00256-x

Volpe, C. M. O., Villar-Delfino, P. H., Dos Anjos, P. M. F., and Nogueira-Machado, J. A. (2018). Cellular death, reactive oxygen species (ROS) and diabetic complications. *Cell Death Dis.* 9:119. doi: 10.1038/s41419-017-0135-z

Vrhovac, I., Eror, D. B., Klessen, D., Burger, C., Breljak, D., Kraus, O., et al. (2015). Localizations of Na+-D-glucose cotransporters SGLT1 and SGLT2 in human kidney and of SGLT1 in human small intestine, liver, lung, and heart. *Pflugers Arch. Eur. J. Physiol.* 467, 1881–1898. doi: 10.1007/s00424-014-1619-7

Walls, A. C., Park, Y.-J., Tortorici, M. A., Wall, A., McGuire, A. T., and Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 181, 281.e6–292.e6. doi: 10.1016/j.cell.2020.02.058

Walls, A. C., Tortorici, M. A., Frenz, B., Snijder, J., Li, W., Rey, F. A., et al. (2016). Glycan shield and epitope masking of a coronavirus spike protein observed by cryo-electron microscopy. *Nat. Struct. Mol. Biol.* 23, 899–905. doi: 10.1038/nsmb.3293

Wang, C., Horby, P. W., Hayden, F. G., and Gao, G. F. (2020). A novel coronavirus outbreak of global health concern. *Lancet* 395, 470–473. doi: 10.1016/S0140-6736(20)30185-9

Watanabe, Y., Allen, J. D., Wrapp, D., McLellan, J. S., and Crispin, M. (2020). Site-specific glycan analysis of the SARS-CoV-2 spike. *Science* 369, 330–333. doi: 10.1126/science.abb9983

WHO (2020). *Coronavirus Disease 2019 (COVID-19) Situation Reports*. 1–19. Available online at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports (accessed October 15, 2020).

Wu, Z., and McGoogan, J. M. (2020). Characteristics of and important lessons fromthe coronavirus disease 2019 (COVID-19) Outbreak in China. *JAMA* 323:1239. doi: 10.1001/jama.2020.2648

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., et al. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med.* 8, 420–422. doi: 10.1016/S2213-2600(20)30076-X

Yang, K., Sheng, Y., Huang, C., Jin, Y., Xiong, N., Jiang, K., et al. (2020). Clinical characteristics, outcomes, and risk factors for mortality in patients with cancer and COVID-19 in Hubei, China: a multicentre, retrospective, cohort study. *Lancet Oncol.* 21, 904–913. doi: 10.1016/S1470-2045(20)30310-7

Ye, Y., and Hogue, B. G. (2007). Role of the coronavirus E Viroporin protein transmembrane domain in virus assembly. *J. Virol.* 81, 3597–3607. doi: 10.1128/jvi.01472-06

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395, 1054–1062. doi: 10.1016/S0140-6736(20)30566-3

Zhou, Y., Hou, Y., Shen, J., Huang, Y., Martin, W., and Cheng, F. (2020). Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. *Cell Discov.* 6, 1–18. doi: 10.1038/s41421-020-0153-3

Zhu, L., She, Z. G., Cheng, X., Qin, J. J., Zhang, X. J., Cai, J., et al. (2020). Association of blood glucose control and outcomes in patients with COVID-19 and Pre-existing Type 2 diabetes. *Cell Metab.* 31, 1068.e3–1077.e3. doi: 10.1016/j.cmet.2020.04.021

Zou, Z., Yan, Y., Shu, Y., Gao, R., Sun, Y., Li, X., et al. (2014). Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. *Nat. Commun.* 5, 1–7. doi: 10.1038/ncomms4594

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

MINI REVIEW
published: 16 December 2020
doi: 10.3389/fphys.2020.596057

COVID-19-Associated Hyper-Fibrinolysis: Mechanism and Implementations

Giris Jacob[1,2], Anat Aharon[2] and Benjamin Brenner[3]*

[1] Medicine F and Recanati Research Center, Tel Aviv Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [2] Hematologic Research Laboratory, Hematologic Department, Tel Aviv Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [3] Coagulation Research Laboratory Unit, Department of Hematology, Rambam Medical Center, Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel

The emerging novel coronavirus disease (COVID-19), which is caused by the SARS-CoV-2 presents with high infectivity, morbidity and mortality. It presenting a need for immediate understanding of its pathogenicity. Inflammation and coagulation systems are over-activated in COVID-19. SARS-CoV-2 damages endothelial cell and pneumocyte, resulting in hemostatic disorder and ARDS. An influential biomarkers of poor outcome in COVID-19 are high circulating cytokines and D-dimer level. This latter is due to hyper-fibrinolysis and hyper-coagulation. Plasmin is a key player in fibrinolysis and is involved in the cleavage of many viruses envelop proteins, including SARS-CoV. This function is similar to that of TMPRSS2, which underpins the entry of viruses into the host cell. In addition, plasmin is involved in the pathophysiology of ARDS in SARS and promotes secretion of cytokine, such as IL-6 and TNF, from activated macrophages. Here, we suggest an out-of-the-box treatment for alleviating fibrinolysis and the ARDS of COVID-19 patients. This proposed treatment is concomitant administration of an anti-fibrinolytic drug and the anticoagulant.

Keywords: SARS-CoV-2, COVID-19, fibrinolysis, coagulation, tranexamic acid

OPEN ACCESS

Edited by:
Georges Leftheriotis, Université Côte d'Azur, France

Reviewed by:
Shisan (Bob) Bao, The University of Sydney, Australia
Denis Doyen, University of Nice Sophia Antipolis, France

****Correspondence:***
Giris Jacob
jacobgi@tlvmc.gov.il

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *18 August 2020*
Accepted: *24 November 2020*
Published: *16 December 2020*

Citation:
Jacob G, Aharon A and Brenner B (2020) COVID-19-Associated Hyper-Fibrinolysis: Mechanism and Implementations. Front. Physiol. 11:596057. doi: 10.3389/fphys.2020.596057

INTRODUCTION

The emerging novel coronavirus disease (COVID-19), which is caused by the SARS-CoV-2, presents with high infectivity, morbidity, and mortality. The pressing need for understanding the virus' pathogenicity and its interaction with the body's biologic defense systems are required (Li et al., 2020). The inflammatory response and the coagulation system frequently join forces to build an effective defense against an assaulting pathogen (Arneth, 2019). Interactions between these two systems offer potential opportunities for novel therapeutic modalities. Unusually high circulating D-dimer (DDI) levels are a main predictor of poor outcome, and indicate that the coagulation and fibrinolytic systems are overactive in COVID-19 (Tang et al., 2020b; Zhou et al., 2020). This review highlights the relationship between virus infectivity and the fibrinolytic system, and suggests a potential new therapeutic modality to mitigate the virus' infectivity in COVID-19.

Etiologic-Pathogenicity of COVID-19

SARS-CoV-2 is a RNA beta-coronavirus of zoonotic origin, and is closely related to the SARS-CoV and MERS-CoV, according to whole genome sequencing (Shirato et al., 2020; Zhou et al., 2020).

The virus is highly infective and respiratory droplets are the main route of its transmission between humans (Ong et al., 2020).

The basic pathogenesis of COVID-19 shares common characteristics with that of SARS and MERS. From a clinical perspective, the airways and lungs are the most affected organs (Pan et al., 2020). Autopsies of COVID-19 patients reveal that the vascular bed is also severely affected (Fox et al., 2020). This specific tropism of SARS-CoV-2 for epithelial cells of the lungs and vascular systems could explain its infectivity.

The spike protein on the surface of the glycoprotein envelope of SARS-CoV-2 comprises two domains: a receptor-binding domain (S1), which binds with high affinity to the angiotensin-converting enzyme 2 (ACE2) receptor on the membranes of human pneumocytes and vascular endothelial cells, (Hamming et al., 2004; Whittaker and Millet, 2020; Zhang et al., 2020) and an S2 domain for anchoring the virus on target host cell membrane (Coutard et al., 2020). Based on homology to SARS-CoV, it has been reported that SARS-CoV-2 requires a host cell protease to achieve infectivity and spread (Hoffmann et al., 2020). After binding to the ACE2 receptor, the S2 protein is proteolytically activated by transmembrane serine protease 2 (TMPRSS2) in order to enter the host cell (Matsuyama et al., 2020).

COVID-19 presents with a wide spectrum of clinical severity, which ranges from a mild pneumonia to a severe disease that could result in acute respiratory distress syndrome disease-like (ARDS) (Guan et al., 2020; Wu and McGoogan, 2020). The ARDS-like feature in COVID-19 is notably different from that seen in septic patients (Yang et al., 2020). The main clinical laboratory findings associated with a poor outcome are lymphopenia, abnormal liver function test, raised serum levels of ferritin and C-reactive protein, and DDI (Shi et al., 2020; Wu et al., 2020; Zhou et al., 2020). High plasma DDI level is consistently advocated as a major predictor of mortality, which suggests that abnormal coagulation plays a key role the pathogenesis of COVID-19 (Chen et al., 2020; Grasselli et al., 2020; Tang et al., 2020a,b; Wang D. et al., 2020).

COAGULATION AND FIBRINOLYSIS: (SEE FIGURE 1)

The extrinsic pathway is triggered by tissue injury, which increases endothelial expression of activated tissue factor (aTF), which in turn activates FVII and the subsequent activation of FX, and formation of the aTF-FVIIa-FXa complex. This complex and the generated thrombin possess the ability to induce intracellular pro-inflammatory signaling via protease-activated receptor 1 and 2 (PAR 1 and 2) (Montagnana et al., 2017). On the other hand, the protein C complex, which comprises thrombin-thrombomodulin and activated protein C, deactivates FVIIIa and FVa (acceleration factors), and results in deceleration of the coagulation process (Vatsyayan et al., 2014; Yau et al., 2015). This anti-coagulation pathway (protein C complex) requires an intact vascular endothelium, which expresses the endothelial cell protein C receptor (EPCR) (Palta et al., 2014; Swieringa et al., 2018). A damaged endothelium frees EPCR (soluble EPCR), which avidly binds to the free activated protein C complex and loss of its anticoagulant moiety, promotes hypercoagulability (Ducros et al., 2012). In addition, the EPCR-Protein C complex exerts a cytoprotective effect under normal conditions (Zelaya et al., 2018). This complex activates PAR1 signaling to generate anti-inflammatory and anti-apoptotic effects (Mosnier et al., 2007; Mohan Rao et al., 2014).

The activated fibrinolytic system on endothelial cells is crucial for dissolving the fibrin clot and facilitating tissue repair. Plasmin, a serine protease, is the key-player in this system and is responsible for degrading fibrin (Olson, 2015; Iba and Levy, 2018).

COVID-19 AND COAGULATION–FIBRINOLYSIS DYSFUNCTION

Reports on patients with COVID-19 emphasize the presence of increased thrombosis and fibrinolysis and less bleeding diathesis (Huang et al., 2020; Tang et al., 2020b). An atypical disseminated intravascular coagulation (DIC) is also seen in COVID-19 (Klok et al., 2020) as thrombocytopenia, hypofibrinogemia, hemolytic anemia, and bleeding are under-represented (Fox et al., 2020; Han et al., 2020; Tang et al., 2020b). Of all the features, the most prominent prognostic factor in patients with COVID-19 is the high plasma DDI levels (Iba et al., 2017; Tang et al., 2020b; Zhou et al., 2020).

Recent clinical observations provide evidence that COVID-19 patients are at high VTE and mortality risks, and anticoagulant therapy might improve their prognosis (Paranjpe et al., 2020; Song et al., 2020; Tang et al., 2020a; Wu et al., 2020; Yin et al., 2020). General agreement exists on the need for thromboprophylaxis in majority of COVID-19 patients, and some suggest that this treatment should be continued after hospital discharge (Kollias et al., 2020).

Coagulation abnormalities include a prolonged prothrombin time, low antithrombin activity, and increased fibrinogen and DDI levels. However, the mechanisms of abnormal coagulation and fibrinolysis in COVID-19 patients are unknown (Han et al., 2020; Tang et al., 2020b).

The finding of a very high plasma DDI level is a hallmark for *hyper-fibrinolysis in COVID-19*. The strong association between high plasma DDI levels and the poor outcome raises several thoughts. Anticoagulation therapy barely reduces mortality, except in a small subgroup of patients, whose plasma DDI levels were six times greater than normal levels (Tang et al., 2020b). Alteplase is a fibrinolytic agent and when administered to patients with COVID-19-related ARDS, all died despite improvements in their oxygenation indices (Wang J. et al., 2020). This treatment was based on the results of animal studies which found that fibrinolytic drugs might improve lung function and alleviate inflammation in ARDS-like animal models (Hardaway et al., 1990; Liu et al., 2018). The present evidence does not support the use of fibrinolytic drugs in COVID-19 patients with ARDS. We can deduce that hyper-fibrinolysis

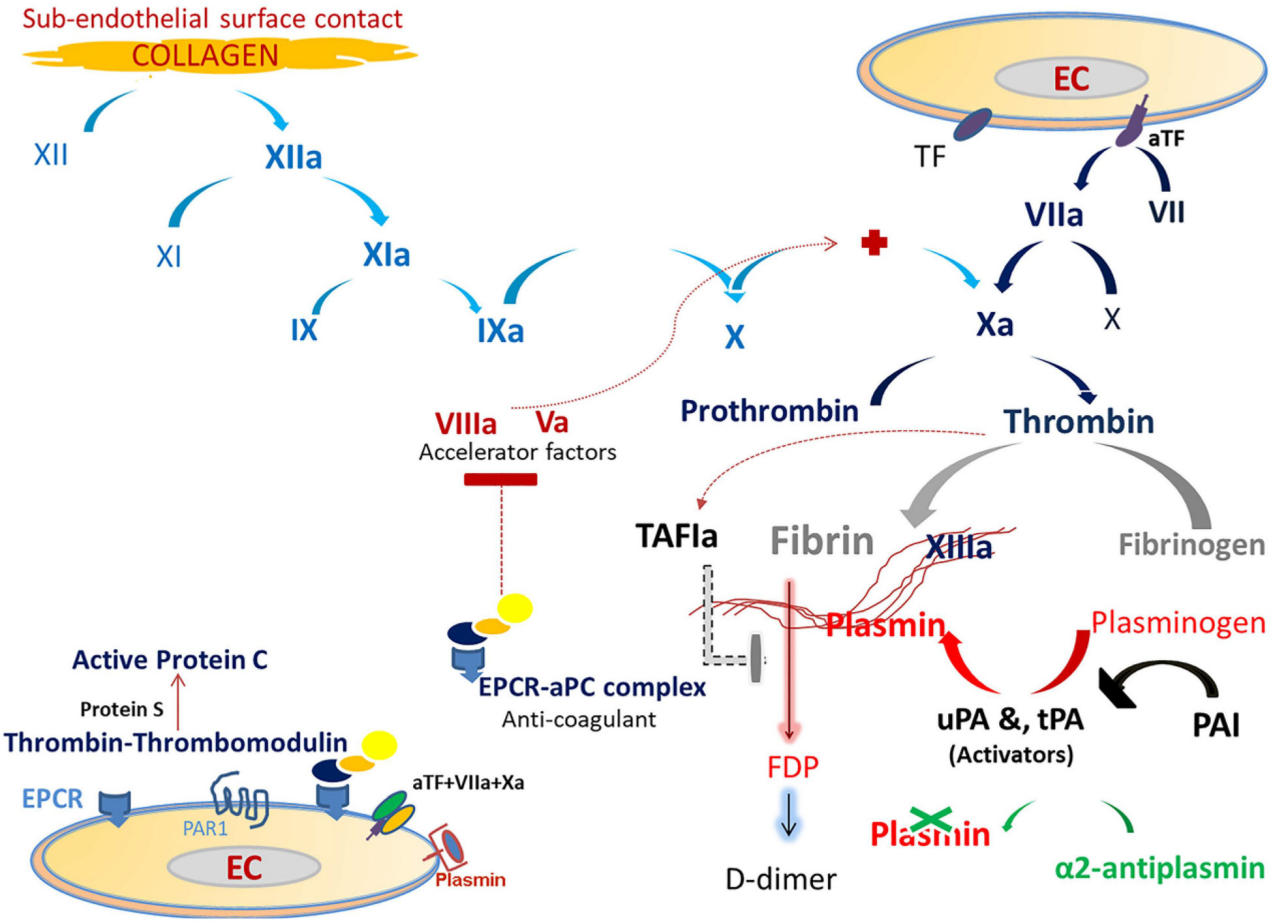


FIGURE 1 | The figure displays the coagulation and fibrinolysis processes. Traditionally, the extrinsic pathway of the main coagulation cascade depends on the release of tissue factor (TF). Injured endothelial cells (EC) by any cause results in increased expression of TF in the cell membrane. This increased expression activates FVII, which then results in TF-FVIIa binding and activating FX. FX is also activated via the intrinsic pathway (collagen path). TF-FVII-FXa is the coagulation initiation complex which binds to EC and stimulates the generation of thrombin from prothrombin. Thrombin triggers many processes, which include fibrin generation, FXIII activation, and activation of protein C. FXIIIa polymerizes fibrin to form the final stable clot. Thrombin binds to the endothelial Protein C receptor (EPCR) and combines with thrombomodulin to activate protein C (aPC) and generate the EPCR-aPC complex. This complex has anti-coagulation activity by blocking the procoagulant factors, FVa and FVIIIa (accelerator factors) and a cytoprotective effect via the protease-activated receptor 1 (PAR1). Fibrinolysis (clot solubilization) is triggered in the presence of fibrin. The conversion of circulating zymogen plasminogen to plasmin is done by endothelial enzymes and the tissue and urokinase plasminogen activators (tPA and uPA, respectively). Plasmin, a key player in fibrinolysis, is a serine protease which cleaves fibrin to generate fibrin degradation products (FDP). Plasminogen activator-inhibitor (PAI) blocks both tPA and uPA. The main plasmin inhibitor is α2-antiplasmin, which is a potent free plasmin (not bounded to fibrin) scavenger. In addition, TAFI (thrombin-activatable fibrinolysis inhibitor) attenuates plasmin generation.

plays a key role in the high pathogenicity and infectivity of SARS-CoV-2.

COMPREHENSIVE ROLE OF FIBRINOLYSIS IN COVID-19 PATHOGENICITY: (FIGURE 2)

SARS-CoV-2 binds with high avidity to the ACE2 receptor. This enzyme exerts a protective function on endothelial cells and pneumocytes, (Tikellis and Thomas, 2012) by virtue of its anti-inflammatory, anti-thrombin and anti-oxidant activity (Pai et al., 2017; Bavishi et al., 2020). A reduction in the protective effects of ACE2, as in aging, diabetes mellitus, and cardiovascular diseases, results in cellular damage and harmful consequences, with increasing oxidative stress and thrombosis (Tikellis and Thomas, 2012). Of note, administering recombinant ACE2 to ACE-deficient mice with induced lung injury protects them from developing an ARDS-like syndrome (Imai et al., 2005). The high mortality in old COVID-19 patients with comorbidities associated with endothelial dysfunction, indicates that this protective effect of ACE2 may be essential for survival (Patel and Verma, 2020; Sunden-Cullberg, 2020). Accordingly, it has been suggested that COVID-19 patients could be treated with human recombinant soluble ACE2 (Batlle et al., 2020; Kruse, 2020). Despite evidence for increased expression of ACE2 in patients with cardiovascular disease who are treated with ACE inhibitors (ACE-I) and angiotensin receptor blockers, the

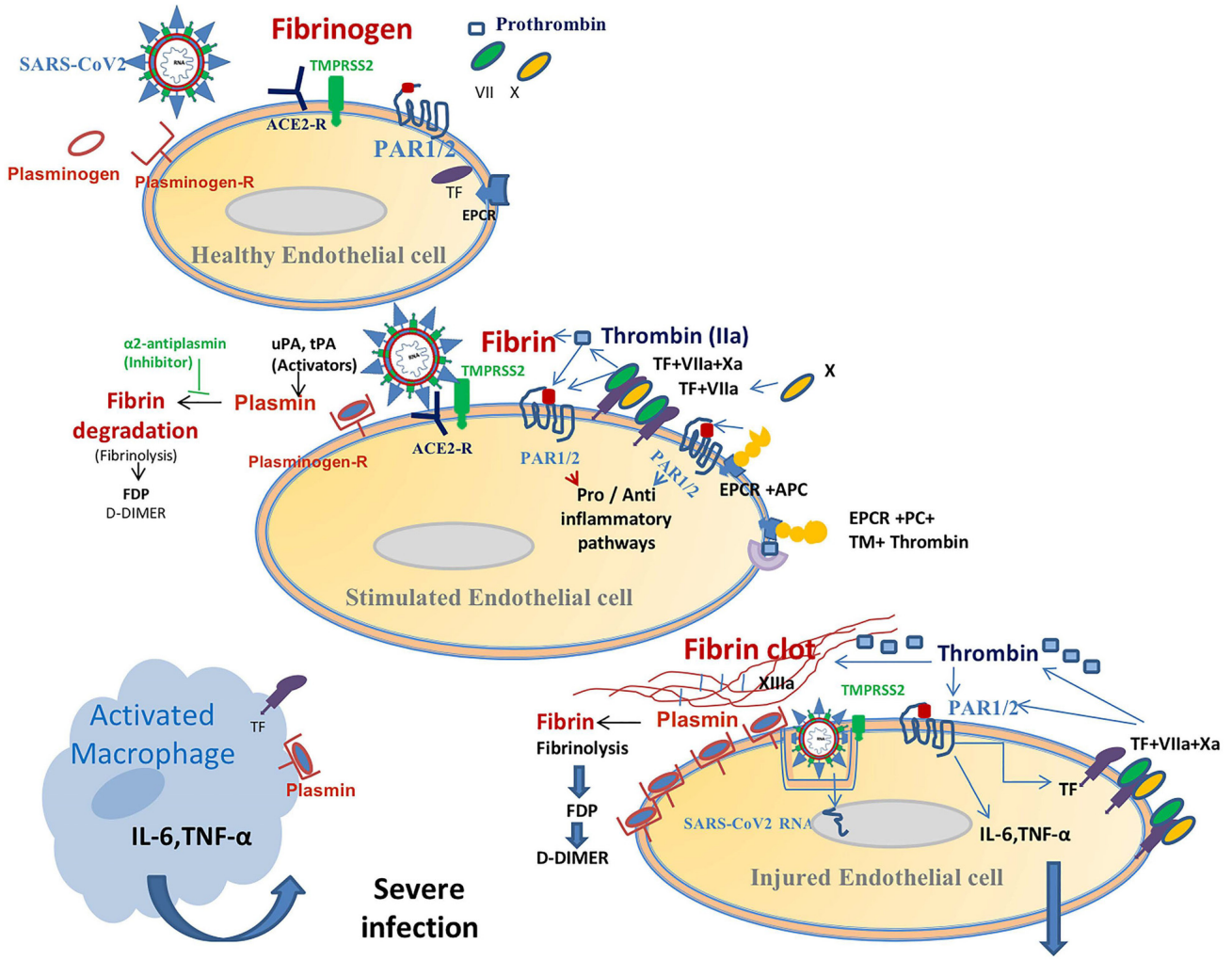


FIGURE 2 | This figure displays a suggested relationship between coagulation and inflammation in patients with a SARS-CoV-2-infection. Spike proteins in the coronavirus' envelope first bind to angiotensin-converting enzyme 2 (ACE2) receptors on the surface of epithelial cells (e.g., EC, endothelial cell). Type II transmembrane serine protease (TMPRSS2) then cleaves the spike protein in order to facilitate viral entry. The SARS-CoV-2 virus damages EC and promotes activation of the coagulation cascade (see **Figure 1**) via the TF-FVIIa (tissue factor pathway) and boosts fibrin generation (hyper-coagulation state). This hyper-activation of the fibrinolytic system results in increased plasmin production. Plasmin binds to the EC-plasmin receptor and degrades fibrin that leads to high plasma DDI levels. Membranous plasmin, which is a potent serine protease, could cleave the virus' protein S hereby aiding its entry into host cells. This shared viral cleavage function with TMPRSS2 contributes to the increased infectivity of the virus and facilitates its spread (this function has been established in many viral infections, see text). In addition, over-production of plasmin (increased plasmin-receptor occupancy) activates the protease-activated receptor 1 and 2 (PAR 1, 2) signaling pathways which are involved in inflammation and immune responses. The EPCR-protein C complex is probably overwhelmed by hypercoagulability, thereby losing its cytoprotective activity. This virus-related pro-coagulation-inflammatory state also involves macrophages. This pathophysiologic paradigm explains the increased infectivity (and replication?) of the virus and the association between high D-dimer production and the cytokine storm (mainly IL-6, IL-10, and TNF) in COVID-19.

actual impact of these drugs on COVID-19 was reported to be controversial (Danser et al., 2020; Mancia et al., 2020). Of note, ACE-Is have an anti-fibrinolytic effect in humans, (Tiryaki et al., 2010) and recent guidance recommends continuing these drugs in patients with cardiovascular diseases.

Transmembrane serine protease 2 has pivotal role in the infectivity of SARS-CoV-2. The cleavage of the coronavirus' S protein by TMPRSS2 is not exclusive for SARS-CoV-1 and 2 (Hoffmann et al., 2020; Matsuyama et al., 2020). Other viruses enter host cells by utilizing this pathway, such as the influenza H1N1 and herpes viruses (Hamming et al., 2004; Matsuyama et al., 2020). Results from *in vitro* studies showed that TMPRSS2 inhibition does not completely block virus entry into host cells (Tang et al., 2020c; Zhang et al., 2020). Camostat mesylate, a potent serine protease inhibitor which efficiently inhibits TMPRSS2, is currently under clinical investigation for reducing the virus infectivity in COVID-19 patients (Kawase et al., 2012; Hoffmann et al., 2020).

Although TMPRSS2 is the major enzyme which facilitates the entry of SARS-CoV-2 into the host cell, other serine proteases

possess this activity (Hoffmann et al., 2020; Walls et al., 2020). The serine proteases, trypsin, elastase and furin, can cleave S protein in the viral envelope of SARS-CoV and MERS-CoV (Ji et al., 2020; Rabi et al., 2020; Tang et al., 2020c). Furin is a part of the *trans*-Golgi network and is highly expressed on endothelial and pneumocyte cells, and it has been recently reported that it also cleaves SARS-CoV-2 (Braun and Sauter, 2019; Lukassen et al., 2020; Walls et al., 2020). Furin can also cleave the S protein of non-coronaviruses, such as the West Nile, Zika, and respiratory syncytial (RS) viruses (Millet and Whittaker, 2015; Coutard et al., 2020; Lukassen et al., 2020).

Plasmin, which is bound to plasmin-Receptor located on cell membranes (**Figure 2**), possesses furin-like cleavage activity (Miles et al., 1986; Kam et al., 2009; Zhao et al., 2020). Plasmin's cleavage activity was first described for the influenza H1N1 virus (Goto et al., 2001; Murakami et al., 2001). However, plasmin's cleavage activity (furin-like) on SARS-CoV-2 requires elucidation (Millet and Whittaker, 2015).

Therefore, cell entry of the virus depends on the specific binding to ACE2 and cleavage by TMPRSS2, but this latter step can be replaced by other serine proteases, such as plasmin (Kawase et al., 2012; Hoffmann et al., 2020; Zhang et al., 2020).

Findings from autopsies of severely affected COVID-19 patients include the presence of abundance of fibrin deposition, (Fox et al., 2020) which requires increased plasmin activity. In addition to its cleavage activity on viruses, plasmin can activate human macrophages promoting production of pro-inflammatory cytokines, such as IL-6, IL-8, IL-10, and TNF (Li et al., 2007). An increased plasma IL-6 level is a marker of the "cytokine release syndrome" in COVID-19 and is associated with poor outcome (Henry et al., 2020). To summarize thus far, high plasmin activity could participate in the perpetuity of virus infectivity and contribute to the excessive inflammatory and immune responses in COVID-19.

Acute respiratory distress syndrome is the most challenging clinical finding and the leading cause of death in patients with COVID-19-associated pneumonia (Wu et al., 2020). The pneumocytes and endothelial cells in the pulmonary alveoli share similar protective biologic mechanisms (Tikellis and Thomas, 2012; Nova et al., 2019). Some patients with COVID-19 present in a procoagulant state with a catastrophic microvascular injury in their lungs (Fox et al., 2020; Magro et al., 2020). The coronaviruses, MERS-CoV, SARS-CoV, and SARS-CoV-2, target cells with high expression of ACE2 and TMPRSS2, such as endothelial cells and pneumocytes (Glowacka et al., 2011). ACE2 has an important protective function in these cells. The lung injury in SARS is reported to be dependent on the balance between coagulation activity and the extent of fibrinolytic process (Gralinski et al., 2013). It is also known that plasminogen-plasmin activity is increased in ARDS (Spadaro et al., 2019). The levels of procoagulant components, in the bronchoalveolar lavage of patients with ARDS, such as plasmin and fibrinolytic degradation products, are markedly higher than in those without ARDS (Fuchs-Buder et al., 1996). A suggestion for the role of the fibrinolytic system in the genesis of ARDS in COVID-19 patients, (Idell, 2003; Spadaro et al., 2019) is supported by a the results of an investigation in plasminogen activator inhibitor-1 (PAI-1) deficient mice. The results indicate that the tPA and uPA contribute to the development the lung injury in coronaviruses infection, and PAI has a protective function in this condition (Gralinski et al., 2013). This suggest that "partial" inhibition of the hyper-fibrinolytic process in COVID-19 might mitigate the development of ARDS. An activated coagulation-plasmin-fibrin pathway in ARDS triggers a various protease secretion, such as elastase, and strong cytokine response, which is manifested by activated leukocytes and macrophages (Gralinski et al., 2013; Spadaro et al., 2019). The cytokine release syndrome has not yet been fully characterized in patients with COVID-19 (Pedersen and Ho, 2020). However, there is evidence that the high viral load in the lungs of COVID-19 patients is associated with an acute inflammatory response comprising epithelial cells and activated macrophages, which are largely responsible for the secretion of the cytokines, such as TNF, IL-6, IL-8, IL-1β, and CXCL10 (Freeman and Swartz, 2020).

Virus-Related Coagulation-Inflammation Interaction

Viruses affect the hemostatic system via activation/deactivation of platelet aggregation, coagulation, and fibrinolysis (Goeijenbier et al., 2012; Koupenova et al., 2018). There is an increasing body of evidence which shows that a viral infection orchestrates a collaborative process which connects coagulation with the inflammatory response (Goeijenbier et al., 2012). Viral infection elicits an inflammatory reaction, which in turn activates the coagulation system (Opal, 2003). Frequently, viral-related hemostasis elicits a procoagulant-thrombotic effect, such as that seen in cytomegalovirus, hepatitis C and HIV infections (van Dam-Mieras et al., 1992; Chiappetta et al., 2016). In contrast, ebola, dengue, and other hemorrhagic viruses, which can also cause endothelial damage, are associated with increased anti-coagulant effects and fatal hemorrhage (Schnittler et al., 1993; Mahanty and Bray, 2004). Viruses which damage endothelial cells can promote the generation of the *TF-VIIa-Xa-EPCR* complex (procoagulant path, **Figures 1**, **2**), which is able to activate PAR2 and trigger an innate immune response (Zelaya et al., 2018). During viral infection, PAR2 activation provokes the toll-like receptor 4 (TLR 4) to modulate the inflammatory response (Antoniak and Mackman, 2014). On the other hand, some viral infections can increase thrombin production and activate the EPCR-aPC complex, which in turn stimulates PAR1 signaling to exert a cytoprotective effect (Mosnier et al., 2007; Antoniak and Mackman, 2014).

In summary, the resultant coagulation abnormalities in viral infections depends on the effect of the virus on the balance between the pro- and anti-coagulant pathways. A virus which mainly activates the procoagulant and fibrinolytic systems could induce in a severe inflammatory response. A virus which activate the anti-coagulant pathway i.e., could induce a mild inflammatory response (Mosnier et al., 2007; Antoniak and Mackman, 2014; Nieman, 2016).

For example, patients with dengue hemorrhagic fever produce antibodies against the virus, which can activate plasminogen and fibrinolysis and contributes to bleeding

diathesis (Chuang et al., 2016). In contrast, SARS-CoV-2 causes hyper-fibrinolysis without any significant bleeding (Chen et al., 2020; Han et al., 2020).

Overview of COVID-19 Treatment

Although several therapeutic agents have been evaluated for the treatment of COVID-19, none have yet been shown to be efficacious (Sanders et al., 2020). The similarity in clinical features of coronaviruses infections offers therapeutic modalities based on SARS and MERS epidemics to clinicians. However, results on the efficacy of reported treatments in SARS and MERS are controversial (Stockman et al., 2006; Morra et al., 2018).

Lopinavir-ritonavir, an aspartate protease inhibitor combined with a CYP450 inhibitor for increasing its half-life, is reported as having no beneficial effects in COVID-19 patients (Cao et al., 2020). Ribavirin, a nucleotide analog, which blocks the viral RNA-dependent RNA polymerase, is also reported as having no beneficial effect (Morra et al., 2018). Remdisivir, a potent RNA polymerase inhibitor and whose use was compassionate, is reported to be effective in shortening the time to recovery in COVID-19 patients (Gordon et al., 2020; Grein et al., 2020).

The antimalarial drugs, chloroquine and hydroxychloroquine, which inhibit lysosomal activity and autophagy, have beneficial immunomodulatory effects (Schrezenmeier and Dorner, 2020). Hydroxychloroquine blocks the endosomal entry of SARS-CoV-2 into host cells and reduces cytokine production *in vitro* (Sinha and Balayla, 2020). Moreover, it has been reported that hydroxychloroquine (a) is not effective in preventing the development of COVID-19 after a moderate to high-risk exposure in out-patients and (b) does not affect the course of the disease in hospitalized patients (Boulware et al., 2020; Cavalcanti et al., 2020; Gautret et al., 2020; Geleris et al., 2020; Vanden Eynde, 2020).

The presence of high plasma IL-6 levels in COVID-19 patients may justify the use of tocilizumab, a monoclonal IL-6 receptor antibody, and offer a protective effect against the cytokine storm. However, its effect on virus replication and infectivity is doubtful (Biran et al., 2020; Buonaguro et al., 2020; Price et al., 2020). More recently, Japanese authors suggested treating COVID-19 patients with heparin and nafamostat mesylate, a synthetic serine protease inhibitor, which possesses anti-trypsin and anti-fibrinolytic effects (Asakura and Ogawa, 2020). Nafamostat is also being investigated because of ability to block MERS-CoV entry into host cells (Yamamoto et al., 2016).

Mechanism-Based Proposed Treatment: (see Figure 2)

In light of the present need, it enables the use of an unconventional treatment for COVID-19 patients. Since a procoagulant state and hyper-fibrinolysis co-exist in COVID-19 (Tang et al., 2020b), we assume *that the increased fibrinolysis boosts the infectivity of SARS-CoV-2* via the *plasmin-mediated pathway.* In addition, plasmin elicits a pro-inflammatory response by activating macrophages (releasing IL-6, and TNF) and increases PAR2-TLR4 signaling (Li et al., 2007; Antoniak and Mackman, 2014). Moreover, the increased mortality in COVID-19 is associated with conditions, which are associated with endothelial dysfunction, low ACE2 expression, and high circulating plasminogen levels (Tikellis and Thomas, 2012; Derhaschnig et al., 2013).

We suggest that pharmacologic interventions whose aim is to reduce plasmin production may decrease virus infectivity and attenuate the associated inflammatory condition in COVID-19 patients.

Tranexamic acid (TA) competitively inhibits the activation of plasminogen (via binding to the kringle domain), thereby reducing the conversion of plasminogen to plasmin, which in turn results in lowering circulating DDI levels. TA is used to treat individuals with bleeding diathesis and can be administered, intravenously, orally, or locally. It can also be administered by inhalation to control pulmonary hemorrhage. TA's half-life is ∼2-3 h, and is mainly eliminated in urine (McCormack, 2012). The efficacy of the inhaled route of administration has been tested in patients with hemoptysis. The investigators reported that inhaled TA was effective and safe for hemoptysis resolution (Wand et al., 2018). Similar results were obtained in patients with hemoptysis who were treated with oral or intravenous TA (Solomonov et al., 2009; Prutsky et al., 2016).

Tranexamic acid is well tolerated and the occurrence of adverse effects, such as mild to moderate headache, muscle cramp and arthralgia, nausea, and diarrhea, are uncommon (Freeman et al., 2011). While inhibition of fibrinolysis could increase thrombotic risk, there is no reported evidence for thrombo-embolism with the use of TA.

For the past two decades, TA has been used in combination with prophylactic anticoagulation (low dose warfarin, LMWH, and DOACs) in elderly patients undergoing major orthopedic surgery with high risk for thrombosis and hemorrhage (Wang et al., 2018; Tang et al., 2019). This combined therapeutic modality offers anticoagulant, anti-fibrinolytic and anti-inflammatory effects, thereby reducing thrombosis, bleeding and indices of inflammation (Gillette et al., 2013; Karampinas et al., 2019). *Hence, our suggestion is to use TA to treat patients with moderate to severe COVID-19-associated pneumonia.* TA could be administered through a systemic route or by using a closed-nebulizer (Wand et al., 2018). All COVID-19 patients should receive intensification of anticoagulant dosing (Barnes et al., 2020; Thachil et al., 2020; Zhai et al., 2020).

The thrombotic burden in COVID-19 patients increases with disease severity. Thus, the suggested intervention with TA should exclude critically ill patients. Future studies should address the timing of the intervention in light of the emerging data on SARS-CoV-2 dynamics and COVID-19 features (Sun et al., 2020; Zheng et al., 2020).

Administering *alpha 2-antiplasmin* (alpha 2-AP) is an alternative treatment to alleviate the respiratory distress of COVID-19 patients. Alpha 2-AP is a potent plasmin scavenger and is usually used as alpha 2-AP replacement therapy for patients with a homozygous alpha 2-AP deficiency. These patients are hemophilia-like and tend to bleed mainly after surgery and alpha 2-AP replacement therapy is the only efficient treatment for these patients (Saes et al., 2018). Therefore, this treatment should be reserved for those critically ill COVID-19 patients with low plasma alpha 2-AP levels.

SUMMARY

This proposed mechanisms and treatment modality are founded on a comprehensive review of reported investigations on the interactions between the coagulation-fibrinolysis and inflammation pathways in coronaviruses diseases. The severity of COVID-19-associated pneumonia places the patient at risk for irreversible ARDS and death. A balanced assessment of the risk-benefit ratio in a deteriorating patient with COVID-19 sometimes requires the implementation of an out-of-the-box treatment in the absence of an alternative proven treatment.

AUTHOR CONTRIBUTIONS

GJ: idea design and writing. AA: protein C expert and consultant. BB: writing and consultant as world expert in homeostasis. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by the Yahel Foundation and Recanati Research Center.

REFERENCES

Antoniak, S., and Mackman, N. (2014). Multiple roles of the coagulation protease cascade during virus infection. *Blood* 123, 2605–2613. doi: 10.1182/blood-2013-09-526277

Arneth, B. (2019). Coevolution of the coagulation and immune systems. *Inflamm. Res.* 68, 117–123. doi: 10.1007/s00011-018-01210-y

Asakura, H., and Ogawa, H. (2020). Potential of heparin and nafamostat combination therapy for COVID-19. *J. Thromb. Haemost.* 18, 1521–1522. doi: 10.1111/jth.14858

Barnes, G. D., Burnett, A., Allen, A., Blumenstein, M., Clark, N. P., Cuker, A., et al. (2020). Thromboembolism and anticoagulant therapy during the COVID-19 pandemic: interim clinical guidance from the anticoagulation forum. *J. Thromb. Thrombolysis* 50, 72–81. doi: 10.1007/s11239-020-02138-z

Batlle, D., Wysocki, J., and Satchell, K. (2020). Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? *Clin. Sci.* 134, 543–545. doi: 10.1042/CS20200163

Bavishi, C., Maddox, T. M., and Messerli, F. H. (2020). Coronavirus Disease 2019 (COVID-19) infection and renin angiotensin system blockers. *JAMA Cardiol.* 5, 745–747. doi: 10.1001/jamacardio.2020.1282

Biran, N., Ip, A., Ahn, J., Go, R. C., Wang, S., Mathura, S., et al. (2020). Tocilizumab among patients with COVID-19 in the intensive care unit: a multicentre observational study. *Lancet Rheumatol.* 2, e603–e612. doi: 10.1016/S2665-9913(20)30277-0

Boulware, D. R., Pullen, M. F., Bangdiwala, A. S., Pastick, K. A., Lofgren, S. M., Okafor, E. C., et al. (2020). A randomized trial of hydroxychloroquine as postexposure prophylaxis for COVID-19. *N. Engl. J. Med.* 383, 517–525. doi: 10.1056/NEJMoa2016638

Braun, E., and Sauter, D. (2019). Furin-mediated protein processing in infectious diseases and cancer. *Clin. Transl. Immunol.* 8, e1073. doi: 10.1002/cti2.1073

Buonaguro, F. M., Puzanov, I., and Ascierto, P. A. (2020). Anti-Il6R role in treatment of COVID-19-related Ards. *J. Transl. Med.* 18:165. doi: 10.1186/s12967-020-02333-9

Cao, B., Wang, Y., Wen, D., Liu, W., Wang, J., Fan, G., et al. (2020). A trial of Lopinavir-Ritonavir in adults hospitalized with severe COVID-19. *N. Engl. J. Med.* 382, 1787–1799. doi: 10.1056/NEJMoa2001282

Cavalcanti, A. B., Zampieri, F. G., Rosa, R. G., Azevedo, L. C. P., Veiga, V. C., Avezum, A., et al. (2020). Hydroxychloroquine with or without Azithromycin in Mild-to-Moderate COVID-19. *N Engl J Med.* 383, 2041–2052. doi: 10.1056/NEJMoa2019014

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395, 507–513. doi: 10.1016/S0140-6736(20)30211-7

Chiappetta, S., Ripa, M., Galli, L., Razzari, C., Longo, V., Galli, A., et al. (2016). Soluble endothelial protein C receptor (sepcr) as an inflammatory biomarker in naive Hiv-infected patients during Art. *J. Antimicrob. Chemother.* 71, 1627–1631. doi: 10.1093/jac/dkw010

Chuang, Y. C., Lin, J., Lin, Y. S., Wang, S., and Yeh, T. M. (2016). Dengue virus nonstructural protein 1-induced antibodies cross-react with human plasminogen and enhance its activation. *J. Immunol.* 196, 1218–1226. doi: 10.4049/jimmunol.1500057

Coutard, B., Valle, C., De Lamballerie, X., Canard, B., Seidah, N. G., and Decroly, E. (2020). The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Res.* 176:104742.

Danser, A. H. J., Epstein, M., and Batlle, D. (2020). Renin-angiotensin system blockers and the COVID-19 pandemic: at present there is no evidence to abandon renin-angiotensin system blockers. *Hypertension* 75, 1382–1385. doi: 10.1161/HYPERTENSIONAHA.120.15082

Derhaschnig, U., Testori, C., Riedmueller, E., Aschauer, S., Wolzt, M., and Jilma, B. (2013). Hypertensive emergencies are associated with elevated markers of inflammation, coagulation, platelet activation and fibrinolysis. *J. Hum. Hypertens* 27, 368–373. doi: 10.1038/jhh.2012.53

Ducros, E., Mirshahi, S. S., Faussat, A. M., Mirshahi, P., Dimicoli, S., Tang, R., et al. (2012). Soluble endothelial protein C receptor (sepcr) is likely a biomarker of cancer-associated hypercoagulability in human hematologic malignancies. *Cancer Med* 1, 261–267. doi: 10.1002/cam4.11

Fox, S. E., Akmatbekov, A., Harbert, J. L., Li, G., Brown, J. Q., and Vander Heide, R. S. (2020). Pulmonary and cardiac pathology in COVID-19: the first autopsy series from new Orleans. *medRxivn* [Preprint]. doi: 10.1101/2020.04.06.20050575

Freeman, E. W., Lukes, A., Vandrie, D., Mabey, R. G., Gersten, J., and Adomako, T. L. (2011). A dose-response study of a novel, oral tranexamic formulation for heavy menstrual bleeding. *Am. J. Obstet. Gynecol.* 205:319.e1-7. doi: 10.1016/j.ajog.2011.05.015

Freeman, T. L., and Swartz, T. H. (2020). Targeting the NLRP3 Inflammasome in Severe COVID-19. *Front. Immunol.* 11:1518. doi: 10.3389/fimmu.2020.01518

Fuchs-Buder, T., De Moerloose, P., Ricou, B., Reber, G., Vifian, C., Nicod, L., et al. (1996). Time course of procoagulant activity and D dimer in bronchoalveolar fluid of patients at risk for or with acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 153, 163–167. doi: 10.1164/ajrccm.153.1.8542111

Gautret, P., Lagier, J. C., Parola, P., Hoang, V. T., Meddeb, L., Mailhe, M., et al. (2020). Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int. J. Antimicrob. Agents* 56:105949. doi: 10.1016/j.ijantimicag.2020.105949

Geleris, J., Sun, Y., Platt, J., Zucker, J., Baldwin, M., Hripcsak, G., et al. (2020). Observational study of hydroxychloroquine in hospitalized patients with COVID-19. *N. Engl. J. Med.* 382, 2411–2418. doi: 10.1056/NEJMoa2012410

Gillette, B. P., Desimone, L. J., Trousdale, R. T., Pagnano, M. W., and Sierra, R. J. (2013). Low risk of thromboembolic complications with tranexamic acid after primary total hip and knee arthroplasty. *Clin. Orthop. Relat. Res.* 471, 150–154. doi: 10.1007/s11999-012-2488-z

Glowacka, I., Bertram, S., Müller, M. A., Allen, P., Soilleux, E., Pfefferle, S., et al. (2011). Evidence that Tmprss3 acitaves the severs respiratory syndrome coronavirus soike protein for membrane fusion and reduces viral control by the humoral immune response. *J Virol.* 85, 4122–4134. doi: 10.1128/JVI.02232-10

Goeijenbier, M., Van Wissen, M., Van De Weg, C., Jong, E., Gerdes, V. E., Meijers, J. C., et al. (2012). Review: viral infections and mechanisms of thrombosis and bleeding. *J. Med. Virol.* 84, 1680–1696. doi: 10.1002/jmv.23354

Gordon, C. J., Tchesnokov, E. P., Woolner, E., Perry, J. K., Feng, J. Y., Porter, D. P., et al. (2020). Remdesivir is a direct-acting antiviral that inhibits Rna-dependent Rna polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J. Biol. Chem.* 295, 6785–6797. doi: 10.1074/jbc.RA120.013679

Goto, H., Wells, K., Takada, A., and Kawaoka, Y. (2001). Plasminogen-binding activity of neuraminidase determines the pathogenicity of influenza A virus. *J. Virol.* 75, 9297–9301. doi: 10.1128/JVI.75.19.9297-9301.2001

Gralinski, L. E., Bankhead, A. III, Jeng, S., Menachery, V. D., Proll, S., Belisle, S. E., et al. (2013). Mechanisms of severe acute respiratory syndrome coronavirus-induced acute lung injury. *mBio* 4, e271–e213. doi: 10.1128/mBio.00271-13

Grasselli, G., Zangrillo, A., Zanella, A., Antonelli, M., Cabrini, L., Castelli, A., et al. (2020). Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to icus of the lombardy region, Italy. *JAMA* 323, 1574–1581. doi: 10.1001/jama.2020.5394

Grein, J., Ohmagari, N., Shin, D., Diaz, G., Asperges, E., Castagna, A., et al. (2020). Compassionate use of remdesivir for patients with severe COVID-19. *N. Engl. J. Med.* 382, 2327–2336. doi: 10.1056/NEJMoa2007016

Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W. H., Ou, C. Q., He, J. X., et al. (2020). Clinical characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* 382, 1708–1720. doi: 10.1056/NEJMoa2002032

Hamming, I., Timens, W., Bulthuis, M. L., Lely, A. T., Navis, G., and Van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203, 631–637. doi: 10.1002/path.1570

Han, H., Yang, L., Liu, R., Liu, F., Wu, K. L., Li, J., et al. (2020). Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. *Clin. Chem. Lab. Med.* 58, 1116–1120. doi: 10.1515/cclm-2020-0188

Hardaway, R. M., Williams, C. H., Marvasti, M., Farias, M., Tseng, A., Pinon, I., et al. (1990). Prevention of adult respiratory distress syndrome with plasminogen activator in pigs. *Crit. Care Med.* 18, 1413–1418. doi: 10.1097/00003246-199012000-00021

Henry, B. M., De Oliveira, M. H. S., Benoit, S., Plebani, M., and Lippi, G. (2020). Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin. Chem. Lab. Med.* 58, 1021–1028. doi: 10.1515/cclm-2020-0369

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271.e8–280.e8. doi: 10.1016/j.cell.2020.02.052

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan. *China. Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Iba, T., and Levy, J. H. (2018). Inflammation and thrombosis: roles of neutrophils, platelets and endothelial cells and their interactions in thrombus formation during sepsis. *J. Thromb. Haemost.* 16, 231–241. doi: 10.1111/jth.13911

Iba, T., Nisio, M. D., Levy, J. H., Kitamura, N., and Thachil, J. (2017). New criteria for sepsis-induced coagulopathy (Sic) following the revised sepsis definition: a retrospective analysis of a nationwide survey. *BMJ Open* 7:e017046. doi: 10.1136/bmjopen-2017-017046

Idell, S. (2003). Coagulation, fibrinolysis, and fibrin deposition in acute lung injury. *Crit. Care Med.* 31, S213–S220. doi: 10.1097/01.CCM.0000057846.21303.AB

Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., et al. (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 436, 112–116. doi: 10.1038/nature03712

Ji, H. L., Zhao, R., Matalon, S., and Matthay, M. A. (2020). Elevated Plasmin(ogen) as a common risk factor for COVID-19 susceptibility. *Physiol. Rev.* 100, 1065–1075. doi: 10.1152/physrev.00013.2020

Kam, Y. W., Okumura, Y., Kido, H., Ng, L. F., Bruzzone, R., and Altmeyer, R. (2009). Cleavage of the SARS coronavirus spike glycoprotein by airway proteases enhances virus entry into human bronchial epithelial cells in vitro. *PLoS One* 4:e7870. doi: 10.1371/journal.pone.0007870

Karampinas, P. K., Megaloikonomos, P. D., Lampropoulou-Adamidou, K., Papadelis, E. G., Mavrogenis, A. F., Vlamis, J. A., et al. (2019). Similar thromboprophylaxis with rivaroxaban and low molecular weight heparin but fewer hemorrhagic complications with combined intra-articular and intravenous tranexamic acid in total knee arthroplasty. *Eur. J. Orthop. Surg. Traumatol.* 29, 455–460. doi: 10.1007/s00590-018-2307-7

Kawase, M., Shirato, K., Van Der Hoek, L., Taguchi, F., and Matsuyama, S. (2012). Simultaneous treatment of human bronchial epithelial cells with serine and cysteine protease inhibitors prevents severe acute respiratory syndrome coronavirus entry. *J. Virol.* 86, 6537–6545. doi: 10.1128/JVI.00094-12

Klok, F. A., Kruip, M. J. H. A., van der Meer, N. J. M., Arbous, M. S., Gommers, D. A. M. P. J., Kant, K. M., et al. (2020). Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb. Res.* 191, 145–147. doi: 10.1016/j.thromres.2020.04.013

Kollias, A., Kyriakoulis, K. G., Dimakakos, E., Poulakou, G., Stergiou, G. S., and Syrigos, K. (2020). Thromboembolic risk and anticoagulant therapy in COVID-19 patients: emerging evidence and call for action. *Br. J. Haematol.* 189, 846–847. doi: 10.1111/bjh.16727

Koupenova, M., Clancy, L., Corkrey, H. A., and Freedman, J. E. (2018). Circulating platelets as mediators of immunity, inflammation, and thrombosis. *Circ. Res.* 122, 337–351. doi: 10.1161/CIRCRESAHA.117.310795

Kruse, R. L. (2020). Therapeutic strategies in an outbreak scenario to treat the novel coronavirus originating in Wuhan, China. *F1000Res* 9:72.

Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., et al. (2020). Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N. Engl. J. Med.* 382, 1199–1207. doi: 10.1056/NEJMoa2001316

Li, Q., Laumonnier, Y., Syrovets, T., and Simmet, T. (2007). Plasmin triggers cytokine induction in human monocyte-derived macrophages. *Arterioscler. Thromb. Vasc. Biol.* 27, 1383–1389. doi: 10.1161/ATVBAHA.107.142901

Liu, C., Ma, Y., Su, Z., Zhao, R., Zhao, X., Nie, H. G., et al. (2018). Meta-analysis of preclinical studies of fibrinolytic therapy for acute lung injury. *Front. Immunol.* 9:1898. doi: 10.3389/fimmu.2018.01898

Lukassen, S., Lorenz Chua, R., Trefzer, T., Kahn, N. C., Schneider, M. A., Muley, T., et al. (2020). SARS-CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. *Embo. J.* 39:e105114. doi: 10.15252/embj.2020105114

Magro, C., Mulvey, J. J., Berlin, D., Nuovo, G., Salvatore, S., Harp, J., et al. (2020). Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. *Transl. Res.* 220, 1–13. doi: 10.1016/j.trsl.2020.04.007

Mahanty, S., and Bray, M. (2004). Pathogenesis of filoviral haemorrhagic fevers. *Lancet Infect. Dis.* 4, 487–498. doi: 10.1016/S1473-3099(04)01103-X

Mancia, G., Rea, F., Ludergnani, M., Apolone, G., and Corrao, G. (2020). Renin-angiotensin-aldosterone system blockers and the risk of COVID-19. *N. Engl. J. Med.* 382, 2431–2440. doi: 10.1056/NEJMoa2006923

Matsuyama, S., Nao, N., Shirato, K., Kawase, M., Saito, S., Takayama, I., et al. (2020). Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. *Proc. Natl. Acad. Sci. U.S.A.* 117, 7001–7003. doi: 10.1073/pnas.2002589117

McCormack, P. L. (2012). Tranexamic acid: a review of its use in the treatment of hyperfibrinolysis. *Drugs* 72, 585–617. doi: 10.2165/11209070-000000000-00000

Miles, L. A., Ginsberg, M. H., White, J. G., and Plow, E. F. (1986). Plasminogen interacts with human platelets through two distinct mechanisms. *J. Clin. Invest.* 77, 2001–2009. doi: 10.1172/JCI112529

Millet, J. K., and Whittaker, G. R. (2015). Host cell proteases: critical determinants of coronavirus tropism and pathogenesis. *Virus Res.* 202, 120–134. doi: 10.1016/j.virusres.2014.11.021

Mohan Rao, L. V., Esmon, C. T., and Pendurthi, U. R. (2014). Endothelial cell protein C receptor: a multiliganded and multifunctional receptor. *Blood* 124, 1553–1562. doi: 10.1182/blood-2014-05-578328

Montagnana, M., Lippi, G., and Danese, E. (2017). An overview of thrombophilia and associated laboratory testing. *Methods Mol. Biol.* 1646, 113–135. doi: 10.1007/978-1-4939-7196-1_9

Morra, M. E., Van Thanh, L., Kamel, M. G., Ghazy, A. A., Altibi, A. M. A., Dat, L. M., et al. (2018). Clinical outcomes of current medical approaches for Middle East respiratory syndrome: a systematic review and meta-analysis. *Rev. Med. Virol.* 28:e1977. doi: 10.1002/rmv.1977

Mosnier, L. O., Zlokovic, B. V., and Griffin, J. H. (2007). The cytoprotective protein C pathway. *Blood* 109, 3161–3172. doi: 10.1182/blood-2006-09-003004

Murakami, M., Towatari, T., Ohuchi, M., Shiota, M., Akao, M., Okumura, Y., et al. (2001). Mini-plasmin found in the epithelial cells of bronchioles triggers infection by broad-spectrum influenza A viruses and Sendai virus. *Eur. J. Biochem.* 268, 2847–2855. doi: 10.1046/j.1432-1327.2001.02166.x

Nieman, M. T. (2016). Protease-activated receptors in hemostasis. *Blood* 128, 169–177. doi: 10.1182/blood-2015-11-636472

Nova, Z., Skovierova, H., and Calkovska, A. (2019). Alveolar-capillary membrane-related pulmonary cells as a target in endotoxin-induced acute lung injury. *Int. J. Mol. Sci.* 20:831. doi: 10.3390/ijms20040831

Olson, J. D. (2015). D-dimer: an overview of hemostasis and fibrinolysis, assays, and clinical applications. *Adv. Clin. Chem.* 69, 1–46. doi: 10.1016/bs.acc.2014.12.001

Ong, S. W. X., Tan, Y. K., Chia, P. Y., Lee, T. H., Ng, O. T., Wong, M. S. Y., et al. (2020). Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) from a symptomatic patient. *JAMA* 323, 1610–1612. doi: 10.1001/jama.2020.3227

Opal, S. M. (2003). Interactions between coagulation and inflammation. *Scand. J. Infect. Dis.* 35, 545–554. doi: 10.1080/00365540310015638

Pai, W. Y., Lo, W. Y., Hsu, T., Peng, C. T., and Wang, H. J. (2017). Angiotensin-(1-7) inhibits thrombin-induced endothelial phenotypic changes and reactive oxygen species production via nadph oxidase 5 downregulation. *Front. Physiol.* 8:994. doi: 10.3389/fphys.2017.00994

Palta, S., Saroa, R., and Palta, A. (2014). Overview of the coagulation system. *Indian J. Anaesth* 58, 515–523. doi: 10.4103/0019-5049.144643

Pan, Y., Zhang, D., Yang, P., Poon, L. L. M., and Wang, Q. (2020). Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect. Dis.* 20, 411–412. doi: 10.1016/S1473-3099(20)30113-4

Paranjpe, I., Fuster, V., Lala, A., Russak, A. J., Glicksberg, B. S., Levin, M. A., et al. (2020). Association of treatment dose anticoagulation with in-hospital survival among hospitalized patients With COVID-19. *J. Am. Coll. Cardiol.* 76, 122–124. doi: 10.1016/j.jacc.2020.05.001

Patel, A. B., and Verma, A. (2020). COVID-19 and angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: what is the evidence? *JAMA* 323, 1769–1770. doi: 10.1001/jama.2020.4812

Pedersen, S. F., and Ho, Y. C. (2020). SARS-CoV-2: a storm is raging. *J. Clin. Invest.* 130, 2202–2205. doi: 10.1172/JCI137647

Price, C. C., Altice, F. L., Shyr, Y., Koff, A., Pischel, L., Goshua, G., et al. (2020). Tocilizumab treatment for cytokine release syndrome in hospitalized patients with Coronavirus Disease 2019: survival and clinical outcomes. *Chest* 158, 1397–1408. doi: 10.1016/j.chest.2020.06.006

Prutsky, G., Domecq, J. P., Salazar, C. A., and Accinelli, R. (2016). Antifibrinolytic therapy to reduce haemoptysis from any cause. *Cochrane Database Syst. Rev.* 11:Cd008711. doi: 10.1002/14651858.CD008711.pub3

Rabi, F. A., Al Zoubi, M. S., Kasasbeh, G. A., Salameh, D. M., and Al-Nasser, A. D. (2020). SARS-CoV-2 and Coronavirus Disease 2019: what we know so far. *Pathogens* 9:231. doi: 10.3390/pathogens9030231

Saes, J. L., Schols, S. E. M., Van Heerde, W. L., and Nijziel, M. R. (2018). Hemorrhagic disorders of fibrinolysis: a clinical review. *J. Thromb. Haemost.* doi: 10.1111/jth.14160 [Epub ahead of print].

Sanders, J. M., Monogue, M. L., Jodlowski, T. Z., and Cutrell, J. B. (2020). Pharmacologic treatments for Coronavirus Disease 2019 (COVID-19): a review. *JAMA* 323, 1824–1836.

Schnittler, H. J., Mahner, F., Drenckhahn, D., Klenk, H. D., and Feldmann, H. (1993). Replication of Marburg virus in human endothelial cells. A possible mechanism for the development of viral hemorrhagic disease. *J. Clin. Invest.* 91, 1301–1309. doi: 10.1172/JCI116329

Schrezenmeier, E., and Dorner, T. (2020). Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. *Nat. Rev. Rheumatol.* 16, 155–166. doi: 10.1038/s41584-020-0372-x

Shi, S., Qin, M., Shen, B., Cai, Y., Liu, T., Yang, F., et al. (2020). Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* 5, 802–810. doi: 10.1001/jamacardio.2020.0950

Shirato, K., Nao, N., Katano, H., Takayama, I., Saito, S., Kato, F., et al. (2020). Development of genetic diagnostic methods for novel Coronavirus 2019 (nCoV-2019) in Japan. *JPN J. Infect. Dis.* 73, 304–307. doi: 10.7883/yoken.JJID.2020.061

Sinha, N., and Balayla, G. (2020). Hydroxychloroquine and COVID-19. *Postgrad. Med. J.* 96, 550–555. doi: 10.1136/postgradmedj-2020-137785

Solomonov, A., Fruchter, O., Zuckerman, T., Brenner, B., and Yigla, M. (2009). Pulmonary hemorrhage: a novel mode of therapy. *Respir. Med.* 103, 1196–1200. doi: 10.1016/j.rmed.2009.02.004

Song, J. C., Wang, G., Zhang, W., Zhang, Y., Li, W. Q., Zhou, Z., et al. (2020). Chinese expert consensus on diagnosis and treatment of coagulation dysfunction in COVID-19. *Mil. Med. Res.* 7:19. doi: 10.1186/s40779-020-00247-7

Spadaro, S., Park, M., Turrini, C., Tunstall, T., Thwaites, R., Mauri, T., et al. (2019). Biomarkers for acute respiratory distress syndrome and prospects for personalised medicine. *J. Inflamm.* 16;1. doi: 10.1186/s12950-018-0202-y

Stockman, L. J., Bellamy, R., and Garner, P. (2006). SARS: systematic review of treatment effects. *PLoS Med.* 3:e343. doi: 10.1371/journal.pmed.0030343

Sun, J., Tang, X., Bai, R., Liang, C., Zeng, L., Lin, H., et al. (2020). The kinetics of viral load and antibodiesto SARS-CoV-2. *Clin. Microbiol. Infect.* 26, 1690.e1–1690.e4. doi: 10.1016/j.cmi.2020.08.043

Sunden-Cullberg, J. (2020). Chronic use of angiotensin-converting enzyme inhibitors and angiotensin ii receptor blockers is high among intensive care unit patients with non-COVID-19 sepsis but carry a moderately increased risk of death. *Hypertension* 75, e15–e16. doi: 10.1161/HYPERTENSIONAHA.120.15178

Swieringa, F., Spronk, H. M. H., Heemskerk, J. W. M., and Van Der Meijden, P. E. J. (2018). Integrating platelet and coagulation activation in fibrin clot formation. *Res. Pract. Thromb. Haemost.* 2, 450–460. doi: 10.1002/rth2.12107

Tang, N., Bai, H., Chen, X., Gong, J., Li, D., and Sun, Z. (2020a). Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J. Thromb. Haemost.* 18, 1520–1521. doi: 10.1111/jth.14851

Tang, N., Li, D., Wang, X., and Sun, Z. (2020b). Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J. Thromb. Haemost.* 18, 844–847. doi: 10.1111/jth.14768

Tang, T., Bidon, M., Jaimes, J. A., Whittaker, G. R., and Daniel, S. (2020c). Coronavirus membrane fusion mechanism offers as a potential target for antiviral development. *Antiviral Res.* 178:104792. doi: 10.1016/j.antiviral.2020.104792

Tang, Y., Wen, Y., Li, W., Li, H., Yang, Y., and Liu, Y. (2019). The efficacy and safety of multiple doses of oral tranexamic acid on blood loss, inflammatory and fibrinolysis response following total knee arthroplasty: a randomized controlled trial. *Int. J. Surg.* 65, 45–51. doi: 10.1016/j.ijsu.2019.03.011

Thachil, J., Tang, N., Gando, S., Falanga, A., Cattaneo, M., Levi, M., et al. (2020). Isth interim guidance on recognition and management of coagulopathy in COVID-19. *J. Thromb. Haemost.* 18, 1023–1026. doi: 10.1111/jth.14810

Tikellis, C., and Thomas, M. C. (2012). Angiotensin-converting enzyme 2 (ACE2) is a key modulator of the renin angiotensin system in health and Disease. *Int. J. Pept.* 2012:256294.

Tiryaki, O., Usalan, C., and Buyukhatipoglu, H. (2010). Effect of combined angiotensin-converting enzyme and aldosterone inhibition on plasma plasminogen activator inhibitor type 1 levels in chronic hypertensive patients. *Nephrology* 15, 211–215. doi: 10.1111/j.1440-1797.2009.01181.x

van Dam-Mieras, M. C., Muller, A. D., Van Hinsbergh, V. W., Mullers, W. J., Bomans, P. H., and Bruggeman, C. A. (1992). The procoagulant response of cytomegalovirus infected endothelial cells. *Thromb. Haemost.* 68, 364–370. doi: 10.1055/s-0038-1656381

Vanden Eynde, J. J. (2020). COVID-19: a brief overview of the discovery clinical trial. *Pharmaceuticals* 13:65. doi: 10.3390/ph13040065

Vatsyayan, R., Kothari, H., Mackman, N., Pendurthi, U. R., and Rao, L. V. (2014). Inactivation of factor Viia by antithrombin in vitro, ex vivo and in vivo: role of tissue factor and endothelial cell protein C receptor. *PLoS One* 9:e103505. doi: 10.1371/journal.pone.0103505

Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., Mcguire, A. T., and Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 181, 281.e6–292.e6. doi: 10.1016/j.cell.2020.02.058

Wand, O., Guber, E., Guber, A., Epstein Shochet, G., Israeli-Shani, L., and Shitrit, D. (2018). Inhaled tranexamic acid for hemoptysis treatment: a randomized controlled trial. *Chest* 154, 1379–1384. doi: 10.1016/j.chest.2018.09.026

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel Coronavirus-infected pneumonia in Wuhan, China. *JAMA* 323, 1061–1069. doi: 10.1001/jama.2020.1585

Wang, J., Hajizadeh, N., Moore, E. E., Mcintyre, R. C., Moore, P. K., Veress, L. A., et al. (2020). Tissue plasminogen activator (tpa) treatment for COVID-19 associated acute respiratory distress syndrome (Ards): a case series. *J Thromb Haemost.* 18, 1752–1755. doi: 10.1111/jth.14828

Wang, D., Luo, Z. Y., Yu, Z. P., Liu, L. X., Chen, C., Meng, W. K., et al. (2018). The antifibrinolytic and anti-inflammatory effects of multiple doses of oral tranexamic acid in total knee arthroplasty patients: a randomized controlled trial. *J. Thromb. Haemost.* 16, 2442–2453. doi: 10.1111/jth.14316

Whittaker, G. R., and Millet, J. K. (2020). Biochemical characterization of middle east respiratory syndrome coronavirus spike protein proteolytic processing. *Methods Mol. Biol.* 2099, 21–37. doi: 10.1007/978-1-0716-0211-9_3

Wu, C., Chen, X., Cai, Y., Xia, J., Zhou, X., Xu, S., et al. (2020). Risk factors associated with acute respiratory distress syndrome and death in patients with Coronavirus disease 2019 Pneumonia in Wuhan. China. *JAMA Intern Med.* 180, 934–943. doi: 10.1001/jamainternmed.2020.0994

Wu, Z., and McGoogan, J. M. (2020). Characteristics of and important lessons from the Coronavirus Disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the chinese center for disease control and prevention. *JAMA* 323, 1239–1242. doi: 10.1001/jama.2020.2648

Yamamoto, M., Matsuyama, S., Li, X., Takeda, M., Kawaguchi, Y., Inoue, J. I., et al. (2016). Identification of nafamostat as a potent inhibitor of middle east respiratory syndrome Coronavirus S protein-mediated membrane fusion using the split-protein-based cell-cell fusion assay. *Antimicrob. Agents Chemother.* 60, 6532–6539. doi: 10.1128/AAC.01043-16

Yang, X., Yu, Y., Xu, J., Shu, H., Xia, J., Liu, H., et al. (2020). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med.* 8, 475–481. doi: 10.1016/S2213-2600(20)30079-5

Yau, J. W., Teoh, H., and Verma, S. (2015). Endothelial cell control of thrombosis. *BMC Cardiovasc Disord.* 15:130. doi: 10.1186/s12872-015-0124-z

Yin, S., Huang, M., Li, D., and Tang, N. (2020). Difference of coagulation features between severe pneumonia induced by SARS-CoV2 and non-SARS-CoV2. *J. Thromb. Thrombolysis.* 3, 1–4. doi: 10.1007/s11239-020-02105-8

Zelaya, H., Rothmeier, A. S., and Ruf, W. (2018). Tissue factor at the crossroad of coagulation and cell signaling. *J. Thromb. Haemost.* 16, 1941–1952. doi: 10.1111/jth.14246

Zhai, Z., Li, C., Chen, Y., Gerotziafas, G., Zhang, Z., Wan, J., et al. (2020). Prevention and treatment of venous thromboembolism associated with Coronavirus Disease 2019 infection: a consensus statement before guidelines. *Thromb. Haemost.* 120, 937–948. doi: 10.1055/s-0040-1710019

Zhang, H., Penninger, J. M., Li, Y., Zhong, N., and Slutsky, A. S. (2020). Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med.* 46, 586–590. doi: 10.1007/s00134-020-05985-9

Zhao, R., Ali, G., Nie, H. G., Chang, Y., Bhattarai, D., Su, X., et al. (2020). Plasmin improves oedematous blood-gas barrier by cleaving epithelial sodium channels. *Br. J. Pharmacol.* 177, 3091–3106. doi: 10.1111/bph.15038

Zheng, S., Fan, J., Yu, F., Feng, B., Lou, B., Zou, Q., et al. (2020). Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. *BMJ* 369:m1443. doi: 10.1136/bmj.m1443

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395, 1054–1062. doi: 10.1016/S0140-6736(20)30566-3

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

MINI REVIEW
published: 22 December 2020
doi: 10.3389/fphys.2020.616552

Physiology of Midkine and Its Potential Pathophysiological Role in COVID-19

Giulia Sanino[1], Martino Bosco[2] and Giuseppe Terrazzano[3,4]**

[1]Farmacia Municipale 2, Azienda Sanitaria Locale (ASL) CN1, Fossano, Italy, [2]Anatomia Patologica, Hospital "Michele e Pietro Ferrero", Verduno, Italy, [3]Department of Science, University of Basilicata, Potenza, Italy, [4]Department of Translational Medical Sciences, University of Naples Federico II, Napoli, Italy

SARS-CoV2 infection not only causes abnormal severe pneumonia but also induces other relevant pathophysiological effects on several tissues and organs. In this regard, the clinical complications observed in COVID-19 include acute coronary syndrome, pulmonary thromboembolism, myocarditis and, in the severe cases, the occurrence of disseminated intravascular coagulation. Literature on COVID-19 highlighted the central role of the Renin Angiotensin Aldosterone System in the determinism of SARS-CoV2 cellular internalization in the target tissues. Lung degeneration and respiratory distress appear to be dependent on the perturbance of physiological mechanisms, such as the uncontrolled release of pro-inflammatory cytokines, a dysregulation of the fibrinolytic coagulative cascade and the hyperactivation of immune effector cells. In this mini review, we address the physiology of Midkine, a growth factor able to bind heparin, and its pathophysiological potential role in COVID-19 determinism. Midkine increases in many inflammatory and autoimmune conditions and correlates with several dysfunctional immune-inflammatory responses that appear to show similarities with the pathophysiological elicited by SARS-CoV2. Midkine, together with its receptor, could facilitate the virus entry, fostering its accumulation and increasing its affinity with Ace2 receptor. We also focus on Netosis, a particular mechanism of pathogen clearance exerted by neutrophils, which under certain pathological condition becomes dysfunctional and can cause tissue damage. Moreover, we highlight the mechanism of autophagy that the new coronavirus could try to escape in order to replicate itself, as well as on pulmonary fibrosis induced by hypoxia and on the release of cytokines and mediators of inflammation, correlating the interplay between Midkine and SARS-CoV2.

Keywords: midkine, SARS-CoV2, COVID-19, neutrophil infiltration, NETs, autophagy, immune responses

OPEN ACCESS

Edited by:
Deborah Schechtman, University of São Paulo, Brazil

Reviewed by:
Jawed Fareed, Loyola University Medical Center, United States
Janos Paloczi, National Institutes of Health (NIH), United States

Correspondence:
Giulia Sanino
giuliasanino84@gmail.com
Giuseppe Terrazzano
giuseppe.terrazzano@unibas.it

Specialty section:
This article was submitted to Clinical and Translational Physiology, a section of the journal Frontiers in Physiology

Received: *12 October 2020*
Accepted: *27 November 2020*
Published: *22 December 2020*

Citation:
Sanino G, Bosco M and Terrazzano G (2020) Physiology of Midkine and Its Potential Pathophysiological Role in COVID-19. Front. Physiol. 11:616552. doi: 10.3389/fphys.2020.616552

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infection not only causes abnormal severe pneumonia but also induces other relevant pathophysiological effects on several tissues and organs. In this regard, the cardiovascular complications observed in Corona Virus Disease of 2019 (COVID-19) include acute coronary syndrome, pulmonary thromboembolism, myocarditis and, in the severe cases, the occurrence of disseminated intravascular coagulation

system (Vallamkondu et al., 2020; Verdecchia et al., 2020). Literature on COVID-19 highlighted the central role of the SAAR in SARS-CoV2 cellular internalization, particularly for the virus binding to angiotensin I converting enzyme 2 (ACE2) receptor expressed on the cell membrane of the tissues targeted by SARS-CoV2 (Hoffmann et al., 2020; Ingraham et al., 2020; Liu et al., 2020; Mycroft-West et al., 2020). Lung degeneration and respiratory distress appear to be dependent on the perturbance of host response mechanisms that could foster the uncontrolled release of pro-inflammatory cytokines, the dysregulation of the fibrinolytic coagulative cascade, as well as the hyper-activation of immune effector cells (Ackermann et al., 2020; Azkur et al., 2020; Becker, 2020; Stephen-Victor et al., 2020; Vallamkondu et al., 2020). Inflammation mediators, endothelial cells, neutrophils, and macrophages are responsible for the amplification of inflammation processes and concur to the cross talk between enzymatic cascades and signal pathways (Ackermann et al., 2020; Becker, 2020; Vallamkondu et al., 2020).

Midkine is a growth factor able to bind heparin and showing a physiological role in embryonic development (Kadomatsu et al., 1988). Midkine is poorly expressed in the adult organism cells, while is highly incremented in cancer cells and correlated with a less favorable prognosis in cancer patients (O'Brien et al., 1996; Maeda et al., 2007). Midkine has a crucial role in the interplay between kidney and lung (Salvati et al., 2011), is involved in inflammation (Weckbach et al., 2011), angiogenesis (Weckbach et al., 2012), tumor growth (Kadomatsu, 2005), vascular stenosis (Weckbach et al., 2011), renal (Sato et al., 2001), neurodegenerative (Kadomatsu, 2005; Takeuchi, 2014), and autoimmune diseases (Takada et al., 1997; Kadomatsu, 2005; **Figure 1**). It is of note that Midkine is significantly involved in inflammation determinism (Weckbach et al., 2011), is induced during inflammation process, and enhances the recruitment of inflammatory cells (Kadomatsu et al., 2013; **Figure 1**). Midkine is expressed in several pathological renal conditions including diabetic nephropathy (**Figure 1**) and can exacerbate several kidney diseases through leukocyte recruitment (Weckbach et al., 2011). Patients with rheumatoid arthritis highly expressed Midkine (Weckbach et al., 2011). Endothelial lesions caused increase expression of Midkine that has been observed in macrophages infiltrated into the injured vascular wall (Weckbach et al., 2011).

Midkine can be easily detected by enzyme-linked immunosorbent assay (ELISA) in serum and urine (Ikematsu et al., 2000; Xia et al., 2016), and its tissue expression in histochemistry has been described (Kim et al., 2017).

Midkine is an important physiological mediator of Renin Angiotensin Aldosterone System (SAAR; Kadomatsu, 2010; **Figure 1**). SAAR regulates the migration and proliferation of smooth muscle cells and the extracellular matrix (ECM) production, the increased expression of adhesion proteins and pro-inflammatory cytokine production (Hoffmann et al., 2020; Ingraham et al., 2020; Liu et al., 2020). Plasma concentration of Midkine dramatically increased in patients with acute respiratory distress syndrome (ARDS; Zhang and Baker, 2017). Midkine appears to be overregulated upon mechanical stress in lung epithelial cells (Zhang et al., 2015; Zhang and Baker, 2017) and induces ACE2 level in the lung (Ezquerra et al., 2005; Kadomatsu, 2010). A recent study showed the interplay between Midkine and ACE2 in mechanically ventilated lung tissue (Huang S. et al., 2020). In addition, the overregulation

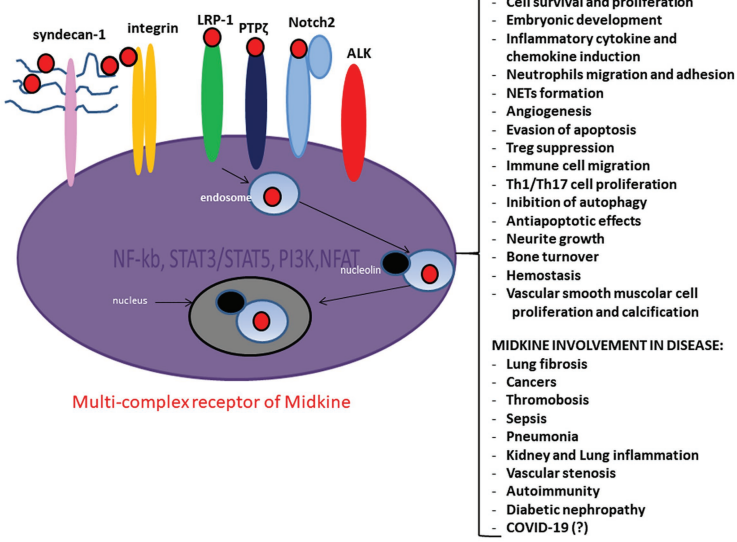


FIGURE 1 | Midkine multi-complex receptor, actions and involvement in disease. Midkine multi-complex receptor includes Syndecan-n-1, low density lipoprotein receptor-related protein 1 (LRP-1), Neurogenic locus notch homolog protein 2 (Notch-2), integrins, protein tyrosine phosphatase zeta (PTP ζ), and Anaplastic lymphoma kinase (ALK). The signaling pathway of Midkine multi-complex receptor involves several molecules as the nuclear factor *kappa*-light-chain-enhancer of activated *B* cells (NF-kB), *signal transducer and activator of transcription 3 and 5 (*STAT3/STAT5), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), and nuclear factor of activated T-cells (NFAT). Midkine promotes several actions ad is involved in various diseases.

of Midkine upon the mechanical stress was found in lung epithelial cells (Zhang et al., 2015; Zhang and Baker, 2017).

In this mini review, we focus the physiology of Midkine and its pathophysiological potential role in COVID-19, and we suggest to investigate Midkine as a putative biomarker of altered physiological conditions and/or a potential therapeutic target in the fight against pandemic COVID-19.

MIDKINE, HEPARAN SULFATE, AND EXTRACELLULAR MATRIX: A ROLE FOR VIRUS ENTRY FACILITATION?

The ECM contains proteoglycans that are very important for the structural integrity and tissue morphogenesis and homeostasis (Frantz et al., 2010). Heparan sulfate proteoglycans (HSPGs) are mainly present in the ECM and in the cell cytoplasmatic membrane and bind the Heparan sulfate (HS) chains (Lu et al., 2011). Syndecans (SDC) are HSPGs acting as regulators of cell migration, endocytosis, and cell signals (Beauvais and Rapraeger, 2003; Afratis et al., 2012; Christianson and Belting, 2014; Gallagher, 2015; Changyaleket et al., 2017). HS chains, according to their different degree of sulfation, can interfere with the growth factors/receptors interplay and promote the signal activation (Lu et al., 2011; Changyaleket et al., 2017). ADAM and ADAMTS metalloproteases and heparanase (Lu et al., 2011; Changyaleket et al., 2017) shed "soluble syndecans," which interact with the microenvironment, where they are released (Lu et al., 2011; Changyaleket et al., 2017). Several viruses use highly sulfated proteoglycans to bind the membrane surface of target cells (Rusnati et al., 2009; Cagno et al., 2019). The negative electrostatic proteoglycans charges interact with glycoproteins basic residues on the viral surface (Rusnati et al., 2009). The SARS-CoV2 spike protein (S-protein) interact with HS (Liu et al., 2020) and the binding affinity increases if HS is added to the S-protein proteolytic cleavage site (Liu et al., 2020). The HSPGs could increase the HCov-NL63 expression and could promote virus entry (Milewska et al., 2014; Kim et al., 2020).

Scientific Literature on COVID-19 highlighted the central role of the SAAR in the mechanisms of SARS-CoV2 cellular internalization, particularly for the occurrence of virus binding to ACE2 receptor expressed on the cell membrane of the tissues targeted by SARS-CoV2 infection (Hoffmann et al., 2020; Ingraham et al., 2020; Liu et al., 2020).

Midkine is a relevant component of heparin releasable endothelial proteins (HREPs) that are bound to the endothelial surface through proteoglycans and exert several specific functions in the vascular homeostasis (Novotny et al., 1993). Midkine strongly binds the hypersulfated structures of HS (Kaneda et al., 1996). Two Cardin and Weintraub (CW) motifs form a binding site based on heparan HS at the Midkine dimerization occurrence (Gallagher, 2015). The interaction with all three Midkine sulfate groups (6-O, 2-O, and n-sulfates) is crucial for the heparin-binding (Muramatsu et al., 1994; Kaneda et al., 1996; Asai et al., 1997; Maeda et al., 1999).

Midkine expression on cell surface strongly needs HS (Gallagher, 2015) and the tri-sulfate unit of HS is the binding site for Midkine itself (Kaneda et al., 1996). Midkine role as neuronal growth factor is impaired when cells are deprived of HS and activity is suppressed by heparin saccharides, which may block the site of interaction between HS and Midkine (Gallagher, 2015). The main receptor complex of Midkine includes Syndecan-1, glycosaminoglycans (GAGs), low density lipoprotein receptor-related protein 1 (LRP-1), Notch-2, integrins, protein tyrosine phosphatase ζ (PTP ζ), and anaplastic lymphoma kinase (ALK; Maeda et al., 1999). Other potential interplay between Midkine and some other extracellular ligands that bind Syndecans and/or interact with the LRP-1, as the tissue factor pathway inhibitor (TFPI), lipoprotein lypase, and several others, could have a relevant role in fostering Midkine activity and in determining other relevant biological functions (Kojima et al., 1996; Tinholt et al., 2015).

We hypothesize that Midkine could be involved in the early stages of viral attack during COVID-19 (**Figure 2**). The S-protein fosters the entry of virus into cells (Hoffmann et al., 2020). The SARS CoV2 S-protein is composed by the S1 and S2 domains that are respectively correlated with the binding and fusion of virus to target cells (Hoffmann et al., 2020). The S1 expresses the receptor-binding domain (RBD) responsible for ACE2 receptor binding (He et al., 2004). S1 subunit of RBD exists in two different conformations, closed and open: the open RBD is able to bind the virus more than closed conformation (Hao et al., 2020). Enzymatic cleavage of protein S at the level of S1/S2 domains supports fusion of viruses to cell membranes *via* the S2 subunit (Liu et al., 2020). SARS-CoV2 S-protein interacts with both the cellular HS and ACE2 through its RBD and can simultaneously engage heparin and ACE2 (Clausen et al., 2020). Positively charged amino acids in a subdomain of RBD are responsible for the binding of heparin/HS complex *via* an interaction site that appears independent on the site involved in ACE2 binding (Clausen et al., 2020). SARS CoV2 protein S appears to bind HS cooperatively with ACE2 receptor on the cell surface (Clausen et al., 2020).

SARS-CoV2 may employ several different promoting factors to infect ACE2 receptor-expressing cells in the upper respiratory tract with greater efficiency than SARS-CoV, and this occurrence may explain the greater transmissibility of SARS-CoV2 compared to SARS-CoV (Hoffmann et al., 2020).

It is reasonable to assume that Midkine could amplify RBD sulfatation sites of S-protein, increasing the binding affinity with ACE2 receptor, and that Midkine would facilitate the open conformation of S1, in such way promoting the subsequent viral attack (Clausen et al., 2020).

MIDKINE AND LIPID RAFTS

Scientific literature suggests the overall role of lipids in viral infection of target cells (Cervin and Anderson, 1991; Lajoie and Nabi, 2007; Li et al., 2007; Lu et al., 2008; Baglivo et al., 2020). Lipid rafts result in microdomains rich in cholesterol, glycosphingolipids, and phospholipids on the plasma membrane, potentially involved in the fusion, internalization, transport, and assembly of viral proteins of

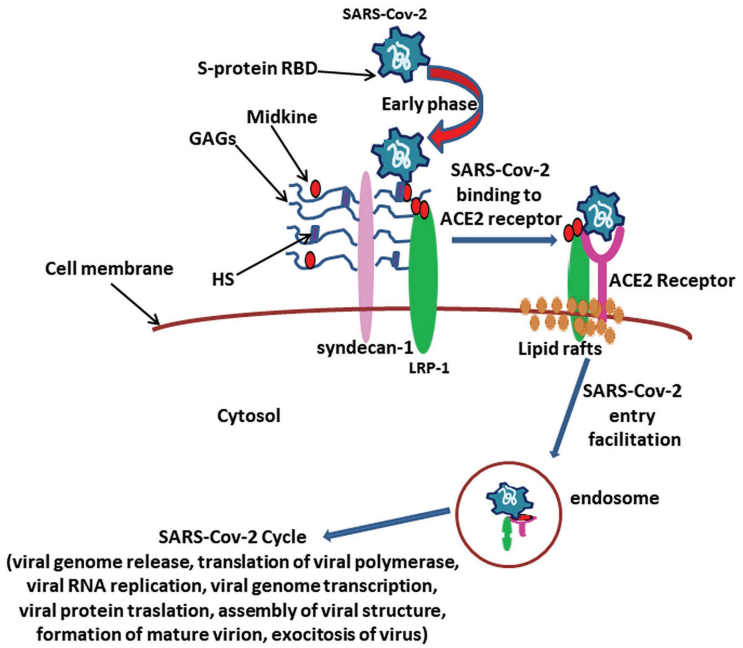


FIGURE 2 | The hypothesis over the role for Midkine in SARS CoV2 viral attack. The complex between Midkine, Syndecan-1, glycosaminoglycans (GAGs), and Heparan sulfate (HS) could play a pivotal role in the early phase of virus attack by amplifying receptor-binding domain (RBD) sulfatation sites of Spike (S)-protein, in such way enhancing the Angiotensin I converting enzyme 2 (ACE2) receptor binding affinity and determining virus localization on the extracellular membrane. After SARS-CoV2/ACE2 receptor binding, Midkine could facilitate virus entry into the cell through LRP-1-mediated endocytosis, allowing the virus cycle as described (V'kovski et al., 2020).

numerous viruses, including coronaviruses (Guo et al., 2017; Fecchi et al., 2020). Cholesterol represents the structural glue of lipid rafts (Fecchi et al., 2020). The ACE2 receptor is precisely located in the lipid rafts and is responsible for the initial phase of the viral infection of SARS-Cov2 (Fecchi et al., 2020). LRP-1 promotes endocytosis, is localized on lipid rafts, promotes the accumulation of cholesterol esters and the lipoproteins absorption (Actis Dato et al., 2020). Midkine is translocated into the nucleus by LRP-1 *via* nucleolin (Muramatsu et al., 2000).

In our hypothesis, the supposed interplay between the virus, Midkine, and HS and the presence of LRP-1 on lipid rafts might reveal new potential features of SARS-CoV2 infection mechanisms (**Figure 2**).

MIDKINE AND IMMUNE REGULATION: A POTENTIAL ROLE IN COVID-19?

Immunological tolerance and immune homeostasis involve regulatory T cells (Tregs; Terrazzano et al., 2020). Tolerogenic dendritic cells (DCregs) influence the inducible Tregs development (Takeuchi, 2014). mTOR (mammalian target of rapamycin) is a protein kinase, involved in apoptosis, cell cycle, metabolic disorders and autoimmunity, carcinogenesis, inflammation and autophagy, immunoregulation, and tolerance (Terrazzano et al., 2020). mTOR forms two complexes: mTORC1 induces the T helper (Th) 1 and Th17 differentiation upon viral antigen presentation by dendritic cells (DC; Omarjee et al., 2020). mTORC2 mediates Th2 differentiation (Omarjee et al., 2020), while both complexes restrict Tregs differentiation. The two mTOR complexes are involved in the regulation of Tregs homeostasis (Omarjee et al., 2020). mTOR-dependent pathways may uncover molecular targets useful for controlling the cellular damage, oxidative stress, and hyperinflammation that occur in COVID-19. Recently, mTOR inhibition therapy has been hypothesized to mitigate the cytokine storm and to reduce hyperactivation of immune responses in COVID-19 (Terrazzano et al., 2020).

COVID-19 patients who undergo ARDS are characterized by highly enhanced pro-inflammatory cytokine production (the cytokine storm) and lung repair dysfunction, which is partially due to reduced or defective Tregs involvement (Gladstone et al., 2020). Midkine suppresses the generation DCregs, which drive the development of inducible Treg (Misa et al., 2017; **Figure 1**), and reduces phosphorylated STAT3 levels in DCregs (Misa et al., 2017). The specific inhibition of Midkine by RNA-based aptamer increased the DCregs and Tregs and decreased the autoreactive Th1 and Th17 cells, and it has been associated with the amelioration of the clinical symptoms in experimental autoimmune encephalomyelitis model (Takeuchi, 2014).

A dysregulation in the signaling pathways of mTOR, hypoxia-inducible factor 1 (HIF-1) alpha, tumor necrosis factor (TNF) has been identified during SARS-CoV2 infection (Appelberg et al., 2020). An increased expression of Midkine in the lung appears to be mediated by HIF-1 alpha (Reynolds et al., 2004).

The respiratory epithelium responds to hypoxia through Midkine dependent HIF-1 alpha regulation (Reynolds et al., 2004). Midkine expression in human polymorphonuclear neutrophils (PMNs), monocytes, and endothelium increased by hypoxia (Weckbach et al., 2019).

Anaplastic lymphoma kinase (ALK) phosphorylates the insulin receptor substrate-1 and activates mitogen-activated protein (MAP) kinase and phosphoinositide 3 (PI3)-kinase leading to transcriptional activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB; Filippou et al., 2020). Filippou et al. (2020) recently reported that Midkine modulates the activity of the protein kinase B (Akt)/mTOR axis, *via* the ALK receptor, to prevent cell death mediated by cannabinoid-induced autophagy. Autophagy is a useful mechanism against viral infection. Autophagy plays a role in innate immunity, in the degradation of viruses or intracellular pathogens, and in the presentation of pathogens to the immune system (Fecchi et al., 2020). Viruses evolved mechanisms to escape the autophagic process (Carmona-Gutierrez et al., 2020).

SARS-CoV2, similarly to MERS-CoV, is able to reduce autophagy in infected cell lines by reducing the mTORC1-pathway, autophagy-related signaling, and the fusion between autophagosome and lysosome (Fecchi et al., 2020). SARS-CoV2 could benefit from reducing autophagy, preventing viral degradation, and improving the availability of double membrane vesicles (DMVs) needed for viral replication (Fecchi et al., 2020).

MIDKINE A KEY FACTOR FOR NEUTROPHIL ACTIVATION IN COVID-19?

The activation of neutrophils is very relevant during COVID-19 occurrence (Leppkes et al., 2020). In the course of inflammatory diseases, neutrophils excrete chromatin, histones and the contents of their own granules in a cellular process described as neutrophil extracellular trap (NET) formation (Leppkes et al., 2020). NET has been correlated to lung disease (Leppkes et al., 2020), neutrophils from pneumonia-associated ARDS undergo NET formation (Leppkes et al., 2020), extracellular histones are elevated in ARDS (Lv et al., 2017), and NET process is described in COVID-19 (Zuo et al., 2020). Furthermore, exacerbated aggregation of NET (NETs) could alter vascular districts and damage tissues (Leppkes et al., 2020). In the vascular system, NETs determine platelet activation and thrombosis, probably due to the release of histones that can be recognized through toll-like receptors (TLRs) on platelets and immune cells (Becker, 2020).

A recent report described that NET formation increases in COVID-19 patients undergoing mechanical ventilation (Zuo et al., 2020).

Patients with severe forms of COVID-19 show a marked increase in neutrophils compared to less severe subjects (Huang C. et al., 2020).

Midkine promotes the trafficking of neutrophils in myocardium and the NET formation in myocarditis (Weckbach et al., 2019). We suggest the occurrence of an important interplay between Midkine, PMN, NETs, and COVID-19. In this regard, we hypothesize that Midkine could promote neutrophil infiltration and NET formation in the myocardium *via* LRP1.

Moreover, it is likely that the Midkine-dependent promotion of neutrophil activation and NETs formation strongly degenerates the complex homeostatic mechanism of coagulation and plays a relevant role in the determinism of thrombotic events correlated to neutrophil hyperactivation (Iba and Levy, 2018). In this regard, neutrophil hyperactivation and NETs formation have been associated with ARDS in influenza pneumonitis (Narasaraju et al., 2011) and with thromboinflammatory response and intravascular thrombosis during sepsis (Iba and Levy, 2018). Finally, the molecules involved in hemostasis, as procoagulant or anticoagulant, should be deeply investigated for their potential relationship with Midkine, such as thrombin and thrombomodulin that are described to interplay each other to determine different effects on hemostasis (Rezaie, 2010) and have associated with NETs occurrence (Toh et al., 2016): Midkine could alter the balance between procoagulant and anticoagulant and could foster thromboinflammatory response and intravascular thrombosis during COVID-19 occurrence.

CONCLUSION

Since December 2019, SARS-Cov2 infection has manifested broad pandemic connotations and several pathophysiological conditions that do not limit COVID-19 to abnormal pneumonia (Cevik et al., 2020; Chen et al., 2020). In this regard, severe phases of COVID-19 present a poor prognosis in those patients underlying clinical conditions such as hypertension, chronic obstructive pulmonary disease, diabetes, and/or cardiovascular disease (Harapan et al., 2020; Nikolich-Zugich et al., 2020). Indeed, such compromised patients incur a greater risk of rapid progression to ARDS, septic-type systemic shock, coagulation dysfunction, arrhythmia and heart failure, renal and/or heart failure, hepatic dysfunction, and the occurrence of secondary infection (Cevik et al., 2020; Chen et al., 2020; Harapan et al., 2020; Nikolich-Zugich et al., 2020).

In this mini review, we suggest the potential and intriguing scenario concerning the interaction between SARS-CoV2 and Midkine, in order to understand the pathophysiological mechanisms occurring in COVID-19.

We highlight a possible involvement of Midkine in the in SARS-CoV2 infection mechanisms. Indeed, Midkine could amplify S-protein RBD sulfatation sites, increasing the binding affinity of SARS-CoV2 with ACE2 receptor. In addition, the interplay between coronavirus, Midkine, HS, LRP-1, and lipid rafts could foster SARS-CoV2 internalization.

The main feature of the immune-mediated involvement in COVID-19 is characterized by neutrophil hyperactivation. In this regard, Midkine signaling could enhance neutrophil proliferation and migration. Several studies have showed that Midkine is involved in neutrophil infiltration and chemokine expression as well as in the Netosis occurrence (**Figure 1**). Moreover, a crucial interplay between Midkine, neutrophils, NET, and COVID-19 might occur. Severe COVID-19 correlates with

TABLE 1 | Brief suggestions for studying the implication as a biomarker of Midkine in SARS-CoV2 infection and in COVID-19 patients.

Disease stages (*)	SARS-CoV2 detection	Midkine detection	Immune response analysis
Mild-Moderate infection (upper respiratory symptoms)	Nasopharyngeal/oropharyngeal swabs and viral RNA levels or viral antigen or anti-SARS-CoV-2 antibodies detection	ELISA (serum or urinary samples)	Basic assessment of leukocyte populations in blood (i.e., total neutrophils, total lymphocytes, and total monocytes)
Pulmonary phase (pneumonia with all its associated symptoms)	Nasopharyngeal/oropharyngeal swabs and viral RNA levels or viral antigen or anti-SARS-CoV-2 antibodies detection	ELISA (serum or urinary samples)	Interleukin-6, Interleukin-17, Interferon-γ detection. Advanced assessment of leukocyte populations in blood (i.e., total neutrophils, total monocytes, Tregs, T and B lymphocytes)
Hyperinflammation phase (with acute respiratory distress syndrome, sepsis, and kidney and other organ failures)	Nasopharyngeal/oropharyngeal swabs and viral RNA levels or viral antigen or anti-SARS-CoV-2 antibodies detection	ELISA (serum or urinary samples)	Interleukin-6, Interleukin-17, Interferon-γ detection. Advanced assessment of leukocyte populations in blood (i.e., total neutrophils, total monocytes, Tregs, T and B lymphocytes)

*The clinical classification is based on Siddiqi and Mehra (2020) and on the "Clinical management of COVID-19" guidance published by the World Health Organization (https://www.who.int/publications/i/item/clinical-management-of-covid-19).

exacerbated neutrophil hyperactivation and NET occurrence. Midkine could promote neutrophil infiltration and NET formation in the myocardium *via* LRP-1. In addition, Midkine could be involved in the pulmonary remodeling and fibrosis, through the collagen deposition and the Nox1, MK, Notch2, and ACE signaling pathway (**Figure 1**). We overviewed literature concerning Midkine-related pathway and its receptors, highlighting a common pathway with mTOR and autophagy that SARS-Cov2 could employ to elude in order to foster virus replication.

Taken in all, we hypothesize a key role of Midkine, particularly in organ dysfunction at the basis of COVID-19 pathogenesis and also propose such protein as a potential biomarker (**Table 1**) of pathophysiological conditions and as a key target for new potential COVID-19 therapeutical strategies by employing anti-Midkine monoclonal antibodies to be specifically prepared for clinical use in humans.

AUTHOR CONTRIBUTIONS

GS and GT equally contributed, conceptualized, and wrote the manuscript. MB contributed to the manuscript reading and editing. All authors contributed to the article and approved the submitted version.

REFERENCES

Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al. (2020). Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in COVID-19. *N. Engl. J. Med.* 383, 120–128. doi: 10.1056/NEJMoa2015432

Actis Dato, V., Benitez-Amaro, A., de Gonzalo-Calvo, D., Vazquez, M., Bonacci, G., Llorente-Cortés, V., et al. (2020). LRP1-mediated AggLDL endocytosis promotes cholesteryl ester accumulation and impairs insulin response in HL-1 cells. *Cell* 9:182. doi: 10.3390/cells9010182

Afratis, N., Gialeli, C., Nikitovic, D., Tsegenidis, T., Karousou, E., Theocharis, A. D., et al. (2012). Glycosaminoglycans: key players in cancer cell biology and treatment. *FEBS J.* 279, 1177–1197. doi: 10.1111/j.1742-4658.2012.08529.x

Appelberg, S., Gupta, S., Svensson, A. S., Ambikan, A. T., Mikaeloff, F., and Saccon, E. (2020). Dysregulation in Akt/mTOR/HIF-1 signaling identified by proteo-transcriptomics of SARS-CoV2 infected cells. *Emerg. Microbes. Infect.* 9, 1–36. doi: 10.1080/22221751.2020.1799723

Asai, T., Watanabe, K., Ichihara-Tanaka, K., Kaneda, N., Kojima, S., and Iguchi, A. (1997). Identification of heparin-binding sites in midkine and their role in neurite-promotion. *Biochem. Biophys. Res. Commun.* 236, 66–70. doi: 10.1006/bbrc.1997.6905

Azkur, A. K., Akdis, M., Azkur, D., Sokolowska, M., van de Veen, W., Brüggen, M. C., et al. (2020). Immune response to SARS-CoV2 and mechanisms of immunopathological changes in COVID-19. *Allergy* 75, 1564–1581. doi: 10.1111/all.14364

Baglivo, M., Baronio, M., Natalini, G., Beccari, T., Chiurazzi, P., Fulcheri, E., et al. (2020). Natural small molecules as inhibitors of coronavirus lipid-dependent attachment to host cells: a possible strategy for reducing SARS-COV2 infectivity? *Acta Biomed* 91, 161–164. doi: 10.23750/abm.v91i1.9402

Beauvais, D. M., and Rapraeger, A. C. (2003). Syndecan-1-mediated cell spreading requires signaling by alphavbeta3 integrins in human breast carcinoma cells. *Exp. Cell Res.* 286, 219–232. doi: 10.1016/s0014-4827(03)00126-5

Becker, R. C. (2020). COVID-19 update: Covid-19-associated coagulopathy. *J. Thromb. Thrombolysis* 50, 54–67. doi: 10.1007/s11239-020-02134-3

Cagno, V., Tseligka, E. D., Jones, S. T., and Tapparel, C. (2019). Heparan sulfate proteoglycans and viral attachment: true receptors or adaptation bias? *Viruses* 11:596. doi: 10.3390/v11070596

Carmona-Gutierrez, D., Bauer, M. A., Zimmermann, A., Kainz, K., Hofer, S. J., Kroemer, G., et al. (2020). Digesting the crisis: autophagy and coronaviruses. *Microb. Cell* 7, 119–128. doi: 10.15698/mic2020.05.715

Cervin, M., and Anderson, R. (1991). Modulation of coronavirus-mediated cell fusion by homeostatic control of cholesterol and fatty acid metabolism. *J. Med.Vir.* 35, 142–149. doi: 10.1002/jmv.1890350213

Cevik, M., Bamford, C., and Ho, A. (2020). COVID-19 pandemic—a focused review for clinicians. *Clin. Microbiol. Infect.* 26, 842–847. doi: 10.1016/j.cmi.2020.04.023

Changyaleket, B., Deliu, Z., Chignalia, A. Z., and Feinstein, D. L. (2017). Heparanase: potential roles in multiple sclerosis. *J. Neuroimmunol.* 310, 72–81. doi: 10.1016/j.jneuroim.2017.07.001

Chen, Y., Liu, Q., and Guo, D. (2020). Emerging coronaviruses: genome structure, replication, and pathogenesis. *J. Med. Virol.* 92, 418–423. doi: 10.1002/jmv.25681

Christianson, H. C., and Belting, M. (2014). Heparan sulfate proteoglycan as a cell-surface endocytosis receptor. *Matrix Biol.* 35, 51–55. doi: 10.1016/j.matbio.2013.10.004

Clausen, T. M., Sandoval, D. R., Spliid, C. B., Pihl, J., Painter, C. D., Thacker, B. E., et al. (2020). SARS-CoV2 infection depends on cellular heparan sulfate and ACE2. *Cell* 183, 1–15. doi: 10.1016/j.cell.2020.09.033

Ezquerra, L., Herradon, G., Nguyen, T., Silos-Santiago, I., and Deuel, T. F. (2005). Midkine, a newly discovered regulator of the renin-angiotensin pathway in mouse aorta: significance of the pleiotrophin/midkine developmental gene family in angiotensin II signaling. *Biochem. Biophys. Res. Commun.* 333, 636–643. doi: 10.1016/j.bbrc.2005.05.113

Fecchi, K., Anticoli, S., Peruzzu, D., Iessi, E., Gagliardi, M. C., Matarrese, P., et al. (2020). Coronavirus interplay with lipid rafts and autophagy unveils promising therapeutic targets. *Front. Microbiol.* 11:1821. doi: 10.3389/fmicb.2020.01821

Filippou, P. S., Karagiannis, G. S., and Constantinidou, A. (2020). Midkine (MDK) growth factor: a key player in cancer progression and a promising therapeutic target. *Oncogene* 39, 2040–2054. doi: 10.1038/s41388-019-1124-8

Frantz, C., Stewart, K. M., and Weaver, V. M. (2010). The extracellular matrix at a glance. *J. Cell Sci.* 123, 4195–4200. doi: 10.1242/jcs.023820

Gallagher, J. (2015). Fell-Muir Lecture: heparan sulphate and the art of cell regulation: a polymer chain conducts the protein orchestra. *Int. J. Exp. Pathol.* 96, 203–231. doi: 10.1111/iep.12135

Gladstone, D. E., Kim, B. S., Mooney, K., Karaba, A. H., and D'Alessio, F. R. (2020). Regulatory T cells for treating patients with COVID-19 and acute respiratory distress syndrome: two case reports. *Ann. Intern. Med.* 173, 852–853. doi: 10.7326/L20-0681

Guo, H., Huang, M., Yuan, Q., Wei, Y., Gao, Y., Mao, L., et al. (2017). The important role of lipid raft-mediated attachment in the infection of cultured cells by coronavirus infectious bronchitis virus beaudette strain. *PLoS One* 12:e0170123. doi: 10.1371/journal.pone.0170123

Hao, W., Ma, B., Li, Z., Wang, X., Gao, X., Li, Y., et al. (2020). Binding of the SARS-CoV2 spike protein to glycans. bioRxiv [Preprint]. doi: 10.1101/2020.05.17.100537

Harapan, H., Itoh, N., Yufika, A., Winardi, W., Keam, S., Te, H., et al. (2020). Coronavirus disease 2019 (COVID-19): a literature review. *J. Infect. Public Health* 13, 667–673. doi: 10.1016/j.jiph.2020.03.019

He, Y., Zhou, Y., Liu, S., Kou, K., Li, W., Farzan, M., et al. (2004). Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunit vaccine. *Biochem. Biophys. Res. Commun.* 324, 773–781. doi: 10.1016/j.bbrc.2004.09.106

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271–280. doi: 10.1016/j.cell.2020.02.052

Huang, S., Kaipainen, A., Strasser, M., and Baranzini, S. (2020). Mechanical ventilation stimulates expression of the SARS-CoV2 receptor ACE2 in the lung and may trigger a vicious cycle [Preprint]. doi: 10.20944/preprints 202005.0429.v1

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Iba, T., and Levy, J. H. (2018). Inflammation and thrombosis: roles of neutrophils, platelets and endothelial cells and their interactions in thrombus formation during sepsis. *J. Thromb. Haemost.* 16, 231–241. doi: 10.1111/jth.13911

Ikematsu, S., Yano, A., Aridome, K., Kikuchi, M., Kumai, H., Nagano, H., et al. (2000). Serum midkine levels are increased in patients with various types of carcinomas. *Br. J. Cancer* 83, 701–706. doi: 10.1054/bjoc.2000.1339

Ingraham, N. E., Barakat, A. G., Reilkoff, R., Bezdicek, T., Schacker, T., Chipman, J. G., et al. (2020). Understanding the renin-angiotensin-aldosterone-SARS-CoV axis: a comprehensive review. *Eur. Respir. J.* 56:2000912. doi: 10.1183/13993003.00912-2020

Kadomatsu, K. (2005). The midkine family in cancer, inflammation and neural development. *Nagoya J. Med. Sci.* 67, 71–82.

Kadomatsu, K. (2010). Midkine regulation of the renin-angiotensin system. *Curr. Hypertens. Rep.* 12, 74–79. doi: 10.1007/s11906-010-0092-8

Kadomatsu, K., Kishida, S., and Tsubota, S. (2013). The heparin-binding growth factor midkine: the biological activities and candidate receptors. *J. Biochem.* 153, 511–521. doi: 10.1093/jb/mvt035

Kadomatsu, K., Tomomura, M., and Muramatsu, T. (1988). cDNA cloning and sequencing of a new gene intensely expressed in early differentiation stages of embryonal carcinoma cells and in mid-gestation period of mouse embryogenesis. *Biochem. Biophys. Res. Commun.* 3, 1312–1318. doi: 10.1016/s0006-291x(88)80505-9

Kaneda, N., Talukder, A., Ishihara, M., Hara, S., Yoshida, K., and Muramatsu, T. (1996). Structural characteristics of heparin-like domain required for interaction of midkine with embryonic neurons. *Biochem. Biophys. Res. Commun.* 220, 108–112. doi: 10.1006/bbrc.1996.0365

Kim, S. Y., Jin, W., Sood, A., Montgomery, D. W., Grant, O. C., Fuster, M. M., et al. (2020). Characterization of heparin and severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV2) spike glycoprotein binding interactions. *Antivir. Res.* 181:104873. doi: 10.1016/j.antiviral.2020.104873

Kim, H. M., Kang, Y. H., Byun, J. H., Jang, S. J., Rho, G. J., Lee, J. S., et al. (2017). Midkine and NANOG have similar immunohistochemical expression patterns and contribute equally to an adverse prognosis of oral squamous cell carcinoma. *Int. J. Mol. Sci.* 18, 2339–2354. doi: 10.3390/ijms18112339

Kojima, T., Katsumi, A., Yamazaki, T., Muramatsu, T., Nagasaka, T., Ohsumi, K., et al. (1996). Human ryudocan from endothelium-like cells binds basic fibroblast growth factor, midkine, and tissue factor pathway inhibitor. *J. Biol. Chem.* 271, 5914–5920. doi: 10.1074/jbc.271.10.5914

Lajoie, P., and Nabi, I. R. (2007). Regulation of raft-dependent endocytosis. *J. Cell. Mol. Med.* 11, 644–653. doi: 10.1111/j.1582-4934.2007.00083.x

Leppkes, M., Knopf, J., Naschberger, E., Lindemann, A., Singh, J., Herrmann, I., et al. (2020). Vascular occlusion by neutrophil extracellular traps in COVID-19. *EBioMedicine* 58:102925. doi: 10.1016/j.ebiom.2020.102925

Li, G. M., Li, Y. G., Yamate, M., Li, S. M., and Ikutaa, K. (2007). Lipid rafts play an important role in the early stage of severe acute respiratory syndrome-coronavirus life cycle. *Microbes Infect.* 9, 96–102. doi: 10.1016/j.micinf.2006.10.015

Liu, L., Chopra, P., Li, X., Wolfert, M. A., Tompkins, S. M., and Boons, G. J. (2020). SARS-CoV2 spike protein binds heparan sulfate in a length- and sequence-dependent manner. bioRxiv [Preprint]. doi: 10.1101/2020.05.10.087288

Lu, Y., Liu, D. X., and Tam, J. P. (2008). Lipid rafts are involved in SARS-CoV entry into Vero E6 cells. *Biochem. Biophys. Res. Commun.* 369, 344–349. doi: 10.1016/j.bbrc.2008.02.023

Lu, P., Takai, K., Weaver, V. M., and Werb, Z. (2011). Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb. Perspect. Biol.* 3:a005058. doi: 10.1101/cshperspect.a005058

Lv, X., Wen, T., Song, J., Xie, D., Wu, L., Jiang, X., et al. (2017). Extracellular histones are clinically relevant mediators in the pathogenesis of acute respiratory distress syndrome. *Respir. Res.* 18:165. doi: 10.1186/s12931-017-0651-5

Maeda, N., Ichihara-Tanaka, K., Kimura, T., Kadomatsu, K., Muramatsu, T., and Noda, M. (1999). A receptor-like protein-tyrosine phosphatase PTPzeta/RPTPbeta binds a heparin-binding growth factor midkine: involvement of arginine 78 of midkine in the high affinity binding to PTPzeta. *J. Biol. Chem.* 274, 12474–12479. doi: 10.1074/jbc.274.18.12474

Maeda, S., Shinchi, H., Kurahara, H., Mataki, Y., Noma, H., Maemura, K., et al. (2007). Clinical significance of midkine expression in pancreatic head carcinoma. *Br. J. Cancer* 97, 405–411. doi: 10.1038/sj.bjc.6603879

Milewska, A., Zarebski, M., Nowak, P., Stozek, K., Potempa, J., and Perlman, K. P. (2014). Human coronavirus NL63 utilizes heparan sulfate proteoglycans for attachment to target cells. *J. Virol.* 88, 13221–13230. doi: 10.1128/JVI.02078-14

Misa, K., Tanino, Y., Wang, X., Nikaido, T., Kikuchi, M., Sato, Y., et al. (2017). Involvement of midkine in the development of pulmonary fibrosis. *Physiol. Rep.* 5:e13383. doi: 10.14814/phy2.13383

Muramatsu, H., Inui, T., Kimura, T., Sakakibara, S., Song, X., Murata, H., et al. (1994). Localization of heparin-binding, neurite outgrowth and antigenic regions in midkine molecule. *Biochem. Biophys. Res. Commun.* 203, 1131–1139. doi: 10.1006/bbrc.1994.2300

Muramatsu, H., Zou, K., Sakaguchi, N., Ikematsu, S., Sakuma, S., and Muramatsu, T. (2000). LDL receptor-related protein as a component of the midkine receptor. *Biochem. Biophys. Res. Commun.* 270, 936–941. doi: 10.1006/bbrc.2000.2549

Mycroft-West, C., Su, D., Elli, S., Guimond, S., Miller, G., Turnbull, J., et al. (2020). The 2019 coronavirus (SARS-CoV2) surface protein (Spike) S1Receptor Binding Domain undergoes conformational change upon heparin binding. bioRxiv [Preprint]. doi: 10.1101/2020.02.29.971093

Narasaraju, T., Yang, E., Samy, R. P., Ng, H. H., Poh, W. P., Liew, A. A., et al. (2011). Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. *Am. J. Pathol.* 179, 199–210. doi: 10.1016/j.ajpath.2011.03.013

Nikolich-Zugich, J., Knox, K. S., Rios, C. T., Natt, B., Bhattacharya, D., and Fain, M. J. (2020). SARS-CoV-2 and COVID-19 in older adults: what we may expect regarding pathogenesis, immune responses, and outcomes. *GeroScience* 42, 505–514. doi: 10.1007/s11357-020-00186-0

Novotny, W. F., Maffi, T., Mehta, R. L., and Milner, P. G. (1993). Identification of novel heparin-releasable proteins, as well as the cytokines midkine and pleiotrophin, in human postheparin plasma. *Arterioscler. Thromb.* 13, 1798–1805. doi: 10.1161/01.ATV.13.12.1798

O'Brien, T., Cranston, D., Fuggle, S., Bicknell, R., and Harris, A. L. (1996). The angiogenic factor midkine is expressed in bladder cancer, and overexpression correlates with a poor outcome in patients with invasive cancers. *Cancer Res.* 56, 2515–2518.

Omarjee, L., Janin, A., Perrot, F., Laviolle, B., Meilhac, O., Mahe, G., et al. (2020). Targeting T-cell senescence and cytokine storm with rapamycin to prevent severe progression in COVID-19. *Clin. Immunol.* 216:108464. doi: 10.1016/j.clim.2020.108464

Reynolds, P. R., Mucenski, M. L., Le Cras, T. D., Nichols, W. C., and Whitsett, J. A. (2004). Midkine is regulated by hypoxia and causes pulmonary vascular remodeling. *J. Biol. Chem.* 279, 37124–37132. doi: 10.1074/jbc.M405254200

Rezaie, A. R. (2010). Regulation of the protein C anticoagulant and antiinflammatory pathways. *Curr. Med. Chem.* 17, 2059–2069. doi: 10.2174/092986710791233706

Rusnati, M., Vicenzi, E., Donalisio, M., Oreste, P., Landolfo, S., and Lembo, D. (2009). Sulfated K5 *Escherichia coli* polysaccharide derivatives: a novel class of candidate antiviral microbicides. *Pharmacol. Ther.* 123, 310–322. doi: 10.1016/j.pharmthera.2009.05.001

Salvati, F., Santoro, A., and Pedone, C. (2011). BPCO e nefropatie croniche. *Ital. J. Med.* 5S, 67–74. doi: 10.1016/j.itjm.2011.01.010

Sato, W., Kadomatsu, K., Yuzawa, Y., Muramatsu, H., Hotta, N., Matsuo, S., et al. (2001). Midkine is involved in neutrophil infiltration into the tubulointerstitium in ischemic renal injury. *J. Immunol.* 167, 3463–3469. doi: 10.4049/jimmunol.167.6.3463

Siddiqi, H. K., and Mehra, M. R. (2020). COVID-19 illness in native and immunosuppressed states: a clinical-therapeutic staging proposal. *J. Heart Lung Transplant.* 39, 405–407. doi: 10.1016/j.healun.2020.03.012

Stephen-Victor, E., Das, M., Karnam, A., Pitard, B., Gautier, J. F., and Bayry, J. (2020). Potential of regulatory T cell-based therapies in the management of severe COVID-19. *Eur. Respir. J.* 56:2002182. doi: 10.1183/13993003.02182-2020

Takada, T., Toriyama, K., Muramatsu, H., Song, X. J., Torii, S., and Muramatsu, T. (1997). Midkine, a retinoic acid-inducible heparin-binding cytokine in inflammatory responses: chemotactic activity to neutrophils and association with inflammatory synovitis. *J. Biochem.* 122, 453–458. doi: 10.1093/oxfordjournals.jbchem.a021773

Takeuchi, H. (2014). Midkine and multiple sclerosis. *Br. J. Pharmacol.* 171, 931–935. doi: 10.1111/bph.12499

Terrazzano, G., Rubino, V., Palatucci, A. T., Giovazzino, A., Carriero, F., and Ruggiero, G. (2020). An open question: is it rational to inhibit the mTor-dependent pathway as COVID-19 therapy? *Front. Pharmacol.* 11:856. doi: 10.3389/fphar.2020.00856

Tinholt, M., Stavik, B., Louch, W., Rein Carlson, C., Sletten, M., Ruf, W., et al. (2015). Syndecan-3 and TFPI Colocalize on the surface of endothelial-, smooth muscle-, and cancer cells. *PLoS One* 10:e0117404. doi: 10.1371/journal.pone.0117404

Toh, C. H., Alhamdi, Y., and Abrams, S. T. (2016). Current pathological and laboratory considerations in the diagnosis of disseminated intravascular coagulation. *Ann. Lab. Med.* 36, 505–512. doi: 10.3343/alm.2016.36.6.505

V'kovski, P., Kratzel, A., Steiner, S., Stalder, H., and Thiel, V. (2020). Coronavirus biology and replication: implications for SARS-CoV-2. *Nat. Rev. Microbiol.* 1–16. doi: 10.1038/s41579-020-00468-6

Vallamkondu, J., John, A., Wani, W. Y., Ramadev, S. P., Jella, K. K., Reddy, P. H., et al. (2020). SARS-CoV2 pathophysiology and assessment of coronaviruses in CNS diseases with a focus on therapeutic targets. *Biochim. Biophys. Acta Mol. basis Dis.* 1866:165889. doi: 10.1016/j.bbadis.2020.165889

Verdecchia, P., Cavallini, P., Spanevello, A., and Angeli, F. (2020). The pivotal link between ACE2 deficiency and SARS-CoV2 infection. *Eur. J. Intern. Med.* 76, 14–20. doi: 10.1016/j.ejim.2020.04.037

Weckbach, L. T., Grabmaier, U., Uhl, A., Gess, S., Boehm, F., Zehrer, A., et al. (2019). Midkine drives cardiac inflammation by promoting neutrophil trafficking and NETosis in myocarditis. *J. Exp. Med.* 216, 350–368. doi: 10.1084/jem.20181102

Weckbach, L. T., Groesser, L., Borgolte, J., Pagel, J. I., Pogoda, F., Schymeinsky, J., et al. (2012). Midkine acts as proangiogenic cytokine in hypoxia-induced angiogenesis. *Am. J. Physio. Heart. Circ. Physiol.* 303, 429–438. doi: 10.1152/ajpheart.00934.2011

Weckbach, L. T., Muramatsu, T., and Walzog, B. (2011). Midkine in inflammation. *ScientificWorldJournal* 11, 2491–2505. doi: 10.1100/2011/517152

Xia, X., Lu, J. J., Zhang, S. S., Su, C. H., and Luo, H. H. (2016). Midkine is a serum and urinary biomarker for the detection and prognosis of non-small cell lung cancer. *Oncotarget* 7, 87462–87472. doi: 10.18632/oncotarget.13865

Zhang, H., and Baker, A. (2017). Recombinant human ACE2: acing out angiotensin II in ARDS therapy. *Crit. Care* 21:305. doi: 10.1186/s13054-017-1882-z

Zhang, R., Pan, Y., Fanelli, V., Wu, S., Luo, A. A., Islam, D., et al. (2015). Mechanical stress and the induction of lung fibrosis via the midkine signaling pathway. *Am. J. Respir. Crit. Care Med.* 192, 315–323. doi: 10.1164/rccm.201412-2326OC

Zuo, Y., Yalavarthi, S., Shi, H., Gockman, K., Zuo, M., Madison, J. A., et al. (2020). Neutrophil extracellular traps in COVID-19. *JCI Insight* 5:e138999. doi: 10.1172/jci.insight.138999

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

MINI REVIEW
published: 08 January 2021
doi: 10.3389/fphys.2020.608788

Pathophysiological Processes Underlying the High Prevalence of Deep Vein Thrombosis in Critically Ill COVID-19 Patients

*Sebastian Voicu[1,2], Chahinez Ketfi[3], Alain Stépanian[4,5], Benjamin G. Chousterman[6,7,8], Nassim Mohamedi[3], Virginie Siguret[4,9], Alexandre Mebazaa[6,7,8], Bruno Mégarbane[1,2] and Philippe Bonnin[3,10]**

[1] Department of Medical and Toxicological Critical Care, Hôpital Lariboisière, APHP, Faculté de Santé, Université de Paris, Paris, France, [2] INSERM UMRS 1144, Université de Paris, Paris, France, [3] Department of Clinical Physiology, Hôpital Lariboisière, APHP, Faculté de Santé, Université de Paris, Paris, France, [4] Laboratory of Biological Hematology, Hôpital Lariboisière, APHP, Faculté de Santé, Université de Paris, Paris, France, [5] EA3518, Université de Paris, Paris, France, [6] Department of Anesthesia and Critical Care, Faculté de Santé, Hôpital Lariboisière, APHP, FHU PROMICE, Paris, France, [7] Université de Paris, Paris, France, [8] INSERM UMR-S 942 – MASCOT, Université de Paris, Paris, France, [9] INSERM UMR-S 1140, Université de Paris, Paris, France, [10] INSERM U1148, LVTS, Université de Paris, Paris, France

OPEN ACCESS

Edited by:
Georges Leftheriotis,
Université Côte d'Azur, France

Reviewed by:
Mariann Gyöngyösi,
Medical University of Vienna, Austria
Mingxing Xie,
Huazhong University of Science and Technology, China

***Correspondence:**
Philippe Bonnin
philippe.bonnin@aphp.fr

Specialty section:
This article was submitted to Clinical and Translational Physiology, a section of the journal Frontiers in Physiology

Received: 23 September 2020
Accepted: 27 November 2020
Published: 08 January 2021

Citation:
Voicu S, Ketfi C, Stépanian A, Chousterman BG, Mohamedi N, Siguret V, Mebazaa A, Mégarbane B and Bonnin P (2021) Pathophysiological Processes Underlying the High Prevalence of Deep Vein Thrombosis in Critically Ill COVID-19 Patients. Front. Physiol. 11:608788. doi: 10.3389/fphys.2020.608788

Coronavirus disease 2019 (COVID-19) predisposes to deep vein thrombosis (DVT) and pulmonary embolism (PE) particularly in mechanically ventilated adults with severe pneumonia. The extremely high prevalence of DVT in the COVID-19 patients hospitalized in the intensive care unit (ICU) has been established between 25 and 84% based on studies including systematic duplex ultrasound of the lower limbs when prophylactic anticoagulation was systematically administrated. DVT prevalence has been shown to be markedly higher than in mechanically ventilated influenza patients (6–8%). Unusually high inflammatory and prothrombotic phenotype represents a striking feature of COVID-19 patients, as reflected by markedly elevated reactive protein C, fibrinogen, interleukin 6, von Willebrand factor, and factor VIII. Moreover, in critically ill patients, venous stasis has been associated with the prothrombotic phenotype attributed to COVID-19, which increases the risk of thrombosis. Venous stasis results among others from immobilization under muscular paralysis, mechanical ventilation with high positive end-expiratory pressure, and pulmonary microvascular network injuries or occlusions. Venous return to the heart is subsequently decreased with increase in central and peripheral venous pressures, marked proximal and distal veins dilation, and drops in venous blood flow velocities, leading to a spontaneous contrast "sludge pattern" in veins considered as prothrombotic. Together with endothelial lesions and hypercoagulability status, venous stasis completes the Virchow triad and considerably increases the prevalence of DVT and PE in critically ill COVID-19 patients, therefore raising questions regarding the optimal doses for thromboprophylaxis during ICU stay.

Keywords: COVID-19, D-dimer, hemostasis disorder, deep vein thrombosis, venous stasis

INTRODUCTION

Coronavirus disease 2019 (COVID-19) patients present with coagulation disorders and marked predisposition to thrombosis (Goshua et al., 2020; Helms et al., 2020; Iba et al., 2020). One major consequence is the high prevalence of deep vein thrombosis (DVT) demonstrated by studies performing duplex ultrasound examination of the lower limbs in patients hospitalized with severe pneumonia (Zhang et al., 2020; Fraissé et al., 2020; Llitjos et al., 2020; Nahum et al., 2020; Ren et al., 2020; Voicu et al., 2020a). The high prevalence of DVT exposes patients to a high risk of pulmonary embolism (PE) (Contou et al., 2020; Poissy et al., 2020), especially when DVT is proximal, at the popliteal level, or above (Konstantinides et al., 2020). DVT in COVID-19 patients may be responsible for approximately 10% of deaths in COVID-19 patients as shown by systematic autopsy findings (Edler et al., 2020). Therefore, understanding the underlying mechanisms of the thrombotic process during the COVID-19 time course is of utmost importance because of the implications in the clinical, biological, and imaging monitoring of the patients, as well as in the anticoagulant prophylaxis and treatment.

High Prevalence of DVT in COVID-19 Patients—Clinical Data

Initial studies reported unusually high rates of thrombotic events including DVT and PE, i.e., 16.7% (Helms et al., 2020), 20.6% (Poissy et al., 2020); DVT, 2% (Helms et al., 2020), 14.8% (Tavazzi et al., 2020); and DVT and/or PE in 27% of the patients (Klok et al., 2020). More recently, studies provided insight into the DVT prevalence by using systematic ultrasound screening of the lower limbs. They established a DVT prevalence up to 85.4% in hospitalized COVID-19 patients (Fraissé et al., 2020; Llitjos et al., 2020; Nahum et al., 2020; Ren et al., 2020; Voicu et al., 2020a; Zhang et al., 2020). Timing and number of ultrasound examinations per patient varied according to the study. The highest DVT prevalence was reported by Ren et al. (2020), who performed at least two ultrasound examinations during the intensive care unit (ICU) stay in a Chinese series of 48 patients under prophylactic enoxaparin and showed an overall DVT prevalence of 84.4% but a prevalence of proximal DTV (defined as popliteal or femoral) of 10.4%. In a study of 143 patients admitted to the medical wards, Zhang et al. (2020) showed that 46.1% had DVT, of which 34.8% were proximal. Approximately 50% of the patients received prophylactic or therapeutic anticoagulation before the ultrasound. The median time from admission to detection of DVT was 9 days (Zhang et al., 2020). Patients with DVT had a worse prognosis requiring more often admission to the ICU, 18.2% of the patients versus 3.9% (p = 0.005) and having a higher mortality, 34.8% versus 11.7%, p = 0.001, compared to patients without DVT. In 56 mechanically ventilated patients, Voicu et al. (2020a) showed a prevalence of 46% of DVT, 50% of them being proximal. Importantly, despite standard unfractionated heparin or enoxaparin prophylaxis, DVT was acquired during ICU stay in 35% of the patients who had a second ultrasound and who were DVT-free on the initial ultrasound (Voicu et al., 2020a). These studies proved that prevalence of DVT was higher that initially suggested, and much higher than in mechanically ventilated influenza patients (i.e., 10.7%) (Obi et al., 2019). However, in non-ICU patients studies performing systematic screening observed lower DVT prevalence of 11.9 with 2.4% being proximal (Santoliquido et al., 2020) and 14.7% among which 1/156 was proximal (Demelo-Rodríguez et al., 2020).

Overall, data from these studies suggested that standard prophylactic anticoagulation was insufficient to prevent DVT and importantly, that ultrasound monitoring should be undertaken to accurately diagnose and treat DVT.

Although routine screening has been performed for DVT using ultrasound, a non-invasive, reliable and cost-effective technique, screening for PE is limited by the difficulties to transport patients in life-threatening conditions to the imaging department and by the adverse effects of computed tomography pulmonary angiography (CTPA) including the high risk of kidney injury and potentially life-threatening allergic reactions. It is not clear how many hospitalized COVID-19 patients present with PE as no study performed routine CTPA. Among retrospective studies reporting PE, a prevalence of 30% was found in 106 patients receiving CTPA for suspicion of PE or other indications, but this high prevalence must be put into perspective with the selection bias inherent to the retrospective nature of the study (Léonard-Lorant et al., 2020). However, one study reporting systematic autopsy studies in patients who died from COVID-19 infection, found that 21% of the patients had PE, and in approximately half of them (8/17), PE was considered the cause of death (Edler et al., 2020).

Several factors can be identified as predisposing to thrombosis in COVID-19 patients. Most patients had underlying conditions including obesity, diabetes, hypertension, ischemic heart disease, associated with hypercoagulable state, endothelial injuries, decreased venous return and immobility, all of which are major factors contributing to thrombosis.

Hypercoagulability Favoring Thrombosis in COVID-19 Patients

The hypercoagulable state underlies the predisposition to thrombosis as indicated in the first studies in China (Tang et al., 2020), especially by the extremely elevated D-dimers. Tang et al. found a correlation between the D-dimer level and mortality and suggested that anticoagulation may improve survival, as subsequently suggested in a retrospective study including 2773 patients showing increased survival in patients treated with versus without anticoagulation in the United States (Paranjpe et al., 2020). The intense inflammatory reaction supported by the elevated interleukin 6 (IL-6), C-reactive protein, and fibrinogen levels (McElvaney et al., 2020) strongly favors thrombosis. Besides the increase in fibrinogen level, one of the most remarkable laboratory features of the hypercoagulable state in COVID-19 patients is the unusually high von Willebrand factor levels, up to 10 times the upper normal limit and higher than the normal range in 100% of the patients (Panigada et al., 2020; Voicu et al., 2020b); factor VIII level is subsequently very

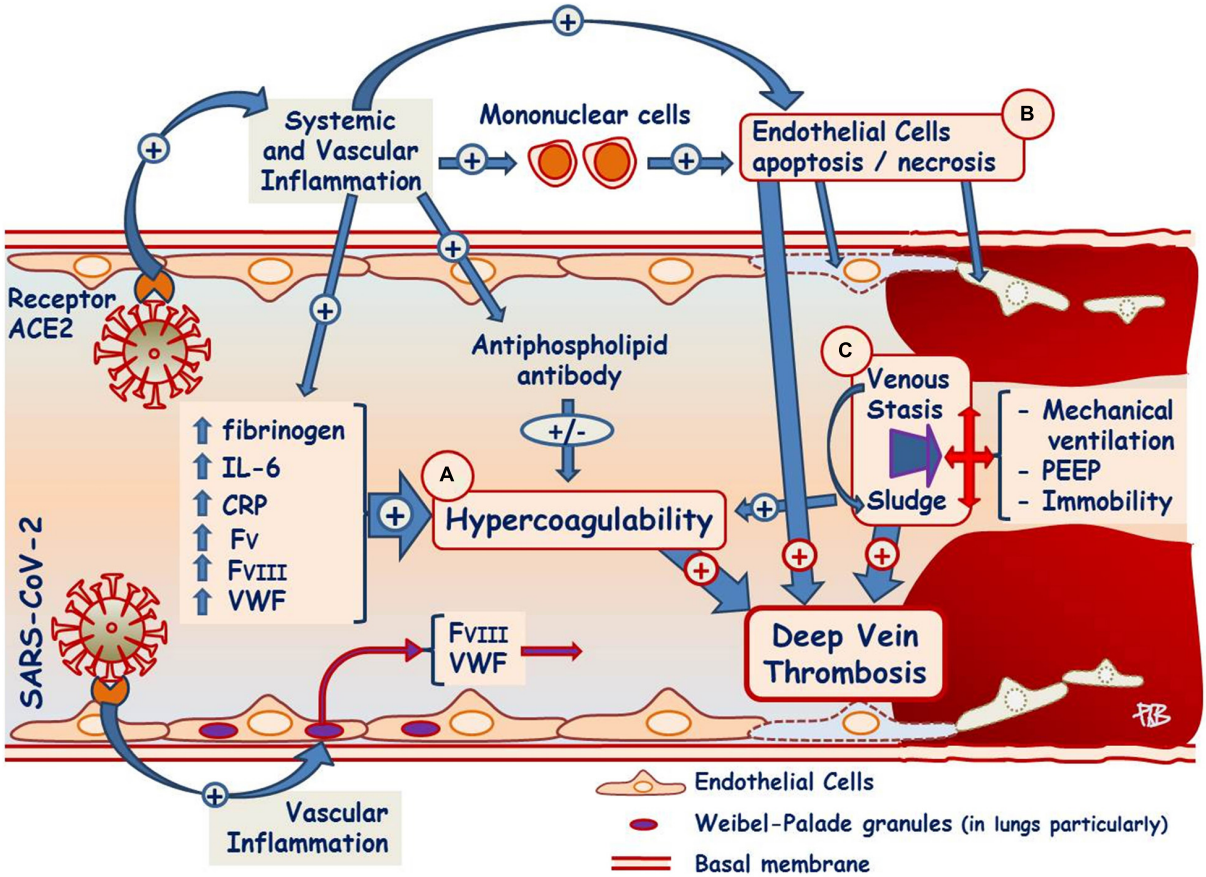


FIGURE 1 | Factors favoring deep vein thrombosis in COVID-19 patients. **(A)** SARS-CoV-2 invades the endothelial cell and produces systemic and vascular inflammation leading to increased fibrinogen, CRP, IL-6, and coagulation factor V, as well as antiphospholipid antibodies possibly through molecular mimicry. Mononuclear cell inflammation, apoptosis, and necrosis were documented in the lungs; in veins, their presence was not specifically studied. SARS-CoV-2 invades the endothelial cell and determines directly and/or through systemic inflammation the exocytosis of von Willebrand factor and factor VIII from Weibel–Palade granules. **(B)** SARS-CoV-2 involves endothelial cells injuries (apoptosis and necrosis). **(C)** Mechanical ventilation, high PEEP, and immobility decrease the blood velocities of the venous return. **(A–C)** Complete the Virchow triad and increase the risk of thrombosis. SARS-CoV-2, severe acute respiratory syndrome–coronavirus 2 virus; IL-6, interleukin-6; CRP, C-reactive-protein; FV, coagulation factor V; FVIII, coagulation factor VIII; VWF, von Willebrand factor; PEEP, positive end-expiratory pressure; ACE2, angiotensin-converting enzyme type 2.

elevated, as factor VIII is transported by von Willebrand factor (Tabatabai et al., 2020; Panigada et al., 2020; Voicu et al., 2020b). Moreover, factor V concentration is also unusually increased (Helms et al., 2020; Voicu et al., 2020b). Both von Willebrand factor and factor VIII are stored in the Weibel–Palade bodies of the endothelial cells, especially in some vascular territories such as lungs, and released during the COVID-19 disease, resulting in high circulating levels (Valentijn et al., 2011). This release may be related to the invasion of the endothelial cell by the virus, which can attach to the angiotensin-converting enzyme type 2, and may lead to endothelial dysfunction or damage and release of the coagulation factors (Panigada et al., 2020; Escher et al., 2020; **Figure 1**). Other specific pathways such as anticoagulant protein C may also play a role, although the extent of its involvement is still under evaluation. Physiologically, thrombin activates protein C through the formation of a thrombin–thrombomodulin complex on the endothelial surface, enhanced by the endothelial protein C receptor. Therefore, endothelial damage may result in protein C pathway disruption. Its protein C activity was found normal in some studies (Panigada et al., 2020), decreased in others (Tabatabai et al., 2020), or appeared deficient despite normal concentrations of protein C due to the saturation or overwhelming of the pathway by high factor V and VIII concentrations (Voicu et al., 2020b). Similarly, ADAMTS13, the von Willebrand factor–specific protease, seems to be slightly decreased, with a subsequent imbalance of von Willebrand factor/ADAMTS13 axis when considering the strikingly elevated von Willebrand factor levels (Blasi et al., 2020; Delrue et al., 2020; Huisman et al., 2020). Hypoxemia associated with COVID-19–related pneumonia (Sherren et al., 2020) may also enhance hypercoagulability through increased synthesis of hypoxia inducible factor-1α (Marchetti, 2020), which increases procoagulant gene expression (Gupta et al., 2019).

Cardiovascular risk factors predispose to COVID-19 but are also associated with increased predisposition to thrombosis. Obesity is associated with increased tissue factor and P-selectin

expression, increased thrombin generation, and platelet activation, whereas antithrombotic pathways such as protein C anticoagulant system, antithrombin, and tissue factor pathway inhibitor are downregulated (Korakas et al., 2020). Diabetes increases the thrombotic risk even further, hyperglycemia increasing the oxidative stress with subsequent thrombin generation and decreasing antithrombin anticoagulant activity, through its non-enzymatic glycation (Ceriello, 2020).

The cytokine storm described in COVID-19 patients is a major pathophysiological bridge between inflammation and thrombosis. Although its complex pathophysiology is beyond the scope of this mini-review, the relationship to thrombosis of one of the main factors involved, IL-6, found in very high levels in COVID-19 patients (Lazzaroni et al., 2020) must be acknowledged. Notably, IL-6 up-regulates fibrinogen levels, activates tissue factor expression, but also increases factor VIII level, through endothelial injury or activation by complex formed by IL-6 and IL6-receptor and subsequent von Willebrand factor release (Joly et al., 2020).

Endothelial Activation and Lesion Favoring Thrombosis in COVID-19 Patients

Thrombosis may also be favored by endothelial cell activation leading to increased expression of adhesion molecules and production of tissue factor by antiphospholipid antibodies, which are occasionally synthesized in viral infections due to the antigen mimicry of the virus (Ruiz-Irastorza et al., 2010). Lupus anticoagulant was positive in 45% of the 56 COVID-19–infected patients, whereas anticardiolipin and/or β2 glycoprotein-I antibodies were found in 10% of the patients (Harzallah et al., 2020). However, their role in the pathogenesis of COVID-19–related thrombosis remains to be determined, since in a study including 74 mechanically ventilated ICU COVID-19 patients receiving systematic ultrasound DVT screening and CTPA if PE was suspected, 12% had positive anticardiolipin and/or β2 glycoprotein-I antibodies, without difference in prevalence between the 28 patients with a thrombotic event and the 46 patients without a thrombotic event, 5 (18%) versus 4 (9%) respectively, $p = 0.30$ (Siguret et al., 2020).

Neutrophils seem to participate actively in the immunothrombotic process by the increased expression of neutrophil extracellular traps (NETs, extracellular traps of chromatin and microbicidal proteins (Zuo et al., 2020) that activate tissue factor expression by endothelial cells in COVID-19 infection) (Skendros et al., 2020). Moreover, complement fraction C5a increases NET expression and tissue factor expression by neutrophils, participating in the thrombotic process (Skendros et al., 2020). Complement is another potential enhancer of thrombosis, the membrane attack complex C5b-9 being increased in COVID-19–infected patients, in parallel with other endothelial activation markers such as von Willebrand factor, tissue plasminogen activator, and plasminogen activator inhibitor-1 (Cugno et al., 2020).

Finally, the endothelium, which also has antithrombotic functions, may be severely damaged (Magalhaes et al., 2020) due to the underlying vascular mononuclear inflammation, as well as apoptosis of endothelial cells documented by pathology studies especially in the lungs or small bowel vessels (Escher et al., 2020; Varga et al., 2020). These features have not been so far described in veins, but may represent another putative thrombosis-favoring mechanism.

Increased thrombosis may lead to increased consumption of platelets and the onset of thrombocytopenia in 5–41.7% of the patients, percentage varying according to the severity of the disease, being more frequent in patients with more severe disease (58–95%) (Wool and Miller, 2020). More severe thrombocytopenia is encountered in cases of disseminated intravascular coagulation (DIC), diagnosed in 2% of the patients in a study including 400 patients, of which 144 were critically ill (Al-Samkari et al., 2020). Coagulation abnormalities, apart from rare cases of DIC, include mildly prolonged prothrombin time (PT) and thrombin time (TT), and increased fibrin degradation products (FDPs)/D-dimers. Noteworthy, PT prolongation associated with normal or elevated coagulation factor concentrations may be most of the time artifactual, due to unusual hyperfibrinogenemia, depending on PT reagents. Similarly, the interpretation of TT prolongation may be difficult because of the presence of FDP high levels and of heparin.

Moreover, microvascular thrombosis promoting kidney injury and lung microvascular thrombosis promoting hypoxemia (Sherren et al., 2020; Vinayagam and Sattu, 2020) may further increase predisposition to thrombosis.

Hemorrheologic Factors Favoring Thrombosis

In its most severe form, immobility related to bed rest during hospitalization is present in mechanically ventilated patients requiring muscular paralysis for the treatment of the acute respiratory distress syndrome (ARDS). These patients are immobile and have no muscular contraction and no or very little muscular tone; therefore, venous return is extremely impaired. The half-seated position used in mechanically ventilated patients to improve ventilation parameters introduces another obstacle to venous return, depending on the angle between lower limbs and the body (Zhang et al., 2018), decreasing the blood flow velocity of the venous return and increasing the diameter of the lower limb veins due to venous pooling.

In addition, intubated critically ill patients are ventilated with high levels of positive end-expiratory pressure (PEEP), the median reported PEEP being 12 cmH_2O (Beloncle et al., 2020) increasing central and peripheral venous pressures. The association of half-seated position and mechanical ventilation increases lower limb venous pressures and decreases the venous return as supported by low maximal velocities in the common femoral vein compared to those in healthy subjects (approximately −45% vs. normal values) (Delis et al., 2004; Needleman et al., 2018). Additionally, increased femoral vein diameters compared to normal (approximately + 31% vs. normal values) consecutive to the increase in hydrostatic pressure in lower limbs veins also contribute to venous stasis and may favor thrombosis. Furthermore, in patients with

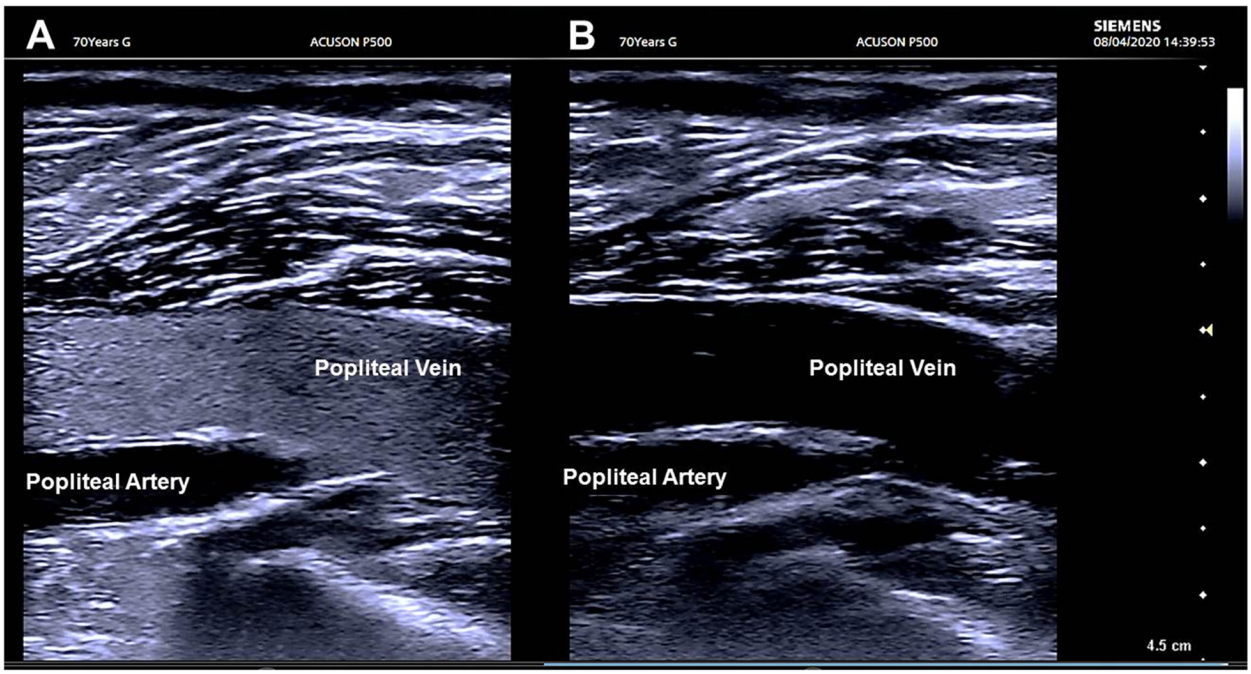


FIGURE 2 | Ultrasound imaging of the popliteal vein. **(A)** The flow is characterized by the presence of a sludge consisting in the increased echogenicity of blood due to the "brightness" of the erythrocyte aggregates occurring in vessels with low blood flow velocities. **(B)** A calf compression is performed that provokes a sudden increase in blood flow velocities in the popliteal vein; blood becomes anechoic with the disappearance of the sludge pattern. The sludge pattern is recovered in less than 20 s after compression. In both panels, the blood is anechoic in the popliteal artery because blood flow velocities are elevated compared to the popliteal vein.

myocarditis and cardiac failure, low cardiac output may increase venous stasis (Shchedrygina et al., 2020). Moreover, during COVID-19 ARDS, pulmonary microcirculatory thrombosis may occur as a consequence of the disease participating in the increased pulmonary arterial resistance and right ventricular pressures and consequently may decrease velocities of venous return to the heart and contributes to peripheral venous stasis (Ackermann et al., 2020; Edler et al., 2020).

Associated peripheral hemodynamic and hemorrheologic alterations have been observed during duplex ultrasound as pronounced sludge patterns particularly in the lower limbs. These impairments may have resulted from the abnormal "brightness" of erythrocyte aggregates occurring in vessels with lowered blood flow velocities (Stuart and Nash, 1990; Knaggs et al., 2005; **Figure 2**). The sludge pattern may be considered as a prothrombotic stage by itself (Delis et al., 2004).

Interpretation of Observations in COVID-19 Patients

Altogether (1) hypercoagulation state of which the most remarkable features are increased fibrinogen, D-dimer, factor VIII, and von Willebrand factor; (2) endothelial lesions due to the viral invasion and vascular inflammation; and (3) immobility, half-seated position, and increased resistance to venous return due to mechanical ventilation PEEP and pulmonary vascular network injuries concur to create the ideal conditions for the occurrence of the Virchow triad (Virchow, 1856; Wolberg et al., 2012) and the consecutive increased DVT prevalence in critically ill COVID-19 patients even under standard prophylactic anticoagulation. COVID-19 plays a major role in thrombus formation in the whole circulatory system by acting on the different actors of the triad of Virchow, Moreover, cytokine storm and organ disorders consecutive to local microvascular thrombosis complete the triad of Virchow to increase the prevalence of DVT. Comorbidities such as diabetes, hypertension, and obesity can increase cytokine storm and hypercoagulability during COVID-19, thus increasing organ dysfunction and predisposition to thrombosis. Therefore, to reduce the thrombotic risk, increased doses of anticoagulants may be required (Marietta et al., 2020; Susen et al., 2020), several regimens being currently evaluated in ongoing randomized trials (Porfidia et al., 2020), whereas some comparative studies showed decreased DVT after increased anticoagulation (Voicu et al., 2020c). Patients presenting low vein blood flow velocities, rising to prethrombotic sludge patterns, do benefit from permanent compression stockings or intermittent leg compression. Anti-inflammatory treatment may also play a role in decreasing endothelial lesions and thus decrease predisposition to thrombosis. Finally, as DVT has a high prevalence, ultrasound screening may be required in order to promptly and accurately diagnose and treat DVT. In case of logistical difficulties and limited availability, ultrasound can be performed according to plasma D-dimer levels as suggested by several studies (Cui et al., 2020; Léonard-Lorant et al., 2020;

Voicu et al., 2020b): the threshold of 3,300 ng/mL (Voicu et al., 2020b) similar to 3,000 ng/mL (Cui et al., 2020) and 2,680 ng/mL (Léonard-Lorant et al., 2020) offering a sensitivity of 78% and specificity of 69% for the onset of a thrombotic event defined as DVT or PE.

CONCLUSION

In conclusion, hypercoagulable state, endothelial lesions, immobility, and reduced venous blood flows explain the extremely high prevalence of thromboembolic events in mechanically ventilated COVID-19 patients. None of the abnormalities alone in any component of Virchow triad fully explain the high DVT prevalence in mechanically ventilated COVID-19 patients. Initiation of a DVT in lower limbs responds to complex, multifactorial, and interactive processes. Careful monitoring and screening with lower limbs ultrasound based on a high index of suspicion may be necessary to promptly diagnose and treat DVT. Studies are ongoing to establish if higher prophylactic regimens could be more effective in preventing thrombotic events and result in an overall benefit in the outcome of COVID-19 patients.

AUTHOR CONTRIBUTIONS

SV and PB wrote the manuscript. CK, AS, BGC, NM, VS, AM, and BM read and amended the final version of the manuscript and provided guidance on the overall direction of the manuscript. All authors critically appraised the final version of the article.

ACKNOWLEDGMENTS

The authors would like to thank Siemens Healthcare France for kindly lending Lariboisière Hospital ultrasound machines for the duration of the pandemic.

REFERENCES

Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al. (2020). Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N. Engl. J. Med.* 383, 120–128. doi: 10.1056/NEJMoa2015432

Al-Samkari, H., Karp Leaf, R. S., Dzik, W. H., Carlson, J. C. T., Fogerty, A. E., Waheed, A., et al. (2020). COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. *Blood* 136, 489–500. doi: 10.1182/blood.2020006520

Beloncle, F. M., Pavlovsky, B., Desprez, C., Fage, N., Olivier, P. Y., Asfar, P., et al. (2020). Recruitability and effect of PEEP in SARS-Cov-2-associated acute respiratory distress syndrome. *Annals Tntensive Care* 10:55. doi: 10.1186/s13613-020-00675-7

Blasi, A., von Meijenfeldt, F. A., Adelmeijer, J., Calvo, A., Ibañez, C., Perdomo, J., et al. (2020). In vitro hypercoagulability and ongoing in vivo activation of coagulation and fibrinolysis in COVID-19 patients on anticoagulation. *J. Thromb. Haemost.* 18, 2646–2653. doi: 10.1111/jth.15043

Ceriello, A. (2020). Diabetes, D-dimer and COVID-19: the possible role of glucose control. *Diabetes Metab. Syndrome* 14:1987. doi: 10.1016/j.dsx.2020.10.011

Contou, D., Pajot, O., Cally, R., Logre, E., Fraissé, M., Mentec, H., et al. (2020). Pulmonary embolism or thrombosis in ARDS COVID-19 patients: a french monocenter retrospective study. *PloS One* 15:e0238413. doi: 10.1371/journal.pone.0238413

Cui, S., Chen, S., Li, X., Liu, S., and Wang, F. (2020). Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. *J. Thromb. Haemost.* 18, 1421–1424. doi: 10.1111/jth.14830

Cugno, M., Meroni, P. L., Gualtierotti, R., Griffini, S., Grovetti, E., Torri, A., et al. (2020). Complement activation and endothelial perturbation parallel COVID-19 severity and activity. *J. Autoimmun.* 29:102560. doi: 10.1016/j.jaut.2020.102560 [Epub ahead of print].

Delis, K. T., Knaggs, A. L., Sonecha, T. N., Zervas, V., Jenkins, M. P., and Wolfe, J. H. (2004). Lower limb venous haemodynamic impairment on dependency: quantification and implications for the "economy class" position. *Thromb. Haemost.* 91, 941–950. doi: 10.1160/TH03-12-0754

Delrue, M., Siguret, V., Neuwirth, M., Joly, B., Beranger, N., Sène, D., et al. (2020). Von willebrand factor / ADAMTS13 axis and venous thromboembolism in moderate-to-severe COVID-19 patients. *Br. J. Haematol.* 2020:17216. doi: 10.1111/bjh.17216

Demelo-Rodríguez, P., Cervilla-Muñoz, E., Ordieres-Ortega, L., Parra-Virto, A., Toledano-Macías, M., Toledo-Samaniego, N., et al. (2020). Incidence of asymptomatic deep vein thrombosis in patients with COVID-19 pneumonia and elevated D-dimer levels. *Thromb. Res.* 192, 23–26. doi: 10.1016/j.thromres.2020.05.018

Edler, C., Schröder, A. S., Aepfelbacher, M., Fitzek, A., Heinemann, A., Heinrich, F., et al. (2020). Dying with SARS-CoV-2 infection-an autopsy study of the first consecutive 80 cases in hamburg, germany. *Int. J. Legal Med.* 134, 1275–1284. doi: 10.1007/s00414-020-02317-w

Escher, R., Breakey, N., and Lämmle, B. (2020). Severe COVID-19 infection associated with endothelial activation. *Thromb. Res.* 190:62. doi: 10.1016/j.thromres.2020.04.014

Fraissé, M., Logre, E., Pajot, O., Mentec, H., Plantefève, G., and Contou, D. (2020). Thrombotic and hemorrhagic events in critically ill COVID-19 patients: a french monocenter retrospective study. *Crit. Care* 24:275. doi: 10.1186/s13054-020-03025-y

Goshua, G., Pine, A. B., Meizlish, M. L., Chang, C. H., Zhang, H., Bahel, P., et al. (2020). Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. Lancet. *Haemat.* 7, e575–e582. doi: 10.1016/S2352-3026(20)30216-7

Gupta, N., Zhao, Y.-Y., and Evans, C. E. (2019). The stimulation of thrombosis by hypoxia. *Thromb. Res.* 181, 77–83. doi: 10.1016/j.thromres.2019.07.013

Harzallah, I., Debliquis, A., and Drénou, B. (2020). Lupus anticoagulant is frequent in patients with Covid-19: response to reply. *J. Thromb. Haemost.* 2020:32608109. doi: 10.1111/jth.14980

Helms, J., Tacquard, C., Severac, F., Leonard-Lorant, I., Ohana, M., Delabranche, X., et al. (2020). High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med.* 46, 1089–1098. doi: 10.1007/s00134-020-06062-x

Huisman, A., Beun, R., Sikma, M., Westerink, J., and Kusadasi, N. (2020). Involvement of ADAMTS13 and von willebrand factor in thromboembolic events in patients infected with SARS-CoV-2. *Int. J. Lab. Hematol.* 42, e211–e212. doi: 10.1111/ijlh.13244

Iba, T., Levy, J. H., Connors, J. M., Warkentin, T. E., Thachil, J., and Levi, M. (2020). The unique characteristics of COVID-19 coagulopathy. *Crit. Care* 24:360. doi: 10.1186/s13054-020-03077-0

Joly, B. S., Siguret, V., and Veyradier, A. (2020). Understanding pathophysiology of hemostasis disorders in critically ill patients with COVID-19. *Intensive Care Med.* 46, 1603–1606. doi: 10.1007/s00134-020-06088-1

Klok, F. A., Kruip, M., van der Meer, N., Arbous, M. S., Gommers, D., Kant, K. M., et al. (2020). Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb. Res.* 191, 145–147. doi: 10.1016/j.thromres.2020.04.013

Knaggs, A. L., Delis, K. T., Mason, P., and Macleod, K. (2005). Perioperative lower limb venous haemodynamics in patients under general anaesthesia. *Br. J. Anaesth.* 94, 292–295. doi: 10.1093/bja/aei049

Konstantinides, S. V., Meyer, G., Becattini, C., Bueno, H., Geersing, G. J., Harjola, V. P., et al. (2020). ESC scientific document group. 2019 ESC guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the european respiratory society (ERS). *Eur. Heart J.* 41, 543–603.

Korakas, E., Ikonomidis, I., Kousathana, F., Balampanis, K., Kountouri, A., Raptis, A., et al. (2020). Obesity and COVID-19: immune and metabolic derangement as a possible link to adverse clinical outcomes. *Am. J. Physiol. Endocrinol. Metab.* 319, E105–E109. doi: 10.1152/ajpendo.00198.2020

Lazzaroni, M. G., Piantoni, S., Masneri, S., Garrafa, E., Martini, G., Tincani, A., et al. (2020). Coagulation dysfunction in COVID-19: the interplay between inflammation, viral infection and the coagulation system. *Blood Rev.* 2020:100745. doi: 10.1016/j.blre.2020.100745

Léonard-Lorant, I., Delabranche, X., Séverac, F., Helms, J., Pauzet, C., Collange, O., et al. (2020). Acute pulmonary embolism in patients with COVID-19 at CT angiography and relationship to d-dimer levels. *Radiology* 296, E189–E191. doi: 10.1148/radiol.2020201561

Llitjos, J. F., Leclerc, M., Chochois, C., Monsallier, J. M., Ramakers, M., Auvray, M., et al. (2020). High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. *J. Thromb. Haemost.* 18, 1743–1746. doi: 10.1111/jth.14869

Magalhaes, G. S., Rodrigues-Machado, M., da, G., Motta-Santos, D., Campagnole-Santos, M. J., and Santos, R. A. S. (2020). Activation of Ang-(1-7)/mas receptor is a possible strategy to treat coronavirus (SARS-CoV-2) infection. *Front. Physiol.* 11:730. doi: 10.3389/fphys.2020.00730

Marchetti, M. (2020). COVID-19-driven endothelial damage: complement, HIF-1, and ABL2 are potential pathways of damage and targets for cure. *Ann. Hematol.* 99, 1701–1707. doi: 10.1007/s00277-020-04138-8

Marietta, M., Ageno, W., Artoni, A., De Candia, E., Gresele, P., Marchetti, M., et al. (2020). COVID-19 and haemostasis: a position paper from italian society on thrombosis and haemostasis (SISET). *Blood Transfusion = Trasfusione del Sangue* 18, 167–169. doi: 10.2450/2020.0083-20

McElvaney, O. J., McEvoy, N., McElvaney, O. F., Carroll, T. P., Murphy, M. P., Dunlea, D. M., et al. (2020). Characterization of the inflammatory response to severe COVID-19 illness. *Am. J. Respir. Crit. Care Med.* 202, 812–821. doi: 10.1164/rccm.202005-1583OC

Nahum, J., Morichau-Beauchant, T., Daviaud, F., Echegut, P., Fichet, J., Maillet, J. M., et al. (2020). Venous thrombosis among critically Ill patients with coronavirus disease 2019 (COVID-19). *JAMA Network open* 3: e2010478. doi: 10.1001/jamanetworkopen.2020.10478

Needleman, L., Cronan, J. J., Lilly, M. P., Merli, G. J., Adhikari, S., Hertzberg, B. S., et al. (2018). Ultrasound for lower extremity deep venous thrombosis: multidisciplinary recommendations from the society of radiologists in ultrasound consensus conference. *Circulation* 137, 1505–1515. doi: 10.1161/CIRCULATIONAHA.117.030687

Obi, A. T., Tignanelli, C. J., Jacobs, B. N., Arya, S., Park, P. K., Wakefield, T. W., et al. (2019). Empirical systemic anticoagulation is associated with decreased venous thromboembolism in critically ill influenza A H1N1 acute respiratory distress syndrome patients. *J. Vasc. Surg. Venous Lymphatic Disord.* 7, 317–324. doi: 10.1016/j.jvsv.2018.08.010

Panigada, M., Bottino, N., Tagliabue, P., Grasselli, G., Novembrino, C., Chantarangkul, V., et al. (2020). Hypercoagulability of COVID-19 patients in intensive care unit: a report of thromboelastography findings and other parameters of hemostasis. *J. Thromb. Haemost.* 18, 1738–1742. doi: 10.1111/jth.14850

Paranjpe, I., Fuster, V., Lala, A., Russak, A. J., Glicksberg, B. S., Levin, M. A., et al. (2020). Association of treatment dose anticoagulation with in-hospital survival among hospitalized patients with COVID-19. *J. Am. Coll. Cardiol.* 76, 122–124. doi: 10.1016/j.jacc.2020.05.001

Poissy, J., Goutay, J., Caplan, M., Parmentier, E., Duburcq, T., Lassalle, F., et al. (2020). Pulmonary embolism in patients with COVID-19: awareness of an increased prevalence. *Circulation* 142, 184–186. doi: 10.1161/CIRCULATIONAHA.120.047430

Porfidia, A., Valeriani, E., Pola, R., Porreca, E., Rutjes, A., and Di Nisio, M. (2020). Venous thromboembolism in patients with COVID-19: systematic review and meta-analysis. *Thromb. Res.* 196, 67–74. doi: 10.1016/j.thromres.2020.08.020

Ren, B., Yan, F., Deng, Z., Zhang, S., Xiao, L., Wu, M., et al. (2020). Extremely high incidence of lower extremity deep venous thrombosis in 48 patients with severe COVID-19 in wuhan. *Circulation* 142, 181–183. doi: 10.1161/CIRCULATIONAHA.120.047407

Ruiz-Irastorza, G., Crowther, M., Branch, W., and Khamashta, M. A. (2010). Antiphospholipid syndrome. *Lancet* 376, 1498–1509. doi: 10.1016/S0140-6736(10)60709-X

Santoliquido, A., Porfidia, A., Nesci, A., De Matteis, G., Marrone, G., Porceddu, E., et al. (2020). Incidence of deep vein thrombosis among non-ICU patients hospitalized for COVID-19 despite pharmacological thromboprophylaxis. *J. Thromb. Haemost.* 18, 2358–2363. doi: 10.1111/jth.14992

Shchedrygina, A., Nagel, E., Puntmann, V. O., and Valbuena-Lopez, S. (2020).). COVID-19 myocarditis and prospective heart failure burden. *Exp. Rev Cardiovasc Ther.* 2020, 1–10. doi: 10.1080/14779072.2021.1844005

Sherren, P. B., Ostermann, M., Agarwal, S., Meadows, C., Ioannou, N., and Camporota, L. (2020). COVID-19-related organ dysfunction and management strategies on the intensive care unit: a narrative review. *Br. J. Anaesth.* 125, 912–925. doi: 10.1016/j.bja.2020.08.050

Siguret, V., Voicu, S., Neuwirth, M., Delrue, M., Gayat, E., Stépanian, A., et al. (2020). Are antiphospholipid antibodies associated with thrombotic complications in critically ill COVID-19 patients? *Thromb. Res.* 195, 74–76. doi: 10.1016/j.thromres.2020.07.016

Skendros, P., Mitsios, A., Chrysanthopoulou, A., Mastellos, D. C., Metallidis, S., Rafailidis, P., et al. (2020). Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. *J. Clin. Investigat.* 130, 6151–6157. doi: 10.1172/JCI141374

Stuart, J., and Nash, G. B. (1990). Technological advances in blood rheology. *Crit. Rev. Clin. Lab. Sci.* 28, 61–93. doi: 10.3109/10408369009105898

Susen, S., Tacquard, C. A., Godon, A., Mansour, A., Garrigue, D., Nguyen, P., et al. (2020). Prevention of thrombotic risk in hospitalized patients with COVID-19 and hemostasis monitoring. *Crit. Care* 24:364. doi: 10.1186/s13054-020-03000-7

Tabatabai, A., Rabin, J., Menaker, J., Madathil, R., Galvagno, S., Menne, A., et al. (2020). Factor VIII and functional protein C activity in critically Ill patients with coronavirus disease 2019: a case series. *A A pract.* 14:e01236. doi: 10.1213/XAA.0000000000001236

Tang, N., Bai, H., Chen, X., Gong, J., Li, D., and Sun, Z. (2020). Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J. Thromb. Haemost.* 18, 1094–1099. doi: 10.1111/jth.14817

Tavazzi, G., Civardi, L., Caneva, L., Mongodi, S., and Mojoli, F. (2020). Thrombotic events in SARS-CoV-2 patients: an urgent call for ultrasound screening. *Intensive Care Med.* 46, 1121–1123. doi: 10.1007/s00134-020-06040-3

Valentijn, K. M., Sadler, J. E., Valentijn, J. A., Voorberg, J., and Eikenboom, J. (2011). Functional architecture of weibel-palade bodies. *Blood* 117, 5033–5043. doi: 10.1182/blood-2010-09-267492

Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., et al. (2020). Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 395, 1417–1418. doi: 10.1016/S0140-6736(20)30937-5

Vinayagam, S., and Sattu, K. (2020). SARS-CoV-2 and coagulation disorders in different organs. *Life Sci.* 260:118431. doi: 10.1016/j.lfs.2020.118431

Virchow, R. (1856). *Gesammalte abhandlungen zur wissenschaftlichen medtzin*. Frankfurt: Medinger Sohn & Co, 219–732.

Voicu, S., Bonnin, P., Stépanian, A., Chousterman, B. G., Le Gall, A., Malissin, I., et al. (2020a). High prevalence of deep vein thrombosis in mechanically ventilated COVID-19 patients. *J. Am. Coll. Cardiol.* 76, 480–482. doi: 10.1016/j.jacc.2020.05.053

Voicu, S., Delrue, M., Chousterman, B. G., Stépanian, A., Bonnin, P., Malissin, I., et al. (2020b). Imbalance between procoagulant factors and natural coagulation inhibitors contributes to hypercoagulability in the critically ill COVID-19 patient: clinical implications. *Eur. Rev. Med. Pharmacol. Sci.* 24, 9161–9168. doi: 10.26355/eurrev_202009_22866

Voicu, S., Chousterman, B. G., Bonnin, P., Deye, N., Malissin, I., Le Gall, A., et al. (2020c). Increased anticoagulation reduces proximal deep vein thrombosis in mechanically ventilated COVID-19 patients. *J. Infect.* [Epub ahead of print].

Wolberg, A. S., Aleman, M. M., Leiderman, K., and Machlus, K. R. (2012). Procoagulant activity in hemostasis and thrombosis: virchow's triad

revisited. *Anesth. Analgesia* 114, 275–285. doi: 10.1213/ANE.0b013e31823a088c

Wool, G. D., and Miller, J. L. (2020). The impact of COVID-19 disease on platelets and coagulation. *Pathobiology* 13, 1–13. doi: 10.1159/000512007 [Epub ahead of print].

Zhang, L., Feng, X., Zhang, D., Jiang, C., Mei, H., Wang, J., et al. (2020). Deep vein thrombosis in hospitalized patients with COVID-19 in wuhan. China Prevalence, Risk Factors, and Outcome. *Circulation* 142, 114–128. doi: 10.1161/CIRCULATIONAHA.120.046702

Zhang, Q., Wang, X., Su, L., Zhang, H., Chai, W., Chao, Y., et al. (2018). Relationship between inferior vena cava diameter ratio and central venous pressure. *J. Clin. Ultrasound* 46, 450–454. doi: 10.1002/jcu.22586

Zuo, Y., Zuo, M., Yalavarthi, S., Gockman, K., Madison, J. A., Shi, H., et al. (2020). Neutrophil extracellular traps and thrombosis in COVID-19. *J. Thromb. Thrombolysis* 2020, 1–8. doi: 10.1007/s11239-020-02324-z

Conflict of Interest: AM reports personal fees from Orion, Servier, Otsuka, Philips, Sanofi, Adrenomed, Epygon, and Fire 1 and grants and personal fees from 4TEEN4, Abbott, Roche, and Sphingotec.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

MINI REVIEW
published: 11 January 2021
doi: 10.3389/fphys.2020.624657

Stimulating the Resolution of Inflammation Through Omega-3 Polyunsaturated Fatty Acids in COVID-19: Rationale for the *COVID-Omega-F* Trial

*Hildur Arnardottir[1,2†], Sven-Christian Pawelzik[1,2†], Ulf Öhlund Wistbacka[3], Gonzalo Artiach[1,2], Robin Hofmann[4,5], Ingalill Reinholdsson[6], Frieder Braunschweig[2], Per Tornvall[4,5], Dorota Religa[3,7] and Magnus Bäck[1,2]**

[1] Translational Cardiology, Karolinska Institutet, Stockholm, Sweden, [2] Department of Cardiology, Theme Heart, Vessels, and Neuro, Karolinska University Hospital, Stockholm, Sweden, [3] Theme Ageing, Karolinska University Hospital, Stockholm, Sweden, [4] Department of Clinical Science and Education, Karolinska Institutet, Stockholm, Sweden, [5] Department of Cardiology, Södersjukhuset, Stockholm, Sweden, [6] Karolinska Trial Alliance, Sabbatsberg's Hospital, Stockholm, Sweden, [7] Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden

OPEN ACCESS

Edited by:
Adriana Castello Costa Girardi, University of São Paulo, Brazil

Reviewed by:
Philip Calder, University of Southampton, United Kingdom
Martin Giera, Leiden University Medical Center, Netherlands

***Correspondence:**
Magnus Bäck
Magnus.Back@ki.se

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: 31 October 2020
Accepted: 09 December 2020
Published: 11 January 2021

Citation:
Arnardottir H, Pawelzik S-C, Öhlund Wistbacka U, Artiach G, Hofmann R, Reinholdsson I, Braunschweig F, Tornvall P, Religa D and Bäck M (2021) Stimulating the Resolution of Inflammation Through Omega-3 Polyunsaturated Fatty Acids in COVID-19: Rationale for the COVID-Omega-F Trial. Front. Physiol. 11:624657. doi: 10.3389/fphys.2020.624657

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). SARS-CoV-2 triggers an immune response with local inflammation in the lung, which may extend to a systemic hyperinflammatory reaction. Excessive inflammation has been reported in severe cases with respiratory failure and cardiovascular complications. In addition to the release of cytokines, referred to as cytokine release syndrome or "cytokine storm," increased pro-inflammatory lipid mediators derived from the omega-6 polyunsaturated fatty acid (PUFA) arachidonic acid may cause an "eicosanoid storm," which contributes to the uncontrolled systemic inflammation. Specialized pro-resolving mediators, which are derived from omega-3 PUFA, limit inflammatory reactions by an active process called resolution of inflammation. Here, the rationale for omega-3 PUFA supplementation in COVID-19 patients is presented along with a brief overview of the study protocol for the trial "Resolving Inflammatory Storm in COVID-19 Patients by Omega-3 Polyunsaturated Fatty Acids - A single-blind, randomized, placebo-controlled feasibility study" (COVID-Omega-F). EudraCT: 2020-002293-28; clinicaltrials.gov: NCT04647604.

Keywords: COVID-19, eicosanoids, omega-3 fatty acids, resolution of inflammation, clinical trial

INTRODUCTION

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) triggers an immune response and a local pulmonary inflammation which may extend to become a systemic inflammation. The leading cause of COVID-19 mortality is respiratory failure from acute respiratory distress syndrome (ARDS), which occurs in 15–29% of cases (Huang et al., 2020). Even in non-fatal cases the excessive inflammation can also extend to for example cardiovascular manifestations (Evans et al., 2020). While anti-inflammatory treatments are effective in dampening

the acute inflammation, there may be a concern that this will cause an immunosuppression to aggravate infection. In this context, it is important to stress that the resolution of inflammation is an active process coordinated to limit and eventually turn off an inflammation while retaining an intact host defense (Panigrahy et al., 2020). Mediators of the resolution phase skew the immune response to clearance mechanisms of apoptotic cells, debris and microbes while limiting inflammatory activation (Serhan, 2014). This process actively promotes healing and repair and drives a tissue back to homeostasis after an acute infection and/or injury (Chiang and Serhan, 2020).

A three-stage classification has been proposed for COVID-19 to illustrate an initial mild infection, a second stage with established pulmonary involvement with or without hypoxia, and a third severe stage with hyperinflammation (Siddiqi and Mehra, 2020). Importantly, an active resolution of inflammation is needed to heal each stage of the disease, as depicted in **Figure 1**. A functional resolution of inflammation may also be crucial for presenting with only mild symptoms, whereas failed resolution may lead to escalating severity in clinical stages of COVID-19 (**Figure 1**). Importantly, the resolution of inflammation is impaired with ageing (Arnardottir et al., 2014), which may explain why younger people are less affected in COVID-19. In addition to age, the level of frailty is a predictor of short-term COVID-19 outcomes in older patient populations (Hägg et al., 2020).

Polyunsaturated fatty acids (PUFA) serve as the substrate for pro-inflammatory, anti-inflammatory, and specialized pro-resolving lipid mediators (SPM) (Chiang and Serhan, 2020). Specifically, the omega-6 PUFA arachidonic acid (AA) is substrate for the lipoxygenase and cyclooxygenase pathways, which generate leukotrienes and prostaglandins, respectively, collectively referred to as eicosanoids (**Figure 1**). In contrast, the omega-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) serve as the substrate for pro-resolving SPM (**Figure 1**). PUFA can also be metabolized by cytochrome (CYP) P450 epoxygenases into their respective epoxides (**Figure 1**), which also regulate the inflammatory reaction.

Increasing omega-3 PUFA and decreasing omega-6 PUFA hence represent a possible mean to skew the immune response toward resolution of inflammation (**Figure 1**). It should however, be considered that AA also gives rise to pro-resolving lipoxins, which is favored by the CYP450-derived AA epoxides (Hammock et al., 2020; **Figure 1**). In addition, another omega-6 PUFA, adrenic acid, has recently been ascribed anti-inflammatory actions (Brouwers et al., 2020). Nevertheless, a low omega-3 to omega-6 ratio is in general indicating a nutritional state favoring inflammation (Artiach et al., 2020b; Xue et al., 2020), which can also be reflected in the ratios of the respective lipid mediators, e.g., the resolvin to leukotriene ratio (Thul et al., 2017).

CYTOKINE STORM IN COVID-19

Increased levels of inflammatory cytokines triggered by SARS-CoV-2 in the peripheral blood cause an uncontrolled systemic inflammation, referred to as cytokine release syndrome or "cytokine storm" (Ragab et al., 2020). In particular, macrophage-related cytokines like interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) were initially reported to be increased

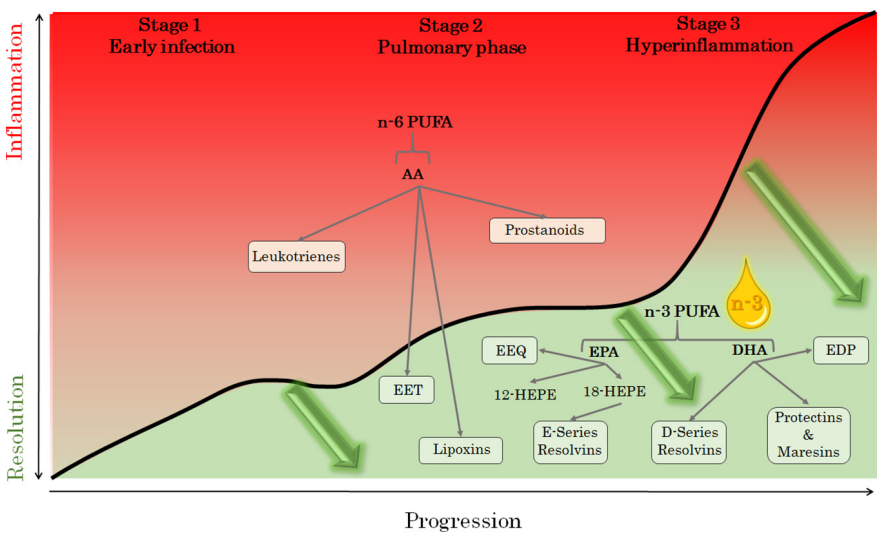


FIGURE 1 | The metabolism of the omega-6 (n-6) and omega-3 (n-3) polyunstaurated fatty acids (PUFA) arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The pathways depicted in red indicate proinflammatory mediators during the three stages of COVID-19 severity: early infection (stage 1), pulmonary phase (stage 2), and hyperinflammation phase (stage 3); adapted from Siddiqi and Mehra (2020). Intravenous administration of n-3 PUFA is anticipated to decrease n-6 PUFA and, in particular, AA-derived proinflammatory lipid mediators, and increase specialized pro-resolving mediators (including resolvins of the E- and D-series, protectins, and maresins), as well as the monohydroxy intermediates hydroxy-EPA (HEPE) and the cytochrome P450 epoxides epoxyeicosatrienoic (EET), epoxyeicosatetraenoic (EEQ), and epoxydocosapentaenoic (EDT) acids. Mediators potentially inducing the resolution of inflammation at the different stages of COVID-19 infection (green arrows), are depicted in green.

in COVID-19 infected patients compared with control subjects, with higher levels of cytokines in severe compared with non-severe infection (Chen et al., 2020; Huang et al., 2020; Ye et al., 2020). Furthermore, plasma concentrations of some cytokines were reported higher in COVID-19 patients admitted to intensive care units (ICU) compared with non-ICU patients (Chen et al., 2020; Huang et al., 2020). The degree of inflammation, measured as IL-6 plasma levels, correlates with the viral load determined as SARS-CoV-2 RNAemia (Huang et al., 2020). IL-6 is also a predictor of mortality for COVID-19 patients in ICU (Huang et al., 2020; Ruan et al., 2020). While both moderate and severe COVID-19 present with an increase in IL-6 (Chen et al., 2020), concentrations $\geq$100 pg/mL are observed exclusively in critically ill patients with high systemic viral nucleic acid detection (Huang et al., 2020). Taken together, these reports support that increased cytokine levels may be associated with worse clinical presentation and outcome in COVID-19, of which IL-6 may be an important driving force of the cytokine storm.

The concept of the cytokine storm has, however, been challenged recently. In a study of 46 patients with COVID-19-related ARDS, the levels of IL-6, IL-8, and TNF-α were lower compared with 51 SARS-CoV-2 negative patients with ARDS as a result of septic shock (Kox et al., 2020). Single cell sequencing has also failed to detect substantial amounts of pro-inflammatory cytokines in peripheral monocytes and lymphocytes from COVID-19 patients (Wilk et al., 2020). Nevertheless, pro-inflammatory, monocyte-derived macrophages are abundant in the bronchoalveolar lavage fluid of severe cases of SARS-CoV-2 infection (Liao et al., 2020), confirming the exaggerated inflammatory response in COVID-19. Taken together, the latter studies reinforce the importance of exploring non-cytokine mediators of inflammation as drivers of the COVID-19 inflammatory storm.

EICOSANOID STORM IN COVID-19

Eicosanoids derived from AA comprise the prostanoids [prostaglandins (PG) E_2, PGD_2, $PGF_{2\alpha}$, PGI_2, and thromboxane (TXA_2)], which are formed by the cyclooxygenase pathway, and the leukotrienes [LTB_4 as well as the cysteinyl leukotrienes (cysLT) LTC_4, LTD_4, and LTE_4], which are formed by the 5-lipoxygenase pathway (**Figure 1**). Eicosanoids contribute to inflammation by several mechanisms, e.g., recruitment of inflammatory cells (LTB_4, PGD_2), vasodilation (PGE_2, PGI_2), broncho- and vasoconstriction (PGE_2, $PGF_{2\alpha}$, cysLT), hyperalgesia and pyrogenicity (PGE_2), or increased vascular permeability (cysLT). Increased levels of pro-inflammatory lipid mediators have been described in uncontrolled immune responses to other severe infections and been coined "eicosanoid storm" (Dennis and Norris, 2015). A targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) lipidomics analysis of serum from 18 moderate and 20 severe COVID-19 patients compared with 19 healthy subjects revealed major changes in AA-derived lipid mediators from both the cyclooxygenase and 5-lipoxygenase pathway, with severe COVID-19 being characterized by an increase in 5-lipoxygenase expressing monocyte/macrophage populations (Schwarz et al., 2020). These findings suggest that in addition to cytokines, an eicosanoid storm of pro-inflammatory lipid mediators may also contribute to the hyperinflammation in COVID-19 (Hammock et al., 2020).

Targeting pro-inflammatory eicosanoid signaling by means of the leukotriene receptor antagonist montelukast could have potential protective effects on pulmonary (Dahlén and Bäck, 2001), cardiovascular (Ingelsson et al., 2012), and inflammatory (Bäck et al., 2014) responses and is currently evaluated in COVID-19 (Pierantonio et al., 2020). Moreover, non-steroidal anti-inflammatory drugs (NSAID) suppress the formation of prostanoids and have been discussed to aggravate COVID-19 infection, but in the lack of evidence to support this notion, regular NSAID users are advised to continue with their therapy (FitzGerald, 2020). It can at present hence not be concluded if eicosanoid targeting during SARS-CoV-2 infection has either adverse or beneficial effects.

Importantly, supplementation with omega-3 PUFA decreases the relative availability of AA for pro-inflammatory eicosanoid synthesis and decreases leukotriene formation (Artiach et al., 2020a) and hence represents another possible means to decrease the eicosanoid storm in COVID-19.

RESOLUTION OF INFLAMMATION

As an alternative to inhibiting pro-inflammatory signaling, acute inflammation could potentially be disrupted by actively stimulating the resolution of inflammation. The enzymatic conversion of omega-3 PUFA into SPMs actively disrupts inflammatory circuits and skews the immune response toward healing and return to homeostasis (Serhan, 2014). Some SPM also inhibit viral replication and reduce the severity of viral pneumonia in experimental models (Morita et al., 2013; Duvall and Levy, 2016). Several observational and experimental studies also support a role of SPM in ARDS and acute lung injury (Darwesh et al., 2020).

The uncontrolled immune response observed in severe cases of COVID-19 with cytokine and eicosanoid release hence represents the archetype for a state of failure in the resolution of inflammation (**Figure 1**).

SPECIALIZED PRO-RESOLVING MEDIATORS IN COVID-19

Lipidomic analysis has importantly showed that subjects with moderate COVID-19 exhibit significantly higher levels of the EPA-derived pro-resolving mediator RvE3 compared with severe cases (Schwarz et al., 2020). This observation provides a first indication that loss of pro-resolving mediators derived from omega-3 PUFA may be associated with more severe COVID-19. Furthermore, the resolvin biosynthetic pathways can be activated by SARS-CoV-2 viral proteins. Monocyte-derived macrophages isolated from subjects with cystic fibrosis and stimulated with the SARS-CoV-2 S and N proteins increase

not only pro-inflammatory cytokines but also the DHA-derived pro-resolving lipid mediator RvD1 (Recchiuti et al., 2020). Increasing the substrates for E- and D series Rv by means of EPA and DHA supplementation hence has the potential to increase the formation of these pro-resolving mediators. Importantly, concomitant treatment of macrophages with SARS-CoV-2 viral proteins and exogenous RvD1 significantly reduces Macrophage Inflammatory Protein (MIP)-1α, TNF-α, and IL-8 (Recchiuti et al., 2020), further reinforcing the potential therapeutic benefit of increasing pro-resolving omega-3 PUFA-derived lipid mediators in COVID-19. Randomized controlled studies (RCT) have supported increased levels of pro-resolving mediators (Elajami et al., 2016) and a decreased inflammation (Mayer et al., 2003; Bhatt et al., 2019) after omega-3 PUFA supplementation.

ANTI-INFLAMMATORY, ANTI-VIRAL AND/OR PRO-RESOLVING TREATMENT OPTIONS IN COVID-19

The sharp increase in numerous pro-inflammatory cytokines and its association to worse clinical presentation and outcome have led to the notion that targeting the cytokine storm may be an important part of rescuing severe COVID-19 patients (Mehta et al., 2020). Therapeutic options currently considered for this purpose include steroids, selective cytokine blockade, and other specific anti-inflammatory treatments (Mehta et al., 2020). Importantly, some anti-inflammatory therapeutic approaches are inherently characterized by a general immunosuppression, which may impede the host defense against the virus, and hence even aggravate the infection.

The omega-3 PUFA-derived pro-resolving mediator protectin D1 (PD1; **Figure 2**) attenuates influenza virus replication in experimental models (Morita et al., 2013). In influenza-infected mice, PD1 improves survival similar to antiviral treatment using peramivir starting 48 hr after infection. Interestingly, the combination of PD1 with peramivir completely rescued the mice from death due to the influenza infection (Morita et al., 2013). Although their clinical implications remain to be established, these preclinical observations indicate a potential additive effect between pro-resolving omega-3 PUFA-derived lipid mediators and antiviral treatment in preventing lethal infectious outcomes.

The benefit of glucocorticoid treatment in COVID-19 was demonstrated by the RECOVERY trial, showing lower 28-day mortality in ventilated and oxygen-treated patients randomized to either dexamethasone or usual care during hospitalization (Recovery Collaborative Group et al., 2020). Importantly, dexamethasone increases the production of pro-resolving lipid mediators from the omega-3 PUFA DHA (**Figure 2**), pointing to possible synergistic effects of omega-3 PUFA and cortisone treatment in COVID-19 (Pyrillou et al., 2018; Andreakos et al., 2020).

OMEGA-3 PUFA SUPPLEMENTATION IN ARDS

Significant improvements in ARDS patients have been reported after omega-3 PUFA administration. A recent meta-analysis of

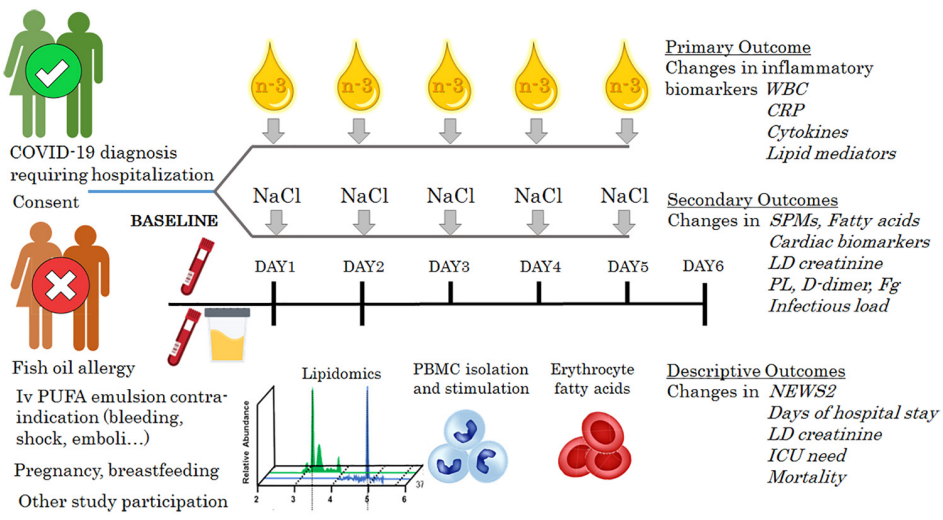


FIGURE 2 | Schematic study protocol for the COVID-Omega-F trial, registered in the European Union Drug Regulating Authorities Clinical Trials (EudraCT) database with number 2020-002293-28 and at ClinicalTrials.gov (clinicaltrials.gov/ct2/show/NCT04647604). An intravenous omega-3 PUFA emulsion (n-3) is compared with placebo (NaCl) for effects on a panel of inflammatory biomarkers; white blood cells (WBC), C-reactive protein (CRP), cytokines and lipid mediators at the end of the treatment period. Secondary endpoints include changes in specialized proresolving mediators (SPMs), fatty acids, cardiac markers, lactate dehydrogenase (LD), D-dimer, fribronogen (Fg), and infectious load. National Early Warning Score 2 (NEWS2), length of hospital stay, intensive cae unit (ICU) need, and mortality will be monitored for a descriptive analysis. The inclusion criteria for COVID-Omega-F comprise female and male patients ≥18 years of age diagnosed with COVID-19 and presenting with a clinical status requiring hospitalization. Exclusion criteria are defined according to i.v. omega-3 PUFA emulsion contraindications, known hypersensitivity, participation in other clinical research study, pregnancy, and breastfeeding.

12 RCT (n = 1280 patients) performed in patients with ARDS concluded that omega-3 PUFA supplementation was associated with improvements in the PaO_2/FiO_2 ratio, and statistical trends toward a shorter ICU stay (p = 0.08) and a shorter duration of mechanical ventilation (p = 0.06) were apparent, whereas infectious complications remained unchanged. In an analysis restricted to enteral omega-3 PUFA supplementation, a significant decrease in ARDS mortality was also seen (Langlois et al., 2019). A Cochrane meta-analysis of 10 studies noted a statistical reduction in mortality from ARDS when omega-3 PUFA were compared with a lipid-rich enteral formula but stated that it is currently uncertain whether omega-3 PUFA supplementation alters mortality, oxygenation, or duration of mechanical ventilation and ICU stays because of large heterogeneity between the studies (Dushianthan et al., 2019). There is hence a need for further studies of omega-3 PUFA supplementation in ARDS.

Omega-3 PUFA supplementation by means of intravenous (i.v.) administration provides an effective strategy of increasing omega-3 PUFA in the setting of acute disease and intensive care. One previous RCT has specifically evaluated the effects of i.v. omega-3 PUFA emulsion in ARDS (of other cause than COVID-19). Whereas the primary outcome of changes in respiratory parameters were not significantly altered in this RCT of 61 ventilated patients with ARDS, the observed fall in PaO_2/FiO_2 ratio from baseline to day 14 was significantly higher in the control group as compared to the n = 31 patients treated with i.v. omega-3 PUFA emulsion at 0.1 g/kg/day (Gupta et al., 2011). The latter study also reported a trend of better survival in the i.v. omega-3 PUFA emulsion group (77%) compared with the control group (56%; p = 0.10) (Gupta et al., 2011). Importantly, no adverse effects were observed, and no safety concerns of i.v. omega-3 PUFA emulsion treatment in ARDS were raised from this (Gupta et al., 2011) and other studies (Sabater et al., 2008), indicating that lipid emulsions enriched with omega-3 PUFA are safe in ARDS.

INTRAVENOUS OMEGA-3 PUFA SUPPLEMENTATION AND INFLAMMATION

The rationale for studying i.v. omega-3 PUFA emulsion treatment in COVID-19 is strengthened by the possibility to reduce the inflammatory storm. In a study of 19 patients in septic shock, the 10 patients randomized to i.v. omega-3 PUFA emulsion (350 mL/day, which is equivalent to 14 g DHA + EPA) for 3 days attained an omega-3/omega-6 ratio of 2.5:1 and remarkably lower levels of TNF-α, IL-6, and IL-8 in *ex vivo* stimulated leukocytes (Mayer et al., 2003). Lower doses of omega-3 PUFA supplementation may, however, not be sufficient, as demonstrated by a larger study of different lipid emulsions in patients with systemic inflammatory response syndrome (SIRS), which resulted in an omega-3/omega-6 ratio of 1:7 in the control group and 1:2 in the supplementation group and did not show any significant differences in circulating IL-6 levels between the groups (Friesecke et al., 2008).

INTRAVENOUS OMEGA-3 PUFA SUPPLEMENTATION IN COVID-19

Based on the above, high dose omega-3 PUFA supplementation by the i.v. route appears as an appealing treatment option in COVID-19 with minimal risks to the patients. Taken together, these observations provide the rationale for actively stimulating the resolution of inflammation to break the inflammatory storm caused by SARS-CoV-2 infection. This is also in line with recent communications from other investigators on the potential benefits of enteral (Calder, 2020) and parenteral (Torrinhas et al., 2020) omega-3 PUFA supplementation in COVID-19 patients. Scientists in the field have stressed the uttermost priority to investigate pro-resolving lipid mediators in COVID-19 (Andreakos et al., 2020).

THE *COVID-Omega-F* TRIAL

To establish whether omega-3 PUFA supplementation by the i.v. route is a possible treatment option in COVID-19, we have initiated the trial "Resolving Inflammatory Storm in ***COVID***-19 Patients by ***Omega***-3 Polyunsaturated Fatty Acids – A single-blind, randomized, placebo-controlled ***F***easibility Study" (COVID-Omega-F). The study received approval from the National Ethics Board on May 25, 2020 (Dnr 2020-02592) and by the Medical Product Agency on May 29, 2020 (Dnr 5.1-2020-42861). Ten patients in each group will be randomized (in total n = 20). An amendment has been approved to increase inclusion up to n = 40 patients in total to achieve comparable groups completing the full study protocol according to the initial sample size calculations (National Ethics Board approval November 25, 2020; Dnr 2020-06137, and Medical Product Agency approval on November 30, 2020; Dnr 5.1-2020-96391). COVID-Omega-F is registered at the European Union Drug Regulating Authorities Clinical Trials (EudraCT) database with the number 2020-002293-28 and at ClinicalTrials.gov (clinicaltrials.gov/ct2/show/NCT04647604).

The study intervention is a single-blind randomization to daily administration of either an i.v. omega-3 PUFA emulsion containing 10 g of fishoil per 100 mL, of which 1.25–2.82 g DHA and 1.44–3.09 g EPA (0.2 g/kg/day at 0.5 mL/kg/h) or placebo (i.v. NaCl at 0.5 mL/kg/h to equivalent volume) for 5 days. The primary objective is to establish the effects of i.v. omega-3 PUFA emulsion on inflammatory biomarkers in COVID-19 patients compared to placebo. The primary endpoint is changes in a panel of inflammatory biomarkers measured in blood samples, urine samples, and released from *ex vivo* stimulated leukocytes at the end of the 5 day treatment period (**Figure 2**). The secondary endpoints are changes in pro-resolving mediators and PUFA levels, including the omega-3 to omega-6 ratio, in the erythrocyte fraction as well as measures of biomarkers for organ damage, thrombosis, and infectious load as indicated in **Figure 2**. Indicators for the clinical course of disease (**Figure 2**) will also be monitored during hospitalization and used for a descriptive analysis only since the study is not powered to detect clinical treatment benefits.

The inclusion criteria for COVID-Omega-F comprise female and male patients $\geq$ 18 years of age found COVID-19 positive or with typical CT image of COVID-19 infection, and clinical status requiring hospitalization. Exclusion criteria are defined according to i.v. omega-3 PUFA emulsion contraindications (serious bleeding disorders and acute life-threatening condition including acute shock, acute myocardial infarction, acute stroke, acute emboli, and coma), known hypersensitivity to the i.v. omega-3 PUFA emulsion or any of its ingredients, participation in any clinical research study evaluating an investigational medicinal product within 3 months prior to screening, pregnancy, and breastfeeding.

Blood samples will be collected for biomarker measures at inclusion before the first dose of treatment (baseline) and then daily until either completing 5 days of treatment or at hospital discharge (whichever comes first). At baseline, 24–48 h after the first dose of omega-3 PUFA emulsion or placebo infusion, and at treatment end, blood and urine samples will be collected for the following experimental procedures: Whole blood will be analyzed by flow cytometry for surface markers and functional assays. Peripheral blood mononuclear cells (PBMCs) will be isolated and stimulated with endotoxin followed by collection of supernatants for biomarker analysis and cells for real time quantitative PCR. The erythrocyte fraction will be used for determination of fatty acid composition by gas chromatography. Serum, plasma, urine, and PBMC supernatants will be used for a comprehensive analysis of inflammatory biomarkers, including cytokines and bioactive lipid mediators from the omega-3 and omega-6 metabolomes using LC-MS/MS. The lipid mediators to be detected include resolvins of the E and D series, lipoxins, leukotrienes, and prostanoids, as well as their intermediates (**Figure 1**), of which 18-HEPE has been previously established as a robust marker for pro-resolving mediator formation from omega-3 PUFA (Laguna-Fernandez et al., 2018).

CONCLUSION

It is anticipated that i.v. omega-3 PUFA administration will decrease inflammatory mediators and that this will be indicative for potential beneficial clinical effects. Importantly, the simultaneous monitoring of pro-inflammatory and pro-resolving mediators will facilitate the understanding of a possible failure of the resolution of inflammation in COVID-19. In addition to obtaining the proof-of-concept for the resolution of inflammation through omega-3 PUFA treatment in COVID-19, this study will provide information on the feasibility of the study protocol. Together, this will lay the groundwork for a larger RCT on i.v. omega-3 PUFA administration on disease outcome in COVID-19.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

Andreakos, E., Papadaki, M., and Serhan, C. N. (2020). Dexamethasone, pro-resolving lipid mediators and resolution of inflammation in COVID-19. *Allergy* [Epub ahead of print]. doi: 10.1111/all.14595

Arnardottir, H. H., Dalli, J., Colas, R. A., Shinohara, M., and Serhan, C. N. (2014). Aging delays resolution of acute inflammation in mice: reprogramming the host response with novel nano-proresolving medicines. *J. Immunol.* 193, 4235–4244. doi: 10.4049/jimmunol.1401313

Artiach, G., Carracedo, M., Plunde, O., Wheelock, C. E., Thul, S., Sjovall, P., et al. (2020a). Omega-3 polyunsaturated fatty acids decrease aortic valve disease through the Resolvin E1 and ChemR23 axis. *Circulation* 142, 776–789. doi: 10.1161/CIRCULATIONAHA.119.041868

Artiach, G., Sarajlic, P., and Bäck, M. (2020b). Inflammation and its resolution in coronary artery disease: a tightrope walk between omega-6 and omega-3 polyunsaturated fatty acids. *Kardiol. Pol.* 78, 93–95. doi: 10.33963/KP.15202

Bäck, M., Powell, W. S., Dahlen, S. E., Drazen, J. M., Evans, J. F., Serhan, C. N., et al. (2014). Update on leukotriene, lipoxin and oxoeicosanoid receptors: IUPHAR review 7. *Br. J. Pharmacol.* 171, 3551–3574. doi: 10.1111/bph.12665

Bhatt, D. L., Steg, P. G., Miller, M., Brinton, E. A., Jacobson, T. A., Ketchum, S. B., et al. (2019). Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N. Engl. J. Med.* 380, 11–22. doi: 10.1056/NEJMoa1812792

Brouwers, H., Jonasdottir, H. S., Kuipers, M. E., Kwekkeboom, J. C., Auger, J. L., Gonzalez-Torres, M., et al. (2020). Anti-Inflammatory and proresolving effects of the Omega-6 polyunsaturated fatty Acid Adrenic Acid. *J. Immunol.* 205, 2840–2849. doi: 10.4049/jimmunol.1801653

Calder, P. (2020). Nutrition, immunity and COVID-19. *BMJ Nutr. Prevent. Health* 3, 74–92. doi: 10.1136/bmjnph-2020-000085

Chen, G., Wu, D., Guo, W., Cao, Y., Huang, D., Wang, H., et al. (2020). Clinical and immunological features of severe and moderate coronavirus disease 2019. *J. Clin. Invest.* 130, 2620–2629. doi: 10.1172/JCI137244

Chiang, N., and Serhan, C. N. (2020). Specialized pro-resolving mediator network: an update on production and actions. *Essays Biochem.* 64, 443–462. doi: 10.1042/ebc20200018

Dahlén, S. E., and Bäck, M. (2001). Leukotriene receptors: functional aspects and future targets. *Clin. Exper. Allergy Rev.* 1, 137–141. doi: 10.1046/j.1472-9725.2001.00025.x

Darwesh, A. M., Bassiouni, W., Sosnowski, D. K., and Seubert, J. M. (2020). Can N-3 polyunsaturated fatty acids be considered a potential adjuvant therapy for COVID-19-associated cardiovascular complications? *Pharmacol. Ther.* 2020:107703. doi: 10.1016/j.pharmthera.2020.107703

Dennis, E. A., and Norris, P. C. (2015). Eicosanoid storm in infection and inflammation. *Nat. Rev. Immunol.* 15, 511–523. doi: 10.1038/nri3859

Dushianthan, A., Cusack, R., Burgess, V. A., Grocott, M. P., and Calder, P. C. (2019). Immunonutrition for acute respiratory distress syndrome (ARDS) in adults. *Cochrane Database Syst. Rev.* 1:CD012041. doi: 10.1002/14651858.CD012041

Duvall, M. G., and Levy, B. D. (2016). DHA- and EPA-derived resolvins, protectins, and maresins in airway inflammation. *Eur. J. Pharmacol.* 785, 144–155. doi: 10.1016/j.ejphar.2015.11.001

Elajami, T. K., Colas, R. A., Dalli, J., Chiang, N., Serhan, C. N., and Welty, F. K. (2016). Specialized proresolving lipid mediators in patients with coronary artery disease and their potential for clot remodeling. *FASEB J.* 30, 2792–2801. doi: 10.1096/fj.201500155R

Evans, P. C., Rainger, G., Mason, J. C., Guzik, T. J., Osto, E., Stamataki, Z., et al. (eds) (2020). Endothelial dysfunction in COVID-19: a position paper of the ESC Working group for atherosclerosis and vascular biology, and the ESC council of basic cardiovascular science. *Cardiovasc. Res.* 116, 2177–2184. doi: 10.1093/cvr/cvaa230

FitzGerald, G. A. (2020). Misguided drug advice for COVID-19. *Science* 367:1434. doi: 10.1126/science.abb8034

Friesecke, S., Lotze, C., Kohler, J., Heinrich, A., Felix, S. B., and Abel, P. (2008). Fish oil supplementation in the parenteral nutrition of critically ill medical

patients: a randomised controlled trial. *Intens. Care Med.* 34, 1411–1420. doi: 10.1007/s00134-008-1072-1

Gupta, A., Govil, D., Bhatnagar, S., Gupta, S., Goyal, J., Patel, S., et al. (2011). Efficacy and safety of parenteral omega 3 fatty acids in ventilated patients with acute lung injury. *Indian J. Crit. Care Med.* 15, 108–113. doi: 10.4103/0972-5229.83019

Hägg, S., Jylhävä, J., Wang, Y., Xu, H., Metzner, C., Annetorp, M., et al. (2020). Age, Frailty, and comorbidity as prognostic factors for short-term outcomes in patients with Coronavirus Disease 2019 in geriatric care. *J. Am. Med. Dir. Assoc.* 21, 1555–1559.e2. doi: 10.1016/j.jamda.2020.08.014

Hammock, B. D., Wang, W., Gilligan, M. M., and Panigrahy, D. (2020). Eicosanoids: the overlooked storm in coronavirus disease 2019 (COVID-19)? *Am. J. Pathol.* 190, 1782–1788. doi: 10.1016/j.ajpath.2020.06.010

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Ingelsson, E., Yin, L., and Back, M. (2012). Nationwide cohort study of the leukotriene receptor antagonist montelukast and incident or recurrent cardiovascular disease. *J. Allergy Clin. Immunol.* 129, 702–707.e2. doi: 10.1016/j.jaci.2011.11.052

Kox, M., Waalders, N. J. B., Kooistra, E. J., Gerretsen, J., and Pickkers, P. (2020). Cytokine levels in critically Ill patients with COVID-19 and other conditions. *JAMA* 324, 1565–1567. doi: 10.1001/jama.2020.17052

Laguna-Fernandez, A., Checa, A., Carracedo, M., Artiach, G., Petri, M. H., Baumgartner, R., et al. (2018). ERV1/ChemR23 signaling protects against atherosclerosis by modifying oxidized low-density lipoprotein uptake and phagocytosis in macrophages. *Circulation* 138, 1693–1705. doi: 10.1161/CIRCULATIONAHA.117.032801

Langlois, P. L., D'Aragon, F., Hardy, G., and Manzanares, W. (2019). Omega-3 polyunsaturated fatty acids in critically ill patients with acute respiratory distress syndrome: A systematic review and meta-analysis. *Nutrition* 61, 84–92. doi: 10.1016/j.nut.2018.10.026

Liao, M., Liu, Y., Yuan, J., Wen, Y., Xu, G., Zhao, J., et al. (2020). Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat. Med.* 26, 842–844. doi: 10.1038/s41591-020-0901-9

Mayer, K., Gokorsch, S., Fegbeutel, C., Hattar, K., Rosseau, S., Walmrath, D., et al. (2003). Parenteral nutrition with fish oil modulates cytokine response in patients with sepsis. *Am. J. Respir. Crit. Care Med.* 167, 1321–1328. doi: 10.1164/rccm.200207-674OC

Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., Manson, J. J., et al. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 395, 1033–1034. doi: 10.1016/S0140-6736(20)30628-0

Morita, M., Kuba, K., Ichikawa, A., Nakayama, M., Katahira, J., Iwamoto, R., et al. (2013). The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. *Cell* 153, 112–125. doi: 10.1016/j.cell.2013.02.027

Panigrahy, D., Gilligan, M. M., Huang, S., Gartung, A., Cortes-Puch, I., Sime, P. J., et al. (2020). Inflammation resolution: a dual-pronged approach to averting cytokine storms in COVID-19? *Cancer Metast. Rev.* 39, 337–340. doi: 10.1007/s10555-020-09889-4

Pierantonio, L. A., Bessa De Sousa, D. F., Michael, J., Schuster, D., and Reitsamer, H. (2020). The leukotriene receptor antagonist montelukast as a potential COVID-19 therapeutic. *Front. Mol. Biosci.* doi: 10.3389/fmolb.2020.610132

Pyrillou, K., Chairakaki, A. D., Tamvakopoulos, C., and Andreakos, E. (2018). Dexamethasone induces omega3-derived immunoresolvents driving resolution of allergic airway inflammation. *J. Allergy Clin. Immunol.* 142, 691–695.e4. doi: 10.1016/j.jaci.2018.04.004

Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R., and Salem, R. (2020). The COVID-19 cytokine storm; what we know so far. *Front. Immunol.* 11:1446. doi: 10.3389/fimmu.2020.01446

Recchiuti, A., Patruno, S., Mattoscio, D., Isopi, E., Pomilio, A., Lamolinara, A., et al. (2020). Resolvin D1 and D2 reduce SARS-Cov-2-induced inflammation in cystic fibrosis macrophages. *bioRxiv* [Preprint], doi: 10.1101/2020.08.28.255463

Recovery Collaborative Group, Horby, P., Lim, W. S., Emberson, J. R., Mafham, M., Bell, J. L., et al. (2020). Dexamethasone in hospitalized patients with Covid-19 - preliminary report. *N. Engl. J. Med.* 2020:NEJMoa2021436. doi: 10.1056/NEJMoa2021436

Ruan, Q., Yang, K., Wang, W., Jiang, L., and Song, J. (2020). Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intens. Care Med.* 324, 603–605. doi: 10.1007/s00134-020-05991-x

Sabater, J., Masclans, J. R., Sacanell, J., Chacon, P., Sabin, P., and Planas, M. (2008). Effects on hemodynamics and gas exchange of omega-3 fatty acid-enriched lipid emulsion in acute respiratory distress syndrome (ARDS): a prospective, randomized, double-blind, parallel group study. *Lipids Health Dis.* 7:39. doi: 10.1186/1476-511X-7-39

Schwarz, B., Sharma, L., Roberts, L., Peng, X., Bermejo, S., Leighton, I., et al. (2020). Severe SARS-CoV-2 infection in humans is defined by a shift in the serum lipidome resulting in dysregulation of eicosanoid immune mediators. *medRxiv* [Preprint]. doi: 10.1101/2020.07.09.20149849

Serhan, C. N. (2014). Pro-resolving lipid mediators are leads for resolution physiology. *Nature* 510, 92–101. doi: 10.1038/nature13479

Siddiqi, H. K., and Mehra, M. R. (2020). COVID-19 illness in native and immunosuppressed states: a clinical-therapeutic staging proposal. *J. Heart Lung Transpl.* 39, 405–407. doi: 10.1016/j.healun.2020.03.012

Thul, S., Labat, C., Temmar, M., Benetos, A., and Bäck, M. (2017). Low salivary resolvin D1 to leukotriene B4 ratio predicts carotid intima media thickness: a novel biomarker of non-resolving vascular inflammation. *Eur. J. Prev. Cardiol.* 24, 903–906. doi: 10.1177/2047487317694464

Torrinhas, R. S., Calder, P. C., Lemos, G. O., and Waitzberg, D. L. (2020). Parenteral fish oil: an adjuvant pharmacotherapy for coronavirus disease 2019? *Nutrition* 81:110900. doi: 10.1016/j.nut.2020.110900

Wilk, A. J., Rustagi, A., Zhao, N. Q., Roque, J., Martinez-Colon, G. J., McKechnie, J. L., et al. (2020). A single-cell atlas of the peripheral immune response in patients with severe COVID-19. *Nat. Med.* 26, 1070–1076. doi: 10.1038/s41591-020-0944-y

Xue, M., Cai, C., Guan, L., Xu, Y., Lin, J., Zeng, Y., et al. (2020). Exploration of n-6 and n-3 polyunsaturated fatty acids metabolites associated with nutritional levels in patients with severe stable chronic obstructive pulmonary disease. *Int. J. Chron. Obstruct. Pulmon. Dis.* 15, 1633–1642. doi: 10.2147/COPD.S245617

Ye, Q., Wang, B., and Mao, J. (2020). The pathogenesis and treatment of the 'Cytokine Storm' in COVID-19. *J. Infect.* 80, 607–613. doi: 10.1016/j.jinf.2020.03.037

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 12 January 2021
doi: 10.3389/fphys.2020.612268

The Impact of Polyphenols-Based Diet on the Inflammatory Profile in COVID-19 Elderly and Obese Patients

Juliana Carvalho Santos[1], Marcelo Lima Ribeiro[2] and Alessandra Gambero[3]**

[1]*Lymphoma Translational Group, Josep Carreras Leukaemia Research Institute (IJC), Badalona, Spain,* [2]*Laboratory of Immunopharmacology and Molecular Biology, Sao Francisco University, Bragança Paulista, Brazil,* [3]*Life Science Center, Pontifical Catholic University of Campinas (PUCCAMP), Campinas, Brazil*

The World Health Organization declared the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)-associated disease (coronavirus disease 2019 – COVID-19) as a pandemic in March 2020. COVID-19 is characterized by cytokine storm, acute respiratory distress syndrome (ARDS), and systemic inflammation–related pathology and already kills more than 1.5 million of people worldwide. Since aged and obese COVID-19 patients exhibit an enhanced inflammatory status, they represent a high-risk cluster for rapidly progressive clinical deterioration. These individuals present comorbid disorders and immunosenescence that may promote viral-induced cytokine storm and expression of molecules acting as virus receptor as angiotensin I converting enzyme 2 (ACE2) and CD26 (dipeptidyl-peptidase 4), resulting in respiratory failure and increased morbidity and mortality. A better knowledge of SARS-CoV-2 infection in inflammatory-associated high-risk population is essential in order to develop the therapies needed to combat or prevent severe COVID-19. Here, we review the pathogenesis and clinical implications of inflammatory disorders and disease markers associated to senescence in COVID-19 patients and the emerging evidence to argue that a high intake of polyphenols may have a protective effect on SARS-CoV-2 illness severity.

Keywords: COVID-19, cytokine storm, inflammation, senescence, polyphenols

OPEN ACCESS

Edited by:
Georges Leftheriotis, Université Côte d'Azur, France

Reviewed by:
Alessandro Tonacci, Italian National Research Council, Italy
Gautham Yepuri, New York University, United States

****Correspondence:***
Marcelo Lima Ribeiro
marcelo.ribeiro@usf.edu.br
Alessandra Gambero
alessandra.gambero@puc-campinas.edu.br;
alegambero@gmail.com

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *01 October 2020*
Accepted: *16 December 2020*
Published: *12 January 2021*

Citation:
Santos JC, Ribeiro ML and Gambero A (2021) The Impact of Polyphenols-Based Diet on the Inflammatory Profile in COVID-19 Elderly and Obese Patients. Front. Physiol. 11:612268. doi: 10.3389/fphys.2020.612268

INTRODUCTION

According to the World Health Organization, as of December 1st coronavirus disease 2019 (COVID-19) had been confirmed in almost 63 million of people worldwide, carrying a mortality of approximately 2.5%, with the vast majority of them (74%) being in people over 65 years (Webmeter, 2020; World Health Organization, 2020). Indeed, age is undoubtedly the most important risk factor for death in COVID-19 patients (Williamson et al., 2020). In addition, it has been reported that the severity of COVID-19 is associated with several comorbidities (i.e., respiratory system diseases, hypertension, diabetes, obesity, and cardiovascular disease; Webmeter, 2020). Although around 80% of confirmed severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) positive cases exhibit mild symptoms or are asymptomatic, the remaining 20% of patients may develop serious symptoms, potentially leading to death (Lai et al., 2020). These patients do

not develop severe clinical manifestations in the early stages of the disease; however, an acute respiratory distress syndrome (ARDS) and multiple-organ failure can occur at later stages. Remarkably, it has been reported that respiratory failure is responsible for 86% of death associated to SARS-CoV-2 infection (Ruan et al., 2020).

The so-called cytokine storm has been pointed out as one of the major player in the process of disease aggravation (Chousterman et al., 2017; Shimabukuro-Vornhagen et al., 2018). Accordingly, *in vitro* data showed that a delayed release of cytokines and chemokines occurs in respiratory epithelial cells, dendritic cells, and macrophages at the early stage of SARS-CoV-2 infection. These cells secrete low levels of interferons (IFNs) and high levels of pro-inflammatory cytokines and chemokines (Cheung et al., 2005; Law et al., 2005; Lau et al., 2013) which attracts inflammatory cells, such as neutrophils and monocytes, resulting in excessive infiltration of the inflammatory cells into lung tissue, an consequent lung injury.

Cellular senescence is a conserved mechanism characterized by cell cycle arrest in response to both, extrinsic and intrinsic stimulation. Although senescent cells no longer replicate, they remain metabolically active and become bigger than non-senescent cells, secrete high levels of inflammatory proteins as part of the senescence associated secretory phenotype (SASP), and acquire cell metabolism changes (van Deursen, 2014). It has been shown that senescent cells may contribute to cell proliferation, inflammation (Freund et al., 2010), angiogenesis (Coppe et al., 2006), epithelial-to-mesenchymal transition (EMT; Laberge et al., 2012), and wound healing (Jun and Lau, 2010). Importantly, cellular senescence is also associated to age-related organ dysfunction and various chronic age-related diseases, such as Alzheimer, atherosclerosis, osteoarthritis, and pulmonary fibrosis (Naylor et al., 2013). Additionally, aging and most of age-related diseases are also related to a chronic systemic condition of inflammation, known as inflammageing (Michaud et al., 2013; Sanada et al., 2018).

The activation of immune system is another important source of chronic inflammation in virus-infected patients. Patients with COVID-19 harbor high levels of inflammatory cytokines, which may activate the T-helper type 1 (Th1) cell response (Huang et al., 2020). The host inflammatory response is driven by binding to toll-like receptors (TLRs), which recognize structural components belonging to viruses, a process known as "pathogen-associated molecular patterns" (PAMPs; Janeway and Medzhitov, 2002). Moreover, neutrophil infiltration in the lungs of individuals infected by SARS-CoV-2 may result in the secretion of damage-associated molecular patterns (DAMPs), as a cell death signal following the viral invasion (Tang et al., 2012; Cicco et al., 2020).

Thus, it has been suggested that the disturbance of inflammatory homeostasis in elderly COVID-19 patients may play a pivotal role in the risk of a cytokine storm and subsequently ARDS, enhancing the mortality risk (Koelman et al., 2019; Williamson et al., 2020). In this review, we discuss the relationship between senescence markers, present in elderly and obese individuals, and the severity of COVID-19. We also highlight the possibility that dietary polyphenols could be beneficial for population most affected by COVID-19 by modifying these senescence markers (Zhou et al., 2020).

PATHOGENESIS OF SARS-CoV-2 IN INFLAMMATORY COMORBIDITIES

In early February 2020, the Chinese Center for Disease Control and Prevention (CDC) reported a large viewpoint (including 72,314 cases) summarizing that the case fatality was 8.0% (312 of 3,918) in patients 70–79 years old and 14.8% in patients aged ≥80 years (208 of 1,408; Wu and McGoogan, 2020). With the expansion of the pandemic throughout the world, it has been widely reported that elderly and geriatric adults are among the highest risk population for death among COVID-19 patients (Covino et al., 2020; Imam et al., 2020; Nguyen et al., 2020; Wang et al., 2020). Indeed, several meta-analysis confirmed that SARS-CoV-2 infection causes the highest morbidity and mortality in patients aged >60 years (Hu et al., 2020; Huang et al., 2020; Wang et al., 2020; Wu et al., 2020; Zheng et al., 2020). The reason for worsening the disease severity may be attributed to the immunosenescence and inflammageing (Chung et al., 2019; Rabi et al., 2020). Moreover, some comorbidities associated with age as hypertension, diabetes, chronic respiratory diseases, dysregulation of immune response, and obesity have been associated with severe COVID-19 (Chen et al., 2020b).

Obesity has previously been associated with hospitalization due to viruses infection, such as influenza and coronavirus (van Kerkhove et al., 2011; Moser et al., 2019). In addition, severe obesity is a risk factor associated with fatalities in hospitalized patients (Louie et al., 2011; Cocoros et al., 2014). In this sense, growing evidences indicate that obesity is also an important risk factor for worst prognosis among COVID-19 patients. The driving hypothesis point out to the axis excess of adipose tissue and inflammation, which exacerbate the cytokine storm associated with virus infection, as described below. Accordingly, several meta-analysis were able to show that comparing with non-obese patients, obese COVID-19 patients have higher risk to die (Santos et al., 2018; Halvatsiotis et al., 2020; Smith et al., 2020; Zheng et al., 2020). In addition, it has been reported that obese aged patients are more likely to be admitted at UCI for ARDS, and have also higher risk for fatality (Caussy et al., 2020; Lighter et al., 2020).

A better understanding of the pathogenesis of SARS-CoV-2 is supported by data generated from previous studies with SARS-CoV and MERS-CoV; however, it is still under construction. Mechanistically, the SARS-CoV-2 virus initially binds to the angiotensin I converting enzyme (ACE)-2 receptor *via* the spike glycoprotein envelope (S-protein) to enter into the target cells (Wan et al., 2020b), a mechanism shared with SARS-CoV (Hofmann et al., 2005) but not by MERS-CoV, that employs dipeptidyl-peptidase 4 (DPP4 or CD26) as a cell entry receptor (Raj et al., 2013). After binding to ACE-2, the virus envelope fuses with membrane epithelial cell, and the RNA strand is released into the cytoplasm of the host cell, initiating viral replication (**Figure 1**).

The ACE-2 receptor is broad and constitutively expressed in various tissues, such as heart and vascular endothelium (Hamming et al., 2004; Burrell et al., 2005), kidneys (Donoghue et al., 2000), gastrointestinal tract (Hamming et al., 2004), lungs, mainly type II alveolar epithelial cells, and immune cells, including monocytes (mainly in the classical subset of $CD14^{++}$ $CD16^{-}$ cells; Rutkowska-Zapała et al., 2015) and macrophages (mainly in the M1 phenotype; He et al., 2006). ACE-2 is the key enzyme in the balance between the production of angiotensin II (AngII) by the classical pathway of the renin angiotensin system (RAS) and production of angiotensin 1–7 (Ang 1–7) that binds to the orphan MAS receptor (MasR) triggering vasodilator, anti-inflammatory and antifibrotic events, characterizing the "anti-RAS" pathway (Rodrigues Prestes et al., 2017). After entering into the cell, SARS-CoV-2 virus represses ACE-2 expression, which results in an increase of AngII, and exacerbation of inflammation and pulmonary fibrosis (Dalan et al., 2020). The infection of lung epithelial cells and resident immune cells, such as macrophages and dendritic cells results in an important production of pro-inflammatory cytokines that contribute to the worsening of the disease.

In an experimental aging model, it was observed that the expression of pulmonary ACE2 was lower in old- versus young-rats (Xudong et al., 2006). However, when the lung injury was induced with LPS in rats of different ages, there was an imbalance of ACE/ACE2 ratio correlated with strong inflammation, which lead to acute respiratory failure in the age-dependent way (Schouten et al., 2016). A clinical study with patients of different age groups with ARDS demonstrated that the activity of ACE1, ACE2, and the ACE2/ACE1 ratio in bronchoalveolar lavage fluid was no different between groups of neonates, children, adults, or elderly (> 65 years; Schouten et al., 2019). These data indicate that we still need to understand the role of alterations in the ACE2/Angiotensin-(1–7)/MasR axis in the lung of elderly individuals. In the SARS-CoV-2 pandemic's context, age-dependent decline of ACE2 expression have been associated with COVID-19 fatality (Chen et al., 2020a; Cristiani et al., 2020).

CYTOKINE STORM IN COVID-19

Cytokine storm syndrome (CSS) is a systemic inflammatory response induced by a wide range of cytokines, resulting in clinical manifestations, such as high fever, lymphadenopathy, hepatosplenomegaly, hyperferritinaemia, and cytopaenia and, if untreated, may progress to multiple organ failure and death (Behrens and Koretzky, 2017; Murthy et al., 2019). The formation of

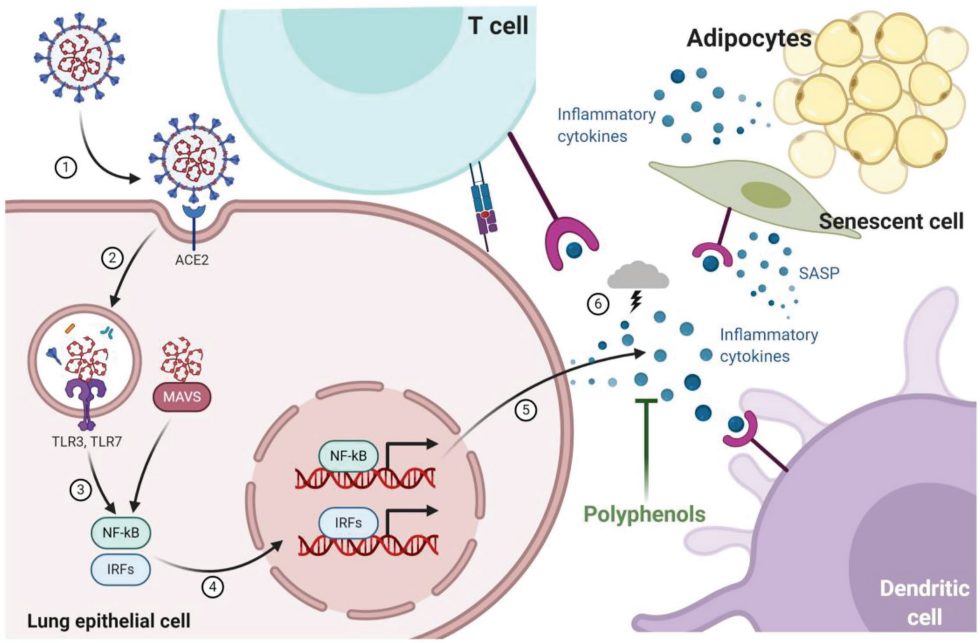


FIGURE 1 | Infection of pulmonary epithelial cells occurs through the interaction of the spike glycoprotein envelope (S-protein) with the angiotensin I converting enzyme (ACE)-2 receptor that allows viral replication and triggers mechanisms to combat infection by the host cells thought toll-like receptors (TLRs) and mitochondrial antiviral-signaling protein (MAVS). Cytokines pro-inflammatory are produced by nuclear factor kappa -B (NF-kB) and interferon-regulatory factors (IRFs) signaling pathways recruiting more immune cells (dendritic cell and T-cell) to lungs. Recruited immune cells increased cytokine production resulting in a cytokine storm that is associated with a worse prognosis of infected patients. During aging and obesity, the production of pro-inflammatory cytokines and the establishment of low-grade systemic inflammation are also observed. The expression of components of the renin-angiotensin-aldosterone system, such as ACE2, is also modified by aging and obesity, which could explain why elderly and obese patients are affected and headed the death statistics by COVID-19. Dietary bioactive substances such as polyphenols are able to block the production of cytokines by senescent cells (senescence-associated secretory phenotype; SASP) and adipocytes, as well as modify the ACE-1/ACE-2 ratio, which can potentially result in beneficial effects in COVID-19.

cytokine storm is characterized by a feedforward activation of host immune that causes an uncontrollable release of a several cytokines, such as IFN-gamma, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and IL-18 resulting in immune regulation disorder (Chousterman et al., 2017; Shimabukuro-Vornhagen et al., 2018). The continuous release of these cytokines triggers a loop reaction characterized by hyperactivation of immune cells, including T cells, macrophages, dendritic, and endothelial cells with further excessive cytokine releasing, which in turn, leads to a self-amplifying hyperinflammatory state known as cytokine storm (Crayne et al., 2019).

Immune response to SARS-CoV-2 infections initially consists in an adaptive immune response necessary to control virus propagation and to prevent disease progression. Once the virus gets to the lung tissue, it will initiate an inflammatory response as part of its immunity to combat the infection (Li et al., 2020). There are strong evidences showing that cytokine storms may participate in the pathogenesis of COVID-19 (Chen et al., 2020d; Huang et al., 2020), similar to prior epidemics such as those caused by SARS and MERS (Channappanavar and Perlman, 2017; Mahallawi et al., 2018). Like many other pathogenic microorganisms, SARS-CoV-2 also evolves mechanisms in order to evade the host immune system. The CSS caused by SARS-CoV-2 enhances the invasion and dissemination of the virus by recruiting different immune cells to the lungs, resulting in an aggressive inflammatory response (**Figure 1**). The rapid onset of spread inflammation in the lungs of patients infected with SARS-CoV-2 could lead to life-threatening respiratory disorders and subsequent death at the severe stage (Xu et al., 2020). Indeed, Wan et al. (2020a) showed a reduction of 47.62% of natural killer (NK) cells in severe COVID-19 patients. Remarkably, the autopsy findings revealed spleen and lymph node atrophy in COVID-19 patients as well as diffused alveolar damage, and macrophages infiltration indicating that macrophages may also play an important role in CSS induced by SARS-CoV-2 (Wei et al., 2020; Yao et al., 2020).

As previously mentioned, mechanistically the binding of the SARS-CoV-2 spike protein to ACE2 host receptor leads to the downregulation of ACE2, which in turn results in excessive secretion of AngII and reduced secretion of vasodilator angiotensins. AngII plays an important role in proinflammatory response through angiotensin receptor 1 (AT1R). This activated pathway further activates nuclear factor kappa B (NF-kB), which stimulates the overexpression of epidermal growth factor receptor (EGFR) ligands and TNF-α (Eguchi et al., 2018). Indeed, higher levels of ACE2 receptors in lung epithelial cells in children and young adults may have a protective effect on severe COVID-19 clinical manifestations. On the other hand, downregulation of ACE2 and unbalanced Ang II/Ang1–7 level during aging can enhance the cytokine storm (Cristiani et al., 2020). In addition, the hyperactivation of both NF-kB and activator of transcription (STAT)3, leads to a hyperinflammatory state mediated by amplification of IL-6, resulting in increased pulmonary vascular permeability (Murakami et al., 2019). The IL-6 is one of the major cytokines involved in acute inflammation (Scheller and Rose-John, 2006) and was already found to be significantly elevated in severe COVID-19 patients (Chen et al., 2020c; Wan et al., 2020a).

Similarly, the cytokine storm caused by unbalanced AngII/Ang1–7 may also explain the direct cardiovascular system injury of SARS-CoV-2 infected patients. Endothelial dysfunction can increase prothrombotic blood activity and myocarditis, which contributes to the high mortality rate observed in COVID-19 patients (Shi et al., 2020a). Moreover, the virus-induced CSS associated with an unbalanced AngII/Ang1–7 in kidney tubules and podocytes is pointed as responsible for acute kidney injury (Ahmadian et al., 2020). The multiple organ injuries characterized by a high incidence of liver dysfunction, gastrointestinal, and neurological injuries, endocrine alterations, and cutaneous manifestation have also been observed in non-surviving patients (D'Errico et al., 2020).

The cytokine storm landscape of COVID-19 patients was further demonstrated in a retrospective study showing higher concentrations of IL-2, IL-7, IL-10, G-CSF, C-X-C motif chemokine ligand (CXCL)-10, C-C motif chemokine ligand (CCL)-2, CCL-3, and TNF-α in the plasma of severe COVID-19 patients (Huang et al., 2020; Lu et al., 2020). Similarly, previous studies also showed higher levels of some cytokines, such as IFN-γ, TGF-β, IL-1, IL-6, IL-8, and IL-12 in the serum of SARS and MERS patients, highlighting the cytokine storm role in the pathogenesis of severe coronaviruses infection (Channappanavar and Perlman, 2017). Thus, the severity and pathogenicity of the viral infection could be directly correlated to the CSS, which implies that the management of hyperinflammation, the major cause of COVID-19 deaths, would significantly avoid fatal complications. Although there is no standard diagnosis recognition of CSS in COVID-19, it has been proposed that a sudden or rapid disease progression with multiple organ involvement, a significant decline of peripheral blood lymphocyte counts, and an increase of multiple cytokines, such as IL-1β, IL-2R, IL-6, IFN-c, CXCL-10, CCL-2, CCL-3, and TNF-α are the main biomarkers of CSS in COVID-19 patients (Gao et al., 2020).

Additionally, it has been proposed that overactivation of NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome also has a central as a trigger of cytokine storm. Mechanistically, the multiprotein complexes form in the cytosol and drive caspase-1 cleavage and the secretion of the pro-inflammatory cytokines IL-1β and IL-18 and other DAMPs (Ros et al., 2020). Moreover, it was recently demonstrated that sirtuin 2 (SIRT2) directly represses the NLRP3 inflammasome activity (He et al., 2020). Accordingly, the well reported age-related decline in the activity of the sirtuins (Massudi et al., 2012) might explain age-dependent increases in NLRP3 inflammasome activation (Stout-Delgado et al., 2016).

It has been demonstrated that a viral protein, called viroporin protein 3a leads to a direct activation of NLRP3 in SARS-CoV (Chen et al., 2019). Similarly, the presence of this protein in SARS-CoV-2 genome also suggest a direct activation of NLRP3 (Mousavizadeh and Ghasemi, 2020). Indeed, patients with a reduced immune capacity demonstrated a dysregulated NLRP3 inflammasome activity, which results in severe COVID-19 with

tissue damage and a cytokine storm (van den Berg and te Velde, 2020). Considering that NLRP3 is frequently over-activated in elderly individuals, it is believed that the NLRP3 inflammasome plays a central role in the increased lethality observed in aged COVID-19 patients (Lara et al., 2020).

Taking into account that COVID-19 is increasingly being recognized as a syndrome of host inflammatory response, the discovery of effective therapy approaches is urgently needed, especially in certain patients with prior inflammatory-related comorbidities, such as older age, specific genetic background, or obesity, where the CSS promotes the progression to severe organ damage (Shi et al., 2020b). Although glucocorticoid, blood purification therapy, and biological agents, such as interleukins inhibitors may be beneficial to improve the outcome of patients with CSS-induced injury, the efficacy and safety of these approaches still needs to be elucidated in further COVID-19 clinical trials (Qiu et al., 2013; Behrens and Koretzky, 2017; Chousterman et al., 2017; Kogelmann et al., 2017; Norelli et al., 2018; Wang et al., 2020).

As COVID-19 still lacks a specific effective-proven therapy, preventive measures could help to fighting off SARS-CoV-2 infection. It is well known that the decrease of fat mass normalizes the systemic inflammatory status of the body by reducing proinflammatory cytokines. Thus, a diet enriched in functional ingredients that have anti-inflammatory, antioxidant, and immunomodulatory properties should be incorporated in the dietary routine, in particular of those individuals with preexisting hyperinflammatory conditions as obesity or elderly. In this sense, there are some speculative studies about the potential association between vitamin D and the survival of COVID-19 patients, which could be ascribe to its anti-inflammatory properties (Daneshkhah et al., 2020; Grant et al., 2020). It has also been shown that vitamin D has immunomodulatory properties and its deficiency is a risk factor for persistent inflammation and the severe course of COVID-19, which might partly explain the geographic variations of COVID-19 mortality rate (Marik et al., 2020; Rhodes et al., 2020). Another vitamin with potential beneficial role in COVID-19 care management is ascorbic acid. Based on a new clinical trial in Wuhan, China, Carr et al. (Carr, 2020) suggested the potential role of high-dose of ascorbic acid for the treatment and prevention of severe COVID-19. Additionally, several studies have shown that vitamin B3 is highly effective in preventing lung tissue damage (Nagai et al., 1994).

Moreover, it has been suggested that the anti-inflammatory effects of polyphenols may help to overcome COVID-19 severity. Considering the global emergency of this pandemic with regard to the cost and availability of treatment especially in poor countries, it would be also interesting to know how effective polyphenols supplementation is in attenuating cytokine storm in comparison to other agents. It is also important to know at which stage of the COVID-19 the polyphenols supplementation would be the most beneficial. Could they be used in the dietary routine as a prophylactic therapy to prevent cytokine storm at early stages of the disease or would their anti-inflammatory and anti-oxidant properties delay viral dissemination?

SENESCENCE AND COVID-19

Cellular senescence was first reported by Hayflick and Moorehead in 1961 (Hayflick and Moorhead, 1961) as a cellular state characterized by replicative arrest and resistance to apoptosis (Kirkland, 1992). Several intra and extracellular signals can activate molecular pathways, such as cyclin dependent kinase inhibitor 2A (CDKN2A aka $p16^{INK4a}$)-Rb, p53, and CDKN 1A (CDKN1A $akap21^{CIP1}$) to induce senescent cell fate (Beauséjour et al., 2003). Besides of the high level of these key regulators, senescent cells can also present increased lysosomal β-galactosidase activity, high DNA damage detected by an accumulation of γH2AX, and telomere-associated foci and are usually larger than non-senescent cells (Kirkland and Tchkonia, 2020). Moreover, terminal telomeric repeats shortening after each cell division during the lifespan is also a hallmark of cellular senescence.

Senescent cells accumulate in different tissues during lifespan e.g., in adipose tissue in conditions like diabetes and obesity, in the hippocampi and frontal cortex in Alzheimer's disease, in the lungs of idiopathic pulmonary fibrosis individuals, in the liver of patients with cirrhosis, and in the kidneys of diabetic kidney disease patients (Kirkland, 2013; Musi et al., 2018; Bian et al., 2019; Justice et al., 2019; Suvakov et al., 2019; Liu and Liu, 2020). Thus, senescence is considered as natural aging process that affects all cell types.

Some senescent cells may also have an hyperinflammatory state caused by secretion of cytokines, chemokines, growth factors, and matrix metalloproteinases, a phenomenon called SASP (Ohtani, 2019). Importantly, cells in SASP can also induce senescence of surrounding cells, and may confer deleterious effects in the tissue microenvironment (Acosta et al., 2013). The aging itself is also associated to the continual production of pro-inflammatory factors, known as "inflammaging" (Sanada et al., 2018; Koelman et al., 2019), which may lead to a chronic inflammation and organ dysfunction. Additionally, other studies suggest that the excess of reactive oxygen species (ROS) production during aging may favor an inflammatory landscape through the increased secretion of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-2, and IL-6 (Garrido et al., 2019). Interestingly, the excess of pro-inflammatory cytokines can also increase the ROS production, sustaining the inflammaging phenotype (Biswas, 2016).

A key process in immunological aging, also known as immunosenescence, is the decrease of thymic activity in about 99% in elderly people compared to newborns (Gruver et al., 2007), declining the competency of the immune system to combat pathogen infections, such as SARS-CoV-2. Although immunosenescence is described as the progressive loss of all immune effectors, Goronzy et al. (2001) have found a correlation specifically between $CD8^{+}CD28^{null}$ T cells and the defective antibody responses to influenza vaccine in elderly adults due to thymic involution. Indeed, the increase in these cells' population has been consistently observed and is currently used as a biomarker of immunosenescence in older individuals (Zanni et al., 2003). Accordantly, lymphopenia, decrease in $CD4^{+}$ and $CD8^{+}$ T cells population, decrease of B cells and NK cells, monocytes, eosinophils, and basophils are common feature in patients with severe COVID-19. Currently, the available data suggest that the accumulation of

senescent T-cells negatively impact the prognosis of COVID-19, as the patients have an ineffective $CD8^+$ response, as well as an excessive cytokine secretion from the senescent cells (Cao, 2020; Huang et al., 2020; Qin et al., 2020).

Adipose tissue is a key player in metabolism and inflammation modulation. The adipose tissue dysfunction during aging is likely associated with chronic inflammation (Stout et al., 2017). As age advances, $CD38^+$ macrophages and senescent cells accumulate in visceral white adipose tissue producing high levels of inflammatory cytokines in the microenvironment (Covarrubias et al., 2019). Indeed, a recent study reported that obese elderly adults have higher susceptibility to more serious complications of COVID-19 as compared to younger patients. The authors have shown that the mortality rate for these COVID-19 patients was approximately 14% (Petrakis et al., 2020). One possible explanation for this observation could be that the increased secretion of pro-inflammatory cytokines by senescent adipocytes could lead to the cytokine storm in poor prognosis COVID-19 patients.

Although the exact mechanisms of SARS-CoV-2 morbidity and mortality in high risk patients still require extensive research, we may speculate some hypothesis based on the previous SARS-CoV infection understanding, given the high (80%) genetic similarity between both viruses (Yan et al., 2020). The strong correlation between obesity and the disease severity was previously reported in SARS-CoV infected patients. Furthermore, it has been reported that obese patients exhibit delayed and blunted antiviral responses to influenza virus infection, and have poor prognosis (Honce and Schultz-Cherry, 2019). Thus, it has been proposed that obesity may also be an important condition that increases the mortality risk of the SARS-CoV-2 infected patients (Petrakis et al., 2020). Indeed, the Centers for CDC advised that people of any age who have serious underlying medical conditions, including severe obesity [body mass index (BMI) > 40], might be at higher risk for COVID-19 complications and severe illness (Centers for Disease Control and Prevention, 2020).

These findings highlight the importance to look for interventions that remove senescent cells as a preventive treatment strategy against SARS-CoV-2 infection. In this sense, some polyphenols (as quercetin and fisetin) and tyrosine kinase inhibitors (as dasatinib) have been used as senolytic therapy (Yousefzadeh et al., 2018; Hickson et al., 2019). Interestingly, polyphenol-based senolytics alleviate dysfunction in murine models of chronic lung diseases (Schafer et al., 2017), and reduced the mortality of mice infected with mouse β-coronavirus and SARS-CoV-2 viral antigens (Kirkland and Tchkonia, 2020). These findings lead to health regulatory agencies around the world to approve a clinical trial to test flavonoids for elderly hospitalized COVID-19 patients to prevent progression to cytokine storm and ARDS (**Table 1**).

POLYPHENOLS AS A PROTECTIVE APPROACH

Polyphenols are key dietary components in preventing inflammatory comorbidities. Interestingly, several plant-derived compounds, such as polyphenols, have been shown to effectively inhibit RNA viruses. Likewise, Zhang et al. (2020) selected biologically proven anti-SARS or MERS coronavirus natural compounds and undertook molecular docking analysis to predict the possible SARS-CoV-2 therapeutic effects of these herbal extracts. They observed that the polyphenols, such as kaempferol, lignan, and quercetin among the 13 anti-inflammatory and anti-oxidant natural compounds potentially suitable for anti-viral usage. Similarly, the results of Singh et al. (2020) suggested that the polyphenols epigallocatechin gallate (EGCG), theaflavin-3-gallate (TF2a), theaflavin-3'-gallate (TF2b), and theaflavin-3,3'-digallate (TF3) can inhibit viral RNA polymerase and may represent an effective therapy for COVID-19.

Even if the consumption of polyphenols is not enough to guarantee a consistent anti-viral effect, many polyphenols have been identified as senolytic agents, which cause the selective death of senescent cells or regulate inflammmageing and immunosenencence. A panel that includes numerous polyphenols in human and murine senescent fibroblasts demonstrated that fisetin (a flavonoid present in fruits and vegetables, such as strawberry, apple, persimmon, grape, onion, and cucumber) and curcumin, were those with the greatest senolitic activity (Khan et al., 2013). Fisetin treatment in mice with progeroid syndrome revealed a reduction in IL-6 levels, which is mainly produced by adipose tissue (Yousefzadeh et al., 2018). Moreover, it has been shown that quercetin, apigenin, wogonin, and kaempferol inhibited the expression of several SASPs markers, including IL-1α, IL-1β, IL-6, IL-8, GM-CSF, CXCL1, monocyte chemoattractant protein-2 (MCP-2), and MMP-3 in senescent fibroblasts model. Considering that apigenin was the most powerful to inhibit IL-6, the *in vivo* approach confirmed that this flavone, found mainly in aromatics as parsley, chamomile, celery, and oregano (Shukla and Gupta, 2010), was able to significantly reduces SASP in the kidneys of aged rats (Lim et al., 2015).

Chronic treatment with resveratrol, found abundantly in the skins of red grapes, wine, peanuts, cocoa, and berries (Burns et al., 2002), in senescent lung fibroblasts (MRC5 fibroblasts) reduced the production of IL-6, IL-8, GROα, and VEGF (Pitozzi et al., 2013). Additionally, it has been demonstrated that senescence markers (e.g., IL-6 production) was counteracted by resveratrol in neuroglial cells (Bigagli et al., 2016), vascular smooth muscle cells (Csiszar et al., 2012). Lastly, olive-derived polyphenols including oleuropein, found at very low level in edible table oil olive (Ben Othman et al., 2008), significantly reduced the senescence in chondrocytes, synovial, and bone cells from osteoarthritic patients, an event that was accompanied by reduced activity of the NF-kB transcription factor and reduced SASP markers, as IL-6, IL-1β, and COX-2 (Varela-Eirín et al., 2020).

To date, there are data showing that mice treated for 18 months with resveratrol presented a significant reduction in the expression of ACE1 and an increase in the expression of ACE2 in the aorta, which translated into an increase in serum levels of Ang (1–7) in parallel with the reduction of AngII (Kim et al., 2018). Similar profile was observed

TABLE 1 | Clinical trials evaluating polyphenols in coronavirus disease 2019 (COVID-19) patients.

Identifier	Study title	Intervention	Status	Primary purpose	Phase study
NCT04400890	Randomized proof-of-concept trial to evaluate the safety and explore the effectiveness of a plant polyphenol for COVID-19	Plant Polyphenol and Vitamin D3	Recruiting	Treatment	Phase 2
NCT04377789	Quercetin on prophylaxis and treatment of COVID-19	Quercetin 500 mg (Prophylaxis) Quercetin 1,000 mg (Treatment)	Recruiting	Prevention	n.a.
NCT04578158	Trial to study the adjuvant benefits of quercetin phytosome in patients with COVID-19	Quercetin 500 mg	Recruiting	Treatment	Phase 2
NCT04468139	The Study of quadruple therapy zinc, quercetin, bromelain, and vitamin C on the clinical outcomes of patients infected with COVID-19	Quercetin (500 mg), bromelain (500 mg), zinc (50 mg), and vitamin c (1,000 mg)	Recruiting	Treatment	Phase 4
NCT04622865	Masitinib combined with Isoquercetin and best supportive care in hospitalized patients with moderate and severe COVID-19	Masitinib, Isoquercetin, and best supportive care	Recruiting	Treatment	Phase 2
NCT04536090	Study of Isoquercetin (IQC-950AN) plus standard of care vs. standard of care only for the treatment of COVID-19	Isoquercetin (IQC-950AN)	Not yet recruiting	Treatment	Phase 2
NCT04404218	The Açaí Berry COVID-19 anti-inflammation trial (ACAI)	1,560 mg/day of Açaí Berry extract	Recruiting	Treatment	Phase 2
NCT04392141	Colchicine plus phenolic monoterpenes to treat COVID-19	Oral administration of Colchicine plus Herbal Phenolic Monoterpene Fractions		Treatment	Phase 2
NCT04542993	Can SARS-CoV-2 viral load and COVID-19 disease severity be reduced by resveratrol-assisted zinc therapy (Reszinate)	Zinc Picolinate (50 mg) and Resveratrol (2 g)	Recruiting	Supportive Care	Phase 2
NCT04507867	Effect of a Nss to reduce complications in patients with COVID-19 and comorbidities in stage III (type 2 DM, SAH, and overweight/obesity with BMI <35)	NSS-1 (Spirulina Maxima 2.5 g), folic acid 5 mg, Glutamine 5 g, Cyanomax Ultra (10 g of powder), ascorbic acid 1 g, zinc 20 mg, selenium 100 mcg, cholecalciferol 2000 IU, resveratrol 200 mg, concentrated omega 3 fatty acids (10 grams of powder), L-Arginine 1.5 g, and magnesium 400 mg	Not yet recruiting	Supportive Care	n.a.
NCT04382040	A Phase II, controlled clinical study designed to evaluate the effect of ArtemiC in patients diagnosed with COVID-19	ArtemiC is a medical spray comprised of Artemisinin (6 mg/ml), Curcumin (20 mg/ml), Frankincense (=Boswellia; 15 mg/ml), and vitamin C (60 mg/ml)	Recruiting	Treatment	Phase 2
NCT04403646	Tannin specific natural extract for COVID-19 infection (TaCOVID)	ARBOX [dry extract of polyphenols (tannins) form quebracho and chestnut 240 mg, B12 vitamin 0.72 µg]	Not yet recruiting	Treatment	n.d.
NCT04410510	P2Et extract in the symptomatic treatment of subjects with COVID-19	P2Et (*Caesalpinia spinosa* extract)	Recruiting	Treatment	Phase 2/3
NCT04446065	Protection of health workers against COVID-19 (HERD)	Previfenon® (patent pending) provides 250 mg EGCG	Not yet recruiting	Prevention	Phase 2/3

n.a., not applicable; n.d., not described.

in aged kidneys and was associated with improvement in oxidative stress, inflammation, and renal fibrosis (Jang et al., 2018), suggesting that polyphenols could increase ACE2 expression in aged subjects and that these alterations in the ACE2/Angiotensin- (1–7)/MasR axis have beneficial results. Experimental data also demonstrated that resveratrol has an organ-protection function, protecting myocardium in peritonitis/sepsis model (Shang et al., 2019), intestine, liver, kidney, and lung injuries in a hemorrhagic shock model (Müller et al., 2017). It has also been shown a protective role of curcumin and green tea polyphenols in a multiple organ dysfunction syndrome model (Di Paola et al., 2006; Liu et al., 2016).

Furthermore, the consumption of a diet rich in polyphenols has often been claimed as a powerful aid in the control of inflammatory response associated with obesity. The use of resveratrol has been proven to be protective in obesity models through the activation of sirtuin-1, mimicking the caloric restriction, which delay age-related diseases and to extend life span in mammals (Fischer-Posovszky et al., 2010). Resveratrol also inhibited the activation of NLRP3 inflammasome in liver of diet-induced obesity mice, reducing IL-1, IL-6, and TNF-α production (Yang and Lim, 2014), as well as, reduced NF-kb signaling and IL-6 expression in adipose tissue of monkeys fed with high caloric diet (Jimenez-Gomez et al., 2013). Consumption of *yerba mate*, rich in flavonoids like quercetin and rutin, and phenolic acids like chlorogenic and caffeic acid can control inflammation in obesity models (for review, see Gambero and Ribeiro, 2015). Quercetin monotherapy or combined with resveratrol also showed anti-inflammatory activity in adipose tissue, reducing the IL-6 release (Zhao et al., 2017). A*çai* seeds extract, which is rich in proanthocyanidins, in addition to controlling the production of inflammatory mediators, also reduced the expression of AT1 in the adipose tissue of obese mice (Santos et al., 2020). Altogether, these data highlight the hypothesis

that these important bioactive dietary components might have modulatory effects on inflammatory pathways present in aging and obesity, as well as, on markers that are been associated with SARS-Cov-2 infection (**Figure 1**).

Based on the literature evidence showing that polyphenols might be helpful in protecting the body from the negative effects of the disease, several clinical trials are ongoing to test such hypothesis. Indeed, a Phase 2 randomized double-blind placebo-controlled study aims to explore the effectiveness of a commercial plant polyphenols supplemented with vitamin D3 in a set of 200 mild COVID-19 patients (NCT04400890). Moreover, it has been suggested that quercetin, a well-characterized antioxidant, anti-inflammatory and immunomodulatory compound would be a good option in COVID-19 therapeutics (Solnier and Fladerer, 2020). Currently, there are five clinical trials evaluating the adjuvant benefits of quercetin (alone or in combination) in patients with COVID-19 (**Table 1**) The antiviral properties of resveratrol have also been shown both *in vitro* and *in vivo* (Marinella, 2020). Thus, a Phase 2 study aiming to evaluate the effects of resveratrol as a means to minimize viral load and severity of resulting COVID-19 disease (NCT04542993) is currently ongoing. In addition, a resveratrol-containing nutritional support system is also under investigation to evaluate its effect in reducing complications and comorbidities in the evolution of patients with COVID-19 (NCT04507867). Considering the protective anti-inflammatory and anti-viral effects of tannins (Ueda et al., 2013), a double-blind, randomized trial will be conducted in 140 COVID-19 patients to study the effect of the treatment with dry extract of tannins + B12 vitamin (NCT04403646). Based on promising unpublished *in vitro* and *in vivo* data, the *Caesalpinia spinosa* standardized polyphenol-rich P2Et extract is currently in a Phase2/3 clinical trial (NCT04410510). The study aims to evaluate the efficacy of the supplement in reducing the hospital stay of COVID-19 patients. A brief summary from the clinical trials including polyphenols is shown in **Table 1**.

CONCLUSION AND PERSPECTIVES

The COVID-19 pandemic brought to light that changes in the cell physiology determined by senescence and inflammation increase substantially the vulnerability of the elderly population and those with comorbidities such as obesity. Indeed, "inflammaged patients" are particularly susceptible to adverse clinical outcomes during SARS-CoV-2 infection and the treatment is challenging. It has been shown that the changes in the expression of the ACE-2 receptor, the imbalance in the angiotensin 1–7/AngII production which increases cardiovascular risk, as well as the increased production of pro-inflammatory cytokines observed in aging and obesity models, can be reversed or controlled by bioactive substances from dietary sources, such as polyphenols. Thus, the data presented here reinforce the hypothesis that polyphenols could have the potential for their use for senescence and inflammation prevention and, therefore for the treatment/management of patients with viral infections such as SARS-CoV-2. It is hoped that the clinical studies under development can add valuable information about this hypothesis and help reduce suffering and mortality imposed by SARS-CoV-2 infection.

AUTHOR CONTRIBUTIONS

The authors contributed equally to the writing and the revision of this article. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 303625/2019-8 and 305402/2019-6).

REFERENCES

Acosta, J. C., Banito, A., Wuestefeld, T., Georgilis, A., Janich, P., Morton, J. P., et al. (2013). A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat. Cell Biol.* 15, 978–990. doi: 10.1038/ncb2784

Ahmadian, E., Hosseiniyan Khatibi, S. M., Razi Soofiyani, S., Abediazar, S., Shoja, M. M., Ardalan, M., et al. (2020). Covid-19 and kidney injury: pathophysiology and molecular mechanisms. *Rev. Med. Virol.* e2176. doi:10.1002/rmv.2176 [Epub ahead of print]

Beauséjour, C. M., Krtolica, A., Galimi, F., Narita, M., Lowe, S. W., Yaswen, P., et al. (2003). Reversal of human cellular senescence: roles of the p53 and p16 pathways. *EMBO J.* 22, 4212–4222. doi: 10.1093/emboj/cdg417

Behrens, E. M., and Koretzky, G. A. (2017). Review: cytokine storm syndrome: looking toward the precision medicine era. *Arthritis Rheum.* 69, 1135–1143. doi: 10.1002/art.40071

Ben Othman, N., Roblain, D., Thonart, P., and Hamdi, M. (2008). Tunisian table olive phenolic compounds and their antioxidant capacity. *J. Food Sci.* 73, C235–C240. doi: 10.1111/j.1750-3841.2008.00711.x

Bian, X., Griffin, T. P., Zhu, X., Islam, M. N., Conley, S. M., Eirin, A., et al. (2019). Senescence marker activin a is increased in human diabetic kidney disease: association with kidney function and potential implications for therapy. *BMJ Open Diabetes Res. Care* 7:e000720. doi: 10.1136/bmjdrc-2019-000720

Bigagli, E., Luceri, C., Scartabelli, T., Dolara, P., Casamenti, F., Pellegrini-Giampietro, D. E., et al. (2016). Long-term neuroglial cocultures as a brain aging model: hallmarks of senescence, microRNA expression profiles, and comparison with in vivo models. *J. Gerontol. A Biol. Sci. Med. Sci.* 71, 50–60. doi: 10.1093/gerona/glu231

Biswas, S. K. (2016). Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxidative Med. Cell. Longev.* 2016:5698931. doi: 10.1155/2016/5698931

Burns, J., Yokota, T., Ashihara, H., Lean, M. E. J., and Crozier, A. (2002). Plant foods and herbal sources of resveratrol. *J. Agric. Food Chem.* 50, 3337–3340. doi: 10.1021/jf0112973

Burrell, L. M., Risvanis, J., Kubota, E., Dean, R. G., MacDonald, P. S., Lu, S., et al. (2005). Myocardial infarction increases ACE2 expression in rat and humans. *Eur. Heart J.* 26, 369–375. doi: 10.1093/eurheartj/ehi114

Cao, X. (2020). COVID-19: immunopathology and its implications for therapy. *Nat. Rev. Immunol.* 20, 269–270. doi: 10.1038/s41577-020-0308-3

Carr, A. C. (2020). A new clinical trial to test high-dose vitamin C in patients with COVID-19. *Crit. Care* 24:133. doi: 10.1186/s13054-020-02851-4

Caussy, C., Pattou, F., Wallet, F., Simon, C., Chalopin, S., Telliam, C., et al. (2020). Prevalence of obesity among adult inpatients with COVID-19 in France. *Lancet Diabetes Endocrinol.* 8, 562–564. doi: 10.1016/S2213-8587(20)30160-1

Centers for Disease Control and Prevention (2020). People who are at higher risk for severe illness|CDC. *Centers Dis. Control Prev.* 68:703. doi: 10.4103/ijo.IJO_843_20

Channappanavar, R., and Perlman, S. (2017). Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin. Immunopathol.* 39, 529–539. doi: 10.1007/s00281-017-0629-x

Chen, J., Jiang, Q., Xia, X., Liu, K., Yu, Z., Tao, W., et al. (2020a). Individual variation of the SARS-CoV-2 receptor ACE2 gene expression and regulation. *Aging Cell* 19:e13168. doi: 10.1111/acel.13168

Chen, J., Kelley, W. J., and Goldstein, D. R. (2020b). Role of aging and the immune response to respiratory viral infections: potential implications for COVID-19. *J. Immunol.* 205, 313–320. doi: 10.4049/jimmunol.2000380

Chen, L., Liu, H. G., Liu, W., Liu, J., Liu, K., Shang, J., et al. (2020c). Analysis of clinical features of 29 patients with 2019 novel coronavirus pneumonia. *Zhonghua Jie He He Hu Xi Za Zhi* 43:E005. doi: 10.3760/cma.j.issn.1001-0939.2020.0005

Chen, I. Y., Moriyama, M., Chang, M. F., and Ichinohe, T. (2019). Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome. *Front. Microbiol.* 10:50. doi: 10.3389/fmicb.2019.00050

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., et al. (2020d). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395, 507–513. doi: 10.1016/S0140-6736(20)30211-7

Cheung, C. Y., Poon, L. L. M., Ng, I. H. Y., Luk, W., Sia, S. -F., Wu, M. H. S., et al. (2005). Cytokine responses in severe acute respiratory syndrome coronavirus-infected macrophages in vitro: possible relevance to pathogenesis. *J. Virol.* 79, 7819–7826. doi: 10.1128/jvi.79.12.7819-7826.2005

Chousterman, B. G., Swirski, F. K., and Weber, G. F. (2017). Cytokine storm and sepsis disease pathogenesis. *Semin. Immunopathol.* 39, 517–528. doi: 10.1007/s00281-017-0639-8

Chung, H. Y., Kim, D. H., Lee, E. K., Chung, K. W., Chung, S., Lee, B., et al. (2019). Redefining chronic inflammation in aging and age-related diseases: proposal of the senoinflammation concept. *Aging Dis.* 10, 367–382. doi: 10.14336/AD.2018.0324

Cicco, S., Cicco, G., Racanelli, V., and Vacca, A. (2020). Neutrophil extracellular traps (NETs) and damage-associated molecular patterns (DAMPs): two potential targets for COVID-19 treatment. *Mediat. Inflamm.* 2020:7527953. doi: 10.1155/2020/7527953

Cocoros, N. M., Lash, T. L., Demaria, A., and Klompas, M. (2014). Obesity as a risk factor for severe influenza-like illness. *Influenza Other Respir. Viruses* 8, 25–32. doi: 10.1111/irv.12156

Coppe, J. P., Kauser, K., Campisi, J., and Beauséjour, C. M. (2006). Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. *J. Biol. Chem.* 281, 29568–29574. doi: 10.1074/jbc.M603307200

Covarrubias, A. J., Kale, A., Perrone, R., Lopez-Dominguez, J. A., Pisco, A. O., Kasler, H. G., et al. (2020). Senescent cells promote tissue NAD+ decline during ageing via the activation of CD38+ macrophages. *Nat. Metab.* 2, 1265–1283. doi: 10.1038/s42255-020-00305-3

Covino, M., De Matteis, G., Santoro, M., Sabia, L., Simeoni, B., Candelli, M., et al. (2020). Clinical characteristics and prognostic factors in COVID-19 patients aged ≥80 years. *Geriatr Gerontol Int* 20, 704–708. doi: 10.1111/ggi.13960

Crayne, C. B., Albeituni, S., Nichols, K. E., and Cron, R. Q. (2019). The immunology of macrophage activation syndrome. *Front. Immunol.* 10:119. doi: 10.3389/fimmu.2019.00119

Cristiani, L., Mancino, E., Matera, L., Nenna, R., Pierangeli, A., Scagnolari, C., et al. (2020). Will children reveal their secret? The coronavirus dilemma. *Eur. Respir. J.* 55:2000749. doi: 10.1183/13993003.00749-2020

Csiszar, A., Sosnowska, D., Wang, M., Lakatta, E. G., Sonntag, W. E., and Ungvari, Z. (2012). Age-associated proinflammatory secretory phenotype in vascular smooth muscle cells from the non-human primate *Macaca mulatta*: reversal by resveratrol treatment. *J. Gerontol. A Biol. Sci. Med. Sci.* 67, 811–820. doi: 10.1093/gerona/glr228

D'Errico, S., Zanon, M., Montanaro, M., Radaelli, D., Sessa, F., Di Mizio, G., et al. (2020). More than pneumonia: distinctive features of SARS-CoV-2 infection. From autopsy findings to clinical implications: a systematic review. *Microorganisms* 8:1642. doi: 10.3390/microorganisms8111642

Dalan, R., Bornstein, S. R., El-Armouche, A., Rodionov, R. N., Markov, A., Wielockx, B., et al. (2020). The ACE-2 in COVID-19: foe or friend? *Horm. Metab. Res.* 52, 257–263. doi: 10.1055/a-1155-0501

Daneshkhah, A., Agrawal, V., Eshein, A., Subramanian, H., Roy, H. K., and Backman, V. (2020). The possible role of Vitamin D in suppressing cytokine storm and associated mortality in COVID-19 patients. MedRxiv [Preprint]. doi: 10.1101/2020.04.08.20058578

Di Paola, R., Mazzon, E., Muià, C., Crisafulli, C., Genovese, T., Di Bella, P., et al. (2006). Green tea polyphenol extract attenuates zymosan-induced non-septic shock in mice. *Shock* 26, 402–409. doi: 10.1097/01.shk.0000191379.62897.1d

Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., et al. (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ. Res.* 87, E1–E9. doi: 10.1161/01.res.87.5.e1

Eguchi, S., Kawai, T., Scalia, R., and Rizzo, V. (2018). Understanding angiotensin II type 1 receptor signaling in vascular pathophysiology. *Hypertension* 71, 804–810. doi: 10.1161/HYPERTENSIONAHA.118.10266

Fischer-Posovszky, P., Kukulus, V., Tews, D., Unterkircher, T., Debatin, K. M., Fulda, S., et al. (2010). Resveratrol regulates human adipocyte number and function in a Sirt1-dependent manner. *Am. J. Clin. Nutr.* 92, 5–15. doi: 10.3945/ajcn.2009.28435

Freund, A., Orjalo, A. V., Desprez, P. Y., and Campisi, J. (2010). Inflammatory networks during cellular senescence: causes and consequences. *Trends Mol. Med.* 16, 238–246. doi: 10.1016/j.molmed.2010.03.003

Gambero, A., and Ribeiro, M. L. (2015). The positive effects of yerba maté (*Ilex paraguariensis*) in obesity. *Nutrients* 7, 730–750. doi: 10.3390/nu7020730

Gao, Y. -M., Xu, G., Wang, B., and Liu, B. -C. (2020). Cytokine storm syndrome in coronavirus disease 2019: a narrative review. *J. Intern. Med.* doi:10.1111/joim.13144 [Epub ahead of print]

Garrido, A., Cruces, J., Ceprián, N., Vara, E., and de la Fuente, M. (2019). Oxidative-inflammatory stress in immune cells from adult mice with premature aging. *Int. J. Mol. Sci.* 20:769. doi: 10.3390/ijms20030769

Goronzy, J. J., Fulbright, J. W., Crowson, C. S., Poland, G. A., O'Fallon, W. M., and Weyand, C. M. (2001). Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals. *J. Virol.* 75, 12182–12187. doi: 10.1128/jvi.75.24.12182-12187.2001

Grant, W. B., Lahore, H., McDonnell, S. L., Baggerly, C. A., French, C. B., Aliano, J. L., et al. (2020). Evidence that vitamin d supplementation could reduce risk of influenza and covid-19 infections and deaths. *Nutrients* 12:988. doi: 10.3390/nu12040988

Gruver, A. L., Hudson, L. L., and Sempowski, G. D. (2007). Immunosenescence of ageing. *J. Pathol.* 211, 144–156. doi: 10.1002/path.2104

Halvatsiotis, P., Kotanidou, A., Tzannis, K., Jahaj, E., Magira, E., Theodorakopoulou, M., et al. (2020). Demographic and clinical features of critically ill patients with COVID-19 in Greece: the burden of diabetes and obesity. *Diabetes Res. Clin. Pract.* 166:108331. doi: 10.1016/j.diabres.2020.108331

Hamming, I., Timens, W., Bulthuis, M. L. C., Lely, A. T., Navis, G. J., and van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203, 631–637. doi: 10.1002/path.1570

Hayflick, L., and Moorhead, P. S. (1961). The serial cultivation of human diploid cell strains. *Exp. Cell Res.* 25, 585–621. doi: 10.1016/0014-4827(61)90192-6

He, M., Chiang, H. H., Luo, H., Zheng, Z., Qiao, Q., Wang, L., et al. (2020). An acetylation switch of the NLRP3 inflammasome regulates aging-associated chronic inflammation and insulin resistance. *Cell Metab.* 31:580.e5–591.e5. doi: 10.1016/j.cmet.2020.01.009

He, L., Ding, Y., Zhang, Q., Che, X., He, Y., Shen, H., et al. (2006). Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2+ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. *J. Pathol.* 210, 288–297. doi: 10.1002/path.2067

Hickson, L. T. J., Langhi Prata, L. G. P., Bobart, S. A., Evans, T. K., Giorgadze, N., Hashmi, S. K., et al. (2019). Senolytics decrease senescent cells in humans: preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. *EBioMedicine* 47, 446–456. doi: 10.1016/j.ebiom.2019.08.069

Hofmann, H., Pyrc, K., van der Hoek, L., Geier, M., Berkhout, B., and Pöhlmann, S. (2005). Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proc. Natl. Acad. Sci. U. S. A.* 102, 7988–7993. doi: 10.1073/pnas.0409465102

Honce, R., and Schultz-Cherry, S. (2019). Impact of obesity on influenza a virus pathogenesis, immune response, and evolution. *Front. Immunol.* 10:1071. doi: 10.3389/fimmu.2019.01071

Hu, H., Du, H., Li, J., Wang, Y., Wu, X., Wang, C., et al. (2020). Early prediction and identification for severe patients during the pandemic of COVID-19:

a severe COVID-19 risk model constructed by multivariate logistic regression analysis. *J. Glob. Health* 10:020510. doi: 10.7189/jogh.10.020510

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Imam, Z., Odish, F., Gill, I., O'Connor, D., Armstrong, J., Vanood, A., et al. (2020). Older age and comorbidity are independent mortality predictors in a large cohort of 1305 COVID-19 patients in Michigan, United States. *J. Intern. Med.* 288, 469–476. doi: 10.1111/joim.13119

Janeway, C. A., and Medzhitov, R. (2002). Innate immune recognition. *Annu. Rev. Immunol.* 20, 197–216. doi: 10.1146/annurev.immunol.20.083001.084359

Jang, I. A., Kim, E. N., Lim, J. H., Kim, M. Y., Ban, T. H., Yoon, H. E., et al. (2018). Effects of resveratrol on the renin-angiotensin system in the aging kidney. *Nutrients* 10:1741. doi: 10.3390/nu10111741

Jimenez-Gomez, Y., Mattison, J. A., Pearson, K. J., Martin-Montalvo, A., Palacios, H. H., Sossong, A. M., et al. (2013). Resveratrol improves adipose insulin signaling and reducestheinflammatoryresponsein adiposetissue of rhesus monkeys on high-fat, high-sugar diet. *Cell Metab.* 18, 533–545. doi: 10.1016/j.cmet.2013.09.004

Jun, J. -I., and Lau, L. F. (2010). The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nat. Cell Biol.* 12, 676–685. doi: 10.1038/ncb2070

Justice, J. N., Nambiar, A. M., Tchkonia, T., LeBrasseur, N. K., Pascual, R., Hashmi, S. K., et al. (2019). Senolytics in idiopathic pulmonary fibrosis: results from a first-in-human, open-label, pilot study. *EBioMedicine* 40, 554–563. doi: 10.1016/j.ebiom.2018.12.052

Khan, N., Syed, D. N., Ahmad, N., and Mukhtar, H. (2013). Fisetin: a dietary antioxidant for health promotion. *Antioxid. Redox Signal.* 19, 151–162. doi: 10.1089/ars.2012.4901

Kim, E. N., Kim, M. Y., Lim, J. H., Kim, Y., Shin, S. J., Park, C. W., et al. (2018). The protective effect of resveratrol on vascular aging by modulation of the renin–angiotensin system. *Atherosclerosis* 270, 123–131. doi: 10.1016/j.atherosclerosis.2018.01.043

Kirkland, J. L. (1992). The biochemistry of mammalian senescence. *Clin. Biochem.* 25, 61–75. doi: 10.1016/0009-9120(92)80047-K

Kirkland, J. L. (2013). Inflammation and cellular senescence: potential contribution to chronic diseases and disabilities with aging. *Public Policy Aging Rep.* 23, 12–15. doi: 10.1093/ppar/23.4.12

Kirkland, J. L., and Tchkonia, T. (2020). Senolytic drugs: from discovery to translation. *J. Intern. Med.* 288, 518–536. doi: 10.1111/joim.13141

Koelman, L., Pivovarova-Ramich, O., Pfeiffer, A. F. H., Grune, T., and Aleksandrova, K. (2019). Cytokines for evaluation of chronic inflammatory status in ageing research: reliability and phenotypic characterisation. *Immun. Ageing* 16:11. doi: 10.1186/s12979-019-0151-1

Kogelmann, K., Jarczak, D., Scheller, M., and Drüner, M. (2017). Hemoadsorption by CytoSorb in septic patients: a case series. *Crit. Care* 21:74. doi: 10.1186/s13054-017-1662-9

Laberge, R. M., Awad, P., Campisi, J., and Desprez, P. Y. (2012). Epithelial-mesenchymal transition induced by senescent fibroblasts. *Cancer Microenviron.* 5, 39–44. doi: 10.1007/s12307-011-0069-4

Lai, C. C., Shih, T. P., Ko, W. C., Tang, H. J., and Hsueh, P. R. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. *Int. J. Antimicrob. Agents* 55:105924. doi: 10.1016/j.ijantimicag.2020.105924

Lara, P. C., Macías-Verde, D., and Burgos-Burgos, J. (2020). Age-induced NLRP3 inflammasome over-activation increases lethality of SARS-CoV-2 pneumonia in elderly patients. *Aging Dis.* 11, 756–762. doi: 10.14336/ad.2020.0601

Lau, S. K. P., Lau, C. C. Y., Chan, K. H., Li, C. P. Y., Chen, H., Jin, D. Y., et al. (2013). Delayed induction of proinflammatory cytokines and suppression of innate antiviral response by the novel Middle East respiratory syndrome coronavirus: implications for pathogenesis and treatment. *J. Gen. Virol.* 94, 2679–2690. doi: 10.1099/vir.0.055533-0

Law, H. K. W., Chung, Y. C., Hoi, Y. N., Sin, F. S., Yuk, O. C., Luk, W., et al. (2005). Chemokine up-regulation in SARS-coronavirus-infected, monocyte-derived human dendritic cells. *Blood* 106, 2366–2374. doi: 10.1182/blood-2004-10-4166

Li, G., Fan, Y., Lai, Y., Han, T., Li, Z., Zhou, P., et al. (2020). Coronavirus infections and immune responses. *J. Med. Virol.* 92, 424–432. doi: 10.1002/jmv.25685

Lighter, J., Phillips, M., Hochman, S., Sterling, S., Johnson, D., Francois, F., et al. (2020). Obesity in patients younger than 60 years is a risk factor for COVID-19 hospital admission. *Clin. Infect. Dis.* 71, 896–897. doi: 10.1093/cid/ciaa415

Lim, H., Park, H., and Kim, H. P. (2015). Effects of flavonoids on senescence-associated secretory phenotype formation from bleomycin-induced senescence in BJ fibroblasts. *Biochem. Pharmacol.* 96, 337–348. doi: 10.1016/j.bcp.2015.06.013

Liu, R. M., and Liu, G. (2020). Cell senescence and fibrotic lung diseases. *Exp. Gerontol.* 132:110836. doi: 10.1016/j.exger.2020.110836

Liu, S., Zhang, J., Pang, Q., Song, S., Miao, R., Chen, W., et al. (2016). The protective role of curcumin in zymosan-induced multiple organ dysfunction syndrome in mice. *Shock* 45, 209–219. doi: 10.1097/SHK.0000000000000502

Louie, J. K., Acosta, M., Samuel, M. C., Schechter, R., Vugia, D. J., Harriman, K., et al. (2011). A novel risk factor for a novel virus: obesity and 2009 pandemic influenza a (H1N1). *Clin. Infect. Dis.* 52, 301–312. doi: 10.1093/cid/ciq152

Lu, L., Zhang, H., Dauphars, D. J., and He, Y. -W. (2020). A potential role of interleukin-10 in COVID-19 pathogenesis. *Trends Immunol.* doi:10.1016/j.it.2020.10.012 [Epub ahead of print]

Mahallawi, W. H., Khabour, O. F., Zhang, Q., Makhdoum, H. M., and Suliman, B. A. (2018). MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. *Cytokine* 104, 8–13. doi: 10.1016/j.cyto.2018.01.025

Marik, P. E., Kory, P., and Varon, J. (2020). Does vitamin D status impact mortality from SARS-CoV-2 infection? *Med. Drug Discov.* 6:100041. doi: 10.1016/j.medidd.2020.100041

Marinella, M. A. (2020). Indomethacin and resveratrol as potential treatment adjuncts for SARS-CoV-2/COVID-19. *Int. J. Clin. Pract.* 74:e13535. doi: 10.1111/ijcp.13535

Massudi, H., Grant, R., Braidy, N., Guest, J., Farnsworth, B., and Guillemin, G. J. (2012). Age-associated changes in oxidative stress and NAD+ metabolism in human tissue. *PLoS One* 7:e42357. doi: 10.1371/journal.pone.0042357

Michaud, M., Balardy, L., Moulis, G., Gaudin, C., Peyrot, C., Vellas, B., et al. (2013). Proinflammatory cytokines, aging, and age-related diseases. *J. Am. Med. Dir. Assoc.* 14, 877–882. doi: 10.1016/j.jamda.2013.05.009

Moser, J. A. S., Galindo-Fraga, A., Ortiz-Hernández, A. A., Gu, W., Hunsberger, S., Galán-Herrera, J. F., et al. (2019). Underweight, overweight, and obesity as independent risk factors for hospitalization in adults and children from influenza and other respiratory viruses. *Influenza Other Respir. Viruses* 13, 3–9. doi: 10.1111/irv.12618

Mousavizadeh, L., and Ghasemi, S. (2020). Genotype and phenotype of COVID-19: their roles in pathogenesis. *J. Microbiol. Immunol. Infect.* doi:10.1016/j.jmii.2020.03.022 [Epub ahead of print]

Müller, T., Kirsch, M., and Petrat, F. (2017). Attenuation of multiple organ damage by continuous low-dose solvent-free infusions of resveratrol after severe hemorrhagic shock in rats. *Nutrients* 9:889. doi: 10.3390/nu9080889

Murakami, M., Kamimura, D., and Hirano, T. (2019). Pleiotropy and specificity: insights from the interleukin 6 family of cytokines. *Immunity* 50, 812–831. doi: 10.1016/j.immuni.2019.03.027

Murthy, H., Iqbal, M., Chavez, J. C., and Kharfan-Dabaja, M. A. (2019). Cytokine release syndrome: current perspectives. *Immunotargets Ther.* 8, 43–52. doi: 10.2147/ITT.S202015

Musi, N., Valentine, J. M., Sickora, K. R., Baeuerle, E., Thompson, C. S., Shen, Q., et al. (2018). Tau protein aggregation is associated with cellular senescence in the brain. *Aging Cell* 17:e12840. doi: 10.1111/acel.12840

Nagai, A., Matsumiya, H., Hayashi, M., Yasui, S., Okamoto, H., and Konno, K. (1994). Effects of nicotinamide and niacin on bleomycin-induced acute injury and subsequent fibrosis in hamster lungs. *Exp. Lung Res.* 20, 263–281. doi: 10.3109/01902149409064387

Naylor, R. M., Baker, D. J., and van Deursen, J. M. (2013). Senescent cells: a novel therapeutic target for aging and age-related diseases. *Clin. Pharmacol. Ther.* 93, 105–116. doi: 10.1038/clpt.2012.193

Nguyen, S., Major, K., Cochet, C., Bizzozzero, T., Barbarossa, L., Bosshard, W., et al. (2020). COVID-19 infection in the elderly in French-speaking Switzerland: an inventory of beliefs, convictions and certainties. *Rev. Med. Suisse* 16, 835–838.

Norelli, M., Camisa, B., Barbiera, G., Falcone, L., Purevdorj, A., Genua, M., et al. (2018). Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nat. Med.* 24, 739–748. doi: 10.1038/s41591-018-0036-4

Ohtani, N. (2019). Deciphering the mechanism for induction of senescence-associated secretory phenotype (SASP) and its role in ageing and cancer development. *J. Infect. Dis.* doi:10.1093/jb/mvz055 [Epub ahead of print]

Petrakis, D., Margină, D., Tsarouhas, K., Tekos, F., Stan, M., Nikitovic, D., et al. (2020). Obesity—a risk factor for increased COVID-19 prevalence, severity and lethality (review). *Mol. Med. Rep.* 22, 9–19. doi: 10.3892/mmr.2020.11127

Pitozzi, V., Mocali, A., Laurenzana, A., Giannoni, E., Cifola, I., Battaglia, C., et al. (2013). Chronic resveratrol treatment ameliorates cell adhesion and mitigates the inflammatory phenotype in senescent human fibroblasts. *J. Gerontol. A Biol. Sci. Med. Sci.* 68, 371–381. doi: 10.1093/gerona/gls183

Qin, C., Zhou, L., Hu, Z., Zhang, S., Yang, S., Tao, Y., et al. (2020). Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin. Infect. Dis.* 71, 762–768. doi: 10.1093/cid/ciaa248

Qiu, P., Cui, X., Sun, J., Welsh, J., Natanson, C., and Eichacker, P. Q. (2013). Antitumor necrosis factor therapy is associated with improvedsurvival in clinical sepsis trials: a meta-analysis. *Crit. Care Med.* 41, 2419–2429. doi: 10.1097/CCM.0b013e3182982add

Rabi, F. A., Al Zoubi, M. S., Al-Nasser, A. D., Kasasbeh, G. A., and Salameh, D. M. (2020). Sars-cov-2 and coronavirus disease 2019: what we know so far. *Pathogens* 9:231. doi: 10.3390/pathogens9030231

Raj, V. S., Mou, H., Smits, S. L., Dekkers, D. H. W., Müller, M. A., Dijkman, R., et al. (2013). Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 495, 251–254. doi: 10.1038/nature12005

Rhodes, J. M., Subramanian, S., Laird, E., and Kenny, R. A. (2020). Editorial: low population mortality from COVID-19 in countries south of latitude 35 degrees north supports vitamin D as a factor determining severity. *Aliment. Pharmacol. Ther.* 51, 1434–1437. doi: 10.1111/apt.15777

Rodrigues Prestes, T. R., Rocha, N. P., Miranda, A. S., Teixeira, A. L., and Simoes-e-Silva, A. C. (2017). The anti-inflammatory potential of ACE2/angiotensin-(1-7)/mas receptor axis: evidence from basic and clinical research. *Curr. Drug Targets* 18, 1301–1313. doi: 10.2174/1389450117666160727142401

Ros, U., Pedrera, L., and Garcia-Saez, A. J. (2020). Partners in crime: the interplay of proteins and membranes in regulated necrosis. *Int. J. Mol. Sci.* 21:2412. doi: 10.3390/ijms21072412

Ruan, Q., Yang, K., Wang, W., Jiang, L., and Song, J. (2020). Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med.* 46, 846–848. doi: 10.1007/s00134-020-05991-x

Rutkowska-Zapała, M., Suski, M., Szatanek, R., Lenart, M., Weglarczyk, K., Olszanecki, R., et al. (2015). Human monocyte subsets exhibit divergent angiotensin I-converting activity. *Clin. Exp. Immunol.* 181, 126–132. doi: 10.1111/cei.12612

Sanada, F., Taniyama, Y., Muratsu, J., Otsu, R., Shimizu, H., Rakugi, H., et al. (2018). Source of chronic inflammation in aging. *Front. Cardiovasc. Med.* 5:12. doi: 10.3389/fcvm.2018.00012

Santos, I. B., de Bem, G. F., da Costa, C. A., de Carvalho, L. C. R. M., de Medeiros, A. F., Silva, D. L. B., et al. (2020). Açaí seed extract prevents the renin-angiotensin system activation, oxidative stress and inflammation in white adipose tissue of high-fat diet–fed mice. *Nutr. Res.* 79, 35–49. doi: 10.1016/j.nutres.2020.05.006

Santos, J. C., Gambeloni, R. Z., Roque, A. T., Oeck, S., and Ribeiro, M. L. (2018). Epigenetic mechanisms of ATM activation after helicobacter pylori infection. *Am. J. Pathol.* 188, 329–335. doi: 10.1016/j.ajpath.2017.10.005

Schafer, M. J., White, T. A., Iijima, K., Haak, A. J., Ligresti, G., Atkinson, E. J., et al. (2017). Cellular senescence mediates fibrotic pulmonary disease. *Nat. Commun.* 8:14532. doi: 10.1038/ncomms14532

Scheller, J., and Rose-John, S. (2006). Interleukin-6 and its receptor: from bench to bedside. *Med. Microbiol. Immunol.* 195, 173–183. doi: 10.1007/s00430-006-0019-9

Schouten, L. R. A., Helmerhorst, H. J. F., Wagenaar, G. T. M., Haltenhof, T., Lutter, R., Roelofs, J. J. T. H., et al. (2016). Age-dependent changes in the pulmonary renin-angiotensin system are associated with severity of lung injury in a model of acute lung injury in rats. *Crit. Care Med.* 44, e1226–e1235. doi: 10.1097/CCM.0000000000002008

Schouten, L. R., van Kaam, A. H., Kohse, F., Veltkamp, F., Bos, L. D., de Beer, F. M., et al. (2019). Age-dependent differences in pulmonary host responses in ARDS: a prospective observational cohort study. *Ann. Intensive Care* 9:55. doi: 10.1186/s13613-019-0529-4

Shang, X., Lin, K., Yu, R., Zhu, P., Zhang, Y., Wang, L., et al. (2019). Resveratrol protects the myocardium in sepsis by activating the phosphatidylinositol 3-kinases (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway and inhibiting the nuclear factor-κB (NF-κB) signaling pathway. *Med. Sci. Monit.* 25, 9290–9298. doi: 10.12659/MSM.918369

Shi, S., Qin, M., Shen, B., Cai, Y., Liu, T., Yang, F., et al. (2020a). Association of Cardiac Injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* 5, 802–810. doi: 10.1001/jamacardio.2020.0950

Shi, Y., Wang, Y., Shao, C., Huang, J., Gan, J., Huang, X., et al. (2020b). COVID-19 infection: the perspectives on immune responses. *Cell Death Differ.* 27, 1451–1454. doi: 10.1038/s41418-020-0530-3

Shimabukuro-Vornhagen, A., Gödel, P., Subklewe, M., Stemmler, H. J., Schlößer, H. A., Schlaak, M., et al. (2018). Cytokine release syndrome. *J. Immunother. Cancer* 6:56. doi: 10.1186/s40425-018-0343-9

Shukla, S., and Gupta, S. (2010). Apigenin: a promising molecule for cancer prevention. *Pharm. Res.* 27, 962–978. doi: 10.1007/s11095-010-0089-7

Singh, S., Sonawane, A., and Sadhukhan, S. (2020). Plant-derived natural polyphenols as potential antiviral drugs against SARS-CoV-2 via RNA-dependent RNA Polymerase (RdRp) Inhibition: An In-Silico Analysis. ChemRxiv [Preprint]. doi:10.26434/CHEMRXIV.12312263.V1

Smith, A. A., Fridling, J., Ibhrahim, D., and Porter, P. S. (2020). Identifying patients at greatest risk of mortality due to COVID-19: a New England perspective. *West. J. Emerg. Med.* 21, 785–789. doi: 10.5811/westjem.2020.6.47957

Solnier, J., and Fladerer, J. P. (2020). Flavonoids: a complementary approach to conventional therapy of COVID-19? *Phytochem. Rev.* doi:10.1007/s11101-020-09720-6 [Epub ahead of print]

Stout, M. B., Justice, J. N., Nicklas, B. J., and Kirkland, J. L. (2017). Physiological aging: links among adipose tissue dysfunction, diabetes, and frailty. *Physiology* 32, 9–19. doi: 10.1152/physiol.00012.2016

Stout-Delgado, H. W., Cho, S. J., Chu, S. G., Mitzel, D. N., Villalba, J., El-Chemaly, S., et al. (2016). Age-dependent susceptibility to pulmonary fibrosis is associated with NLRP3 inflammasome activation. *Am. J. Respir. Cell Mol. Biol.* 55, 252–263. doi: 10.1165/rcmb.2015-0222OC

Suvakov, S., Cubro, H., White, W. M., Butler Tobah, Y. S., Weissgerber, T. L., Jordan, K. L., et al. (2019). Targeting senescence improves angiogenic potential of adipose-derived mesenchymal stem cells in patients with preeclampsia. *Biol. Sex Differ.* 10:49. doi: 10.1186/s13293-019-0263-5

Tang, D., Kang, R., Coyne, C. B., Zeh, H. J., and Lotze, M. T. (2012). PAMPs and DAMPs: signal 0s that spur autophagy and immunity. *Immunol. Rev.* 249, 158–175. doi: 10.1111/j.1600-065X.2012.01146.x

Ueda, K., Kawabata, R., Irie, T., Nakai, Y., Tohya, Y., and Sakaguchi, T. (2013). Inactivation of pathogenic viruses by plant-derived tannins: strong effects of extracts from persimmon (*Diospyros kaki*) on a broad range of viruses. *PLoS One* 8:e55343. doi: 10.1371/journal.pone.0055343

van den Berg, D. F., and te Velde, A. A. (2020). Severe COVID-19: NLRP3 inflammasome dysregulated. *Front. Immunol.* 11:1580. doi: 10.3389/fimmu.2020.01580

van Deursen, J. M. (2014). The role of senescent cells in ageing. *Nature* 509, 439–446. doi: 10.1038/nature13193

van Kerkhove, M. D., Vandemaele, K. A. H., Shinde, V., Jaramillo-Gutierrez, G., Koukounari, A., Donnelly, C. A., et al. (2011). Risk factors for severe outcomes following 2009 influenza a (H1N1) infection: a global pooled analysis. *PLoS Med.* 8:e1001053. doi: 10.1371/journal.pmed.1001053

Varela-Eirín, M., Carpintero-Fernández, P., Sánchez-Temprano, A., Varela-Vázquez, A., Paíno, C. L., Casado-Díaz, A., et al. (2020). Senolytic activity of small molecular polyphenols from olive restores chondrocyte redifferentiation and promotes a pro-regenerative environment in osteoarthritis. *Aging* 12, 15882–15905. doi: 10.18632/aging.103801

Wan, Y., Shang, J., Graham, R., Baric, R. S., and Li, F. (2020b). Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J. Virol.* 94, e00127–e00120. doi: 10.1128/jvi.00127-20

Wan, S., Yi, Q., Fan, S., Lv, J., Zhang, X., Guo, L., et al. (2020a). Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). medRxiv [Preprint]. doi: 10.1101/2020.02.10.20021832

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 323, 1061–1069. doi: 10.1001/jama.2020.1585

Wei, P.-F. (Released by National Health Commission & National Administration of Traditional Chinese Medicine on March 3, 2020) (2020). Diagnosis and treatment protocol for novel coronavirus pneumonia (Trial Version 7). *Chin. Med. J.* 133, 1087–1095. doi: 10.1097/CM9.0000000000000819

Webmeter (2020). Coronavirus Age, Sex, Demographics (COVID-19)—Worldometer. Available at: www.worldometers.info (Accessed December 01, 2020).

Williamson, E. J., Walker, A. J., Bhaskaran, K., Bacon, S., Bates, C., Morton, C. E., et al. (2020). OpenSAFELY: factors associated with COVID-19 death in 17 million patients. *Nature* 584, 430–436. doi: 10.1038/s41586-020-2521-4

World Health Organization (2020). WHO coronavirus disease (COVID-19) Dashboard. World Heal. Organ.

Wu, J. T., Leung, K., Bushman, M., Kishore, N., Niehus, R., de Salazar, P. M., et al. (2020). Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China. *Nat. Med.* 26, 506–510. doi: 10.1038/s41591-020-0822-7

Wu, Z., and McGoogan, J. M. (2020). Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China. *JAMA* 323, 1239–1242. doi: 10.1001/jama.2020.2648

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., et al. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med.* 8, 420–422. doi: 10.1016/S2213-2600(20)30076-X

Xudong, X., Junzhu, C., Xingxiang, W., Furong, Z., and Yanrong, L. (2006). Age- and gender-related difference of ACE2 expression in rat lung. *Life Sci.* 78, 2166–2171. doi: 10.1016/j.lfs.2005.09.038

Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., and Zhou, Q. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* 367, 1444–1448. doi: 10.1126/science.abb2762

Yang, S. J., and Lim, Y. (2014). Resveratrol ameliorates hepatic metaflammation and inhibits NLRP3 inflammasome activation. *Metabolism* 63, 693–701. doi: 10.1016/j.metabol.2014.02.003

Yao, X. H., Li, T. Y., He, Z. C., Ping, Y. F., Liu, H. W., Yu, S. C., et al. (2020). A pathological report of three COVID-19 cases by minimally invasive autopsies. *Zhonghua Bing Li Xue Za Zhi* 49, 411–417. doi: 10.3760/cma.j.cn112151-20200312-00193

Yousefzadeh, M. J., Zhu, Y., McGowan, S. J., Angelini, L., Fuhrmann-Stroissnigg, H., Xu, M., et al. (2018). Fisetin is a senotherapeutic that extends health and lifespan. *EBioMedicine* 36, 18–28. doi: 10.1016/j.ebiom.2018.09.015

Zanni, F., Vescovini, R., Biasini, C., Fagnoni, F., Zanlari, L., Telera, A., et al. (2003). Marked increase with age of type 1 cytokines within memory and effector/cytotoxic CD8+ T cells in humans: a contribution to understand the relationship between inflammation and immunosenescence. *Exp. Gerontol.* 38, 981–987. doi: 10.1016/S0531-5565(03)00160-8

Zhang, D. -H., Wu, K. -L., Zhang, X., Deng, S. -Q., and Peng, B. (2020). In silico screening of Chinese herbal medicines with the potential to directly inhibit 2019 novel coronavirus. *J. Integr. Med.* 18, 152–158. doi: 10.1016/j.joim.2020.02.005

Zhao, L., Cen, F., Tian, F., Li, M. J., Zhang, Q., Shen, H. Y., et al. (2017). Combination treatment with quercetin and resveratrol attenuates high fat diet-induced obesity and associated inflammation in rats via the AMPKα1/SIRT1 signaling pathway. *Exp. Ther. Med.* 14, 5942–5948. doi: 10.3892/etm.2017.5331

Zheng, Z., Peng, F., Xu, B., Zhao, J., Liu, H., Peng, J., et al. (2020). Risk factors of critical & mortal COVID-19 cases: a systematic literature review and meta-analysis. *J. Inf. Secur.* 81, e16–e25. doi: 10.1016/j.jinf.2020.04.021

Zhou, P., Yang, X. -L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273. doi: 10.1038/s41586-020-2012-7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 15 January 2021
doi: 10.3389/fphys.2020.622179

Serum Renin Levels Increase With Age in Boys Resulting in Higher Renin Levels in Young Men Compared to Young Women, and Soluble Angiotensin-Converting Enzyme 2 Correlates With Renin and Body Mass Index

Lars Jehpsson[1†], Jiangming Sun[2†], Peter M. Nilsson[3], Andreas Edsfeldt[2,4,5] and Per Swärd[1]*

[1] Clinical and Molecular Osteoporosis Research Unit, Departments of Orthopedics and Clinical Sciences, Skåne University Hospital, Lund University, Malmö, Sweden, [2] Department of Cardiovascular Research-Translational Studies and Cardiology, Skåne University Hospital, Lund University, Malmö, Sweden, [3] Internal Medicine–Epidemiology, Department of Clinical Sciences, Skåne University Hospital, Lund University, Malmö, Sweden, [4] Wallenberg Center for Molecular Medicine, Lund University, Malmö, Sweden, [5] Department of Cardiology, Skåne University Hospital, Malmö, Sweden

OPEN ACCESS

Edited by:
Adriana Castello Costa Girardi, University of São Paulo, Brazil

Reviewed by:
Ravindra K. Sharma, University of Florida, United States
Eliana Hiromi Akamine, University of São Paulo, Brazil

***Correspondence:**
Per Swärd
per.sward@skane.se

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Vascular Physiology, a section of the journal Frontiers in Physiology

Received: 27 October 2020
Accepted: 18 December 2020
Published: 15 January 2021

Citation:
Jehpsson L, Sun J, Nilsson PM, Edsfeldt A and Swärd P (2021) Serum Renin Levels Increase With Age in Boys Resulting in Higher Renin Levels in Young Men Compared to Young Women, and Soluble Angiotensin-Converting Enzyme 2 Correlates With Renin and Body Mass Index. Front. Physiol. 11:622179. doi: 10.3389/fphys.2020.622179

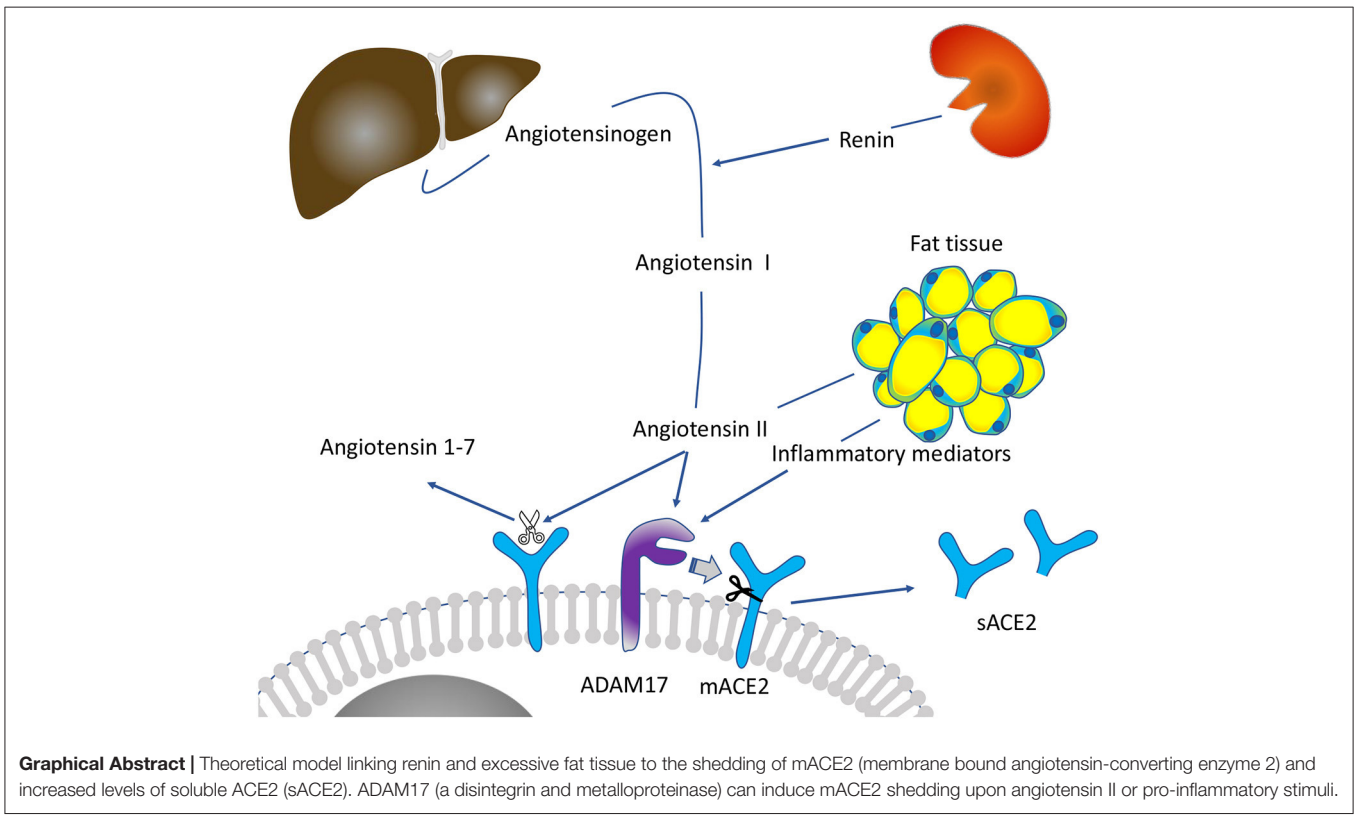
Background: Age, sex, and body constitution may affect the shedding of membrane bound angiotensin-converting enzyme 2 (mACE2) and lead to a relative mACE2 deficiency. However, it is unclear if differences, reflected by serum renin levels, exist in the basal renin-angiotensin-system (RAS) between children and adults, boys, and girls as well as young women and young men. Furthermore, it remains to be investigated if renin and soluble ACE2 (sACE2) levels are correlated with body mass index (BMI) in children and young adults. The aim of this observational study was to assess age-and sex differences in serum renin, and the relationship between renin, soluble angiotensin-converting enzyme 2, and body mass index in a prospectively followed population-based cohort of children which were followed into young adulthood.

Study Design: We analyzed renin and sACE2 in serum in a prospectively followed population-based cohort at 9.9 (0.6) [mean (SD)] ($n = 173$), 11.7 (0.6) ($n = 156$), 14.8 (0.8) ($n = 149$), 18.8 (0.3) ($n = 93$), and 23.5 (0.7) ($n = 152$) years of age. Height (cm) and weight (kg) was measured and body mass index (BMI) was calculated as weight (kg)/height (m)^2. Sex-related differences in renin levels were calculated using analysis of covariance, adjusted for age. Correlations were assessed by calculating the correlation coefficient (R^2) using a multivariable linear mixed model.

Results: Both sexes had low renin levels up to 12 years of age. Thereafter renin levels increased more in boys than in girls. Males from the age of 15 had significantly higher levels than females ($p < 0.001$). There was a positive linear relationship between renin and sACE2 levels in male and female subjects ($p < 0.001$), and between sACE2 levels and BMI in males ($p < 0.001$).

Conclusion: Renin levels increase with age, are higher in men than in women since around puberty, and are correlated with sACE2 levels. Furthermore, sACE2 levels are correlated with body mass index in males. These findings indicate that high renin levels in males and females and a high BMI in males may activate pathways which increase the shedding of mACE2, with possible implications for the risk of severe coronavirus disease 2019.

Keywords: angiotensin-converting enzyme 2, body mass index, coronavirus–COVID-19, renin, renin–angiotensin–aldosterone system, severe acute respiratory coronavirus 2



Graphical Abstract | Theoretical model linking renin and excessive fat tissue to the shedding of mACE2 (membrane bound angiotensin-converting enzyme 2) and increased levels of soluble ACE2 (sACE2). ADAM17 (a disintegrin and metalloproteinase) can induce mACE2 shedding upon angiotensin II or pro-inflammatory stimuli.

INTRODUCTION

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has during 2020 caused a pandemic of Coronavirus disease 2019 (COVID-19) (Dong et al., 2020). Severe COVID-19 infection is most common among elderly men and rare among children (Grasselli et al., 2020; Suleyman et al., 2020). In young adults, the proportion with severe COVID-19 is higher among men than women (Grasselli et al., 2020). Comorbidities, including obesity, hypertension, cardiovascular disease and diabetes, have been identified as important factors for developing severe COVID-19 and COVID-19 associated mortality (Grasselli et al., 2020; Guan et al., 2020; Popkin et al., 2020). The pathophysiology behind these clinical observations is still unclear. However, a relative deficiency of membrane-bound angiotensin-converting enzyme 2 (mACE2) has been implicated as a link between cardiovascular disease, diabetes, old age, and male sex (Xie et al., 2006; Oudit and Pfeffer, 2020; Verdecchia et al., 2020; Wang et al., 2020). In line with this, it has been proposed that a pre-existing deficiency of mACE2 in the lung and other organs (which can be infected by SARS-CoV-2) (Gupta et al., 2020) may corroborate with increased risk to develop severe COVID-19 (Oudit and Pfeffer, 2020; Verdecchia et al., 2020; Wang et al., 2020).

In the basal renin-angiotensin-system (RAS) state, renin cleaves angiotensinogen into angiotensin I (ANGI), which is subsequently cleaved into ANGII by angiotensin-converting enzyme, a membrane-bound metalloproteinase highly expressed in the pulmonary circulation (Paul et al., 2006). ANGII interacts with the angiotensin II type 1 receptor subtype (AT1R), which leads to vasoconstriction and activation of pro-inflammatory and pro-fibrotic pathways (Skurk et al., 2004; Oudit and Pfeffer, 2020; Wang et al., 2020). Regulating ANGII signaling, mACE2 cleaves ANGII, generating the vasodilator angiotensin 1-7, which

induces anti-inflammatory and anti-thrombotic pathways (Oudit and Pfeffer, 2020; Wang et al., 2020). These observations have led to the hypothesis that individual differences in the basal RAS signaling, which are associated with higher circulating renin levels, may lead to increased ANGII/a disintegrin and metalloproteinase-17 (ADAM-17) induced mACE2 shedding. An increased shedding could then potentially contribute to a pre-existing mACE2 deficiency with increasing age from childhood until adulthood, preferentially in men.

However, if underlying differences in the basal RAS signaling can explain why individuals with high age, male gender, and overweight/obesity, have a higher risk to develop severe COVID-19 upon SARS-CoV-2 infection is still unclear. Although previous studies have found that circulating renin levels are higher in middle-aged men compared to middle-aged women (Schunkert et al., 1997) and that circulating renin levels, and plasma renin activity correlate with plasma ANGII (Kosunen and Pakarinen, 1978; Nystrom et al., 1997), a deeper knowledge on differences in renin levels between children and young adults as well as between young men and women, is lacking.

We have shown in a recent publication that soluble ACE2 (sACE2) increases with increasing age so that young adult men have higher sACE2 compared to young women and children (Sward et al., 2020). Complementing our findings, others have shown that sACE2 levels are higher in elderly men compared to women (Kornilov et al., 2020; Sama et al., 2020) and that sACE2 levels are higher among individuals with higher body mass index (BMI) and the metabolic syndrome (Kornilov et al., 2020). However, we are lacking studies investigating the relationship between sACE2 and lung mACE2 protein levels, which is of great importance (Vaduganathan et al., 2020). Hence, it is still unclear if there are differences in the rate of ADAM-17 induced mACE2 shedding between children and young adults and between the sexes, yet such differences are potentially of importance to improve current treatment of COVID-19.

The *aim* of this observational study was to assess age-and sex differences in serum renin, and the relationship between renin and sACE2, and between renin, sACE2 and BMI in a prospectively followed population-based cohort of children, followed into young adulthood. We hypothesized that in groups with high risk to develop severe COVID-19 (adults > children and men > women), (i) renin levels would increase with age and reach higher levels in adult men compared to adult women, that (ii) renin and sACE2 levels would be positively correlated, and that (iii) renin and sACE2 would be positively correlated with BMI. Such findings would support the hypothesis that there are differences in RAS signaling between adults and children, and between men and women, and that renin levels, by affecting ANGII levels, may induce ADAM-17 activity toward mACE2.

SUBJECTS AND METHODS

Study Population Pediatric Osteoporosis Prevention Study

The Pediatric Osteoporosis Prevention (POP) study is a prospective study that annually follow a population-based cohort of children through the nine compulsory school years, primary to evaluate development in musculoskeletal traits, fracture incidence and academic achievement in relation to physical activity. The study design has been reported in detail in previous publications (Linden et al., 2007; Detter et al., 2014; Coster et al., 2017). In short, the POP cohort includes children from four community-based and government-funded, neighboring elementary schools in the community of Bunkeflo, Malmö, Sweden. All schools were located in the same city area with similar socioeconomic status. The children were allocated to the schools depending on their residential address. The only registered difference between the four schools was that one of the schools had 40 min daily physical education (200 min/week) whereas the other three had only 60min/week (provided in 1–2 lessons).

Children who started grade 1 (1998–2000) were invited to participate at the time of school start. Of the children who agreed to participate in the study, 98% were of Caucasian ethnicity. The children were then 7.7 (0.6) years [mean (SD)] (Cronholm et al., 2020). At baseline 349 children were included in the study and the children were followed by annual measurements from baseline to grade nine, corroborating with termination of compulsory school (Cronholm et al., 2020). The POP cohort was also evaluated by measurements at mean age 19 and 24 years. Height (cm) was measured with a Holtain Stadiometer (Holtain LTD, Pembrokeshire, UK) and weight (kg) with an electric scale (Avery Berkel HL 120 Electric Scale, Avery Berkel, West Midlands, UK). Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Blood samples were collected at a mean age of 9.9 (0.6; $n = 173$), 11.7 (0.6; $n = 156$), 14.8 (0.8; $n = 149$), 18.8 (0.3; $n = 93$), and 23.5 (0.7) ($n = 152$) years of age (Sward et al., 2020) (**Figure 1**). Ethical approval for the POP study was obtained at the Regional Ethics Committee at Lund University, Lund, Sweden (LU 471-95, LU 486-96, and 2015/118). Written informed consent was gained from parent(s) of all children included in the study.

In a previous drop-out analysis, utilizing the general school health data register, we found at baseline similar body weight, body height and body mass index (BMI; kg/m^2) in children who agreed or disagreed to participate at baseline (Dencker et al., 2006). A second drop-out analysis, comparing children who left blood samples at 9.9 (0.6), 11.7 (0.6), 14.8 (0.8), 18.8 (0.3), 327 and 23.5 (0.6) years of age (assessments 1–5) with those who attended at baseline but who did not donate blood is presented in **Supplementary Figures 1–5**.

Laboratory Methods

Serum was prepared by letting the blood clot for 30 min at 8°C, followed by centrifugation at 1430 G for 10 min. The serum was then stored at −70°C until analysis. All samples were analyzed in the same batch, with subject's serum from the four schools randomized between the plates. Renin and sACE2 were measured using the Olink® panels (Olink Proteomics AB, Uppsala, Sweden) according to the manufacturer's instructions. The Proximity Extension Assay (PEA) technology used for the Olink protocol has been described in detail (Assarsson et al., 2014). Biomarker levels were normalized using an

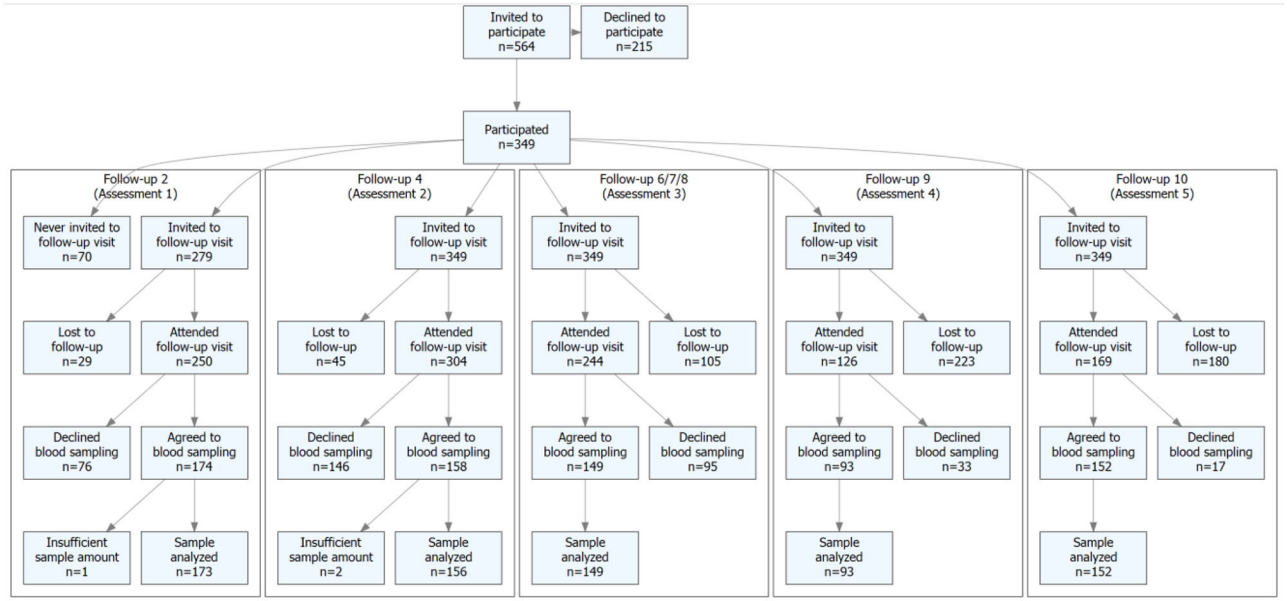


FIGURE 1 | Flow-chart of subjects included in the study, and lost to follow-up.

internal extension control and an inter-plate control, to adjust for intra- and inter-run variation. The final assay read-out is presented in Normalized Protein eXpression (NPX) values, which is an arbitrary unit on a log2-scale where a high value corresponds to a high protein expression. Detection limits, intra- and inter-assay precision data are available on manufacturer's website (www.olink.com).

STATISTICAL ANALYSIS

We performed a drop-out analysis at each follow-up visit, comparing the distribution of standardized height, weight and BMI between subjects included in the study, and subjects lost during follow-up. Data from the most recent visit was used in the comparisons and distributions were compared visually. Differences in renin levels between male and female subjects were calculated using analysis of covariance, adjusted for age. Outlier observations which deviated more than 3 SD from the sex-specific mean ($n = 8$) were excluded from the analysis. To assess the relationship between renin and sACE2 we calculated the correlation coefficient (R^2) using a linear mixed model taking age, sex, and repeated measures into accounts, i.e., subjects as random effects. Because that data from Olink's platform have an S-curve (sigmoid) relationship with the true protein concentration in a sample (www.olink.com), the rank-based inverse normal transformation was applied on the protein levels of renin and sACE2 prior to correlation analysis. Data is presented as mean with 95% confidence intervals (CI). The level of significance was set at $p < 0.05$, and analyses were performed using R statistical software (version 4.0.2, The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Drop-out analysis showed that there were no major discernable differences when comparing the distribution of height, weight, and BMI between subjects included in the analyses and subjects lost during follow-up (**Supplementary Figures 1–5**). Baseline characteristics are presented in **Table 1**.

Renin in Relation to Age and Sex

Sex explained 5.8% of the variance in renin ($p = 1.6 \times 10^{-6}$). Renin levels were similar in both sexes up until the age of 12 years. Among male subjects, renin levels increased with age and the renin levels were significantly higher among males from 15 years of age compared to males younger than 15 years of age (**Figure 2**). Also, whereas renin levels increased in males from age 12, they decreased in females with growth/aging and males older then 15 years of age had significantly higher renin levels compared to females (**Figure 2**). The mean (95% CI) renin Normalized Protein eXpression (NPX) levels were for male and female subjects at a mean age of 14.8 [7.1 (7.0–7.2) vs. 6.8 (6.7–6.9), $p = 1.8 \times 10^{-4}$], at mean age 18.8 [7.1 (7.0–7.2) vs. 6.7 (6.5–6.9), $p = 4.6 \times 10^{-5}$], and at mean age 23.5 [7.1 (7.0–7.2) vs. 6.6 (6.5–6.8), $p = 3.0 \times 10^{-7}$] years, corresponding to 23, 32, and 41% higher renin levels in male compared to female subjects at ages 14.8, 18.8, and 23.5 years, respectively.

Correlations Between Renin, sACE2, and BMI

No significant interaction effect for sACE2 levels and sex was found in the association with renin ($p = 0.37$). Overall, renin showed a positive correlation with sACE2 levels ($R^2 = 0.072$, 95% CI 0.04–0.112, $p = 9.7 \times 10^{-12}$, **Figure 3**). The correlation

TABLE 1 | Subject background data in relation to age and sex at repeated health examinations.

	Baseline, Age 7.7 (SD 0.6) years		Age 9.9 (SD 0.6) years		Age 11.7 (SD 0.6) years	
	Boys (*n* = 191)	**Girls (*n* = 158)**	**Boys (*n* = 92)**	**Girls (*n* = 80)**	**Boys (*n* = 88)**	**Girls (*n* = 67)**
BACKGROUND DATA						
Age, years (SD)	7.7 (0.6)	7.7 (0.6)	10.0 (0.6)	9.8 (0.6)	11.8 (0.6)	11.7 (0.6)
Height, cm (SD)	128.8 (6.5)	128.0 (7.0)	140.6 (6.8)	140.1 (7.3)	152.5 (8.0)	152.6 (10.0)
Weight, kg (SD)	27.7 (5.3)	27.3 (5.3)	34.3 (6.9)	34.4 (6.6)	43.4 (9.2)	43.8 (9.6)
BMI, kg/m^2 (SD)	16.6 (2.3)	16.6 (2.4)	17.3 (2.6)	17.5 (2.7)	18.5 (2.9)	18.6 (3.3)
	Age 14.8 (SD 0.8) years		**Age 18.8 (SD 0.3) years**		**Age 23.5 (SD 0.7) years**	
	Boys (*n* = 82)	**Girls (*n* = 66)**	**Boys (*n* = 48)**	**Girls (*n* = 44)**	**Men (*n* = 75)**	**Women (*n* = 74)**
BACKGROUND DATA						
Age, years (SD)	14.9 (0.7)	14.7 (0.8)	18.8 (0.3)	18.8 (0.3)	23.5 (0.7)	23.5 (0.7)
Height, cm (SD)	173.2 (8.2)	165.7 (6.7)	181.8 (6.5)	168.5 (5.0)	180.6 (6.9)	168.6 (5.9)
Weight, kg (SD)	61.8 (13.2)	57.6 (11.0)	75.9 (11.9)	64.0 (10.3)	78.9 (11.8)	66.4 (12.4)
BMI, kg/m^2 (SD)	20.5 (3.5)	20.9 (3.6)	23.0 (3.4)	22.5 (3.2)	24.1 (3.0)	23.3 (4.1)

BMI, body mass index; SD, standard deviation.

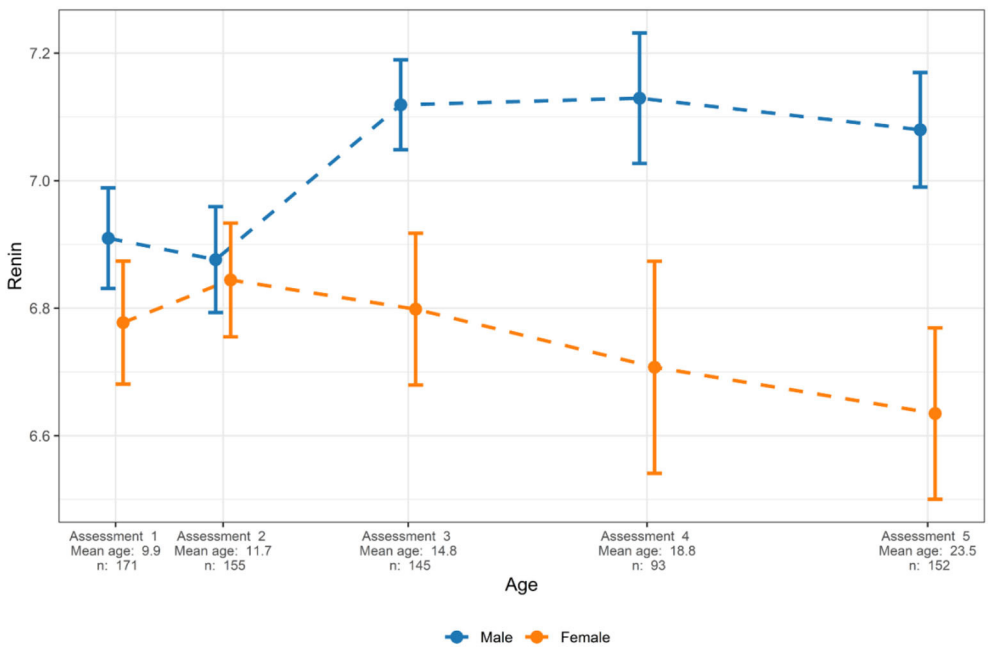


FIGURE 2 | Sex-specific levels of renin in relation to age. Mean and 95% confidence intervals are presented. Renin levels are presented as normalized protein expression on a Log^2 scale.

between renin and sACE2 was also tested separately in males and females. We observed a positive correlation between renin and sACE2 in males (R^2 = 0.072, 95% CI 0.031–0.128, $P = 1.7 \times 10^{-6}$; data not shown), and a positive correlation between renin and sACE2 in females (R^2 = 0.056, 95% CI: 0.018–0.112, $P = 2.8 \times 10^{-5}$; data not shown).

There was a significant interaction effect for sACE2 levels and sex in the association with BMI ($p = 7.0 \times 10^{-8}$). Therefore, the association between sACE2 levels and BMI was tested separately in males and females. We observed a significant correlation between BMI and sACE2 levels for males (R^2 = 0.074, 95% CI 0.032–0.130, $p = 8.8 \times 10^{-6}$, **Figure 4)** whereas no such correlation was identified among females (R^2 = 0.009, 95% CI 0–0.041, $p = 0.14$; data not shown).

Furthermore, we identified an interaction effect for renin levels and sex in the association with BMI (p = 0.001) but no significant correlation between renin levels and BMI in males (R^2 = 0.0002, 95% CI 0–0.014, p = 0.79) nor in females

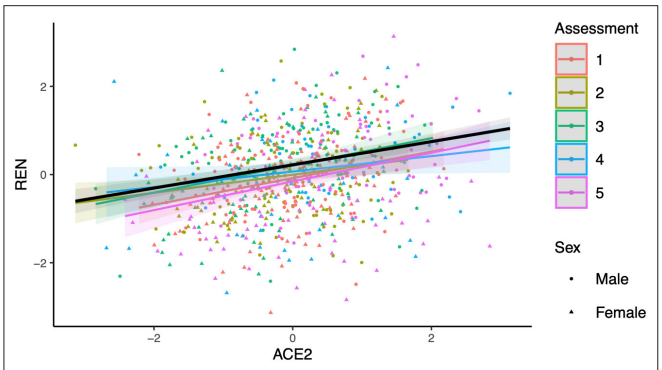


FIGURE 3 | Relationship between renin (REN) and soluble angiotensin-converting enzyme 2 (ACE2). The slope (black line) and 95% confidence intervals (shaded regions) were estimated from a linear mixed model using age and sex as covariates as well subjects as random effects. The obtained R^2 between renin and sACE2 is 0.072 ($p = 9.7 \times 10^{-12}$). Regression lines for respective groups of assessment (assessments 1–5: mean age 9.9, 11.7, 14.8, 18.8, and 23.5 years) are also shown.

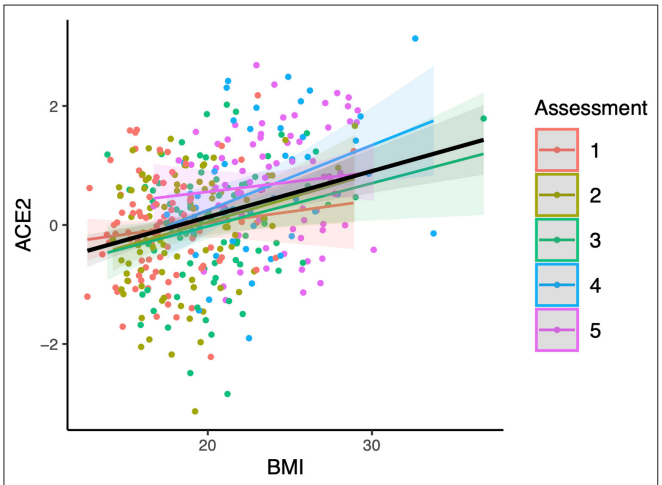


FIGURE 4 | Relationship between soluble angiotensin-converting enzyme 2 (ACE2) and body mass index (BMI) in males. The slope (black line) and 95% confidence intervals (shaded regions) were estimated from a linear mixed model using age and sex as covariates as well subjects as random effects. The obtained R^2 between sACE2 and BMI in males is 0.074 ($p = 8.8 \times 10^{-6}$). Regression lines for respective groups of assessment (assessments 1–5: mean age 9.9, 11.7, 14.8, 18.8, and 23.5 years) are also shown.

were identified ($R^2 = 0.006$, 95% CI 0–0.034, $p = 0.23$; data not shown).

DISCUSSION

The main finding of the present longitudinal study on a population-based cohort followed from childhood until young adulthood was that renin levels increased with age in boys, reaching higher levels in young men compared to young women from age 15 and onwards. This could imply underlying differences in RAS signaling between young men, children, and young women from adolescence, confirming sex-related differences in renin levels in middle-aged subjects (Schunkert et al., 1997). That renin levels in boys increase from around puberty, could suggest a positive link between renin levels, sexual maturation and increased testosterone levels. Studies in mice and rats indicate that testosterone increases pro-renin expression, and that anti-androgen treatment associates with reduced renin levels (White et al., 2019), findings which adhere with those of the present study. Also, that serum renin levels in females decrease from around puberty could be related to a downregulation of renin by estrogen. In line with these findings, estrogen therapy administered to post-menopausal women, leads to decreased plasma renin levels (White et al., 2019).

We also found that renin levels were positively correlated with sACE2 levels. This could imply that the higher sACE2 levels observed in men, by us and others (Sama et al., 2020; Sward et al., 2020), at least partly reflects increased RAS signaling/ADAM-17 activity toward mACE2 in men compared to women. Hence, underlying age- and sex differences between children and adults, and between men and women in the RAS (Fischer et al., 2002), could thereby theoretically contribute to a pre-existing mACE2 deficiency in young men compared to women and children, similar to findings in rats (Xie et al., 2006). However, this conclusion is speculative, and needs to be confirmed in other studies.

Interestingly, in the present study we showed a tendency toward decreasing levels of renin among female subjects from puberty and onwards whereas we previously showed that sACE2 tends to increase among female subjects in the same age group (Sward et al., 2020). Nevertheless, we found positive correlations between renin and sACE2 in females. Thus, although several factors (including estrogen levels) may affect the protein levels of circulating renin and mACE2 (Bukowska et al., 2017; White et al., 2019), positive correlations between serum renin and sACE2 levels in females were identified. Together this could indicate that enhanced RAS signaling/ADAM-17 dependent mACE2 shedding is reflected by increased levels of circulating sACE2.

One of the most prominent risk factors for severe COVID-19 in both children and adults is obesity. Obesity has been associated with increased risk of pneumonia, severe disease among children (Leon-Abarca, 2020; Zachariah et al., 2020), risk of hospitalization, intensive care unit admission and mortality in adults (Popkin et al., 2020). Hence, our findings of a positive correlation between sACE2 and BMI in young males may have clinical relevance which needs to be explored further.

Furthermore, our findings are in line with a previous study by Kornilov et al., where they showed that sACE2 correlated to BMI and the metabolic syndrome (Kornilov et al., 2020). Interestingly, they also found that the association between sACE2 and the metabolic syndrome was stronger among men compared to women (Kornilov et al., 2020). On the other hand, in contrast to a study by Goncalves et al. (2016), we did not find a significant correlation between renin levels and BMI. The two study cohorts are not comparable in age which could explain this difference. If the observed association between sACE2 and BMI in boys and young males is related to that RAS components (other than renin), including ANG II and inflammatory mediators can be

generated in adipose tissue (Schutten et al., 2017; Delaney et al., 2018; Da Silva-Bertani et al., 2020), and thereby contribute to mACE2 shedding (Jia et al., 2009; Patel et al., 2014), needs to be explored in future studies. A complementary explanation to the association between sACE2 and BMI in males could be that ACE2 gene expression may be upregulated in obesity. In a recent publication, ACE2 gene expression was found to be higher in the lung of obese mice, and in lung epithelial cells of obese subjects, compared to non-obese subjects (Al Heialy et al., 2020).

It has been shown that RAS blockage can induce higher cardiac ACE2 mRNA and ACE2 activity in a rat model (Ferrario et al., 2005). Based on these findings, there were initial concerns that RAS blockers could increase COVID-19 risk by upregulation of the number of SARS-CoV-2 receptors. However, if these findings can be translated to the lung and to humans is unclear (Vaduganathan et al., 2020; Wysocki et al., 2020). Also, several publications have found that the treatment with ACE inhibitors or angiotensin II receptor blockers (ARBs) is not associated with the likelihood of a positive COVID-19 test, the risk of severe COVID-19 nor associated mortality (Chung et al., 2020; Lo et al., 2020; Mehta et al., 2020; Reynolds et al., 2020). Ongoing randomized trials, on the effects of RAS inhibition on outcome for SARS-CoV-2 infected patients requiring/not requiring hospital admission will provide more clarity (NCT04312009, NCT04311177).

The cumulative findings of the present study could imply higher RAS signaling and increased mACE2 shedding in males compared to females from adolescence and into young adulthood. This could be of relevance for the observed higher incidence of hypertension in young men compared to young women (Everett and Zajacova, 2015). Also, with potential importance for COVID-19, differences in mACE2 could contribute to differences in response to SARS-CoV-2 infection. The SARS-CoV-1 and SARS-CoV-2 viruses enter lung cells by binding to mACE2 (Hoffmann et al., 2020), which upon SARS-CoV-1 infection has been shown to lead to ADAM-17 induced shedding of mACE2, and increased cellular release of tumor necrosis factor (Haga et al., 2008). This may be associated with increased levels of ANGII and dysregulated RAS signaling (Kuba et al., 2005). That these events are involved in the severity of COVID-19 was implied by studies showing that ANGII levels correlate with COVID-19 lung injury in patients (Liu et al., 2020). Therefore, it was speculated that individuals with a pre-existing mACE2 deficiency, upon SARS-CoV-2 infection, may be at higher risk to develop critical mACE2 deficiency in the lung, which may lead to increased risk of acute lung injury and mortality (Verdecchia et al., 2020).

However, the role of sACE2 levels as a potential risk marker of severe COVID-19 is controversial (Rieder et al., 2020; Rojas et al., 2020). sACE2 levels are higher in males, increase with age and are associated with BMI and the metabolic syndrome (Kornilov et al., 2020; Sama et al., 2020; Sward et al., 2020), which are both described as risk factors for severe COVID-19 (Grasselli et al., 2020; Suleyman et al., 2020). Nevertheless, it has been suggested that elevated sACE2 levels may have beneficial effects on COVID-19 explained by the role of sACE2 working as a decoy receptor and thereby theoretically inhibiting the binding between SARS-COV-2 and mACE2 on host cells (Lei et al., 2020). A recent large-scaled study found that sACE2 levels were higher in patients with severe compared to non-severe COVID-19, suggesting that the relevance of this mechanism as a protective factor for severe COVID-19 may be insufficient (Filbin et al., 2020).

Study strengths include the longitudinal study design, and repeated sampling of serum in the prospectively followed population-based cohort included in the present study. Study limitations include that the cohort was only followed into young adulthood, the lack of longitudinal blood pressure data and that we cannot asses mACE2 and cell ADAM-17 activity in serum. Therefore, any conclusions with regards to ADAM-17 activity and mACE2 protein expression in the present study are speculative, but our findings highlight the need of studies designed to address age- and sex-related differences in mACE2 tissue protein levels. Although ADAM-17 is believed to be of particular importance for the shedding of mACE2 (Haga et al., 2008; Jia et al., 2009; Patel et al., 2014), other sheddases, including ADAM-10 can also cleave mACE2 (Jia et al., 2009). However, *in vitro* studies of human airway epithelial cells showed that whereas inhibition of ADAM-17 decreased the basal sACE2 release into medium, ADAM-10 inhibition did not (Jia et al., 2009), and the shedding of mACE2 by ADAM-17 after ANGII stimulation has been shown by others (Patel et al., 2014).

In conclusion, the present longitudinal study presents data showing that renin levels increase with age in children, are higher in men than in women since around puberty, and are correlated with sACE2 levels. These data could suggest that higher renin levels in men compared to women and in men compared to children, could lead to increased angiotensin II/ADAM-17 induced mACE2 shedding in men. We also identified a correlation between increasing sACE2 and BMI in males, but not in females. This could theoretically be related to increased production of RAS and inflammatory mediators in male adipose tissue, possibly combined with an adiposity-induced increase in ACE2 gene expression. In the COVID-19 context, increased RAS signaling could lead to a pre-existing mACE2 deficiency developing with increasing age, and more in men than women, with possible implications for the risk of severe COVID-19. We recommend that studies further explore this potential relationship.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Regional Ethics Committee at Lund University, Lund, Sweden. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

The present study was designed and data analyzed by LJ, JS, PN, AE, and PS. Statistical analysis was performed by LJ and JS. PS wrote the first draft of the manuscript. All authors critically assessed the manuscript and approved the final manuscript.

FUNDING

The research was funded by the Swedish Research Council for Sport Science, the Herman Järnhardt Foundation, Skåne Regional Foundations, Kockska foundation (Fromma), and ALF Foundations. None of the funders had any influence on study design, data collection, interpretation or writing of the manuscript.

ACKNOWLEDGMENTS

The subjects which have participated in the POP study and all personnel involved in in the recruitment and follow-up of subjects in the POP study are gratefully acknowledged.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.622179/full#supplementary-material

REFERENCES

Al Heialy, S., Hachim, M. Y., Senok, A., Gaudet, M., Abou Tayoun, A., Hamoudi, R., et al. (2020). Regulation of angiotensin-converting enzyme 2 in obesity: implications for COVID-19. *Front. Physiol.* 11:555039. doi: 10.1101/2020.04.17.046938

Assarsson, E., Lundberg, M., Holmquist, G., Bjorkesten, J., Thorsen, S. B., Ekman, D., et al. (2014). Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS ONE* 9:e95192. doi: 10.1371/journal.pone.0095192

Bukowska, A., Spiller, L., Wolke, C., Lendeckel, U., Weinert, S., Hoffmann, J., et al. (2017). Protective regulation of the ACE2/ACE gene expression by estrogen in human atrial tissue from elderly men. *Exp. Biol. Med. (Maywood)* 242, 1412–1423. doi: 10.1177/1535370217718808

Chung, M. K., Karnik, S., Saef, J., Bergmann, C., Barnard, J., Lederman, M. M., et al. (2020). SARS-CoV-2 and ACE2: the biology and clinical data settling the ARB and ACEI controversy. *EBioMedicine* 58:102907. doi: 10.1016/j.ebiom.2020.102907

Coster, M. E., Fritz, J., Nilsson, J. A., Karlsson, C., Rosengren, B. E., Dencker, M., et al. (2017). How does a physical activity programme in elementary school affect fracture risk? a prospective controlled intervention study in Malmo, Sweden. *BMJ Open* 7:e012513. doi: 10.1136/bmjopen-2016-012513

Cronholm, F., Lindgren, E., Rosengren, B. E., Dencker, M., Karlsson, C., and Karlsson, M. K. (2020). Daily school physical activity from before to after puberty improves bone mass and a musculoskeletal composite risk score for fracture. *Sports (Basel)* 8:40. doi: 10.3390/sports8040040

Da Silva-Bertani, D. C. T., Vileigas, D. F., Mota, G. A. F., De Souza, S. L. B., Sant'ana, P. G., Freire, P. P., et al. (2020). Increased angiotensin II from adipose tissue modulates myocardial collagen I and III in obese rats. *Life Sci.* 252:117650. doi: 10.1016/j.lfs.2020.117650

Delaney, K. Z., Vanstone, C. A., Weiler, H. A., and Santosa, S. (2018). Regional adiposity and markers of inflammation in pre-school age children. *Sci. Rep.* 8:15204. doi: 10.1038/s41598-018-33054-1

Dencker, M., Thorsson, O., Karlsson, M. K., Linden, C., Eiberg, S., Wollmer, P., et al. (2006). Daily physical activity related to body fat in children aged 8-11 years. *J. Pediatr.* 149, 38–42. doi: 10.1016/j.jpeds.2006.02.002

Detter, F., Nilsson, J. A., Karlsson, C., Dencker, M., Rosengren, B. E., and Karlsson, M. K. (2014). A 3-year school-based exercise intervention improves muscle strength–a prospective controlled population-based study in 223 children. *BMC Musculoskelet. Disord.* 15:353. doi: 10.1186/1471-2474-15-353

Dong, E., Du, H., and Gardner, L. (2020). An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect. Dis.* 20, 533–534. doi: 10.1016/S1473-3099(20)30120-1

Everett, B., and Zajacova, A. (2015). Gender differences in hypertension and hypertension awareness among young adults. *Biodemography. Soc. Biol.* 61, 1–17. doi: 10.1080/19485565.2014.929488

Ferrario, C. M., Jessup, J., Chappell, M. C., Averill, D. B., Brosnihan, K. B., Tallant, E. A., et al. (2005). Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation* 111, 2605–2610. doi: 10.1161/CIRCULATIONAHA.104.510461

Filbin, M. R., Mehta, A., Schneider, A. M., Kays, K. R., Guess, J. R., Gentili, M., et al. (2020). Plasma proteomics reveals tissue-specific cell death and mediators of cell-cell interactions in severe COVID-19 patients. *bioRxiv [Preprint]*. doi: 10.1101/2020.11.02.365536

Fischer, M., Baessler, A., and Schunkert, H. (2002). Renin angiotensin system and gender differences in the cardiovascular system. *Cardiovasc. Res.* 53, 672–677. doi: 10.1016/S0008-6363(01)00479-5

Goncalves, I., Edsfeldt, A., Colhoun, H. M., Shore, A. C., Palombo, C., Natali, A., et al. (2016). Association between renin and atherosclerotic burden in subjects with and without type 2 diabetes. *BMC Cardiovasc. Disord.* 16:171. doi: 10.1186/s12872-016-0346-8

Grasselli, G., Zangrillo, A., Zanella, A., Antonelli, M., Cabrini, L., Castelli, A., et al. (2020). Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. *JAMA* 323, 1574–1581. doi: 10.1001/jama.2020.5394

Guan, W. J., Liang, W. H., Zhao, Y., Liang, H. R., Chen, Z. S., Li, Y. M., et al. (2020). Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. *Eur. Respir. J.* 55:2000547. doi: 10.1183/13993003.00547-2020

Gupta, A., Madhavan, M. V., Sehgal, K., Nair, N., Mahajan, S., Sehrawat, T. S., et al. (2020). Extrapulmonary manifestations of COVID-19. *Nat. Med.* 26, 1017–1032. doi: 10.1038/s41591-020-0968-3

Haga, S., Yamamoto, N., Nakai-Murakami, C., Osawa, Y., Tokunaga, K., Sata, T., et al. (2008). Modulation of TNF-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-alpha production and facilitates viral entry. *Proc. Natl. Acad. Sci. U.S.A.* 105, 7809–7814. doi: 10.1073/pnas.0711241105

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 Cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271–280. doi: 10.1016/j.cell.2020.02.052

Jia, H. P., Look, D. C., Tan, P., Shi, L., Hickey, M., Gakhar, L., et al. (2009). Ectodomain shedding of angiotensin converting enzyme 2 in human airway epithelia. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 297, L84–L96. doi: 10.1152/ajplung.00071.2009

Kornilov, S. A., Lucas, I., Jade, K., Dai, C. L., Lovejoy, J. C., and Magis, A. T. (2020). Plasma levels of soluble ACE2are associated with sex, metabolic syndrome, and its biomarkers in a large cohort, pointing to a possible mechanism for increased severity in COVID-19. *Crit. Care* 24:452. doi: 10.1186/s13054-020-03141-9

Kosunen, K. J., and Pakarinen, A. (1978). Correlations between plasma renin activity, angiotensin II, and aldosterone. *J. Clin. Endocrinol. Metab.* 47, 665–666. doi: 10.1210/jcem-47-3-665

Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., et al. (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.*11, 875–879. doi: 10.1038/nm1267

Lei, C., Qian, K., Li, T., Zhang, S., Fu, W., Ding, M., et al. (2020). Neutralization of SARS-CoV-2 spike pseudotyped virus by recombinant ACE2-Ig. *Nat. Commun.* 11:2070. doi: 10.1038/s41467-020-16048-4

Leon-Abarca, J. A. (2020). Obesity and immunodeficiencies are the main pre-existing conditions associated with mild to moderate COVID-19 in children. *Pediatr. Obes.* 15:e12713. doi: 10.1111/ijpo.12713

Linden, C., Alwis, G., Ahlborg, H., Gardsell, P., Valdimarsson, O., Stenevi-Lundgren, S., et al. (2007). Exercise, bone mass, and bone size in prepubertal boys: one-year data from the pediatric osteoporosis prevention study. *Scand. J. Med. Sci. Sports* 17, 340–347. doi: 10.1111/j.1600-0838.2006.00568.x

Liu, Y., Yang, Y., Zhang, C., Huang, F., Wang, F., Yuan, J., et al. (2020). Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci. China Life Sci.* 63, 364–374. doi: 10.1007/s11427-020-1643-8

Lo, K. B., Bhargav, R., Salacup, G., Pelayo, J., Albano, J., Mccullough, P. A., et al. (2020). Angiotensin converting enzyme inhibitors and angiotensin II receptor blockers and outcomes in patients with COVID-19: a systematic review and meta-analysis. *Expert Rev. Cardiovasc. Ther.* 18, 919–930. doi: 10.1080/14779072.2020.1826308

Mehta, N., Kalra, A., Nowacki, A. S., Anjewierden, S., Han, Z., Bhat, P., et al. (2020). Association of use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers with testing positive for coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 5, 1020–1026. doi: 10.1001/jamacardio.2020.1855

Nystrom, F., Karlberg, B. E., and Ohman, K. P. (1997). Serum angiotensin-converting enzyme activity correlates positively with plasma angiotensin II: a population-based study of ambulatory blood pressure and the renin-angiotensin system. *J. Hum. Hypertens.* 11, 301–306. doi: 10.1038/sj.jhh.1000433

Oudit, G. Y., and Pfeffer, M. A. (2020). Plasma angiotensin-converting enzyme 2: novel biomarker in heart failure with implications for COVID-19. *Eur. Heart J.* 41, 1818–1820. doi: 10.1093/eurheartj/ehaa414

Patel, V. B., Clarke, N., Wang, Z., Fan, D., Parajuli, N., Basu, R., et al. (2014). Angiotensin II induced proteolytic cleavage of myocardial ACE2 is mediated by TACE/ADAM-17: a positive feedback mechanism in the RAS. *J. Mol. Cell. Cardiol.* 66, 167–176. doi: 10.1016/j.yjmcc.2013.11.017

Paul, M., Poyan Mehr, A., and Kreutz, R. (2006). Physiology of local renin-angiotensin systems. *Physiol. Rev.* 86, 747–803. doi: 10.1152/physrev.00036.2005

Popkin, B. M., Du, S., Green, W. D., Beck, M. A., Algaith, T., Herbst, C. H., et al. (2020). Individuals with obesity and COVID-19: a global perspective on the epidemiology and biological relationships. *Obes. Rev.* 21:e13128. doi: 10.1111/obr.13128

Reynolds, H. R., Adhikari, S., Pulgarin, C., Troxel, A. B., Iturrate, E., Johnson, S. B., et al. (2020). Renin-angiotensin-aldosterone system inhibitors and risk of covid-19. *N. Engl. J. Med.* 382, 2441–2448. doi: 10.1056/NEJMoa2008975

Rieder, M., Wirth, L., Pollmeier, L., Jeserich, M., Goller, I., Baldus, N., et al. (2020). Serum ACE-2, angiotensin II, and aldosterone levels are unchanged in patients with COVID-19. *Am. J. Hypertens.* doi: 10.1093/ajh/hpaa169. [Epub ahead of print].

Rojas, M., Acosta-Ampudia, Y., Monsalve, D. M., Ramirez-Santana, C., Anaya, J. M., and CP-COVID-19 group (2020). How important is the assessment of soluble ACE-2 in COVID-19? *Am. J. Hypertens.* doi: 10.1093/ajh/hpaa178. [Epub ahead of print].

Sama, I. E., Ravera, A., Santema, B. T., Van Goor, H., Ter Maaten, J. M., Cleland, J. G. F., et al. (2020). Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin-angiotensin-aldosterone inhibitors. *Eur. Heart J.* 41, 1810–1817. doi: 10.1093/eurheartj/ehaa373

Schunkert, H., Danser, A. H., Hense, H. W., Derkx, F. H., Kurzinger, S., and Riegger, G. A. (1997). Effects of estrogen replacement therapy on the renin-angiotensin system in postmenopausal women. *Circulation* 95, 39–45. doi: 10.1161/01.CIR.95.1.39

Schutten, M. T., Houben, A. J., De Leeuw, P. W., and Stehouwer, C. D. (2017). The link between adipose tissue renin-angiotensin-aldosterone system signaling and obesity-associated hypertension. *Physiology (Bethesda)* 32, 197–209. doi: 10.1152/physiol.00037.2016

Skurk, T., Van Harmelen, V., and Hauner, H. (2004). Angiotensin II stimulates the release of interleukin-6 and interleukin-8 from cultured human adipocytes by activation of NF-kappaB. *Arterioscler. Thromb. Vasc. Biol.* 24, 1199–1203. doi: 10.1161/01.ATV.0000131266.38312.2e

Suleyman, G., Fadel, R. A., Malette, K. M., Hammond, C., Abdulla, H., Entz, A., et al. (2020). Clinical characteristics and morbidity associated with coronavirus disease 2019 in a series of patients in metropolitan detroit. *JAMA Netw. Open* 3:e2012270. doi: 10.1001/jamanetworkopen.2020.12270

Sward, P., Edsfeldt, A., Reepalu, A., Jehpsson, L., Rosengren, B. E., and Karlsson, M. K. (2020). Age and sex differences in soluble ACE2 may give insights for COVID-19. *Crit. Care* 24:221. doi: 10.1186/s13054-020-02942-2

Vaduganathan, M., Vardeny, O., Michel, T., Mcmurray, J. J. V., Pfeffer, M. A., and Solomon, S. D. (2020). Renin-angiotensin-aldosterone system inhibitors in patients with covid-19. *N. Engl. J. Med.* 382, 1653–1659. doi: 10.1056/NEJMsr2005760

Verdecchia, P., Cavallini, C., Spanevello, A., and Angeli, F. (2020). The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur. J. Intern. Med.* 76, 14–20. doi: 10.1016/j.ejim.2020.04.037

Wang, K., Gheblawi, M., and Oudit, G. Y. (2020). Angiotensin converting enzyme 2: a double-edged sword. *Circulation* 142, 426–428. doi: 10.1161/CIRCULATIONAHA.120.047049

White, M. C., Fleeman, R., and Arnold, A. C. (2019). Sex differences in the metabolic effects of the renin-angiotensin system. *Biol. Sex Differ.* 10:31. doi: 10.1186/s13293-019-0247-5

Wysocki, J., Lores, E., Ye, M., Soler, M. J., and Batlle, D. (2020). Kidney and lung ACE2 expression after an ACE inhibitor or an Ang II receptor blocker: implications for COVID-19. *J. Am. Soc. Nephrol.* 31, 1941–1943. doi: 10.1681/ASN.2020050667

Xie, X., Chen, J., Wang, X., Zhang, F., and Liu, Y. (2006). Age- and gender-related difference of ACE2 expression in rat lung. *Life Sci.* 78, 2166–2171. doi: 10.1016/j.lfs.2005.09.038

Zachariah, P., Johnson, C. L., Halabi, K. C., Ahn, D., Sen, A. I., Fischer, A., et al. (2020). Epidemiology, clinical features, and disease severity in patients with coronavirus disease 2019 (COVID-19) in a Children's Hospital in New York City, New York. *JAMA Pediatr.* 174:e202430. doi: 10.1001/jamapediatrics.2020.2430

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

PERSPECTIVE
published: 18 January 2021
doi: 10.3389/fphys.2020.614590

Hyperventilation: A Possible Explanation for Long-Lasting Exercise Intolerance in Mild COVID-19 Survivors?

Justina Motiejunaite[1,2], Pauline Balagny[1,3], Florence Arnoult[1], Laurence Mangin[1,4], Catherine Bancal[1], Marie-Pia d'Ortho[1,2] and Justine Frija-Masson[1,2]*

[1] Service de Physiologie-Explorations Fonctionnelles, FHU APOLLO, Assistance Publique Hôpitaux de Paris, Hôpital Bichat-Claude Bernard, Paris, France, [2] INSERM, UMR 1141 NeuroDiderot, Université de Paris, Paris, France, [3] INSERM, UMS 011, Population-based Epidemiological Cohorts Unit, Villejuif, France, [4] UMR 7057, CNRS, Laboratoire Matière et Système Complexes, Paris, France

Since the outbreak of the coronavirus (COVID-19) pandemic, most attention has focused on containing transmission and addressing the surge of critically ill patients in acute care settings. As we enter the second phase of the pandemic, emphasis must evolve to post-acute care of COVID-19 survivors. Persisting cardiorespiratory symptoms have been reported at several months after the onset of the infection. Information is lacking on the pathophysiology of exercise intolerance after COVID-19. Previous outbreaks of coronaviruses have been associated with persistent dyspnea, muscle weakness, fatigue and reduced quality of life. The extent of Covid-19 sequelae remains to be evaluated, but persisting cardiorespiratory symptoms in COVID-19 survivors can be described as two distinct entities. The first type of post-Covid symptoms are directly related to organ injury in the acute phase, or the complications of treatment. The second type of persisting symptoms can affect patients even with mild initial disease presentation without evidence of organ damage. The mechanisms are still poorly qualified to date. There is a lack of correlation between initial symptom severity and residual symptoms at exertion. We report exercise hyperventilation as a major limiting factor in COVID-19 survivors. The origin of this hyperventilation may be related to an abnormality of ventilatory control, by either hyperactivity of activator systems (automatic and cortical ventilatory control, peripheral afferents, and sensory cortex) or failure of inhibitory systems (endorphins) in the aftermath of pulmonary infection. Hyperventilation-induced hypocapnia can cause a multitude of extremely disabling symptoms such as dyspnea, tachycardia, chest pain, fatigue, dizziness and syncope at exertion.

Keywords: COVID-19, hyperventilation syndrome, dyspnea, cardiopulmonary exercise testing, SARS-CoV-2, exercise hyperventilation, persisting symptoms

OPEN ACCESS

Edited by:
Adriana Castello Costa Girardi, University of São Paulo, Brazil

Reviewed by:
Marli Maria Knorst, Federal University of Rio Grande do Sul, Brazil
Ren-Jay Shei, Mallinckrodt (United States), United States

****Correspondence:***
Justina Motiejunaite
justina.motiejunaite@aphp.fr
orcid.org/0000-0001-8385-528X

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *06 October 2020*
Accepted: *24 December 2020*
Published: *18 January 2021*

Citation:
Motiejunaite J, Balagny P, Arnoult F, Mangin L, Bancal C, d'Ortho M-P and Frija-Masson J (2021) Hyperventilation: A Possible Explanation for Long-Lasting Exercise Intolerance in Mild COVID-19 Survivors? Front. Physiol. 11:614590. doi: 10.3389/fphys.2020.614590

INTRODUCTION

The infection with severe respiratory syndrome coronavirus 2 (SARS-CoV-2) was declared a pandemic on March 11, 2020. As of November 2020, the novel coronavirus, hereafter referred to as COVID-19, has affected more than 60 million people worldwide (Center, 2020; Simpson and Robinson, 2020). For the majority (81%), infection with COVID-19 manifests as a mild

disease. Fever (88.7%), cough (57.6%), and dyspnea (45.6%) were the most commonly reported symptoms in a recent systematic review (Rodriguez-Morales et al., 2020). However, for a significant minority, and particularly those older than 65 years and with comorbidities, the infection requires management in an intensive care unit due to acute respiratory distress syndrome (ARDS) (Pascarella et al., 2020). Since the outbreak of the pandemic, most attention has focused on containing transmission and managing the wave of critically ill patients in acute care settings. As we enter the second phase of the pandemic, emphasis must evolve to post-acute care of COVID-19 survivors. For these patients, having defeated the virus is just the beginning of an unknown recovery path. There are increasing reports of persisting and recurrent symptoms at several months after the onset of the infection (Carfì et al., 2020; Goërtz et al., 2020), dyspnea at exertion being one of the most common complaints. The extent and severity of the lasting sequelae remain to be evaluated, but persisting cardiorespiratory symptoms in COVID-19 survivors can be described as two distinct entities. The first type of post-Covid consequences are directly related to organ damage in the acute phase, either due to the viral infection and the ensuing inflammatory response, or the complications of treatment in the intensive care setting (Li et al., 2006; Zhou et al., 2014; Mart and Ware, 2020). The persisting cardiorespiratory symptoms related to cardiac and lung injury in COVID-19 will be summarized below. The second type of persisting symptoms can affect patients even with mild initial disease presentation without evidence of severe organ damage. Sustained fatigue and exercise intolerance are the most frequent complaints in patients not requiring hospitalization (Goërtz et al., 2020; Tenforde et al., 2020). In these patients, there is a striking lack of correlation between initial symptom severity and residual symptoms at exertion. The mechanisms behind post-Covid exercise intolerance are most likely complex and still poorly qualified to date. In this article, we aimed to review the available evidence on persisting exercise intolerance experienced by COVID-19 survivors. We also report exercise hyperventilation as a major limiting factor that can cause a multitude of extremely disabling symptoms such as dyspnea, tachycardia, chest pain, fatigue, dizziness, and syncope at exertion. To our knowledge, this is the first report of a possible explanation for prolonged exercise intolerance in long-lasting COVID-19.

PERSISTING CARIORESPIRATORY SYMPTOMS IN CORONAVIRUS SURVIVORS: CURRENT STATE OF KNOWLEDGE

Around 20% of patients with severe COVID-19 require in-hospital management (Simpson and Robinson, 2020). For the patients presenting with severe initial illness, the long-term consequences are yet to be elucidated. However, emerging studies show evidence of persistent cardiorespiratory symptoms months after hospital discharge. Weerahandi et al. (2020) studied severe COVID-19 patients who required at least 6 l/min of oxygen during hospital stay. At one-month follow-up, 74% of participants reported shortness of breath. Carfì et al. (2020) also assessed persistent symptoms in patients discharged from the hospital. At 2 months follow-up, half of the patients reported persistent fatigue, whereas dyspnea (43%) and chest pain (22%) were also highly prevalent. These findings are in line with the studies by Wong et al. (2020) and Garrigues et al. (2020), both of which found nearly half of the patients complaining of breathlessness at 3 months after hospital discharge.

The persistence of cardiorespiratory symptoms in survivors of severe COVID-19 can be partly explained by the pathophysiology of organ damage during the initial phase of the disease. The SARS-CoV-2 virus predominantly affects the respiratory system, although other organ systems can be compromised as well. The virus uses angiotensin-converting enzyme-2 (ACE2) receptors in pneumocytes of the epithelial alveolar lining to infect the host, thus causing lung injury (Varga et al., 2020). Diffuse alveolar damage was showed by several post-mortem studies (Carsana et al., 2020; Schaller et al., 2020), leading to hypotheses of residual pulmonary function impairment at long term. These hypotheses are supported by the available evidence on previous coronavirus outbreaks. A recent meta-analysis (Ahmed et al., 2020) of studies on SARS-CoV and MERS-CoV reported that approximately one third of hospitalized patients had persisting lung abnormalities after their acute illness. Prospective studies on SARS-CoV, MERS-CoV as well as short-term follow-up studies on the current SARS-CoV-2 epidemic are summarized in **Table 1**. These studies demonstrate mostly a mild pulmonary function impairment and the functional disability appears out of proportion to the degree of lung function impairment. For example, a 2005 study on SARS-CoV by Hui et al. (2005) found that the exercise capacity 6 months after disease onset was considerably lower than that of normal controls in the same age groups. However, significant impairment of surface area gas exchange was only found in 15% of the patients. Along with Hui' study, Ong et al. (2004) performed cardiopulmonary exercises testing in 46 SARS-CoV patients at 3 months after hospital discharge. Half of the patients had abnormalities in the pulmonary function tests, but the impairment was mild in almost all cases. However, 41% of the patients had impairment of exercise capacity not due to ventilatory limitation. The discordance of the results of pulmonary function and exercise testing has been attributed to several factors, such as physical deconditioning, muscle weakness and poor motivation. The recent data in SARS-CoV-2 by Arnold et al. (2020) demonstrates that even 74% of survivors complained of persistent breathlessness and excessive fatigue at 3 months after hospital discharge. Yet abnormal pulmonary function was found in only 10% of the patients. Major limitations have to be taken into account when considering available evidence on long-term pulmonary function abnormalities. The majority of information is derived from single-center studies with a small sample of patients and a relatively short-term follow-up. However, most of the data support the idea that persistent cardiorespiratory symptoms in COVID-19 survivors cannot be accounted for by impairment of pulmonary function alone.

Multisystem involvement is the key pathophysiological feature of SARS-CoV-2 infection and can help explain the

TABLE 1 | prospective studies on residual symptoms, pulmonary function impairment and exercise intolerance following SARS-CoV, MERS-CoV and SARS-CoV-2 viral infections.

	Study	Year	Number of patients	Assessment modality	Duration of follow-up	Main findings
SARS-CoV	Zhang et al., 2020	2018	71	PFT	15 years	No data on residual symptoms Mildly reduced D_LCO in 38% of patients Reduced FEV_1/FVC ratio in patients with residual chest CT abnormalities
	Ngai et al., 2010	2010	123	PFT and 6MWD	2 years	Lower quality of life compared to normal controls Persistent impairment of D_LCO in 52% of survivors Reduced 6MWD compared to controls
	Li et al., 2006	2006	36	PFT and 6MWD	1 year	Reduced quality of life in patients older than 40 years Mild impairment of D_LCO with near normal PFT Near-normal 6MWD
	Hui et al., 2005	2005	110	PFT and 6MWD	6 months	Impairment of health-related quality of life D_LCO impairment in 15.5% of survivors Reduced 6MWD compared to controls
	Ong et al., 2004	2004	46	PFT and CPET	3 months	Shortness of breath in 50% of patients Abnormalities on PFT in 50% of patients Reduced maximum aerobic capacity in 41% of patients
MERS-CoV	Park et al., 2018	2018	73	PFT and 6MWD	1 year	No data on residual symptoms 8% of patients had lung function parameters <80% of predicted values Impairment of D_LCO in 34% of all patients Preserved 6MWD
SARS-CoV-2	Zhao et al., 2020	2020	55	PFT	3 months	Persisting symptoms in 60% of patients Lung function abnormalities in 25%
	Clavario et al., 2020	2020	150	PFT and CPET	3 months	Normal PFT Functional capacity limitation in 50% of patients, mainly explained by muscular impairment
	Raman et al., 2020	2020	58	PFT, 6MWD, and CPET	2–3 months	Persistent breathlessness in 64% of patients Shorter 6MWD compared to controls Reduced exercise capacity in 55% of patients
	Arnold et al., 2020	2020	163	PFT	2–3 months	Persistent breathlessness and excessive fatigue in 74% of patients Restrictive syndrome in 10%
	van Gassel et al., 2020	2020	48	PFT and 6MWD	2 months	No data on persistent symptoms Diminished total lung capacity and diffusion capacity in half of the participants 6MWD result was 82% of predicted distance
	Frija-Masson et al., 2020	2020	50	PFT	1 month	Abnormal lung function in half of the patients, without a clear relationship with CCT findings
	Huang Y. et al., 2020	2020	57	PFT and 6MWD	1 month	Impaired D_LCO and lung imaging abnormalities in 50% of patients, in relation to initial disease severity
	Mo et al., 2020	2020	110	PFT	1 month	Anomalies in D_LCO in 47% of patients, in relation to pneumonia severity

CCT, chest computed tomography; PFT, pulmonary function tests; 6MWD, 6-min walking distance; CPET, cardiopulmonary exercise testing; MRI, magnetic resonance imaging; D_LCO, diffusing capacity of the lung for carbon monoxide; FEV_1/FVC, forced expiratory volume in 1 s to forced vital capacity.
As the COVID-19 outbreak continues to evolve and the scientific evidence rapidly expands, the information provided in this table is only current as of the date of submission.

heterogeneous symptoms experienced by patients, as well as the complex mechanisms of long-lasting exercise intolerance. As described above, the virus binds to ACE2 receptors, which are present not only in the lungs, but are widely distributed in endothelial cells. Endothelial injury recruits inflammatory leukocytes, and contributes to tissue damage and cytokine release, as well as thrombosis and disseminated intravascular coagulation (Evans et al., 2020). Autopsy studies (Varga et al., 2020) show severe endothelial injury that is associated with the presence of intracellular virus. Widespread thrombosis with microangiopathy were also described (Ackermann et al., 2020). It has been postulated that endothelial injury and microangiopathy might be the reasons behind autonomic dysfunction, which is compatible with residual symptoms such as fatigue, palpitations and chest pain, among others. Cardiac injury has also been documented in patients hospitalized with moderate or severe COVID-19 (Clerkin et al., 2020; Driggin et al., 2020; Huang C. et al., 2020). Inflammatory myocarditis is frequently diagnosed in severe COVID-19 patients and may later lead to left ventricular failure. Inflammation induced pro-coagulatory state might lead to type 1 myocardial infarction (MI) due to plaque rupture or intracoronary thrombosis (Chieffo et al., 2020;

Clerkin et al., 2020). Type 2 MI (myocardial injury in the absence of obstructive coronary artery disease) might be induced by prolonged hypoxia. Right ventricular failure secondary to acute pulmonary embolisms is also possible. Myocardial injury can persist after the acute phase and clinically manifest as dyspnea or chest pain at exertion. Therefore, appropriate follow-up to detect these complications is warranted. Two recent studies with cardiac magnetic resonance imaging (MRI) (Huang L. et al., 2020; Puntmann et al., 2020) showed ongoing cardiac involvement in a majority of patients months after a COVID-19 diagnosis. Another study by Raman et al. (2020) describes persistent MRI abnormalities seen in the lungs (60%) and the heart (26%) of COVID-19 patients at 2–3 months after hospital discharge, in relation to ongoing symptoms of breathlessness and excessive fatigue (in 64 and 55% of patients respectively). In the same study, cardiopulmonary exercise testing showed that 55% of patients had peak oxygen uptake (VO_2 max) values lower than 80% of the predicted. In contrast, only 12% of patients had impaired pulmonary function tests.

Persisting cardiorespiratory symptoms may be in part related to initial disease severity and residual organ damage. In critically ill patients, acute respiratory distress (ARDS) – related consequences have been described previously (Desai et al., 1999; Mart and Ware, 2020) and are not specific to COVID-19. Other ICU-related complications such as ventilator-induced lung injury or critical illness-associated poly-neuropathy are well established (Zhou et al., 2014; Beitler et al., 2016) and are out of the scope of this article. The routine administration of high dose steroids to many patients with ARDS might lead to steroid myopathy and muscle wasting, resulting in residual exercise intolerance and excessive fatigue.

Most of the studies that have reported on sequelae of COVID-19 included participants whose illness was severe enough to require hospitalization. However, the majority of patients with COVID-19 are managed in outpatient settings. Long term outcomes might not be comparable due to multiple factors: varying degree of organ damage, different treatment and distinct patient demographics, as many patients admitted to hospital with COVID-19 are older, have more comorbidities and frailty. Despite mild initial presentation, emerging evidence indicates that it might take weeks for resolution of symptoms and return to usual health. Tenforde et al. (2020) reported that out of 274 patients tested for COVID-19 in an outpatient setting, one third complained of fatigue and dyspnea 2 weeks after symptom onset. Even among young adults aged 18–34 years with no chronic medical conditions, nearly one in five reported that they had not returned to their usual state of health. Thus it seems that the persistence of symptoms might not necessarily be related to patient age or initial clinical severity of the disease.

These findings were further confirmed by Goërtz et al. (2020), who performed a survey in ambulatory COVID-19 patients at 3 months after symptom onset. They showed that the majority of patients were still symptomatic, and reported a multitude of symptoms, ranging from cough, sore throat, muscle pain, dizziness, chest tightness, palpitations, weight loss, etc. Fatigue and dyspnea were the two most prevalent symptoms, described by 87 and 71% of patients respectively. These findings are especially alarming for such a young population (median age of 47 years) mostly without serious comorbidities and normal physical examination.

To date, the etiology of these heterogeneous long-Covid symptoms is poorly understood. It is possible that endothelial injury might play a role in the persistence of dysautonomic symptoms in COVID-19 survivors. Davido et al. (2020) described their experience working with outpatients who experienced mostly mild symptoms attributable to COVID-19. Subsequently they observed multiple persistent symptoms, especially intense fatigue, shortness of breath, chest tightness and tachycardia. Authors suggest that these symptoms are compatible with dysautonomia due to microangiopathy and endothelial injury. Miglis et al. (2020a,b) also described a subset of COVID-19 survivors presenting symptoms of autonomic dysfunction such as orthostatic intolerance and postural orthostatic tachycardia. Such symptoms are frequently reported after other viral infections and might be related to gastrointestinal fluid loss, prolonged bed rest and deconditioning of the cardiovascular system. However, further research is needed to further characterize the dysautonomic syndromes in COVID-19 survivors.

In contrast to hospitalized COVID-19 patients, conventional risk factors such as age and the presence of comorbidities do not seem to have an impact on the duration and severity of persistent symptoms. A study by O'Keefe et al. (2020) analyzed 273 non-hospitalized patients recovering from COVID-19. Interestingly, the authors found no correlation between symptom duration and patient factors such as age or comorbidities.

All in all, the pathophysiology of persistent cardiorespiratory symptoms in COVID-19 outpatients are not clearly understood to date. Even though these symptoms seem to be benign, their importance should not be underestimated. Persisting exercise intolerance can result in worsened quality of life, inability to return to work and increased use of health care systems, constituting a worldwide public health problem.

HYPERVENTILATION AS A POSSIBLE CAUSE OF PERSISTING EXERCISE INTOLERANCE

As a French Reference Center for Infectious Diseases, Bichat hospital has had an important number of COVID-19 patients, treated both in and out of hospital settings. All patients are systematically offered follow-up and undergo a thorough cardio-pulmonary exploration, including pulmonary function tests (PFT), a chest CT scan, a trans-thoracic echocardiogram, and cardiopulmonary exercise testing (CPET). Ethics committee approvals were obtained according to local requirements.

We report a case series of eight patients (seven women and one man) with important exertional dyspnea at 3 months after the onset of COVID-19 symptoms. All patients were aged 31–73 [median age 39 years (interquartile range 33–49)] and had no previous medical history (notably, no chronic cardiovascular or pulmonary diseases). All of the patients had a relatively mild course of COVID-19 and received ambulatory treatment without indications for hospitalization or oxygen therapy. Nijmegen

test was available in five patients and median score was 35 (27–38). At 3 months after the symptom onset, all patients had near-normal pulmonary function tests and normal chest CT scans. Transthoracic echocardiogram showed preserved left ventricular ejection fraction as well as the absence of pulmonary hypertension (see **Table 2**). During CPET, dyspnea, palpitations, and dizziness were reproduced in all patients. For two patients CPET had to be interrupted due to syncope at exertion. All of the patients showed a significant impairment of exercise capacity: seven out of eight were incapable of reaching 100% of predicted workload and none of the patients reached their predicted VO_2 max values. An elevated VE/VCO_2 ratio was observed in five patients, suggesting exercise hyperventilation. All patients increased their ventilation and their tidal volume as soon as the effort started. However, we were not able to define the generic ventilatory profile since two patients increased their respiratory rate (RR) only after the aerobic threshold and one patient had no significant increase in RR. Unfortunately, we were not able to collect data on inspiratory capacity. Arterial blood gasses were also analyzed at rest as well as at maximum effort. The apparition of respiratory alkalosis with low arterial CO_2 levels and significant base excess at exertion was observed in three patients. We hypothesized that hyperventilation-induced hypocapnia following COVID-19 infection and prolonged bed

TABLE 2 | Characteristics of patients presenting with hyperventilation syndrome in the aftermath of COVID-19, and findings of cardiopulmonary exercise testing.

Patient No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Gender	F	F	F	F	F	F	F	M
Age, years	73	32	35	48	49	31	35	39
BMI, kg/m2	25.0	18.7	20.4	21.5	26.2	18.0	30.1	33.3
Cardiorespiratory parameters at rest								
RR at rest, rpm	52	16	31	19	22	31	10	24
Tidal volume at rest, L	1.14	0.45	0.40	0.72	0.60	0.36	1.37	0.83
FEV_1,%	85	88	116	107	109	75	101	88
FEV_1/VC	0.68	0.84	0.81	0.80	0.85	0.89	0.80	0.7
TLC,%	103	97	113	90	89	85	103	73
D_LCO,%	79	83	82		62	67	60	68
HR at rest, bpm	121	82	88	104	73	101	78	107
BP at rest, mmHg	110/70	152/99	101/73	119/87	93/70	89/63	106/63	127/88
LVEF,%	63	70	75	68	68	56	64	66
Cardiorespiratory parameters during exercise								
Load reached (W)	47	113	147	100	90	78	70	50
% of predicted load	82	87	119	96	95	63	54	22
% of target heart rate	84	98	82	90	97	87	59	67
Respiratory rate at peak exercise	44	45	46	25	61	48	32	38
Tidal volume at peak exercise, L	0.87	1.08	1.48	1.75	1.26	0.85	1.54	1.50
Minute ventilation at peak exercise, L/min	45	48	69	45	75	44	52	55
Peak respiratory exchange ratio	1.08	1.29	1.17	1.19	1.22	1.06	1.17	1.24
Breathing reserve,%	48	59	51	77	23	53	58	46
VO_2 max, ml/kg/min	10.4	21.2	28.6	17.6	22.8	17.3	11	8.2
% of predicted VO_2 max	62	64	93	70	96	47	49	29
VE/VCO_2	49	32	37	34	44	48	46	51
Symptoms at exertion	dyspnea	dyspnea, palpitations, chest pain	dyspnea, dizziness, tingling	dyspnea	dyspnea	dyspnea, dizziness, syncope	dyspnea	dyspnea, chest pain, syncope
Arterial blood gasses at rest and at peak exercise								
pH at rest	7.55	7.41	7.45	7.47	7.46	7.45	7.44	7.41
pH at exertion	7.42	7.33	7.38	7.41	7.42	7.47	7.47	7.51
PaO2 at rest, mmHg	90	94	100	98	99	100	81	91
PaO2 at exertion, mmHg	92	92	102	104	102	120	116	117
PaCO2 at rest, mmHg	23	39	36	34	32	30	32	39
PaCO2 at exertion, mmHg	31	37	35	32	33	23	27	26
HCO3 at rest, mmol/l	20	25	25	25	23	21	22	25
HCO3 at exertion, mmol/l	20	19	20	20	22	16	19	21
Nijmegen score		20	34	37			40	35

BMI, body mass index; RR, respiratory rate; HR, heart rate; BP, blood pressure; LVEF, left ventricular ejection fraction; FEV_1, forced expiratory volume in 1 s; FEV_1/VC, the ratio of forced expiratory volume on vital capacity; TLC, total lung capacity; D_LCO, diffusing capacity for carbon monoxide.
All pulmonary function values are presented as predicted percentage considering age, sex, height, body weight, and race.

rest might be responsible for a multitude of extremely disabling symptoms such as dyspnea, tachycardia, chest pain, fatigue, dizziness and syncope at exertion. Indeed, clinical follow-up of patients showed progressive resolution of symptoms. Interestingly, most patients described paroxysmal dyspnea that could also occur at rest, which could indicate a wider breathing dysfunction. Normality of PFT is in accordance with our hypothesis that alteration of lung function does not solely account for residual symptoms.

Exercise hyperventilation is a condition defined by inappropriate alveolar hyperventilation, in regards to metabolic needs and mechanical stress in the body (Brat et al., 2019). It generally affects women more frequently than men, especially between the ages of 15 and 55 (Lum, 1975). The variety of symptoms experienced by the patient might take them to a wide range of specialist consultations, numerous investigations and inappropriate treatment.

The origin of this hyperventilation is unknown. The hypothesis of an abnormality of ventilatory control has been proposed, by either a stimulation of activator systems (automatic and cortical ventilatory control, peripheral afferents, and sensory cortex) or a suppression of inhibitory systems (endorphins) in the aftermath of a pulmonary infection (Gardner, 1994). Deciphering the mechanisms underlying this hyperventilation would require to examine chemo responsiveness by hypercapnic ventilatory challenge in patients recovering from COVID-19 and compare patients with and without hyperventilation.

Various forms of primary lung disease can influence the ventilatory control mechanisms and alter the respiratory center output. Hypoxemia-induced activation of peripheral chemoreceptors or stimulation of receptors by diseases affecting the airways or pulmonary interstitium can induce the respiratory center to increase its output, resulting in respiratory alkalosis. Similarly, patients recovering from acute pulmonary embolism, pneumonia, or chronic interstitial lung disease often hyperventilate, probably as a result of stimulation of one or more types of intrathoracic receptors, with or without the additional ventilatory stimulus induced by hypoxemia (Jack et al., 2003).

Even though the mechanisms of hyperventilation are not quite clear, the consequences of alveolar hyperventilation are well known (Laffey and Kavanagh, 2002). The most important physiological consequence of alveolar hyperventilation is the decrease in depolarization threshold of cell membranes. In case of respiratory alkalosis, H^+ ions do not participate in membrane potential, and are transported out of the cell to decrease the blood pH, whereas K^+ ions are transported into the cell. A relative excess of positive ions inside the membrane increases its potential, therefore decreasing the depolarization threshold. Neuronal hyperexcitability causes the activation of autonomous nervous system which in turn is responsible for neurovegetative symptoms described in hyperventilation syndrome. Muscular hyperexcitability results in increased muscular tone and arterial vasoconstriction due to smooth muscle cell contraction in the arterial wall. Resulting hypoperfusion of different organs might cause various symptoms related to ischemia. Hyperventilation related symptoms can range from dyspnea, palpitations, chest pain, muscle cramps, syncope to paresthesia, dizziness, headache, abdominal pain, nausea, fatigue, and anxiety.

There is no gold standard for the diagnosis of hyperventilation syndrome. Diagnostic tools include arterial blood gas measurement and various provocation tests in order to reproduce the symptoms. The Nijmegen Questionnaire (van Dixhoorn and Duivenvoorden, 1985) may be used as a screening instrument for early detection of hyperventilation syndrome, as well as an aid in diagnosis and therapy planning (with a sensibility of 91% and specificity 95%). Treatment options include respiratory physiotherapy with an experienced respiratory therapist, focusing on patient education and various techniques to control breathing. Fortunately, spontaneous resolution is common, especially with the gradual resumption of physical activity.

CONCLUSION

Persisting cardiorespiratory symptoms are rather common at several months after the onset of the COVID-19 infection. Currently, data is lacking on the pathophysiology of this post-COVID entity. In patients with severe initial disease presentation, persisting symptoms might be directly related to target organ damage as well as treatment complications. New studies have emerged describing the post-COVID syndrome even in patients with mild initial presentation, without a clear relationship with age and comorbidities. Persistent cardiorespiratory symptoms in these patients are most likely benign and unrelated to long-term organ damage. Muscle deconditioning, dysautonomia and exercise hyperventilation might partly explain the disabling symptoms in COVID-19 survivors. However, more studies are needed to further clarify the mechanisms behind this prolonged path to recovery.

AUTHOR CONTRIBUTIONS

JM, PB, FA, LM, CB, and JF-M performed the cardiopulmonary function tests. JM and PB performed a literature review. JM and JF-M drafted the manuscript. PB, FA, LM, CB, and M-Pd'O contributed significantly to manuscript correction and finalization. All authors contributed to the article and approved the submitted version.

REFERENCES

Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al. (2020). Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in COVID-19. *N. Eng. J. Med.* 383, 120–128. doi: 10.1056/nejmoa2015432

Ahmed, H., Patel, K., Greenwood, D., Halpin, S., Lewthwaite, P., Salawu, A., et al. (2020). Long-term clinical outcomes in survivors of coronavirus outbreaks after hospitalization or icu admission: a systematic review and meta-analysis of follow-up studies. *medRxiv [preprint]* doi: 10.1101/2020.04.16.20067975

Arnold, D. T., Hamilton, F. W., Milne, A., Morley, A., Viner, J., Attwood, M., et al. (2020). Patient outcomes after hospitalisation with COVID-19 and implications for follow-up; results from a prospective UK cohort. *medRxiv [preprint]* doi: 10.1101/2020.08.12.20173526

Beitler, J. R., Malhotra, A., and Thompson, B. T. (2016). Ventilator-induced lung injury. *Clin. Chest Med.* 37, 633–646. doi: 10.1016/j.ccm.2016.07.004

Brat, K., Stastna, N., Merta, Z., Olson, L. J., Johnson, B. D., and Cundrle, I. Jr. (2019). Cardiopulmonary exercise testing for identification of patients with hyperventilation syndrome. *PLoS One* 14:e0215997. doi: 10.1371/journal.pone.0215997

Carfi, A., Bernabei, R., Landi, F., and Gemelli Against, C.-P.-A. C. S. G. (2020). Persistent symptoms in patients after acute COVID-19. *JAMA* 324, 603–605. doi: 10.1001/jama.2020.12603

Carsana, L., Sonzogni, A., Nasr, A., Rossi, R. S., Pellegrinelli, A., Zerbi, P., et al. (2020). Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. *Lancet Infect. Dis.* 20, 1135–1140. doi: 10.1016/s1473-3099(20)30434-5

Center, J. H. C. R. (2020). *COVID-19 Map - Johns Hopkins Coronavirus Resource Center. [online]*. Available online at: https://coronavirus.jhu.edu/map.html (accessed November 26, 2020).

Chieffo, A., Stefanini, G. G., Price, S., Barbato, E., Tarantini, G., Karam, N., et al. (2020). EAPCI position statement on invasive management of acute coronary syndromes during the COVID-19 pandemic. *Eur. Heart J.* 41, 1839–1851. doi: 10.1093/eurheartj/ehaa381

Clavario, P., De Marzo, V., Lotti, R., Barbara, C., Porcile, A., Russo, C., et al. (2020). Assessment of functional capacity with cardiopulmonary exercise testing in non-severe COVID-19 patients at three months follow-up. *medRxiv [preprint]* doi: 10.1101/2020.11.15.20231985

Clerkin, K. J., Fried, J. A., Raikhelkar, J., Sayer, G., Griffin, J. M., Masoumi, A., et al. (2020). COVID-19 and cardiovascular disease. *Circulation* 141, 1648–1655. doi: 10.1161/CIRCULATIONAHA.120.046941

Davido, B., Seang, S., Tubiana, R., and de Truchis, P. (2020). Post-COVID-19 chronic symptoms: a postinfectious entity? *Clin. Microbiol. Infect.* 26, 1448–1449. doi: 10.1016/j.cmi.2020.07.028

Desai, S. R., Wells, A. U., Rubens, M. B., Evans, T. W., and Hansell, D. M. (1999). Acute respiratory distress syndrome: CT abnormalities at long-term follow-up. *Radiology* 210, 29–35. doi: 10.1148/radiology.210.1.r99ja2629

Driggin, E., Madhavan, M. V., Bikdeli, B., Chuich, T., Laracy, J., Biondi-Zoccai, G., et al. (2020). Cardiovascular considerations for patients, health care workers, and health systems during the COVID-19 Pandemic. *J. Am. Coll. Cardiol.* 75, 2352–2371. doi: 10.1016/j.jacc.2020.03.031

Evans, P. C., Rainger, G., Mason, J. C., Guzik, T. J., Osto, E., Stamataki, Z., et al. (eds) (2020). Endothelial dysfunction in COVID-19: a position paper of the ESC working group for atherosclerosis and vascular biology, and the esc council of basic cardiovascular science. *Cardiovasc. Res.* 116, 2177–2184. doi: 10.1093/cvr/cvaa230

Frija-Masson, J., Debray, M. P., Gilbert, M., Lescure, F. X., Travert, F., Borie, R., et al. (2020). Functional characteristics of patients with SARS-CoV-2 pneumonia at 30 days post-infection. *Eur. Respir. J.* 56:2001754. doi: 10.1183/13993003.01754-2020

Gardner, W. N. (1994). "Hyperventilation: diagnosis and therapy," in *Behavioral and Psychological Approaches to Breathing Disorders*, eds H. Beverly and R. L. Timmons (New York: Springer Science + Business Media, LLC), 99–111. doi: 10.1007/978-1-4757-9383-3_7

Garrigues, E., Janvier, P., Kherabi, Y., Le Bot, A., Hamon, A., Gouze, H., et al. (2020). Post-discharge persistent symptoms and health-related quality of life after hospitalization for COVID-19. *J. Infect.* 81, e4–e6. doi: 10.1016/j.jinf.2020.08.029

Goërtz, Y. M. J., Van Herck, M., Delbressine, J. M., Vaes, A. W., Meys, R., and Machado, F. V. C. (2020). Persistent symptoms 3 months after a SARS-CoV-2 infection: the post-COVID-19 syndrome? *ERJ Open Res.* 6, 00542–2020. doi: 10.1183/23120541.00542-2020

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan. *China Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Huang, L., Zhao, P., Tang, D., Zhu, T., Han, R., Zhan, C., et al. (2020). Cardiac involvement in patients recovered from COVID-2019 identified using magnetic resonance imaging. *JACC Cardiovasc. Imaging* 13, 2330–2339. doi: 10.1016/j.jcmg.2020.05.004

Huang, Y., Tan, C., Wu, J., Chen, M., Wang, Z., Luo, L., et al. (2020). Impact of coronavirus disease 2019 on pulmonary function in early convalescence phase. *Respir. Res.* 21:163. doi: 10.1186/s12931-020-01429-6

Hui, D. S., Joynt, G. M., Wong, K. T., Gomersall, C. D., Li, T. S., Antonio, G., et al. (2005). Impact of severe acute respiratory syndrome (SARS) on pulmonary function, functional capacity and quality of life in a cohort of survivors. *Thorax* 60, 401–409. doi: 10.1136/thx.2004.030205

Jack, S., Rossiter, H. B., Warburton, C. J., and Whipp, B. J. (2003). Behavioral influences and physiological indices of ventilatory control in subjects with idiopathic hyperventilation. *Behav. Modif.* 27, 637–652. doi: 10.1177/0145445503256318

Laffey, J. G., and Kavanagh, B. P. (2002). Hypocapnia. *N. Engl. J. Med.* 347, 43–53. doi: 10.1056/NEJMra012457

Li, T. S., Gomersall, C. D., Joynt, G. M., Chan, D. P., Leung, P., and Hui, D. S. (2006). Long-term outcome of acute respiratory distress syndrome caused by severe acute respiratory syndrome (SARS): an observational study. *Crit. Care Med.* 8, 302–308.

Lum, L. C. (1975). Hyperventilation: the tip and the iceberg. *J. Psychosom. Res.* 19, 375–383. doi: 10.1016/0022-3999(75)90017-3

Mart, M. F., and Ware, L. B. (2020). The long-lasting effects of the acute respiratory distress syndrome. *Exp. Rev. Respir. Med.* 14, 577–586. doi: 10.1080/17476348.2020.1743182

Miglis, M. G., Goodman, B. P., Chemali, K. R., and Stiles, L. (2020a). Re: 'post-COVID-19 chronic symptoms' by Davido et al. *Clin. Microbiol. Infect.* doi: 10.1016/j.cmi.2020.08.028 [Epub ahead of print].

Miglis, M. G., Prieto, T., Shaik, R., Muppidi, S., Sinn, D. I., and Jaradeh, S. (2020b). A case report of postural tachycardia syndrome after COVID-19. *Clin. Auton. Res.* doi: 10.1007/s10286-020-00727-9 [Epub ahead of print].

Mo, X., Jian, W., Su, Z., Chen, M., Peng, H., Peng, P., et al. (2020). Abnormal pulmonary function in COVID-19 patients at time of hospital discharge. *Eur. Respir. J.* 55:2001217. doi: 10.1183/13993003.01217-2020

Ngai, J. C., Ko, F. W., Ng, S. S., To, K. W., Tong, M., and Hui, D. S. (2010). The long-term impact of severe acute respiratory syndrome on pulmonary function, exercise capacity and health status. *Respirology* 15, 543–550. doi: 10.1111/j.1440-1843.2010.01720.x

O'Keefe, J. B., Tong, E. J., Datoo, O., Keefe, G. A., and Tong, D. C. (2020). Predictors of disease duration and symptom course of outpatients with acute COVID-19: a retrospective cohort study. *medRxiv [preprint]* doi: 10.1101/2020.06.05.20123471

Ong, K. C., Ng, A. W., Lee, L. S., Kaw, G., Kwek, S. K., Leow, M. K., et al. (2004). Pulmonary function and exercise capacity in survivors of severe acute respiratory syndrome. *Eur. Respir. J.* 24, 436–442. doi: 10.1183/09031936.04.00007104

Park, W. B., Jun, K. I., Kim, G., Choi, J. P., Rhee, J. Y., Cheon, S., et al. (2018). Correlation between pneumonia severity and pulmonary complications in middle east respiratory syndrome. *J. Korean Med. Sci.* 33:e169. doi: 10.3346/jkms.2018.33.e169

Pascarella, G., Strumia, A., Piliego, C., Bruno, F., Del Buono, R., Costa, F., et al. (2020). COVID-19 diagnosis and management: a comprehensive review. *J. Intern. Med.* 288, 192–206. doi: 10.1111/joim.13091

Puntmann, V. O., Carerj, M. L., Wieters, I., Fahim, M., Arendt, C., Hoffmann, J., et al. (2020). Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 5, 1265–1273. doi: 10.1001/jamacardio.2020.3557

Raman, B., Cassar, M. P., Tunnicliffe, E. M., Filippini, N., Griffanti, L., Alfaro-Almagro, F., et al. (2020). Medium-term effects of SARS-CoV-2 infection on multiple vital organs, exercise capacity, cognition, quality of life and mental health, post-hospital discharge. *medRxiv [preprint]* doi: 10.1101/2020.10.15.20205054

Rodriguez-Morales, A. J., Cardona-Ospina, J. A., Gutierrez-Ocampo, E., Villamizar-Pena, R., Holguin-Rivera, Y., Escalera-Antezana, J. P., et al. (2020). Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. *Travel Med. Infect. Dis.* 34:101623. doi: 10.1016/j.tmaid.2020.101623

Schaller, T., Hirschbuhl, K., Burkhardt, K., Braun, G., Trepel, M., Markl, B., et al. (2020). Postmortem examination of patients with COVID-19. *JAMA* 323, 2518–2520. doi: 10.1001/jama.2020.8907

Simpson, R., and Robinson, L. (2020). Rehabilitation after critical illness in people with COVID-19 infection. *Am. J. Phys. Med. Rehabil.* 99, 470–474. doi: 10.1097/PHM.0000000000001480

Tenforde, M. W., Kim, S. S., Lindsell, C. J., Billig Rose, E., Shapiro, N. I., Files, D. C., et al. (2020). Symptom duration and risk factors for delayed return to usual health among outpatients with COVID-19 in a multistate health care systems network - united states, march-june. *MMWR Morb. Mortal. Wkly. Rep.* 69, 993–998. doi: 10.15585/mmwr.mm6930e1

van Dixhoorn, J., and Duivenvoorden, H. J. (1985). Efficacy of nijmegen questionnaire in recognition of the hyperventilation syndrome. *J. Psychosom. Res.* 29, 199–206. doi: 10.1016/0022-3999(85)90042-x

van Gassel, R. J. J., Bels, J. L. M., Raafs, A., van Bussel, B. C. T., van de Poll, M. C. G., Simons, S. O., et al. (2020). High Prevalence of Pulmonary Sequelae at 3 Months After Hospital Discharge in Mechanically Ventilated COVID-19 Survivors. *Am. J. Respir. Crit. Care Med.* doi: 10.1164/rccm.202010-3823LE Online ahead of print.

Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., et al. (2020). Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 395, 1417–1418. doi: 10.1016/s0140-6736(20)30937-5

Weerahandi, H., Hochman, K. A., Simon, E., Blaum, C., Chodosh, J., Duan, E., et al. (2020). Post-discharge health status and symptoms in patients with severe COVID-19. *medRxiv [preprint]* doi: 10.1101/2020.08.11.20172742

Wong, A. W., Shah, A. S., Johnston, J. C., Carlsten, C., and Ryerson, C. J. (2020). Patient-reported outcome measures after COVID-19: a prospective cohort study. *Eur. Respir. J.* 56:2003276. doi: 10.1183/13993003.03276-2020

Zhang, P., Li, J., Liu, H., Han, N., Ju, J., Kou, Y., et al. (2020). Long-term bone and lung consequences associated with hospital-acquired severe acute respiratory syndrome: a 15-year follow-up from a prospective cohort study. *Bone Res.* 8:8. doi: 10.1038/s41413-020-0084-5

Zhao, Y. M., Shang, Y. M., Song, W. B., Li, Q. Q., Xie, H., Xu, Q. F., et al. (2020). Follow-up study of the pulmonary function and related physiological characteristics of COVID-19 survivors three months after recovery. *E Clin. Med.* 25:100463. doi: 10.1016/j.eclinm.2020.100463

Zhou, C., Wu, L., Ni, F., Ji, W., Wu, J., and Zhang, H. (2014). Critical illness polyneuropathy and myopathy: a systematic review. *Neural Regen. Res.* 9, 101–110. doi: 10.4103/1673-5374.125337

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

HYPOTHESIS AND THEORY
published: 20 January 2021
doi: 10.3389/fphys.2020.588248

A Potential Role of the Renin-Angiotensin-System for Disturbances of Respiratory Chemosensitivity in Acute Respiratory Distress Syndrome and Severe Acute Respiratory Syndrome

Swen Hülsmann[1], Sepideh Khabbazzadeh[1], Konrad Meissner[1] and Michael Quintel[1,2]*

[1]Universitätsmedizin Göttingen, Klinik für Anästhesiologie, Georg-August-Universität, Göttingen, Germany, [2]DONAUISAR Klinikum Deggendorf, Deggendorf, Germany

OPEN ACCESS

Edited by:
Deborah Schechtman, University of São Paulo, Brazil

Reviewed by:
Carmen Silvia Valente Barbas, University of São Paulo, Brazil
Rishi R. Dhingra, University of Melbourne, Australia

***Correspondence:**
Swen Hülsmann
shuelsm2@uni-goettingen.de

Specialty section:
This article was submitted to Respiratory Physiology, a section of the journal Frontiers in Physiology

Received: *28 July 2020*
Accepted: *07 December 2020*
Published: *20 January 2021*

Citation:
Hülsmann S, Khabbazzadeh S, Meissner K and Quintel M (2021) A Potential Role of the Renin-Angiotensin-System for Disturbances of Respiratory Chemosensitivity in Acute Respiratory Distress Syndrome and Severe Acute Respiratory Syndrome. Front. Physiol. 11:588248. doi: 10.3389/fphys.2020.588248

Acute respiratory distress syndrome (ARDS) represents an acute diffuse inflammation of the lungs triggered by different causes, uniformly leading to a noncardiogenic pulmonary edema with inhomogeneous densities in lung X-ray and lung CT scan and acute hypoxemia. Edema formation results in "heavy" lungs, inducing loss of compliance and the need to spend more energy to "move" the lungs. Consequently, an ARDS patient, as long as the patient is breathing spontaneously, has an increased respiratory drive to ensure adequate oxygenation and CO_2 removal. One would expect that, once the blood gases get back to "physiological" values, the respiratory drive would normalize and the breathing effort return to its initial status. However, in many ARDS patients, this is not the case; their respiratory drive appears to be upregulated and fully or at least partially detached from the blood gas status. Strikingly, similar alteration of the respiratory drive can be seen in patients suffering from SARS, especially SARS-Covid-19. We hypothesize that alterations of the renin-angiotensin-system (RAS) related to the pathophysiology of ARDS and SARS are involved in this dysregulation of chemosensitive control of breathing.

Keywords: acute lung damage, respiratory chemoreflexes, neuronal control of breathing, brainstem, homeostasis

INTRODUCTION

Per definition, acute respiratory distress syndrome (ARDS) is characterized by an inhomogeneously distributed, noncardiogenic pulmonary edema and acute hypoxemia. Its presence is still associated with a high mortality. ARDS is triggered by various stimuli, such as sepsis, major trauma, and pneumonia. The underlying pathophysiology involves activation of the immune system, pneumocyte injury, surfactant dysfunction, and coagulopathies. It markedly impairs adequate exchange and consecutively oxygenation and carbon dioxide removal (Balibrea and Arias-Diaz, 2003; Ranieri et al., 2012; Fanelli and Ranieri, 2015). Patients with ARDS may present with alterations of the breathing pattern, and its regulation might not directly correlate with the O_2 or CO_2 partial pressures measured in the arterial blood (Spinelli et al., 2020).

Of note, despite normalizing arterial pO_2 and pCO_2 by mechanical ventilation and/or extracorporeal lung support, patients might still present with respiratory rates far higher than expected or needed (Crotti et al., 2017). These patients might require high doses of sedation or even muscle relaxants and controlled ventilation to prevent patient self-inflicted lung injury (P-SILI). Interestingly, in acute cases of COVID-19 pneumonia (SARS), similar observations were made. Despite normalization of the arterial blood gases, COVID-19 patients continued to show forced breathing patterns that might additionally harm the already virus-altered lungs (Cruces et al., 2020; de Vries et al., 2020; Li et al., 2020; Marini and Gattinoni, 2020; Smit et al., 2020).

In this hypothesis and theory paper, we discuss potential mechanisms that might disturb respiratory chemosensitivity in patients with ARDS or SARS.

THE RENIN-ANGIOTENSIN-SYSTEM IN ARDS

The renin-angiotensin-system (RAS; **Figure 1**) or renin-angiotensin-aldosterone system (RAAS) appears, apart from regulation of blood pressure, to be also involved in the pathogenesis of ARDS (Magalhães et al., 2019). Its main mediator, Angiotensin II (Ang II), is involved in inflammatory and fibrogenic processes in the lungs (Marshall et al., 2004; Hagiwara et al., 2009; Fletcher et al., 2017). Animal experiments in ARDS models demonstrate that the reduction of Ang II formation by inhibition of ACE exerts a protective effect (Imai et al., 2005, 2008; Shen et al., 2009). For example, the ACE inhibitor captopril is able to diminish oleic acid-induced severe acute lung injury in rats (He et al., 2007). Likewise, pharmacological inhibition or genetic deletion of AT1a receptors significantly mitigates lung injury (Raiden et al., 2002; Imai et al., 2005, 2008).

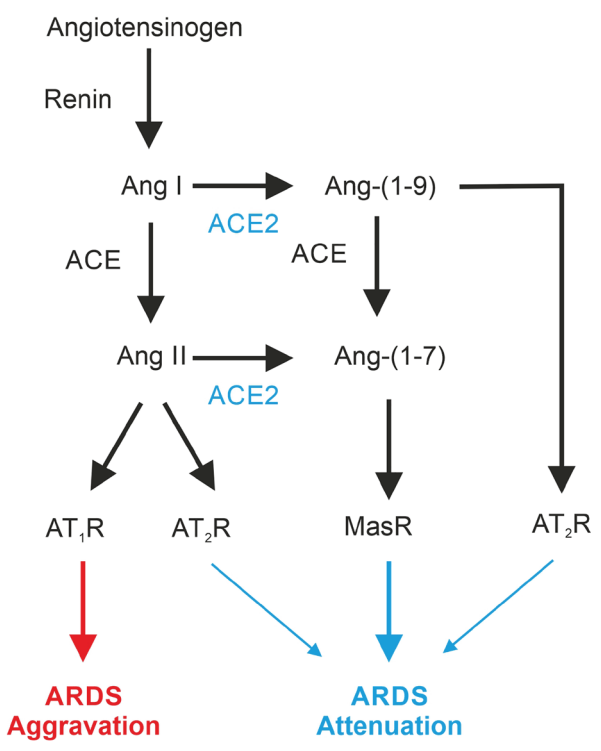


FIGURE 1 | Differential effects of mediators of the renin-angiotensin system involved in acute respiratory distress syndrome (ARDS). ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; AT1-R, angiotensin II receptor type 1; AT2-R, angiotensin II receptor type 2; Mas-R, Mas-Receptor.

The angiotensin-converting enzyme 2 (ACE2; Donoghue et al., 2000; Tipnis et al., 2000), a homolog to the classical ACE, is also expressed in the lung (Hamming et al., 2004; Jia, 2016). The lack of ACE2 expression in ACE2-KO animals increases ARDS susceptibility, and moreover, inactivation of ACE in ACE2-deficient mice attenuates ARDS (Imai et al., 2005). ACE2 catalyzes the formation of angiotensin Ang-(1–7), which acts *via* the Mas-Receptor (Mas-R; Zambelli et al., 2012). Pharmacological activation of Mas-Rs or administration of recombinant ACE2 has been shown to exert lung-protective effects (Imai et al., 2005; Wosten-van Asperen et al., 2011). In addition, ACE activity is increased in ARDS-lungs, and ACE2 activity is reduced (Li et al., 2008; Wosten-van Asperen et al., 2011).

Taken together, these observations suggest that the ACE2-product Ang-(1–7) *via* the Mas-Receptor promotes protective effects in the lung, and shifting the RAS toward ACE/Ang II/AT_1R has deleterious effects (Wang et al., 2019). Finally, ACE2 also cleaves Ang-(1–10) to angiotensin 1–9 acting *via* the AT_2R, which has been shown to exert protective effects on ARDS development (Imai et al., 2005) and pulmonary hypertension (Cha et al., 2018).

Although in ARDS mice Ang II serum levels are elevated (Imai et al., 2005; Chen et al., 2013; Zou et al., 2014), data for humans are less clear. The *Ace* gene insertion/deletion (I/D) polymorphisms correlate with the susceptibility for and severity of ARDS (Marshall et al., 2002; Jerng et al., 2006; Adamzik et al., 2007; Tsantes et al., 2013) with those patients carrying a lower risk that are homozygous for the insertion (II) genotype (Adamzik et al., 2007). Since the ACE II genotype is associated with a lower serum ACE concentration (Rigat et al., 1990), one would expect lower ANG II serum levels. However, serum Ang II levels in humans are quite variable in ARDS as well as in control patients. Significantly higher Ang II serum levels in ARDS patients have never been reported (Wiberg-Jorgensen et al., 1983; Reddy et al., 2019). Nevertheless, a significantly higher Ang-(1–7) to Angiotensinogen [Ang-(1–10)] ratio as well as Ang-(1–9) to Ang-(1–10) ratio in ARDS survivors (Reddy et al., 2019) gives a hint of a protective effect of the ACE2. In addition, a pilot clinical trial using recombinant human angiotensin-converting enzyme 2 in ARDS revealed increased Ang-(1–7) levels but "did not result in improvement in physiological or clinical measures of ARDS in this small study" (Khan et al., 2017). Unfortunately, in this study, Ang-(1–9) levels were not tested.

THE RENIN-ANGIOTENSIN-SYSTEM IN SARS

Coronavirus disease 2019 (COVID-19[1]) is a zoonotic disease caused by the novel SARS-CoV2 (Zhu et al., 2020). Although causing, in many cases, only mild symptoms, some patients develop a severe acute respiratory syndrome (SARS), which resembles ARDS in some but not all aspects (Gattinoni et al., 2020b,c; Marini and Gattinoni, 2020). The angiotensin-converting enzyme 2 is the receptor for SARS-CoV (Li et al., 2003) and SARS-CoV2 (Hoffmann et al., 2020).

In the initial phase of the COVID-19 pandemic, concerns about an increased risk for patients treated with ACE-inhibitors or angiotensin-receptor-blockers (ARBs) were raised (Kuster et al., 2020). Meanwhile, this topic has been studied extensively. In brief, no increase in the severity of COVID-19 or SARS-CoV2 infections have been found (Reynolds et al., 2020); in contrast, studies confirm a potential protective effect (Hippisley-Cox et al., 2020).

Interestingly, a considerable number of patients do not experience shortness of breath or dyspnea in the early phase of COVID-19 despite an already markedly impaired gas exchange, a status called silent hypoxia or silent or happy hypoxemia (Couzin-Frankel, 2020; Dhont et al., 2020; Ottestad et al., 2020). This phenomenon appears when lung compliance is still near normal but gas exchange is already impaired by ventilation/perfusion mismatch and functional shunt [non-ARDS type 1 (or type L); Gattinoni et al., 2020b]. SARS-CoV2 does not only infect the pulmonary epithelium, but heavily alters the vascular endothelium, causing impairment of its antithrombotic properties (McFadyen et al., 2020; Teuwen et al., 2020); thus micro-angiopathy and micro-embolisms can explain the alteration of the ventilation/perfusion ratio that is caused (Merrill et al., 2020). Moreover, pulmonary vasoplegia suspending partially or totally hypoxic pulmonary vasoconstriction leads to reasonable functional shunt (Chau et al., 2020).

However, these patients show mostly tachypnea (Chandra et al., 2020; Ottestad et al., 2020), clearly favoring the concept of an already increased respiratory drive and conflicting with the concept of a "failure to trigger the centrally mediated increase in respiratory rate" as put forward by Soliz (Soliz et al., 2020). The nearly normal compliance of the type L lung can explain the lack of dyspnea: As long as breathing efforts are not limited by the lungs' elastance or external factors (Albashir, 2020). However, the increased respiratory drive can lead to severe hyperventilation with breathing efforts that create large negative pressure swings that lead to self-inflicted lung injury (P-SILI), thus promoting a shift to the H-type of COVID-19 pneumonia (Cruces et al., 2020; Gattinoni et al., 2020a; Smit et al., 2020).

Apart from this clinical alteration, it has been shown that plasma levels of angiotensin II of SARS-CoV2 infected patients were elevated (Liu et al., 2020; Wu et al., 2020), and moreover, plasma levels correlated to the viral load as well as to the degree of lung injury (Liu et al., 2020). An explanation for this is that the binding of SARS-CoV2 to virus-receptor ACE2 led to a downregulation of enzyme ACE2 in the lung tissue (Silhol et al., 2020), a mechanism that had been described already for SARS-CoV1 (Kuba et al., 2005).

[1]Novel Coronavirus (2019-nCoV). Situation Report – 22, WHO, February 12th, 2020, PDF downloaded July 28th 2020.

RAS AND THE REGULATION BREATHING

Ang II and Ang-(1–7) exert differential effects on the carotid body (CB) glomus cells. In CB glomus cells, Ang II increases the respiratory drive by activation of NADPH oxidase (NOX) and mitochondrial-mediated O_2-production with the consequence that K^+-channels are inhibited and voltage-gated Ca^{2+} channels are activated (Allen, 1998; Schultz, 2011). In contrast, Ang-(1–7) exerts an inhibitory influence on glomus cells *via* activation of nNOS and NO-mediated activation of K^+ channels (Schultz, 2011; Fung, 2014). It is of note that chronic hypoxia upregulates the expression and function of AT1-receptors in the carotid body (Leung et al., 2000).

However, the stimulation of breathing by i.v. application of Ang II in dogs could not solely be attributed to alterations in the carotid body activity (Potter and McCloskey, 1979), thus suggesting a role of central chemosensory pathways. Injection of Ang II into the nucleus of the solitary tract (NTS), which relays the chemosensitive information from the CB, is able to increase the respiratory rate (Paton and Kasparov, 1999). Moreover, Ang II receptors are expressed on many neurons, including serotonergic neurons in the raphe nuclei (Allen et al., 1991), which contain central CO_2-chemosensor neurons (Severson et al., 2003; Richerson, 2004; Bhandare et al., 2020). Although the mechanism of Ang II action in these neurons is not yet completely understood, it is known that Ang II regulates release and synthesis of serotonin in raphe neurons (Nahmod et al., 1978) and that Ang II decreases the resting K^+ conductance in other types of brainstem neurons (Li and Guyenet, 1996).

ACE2 is also expressed in the mouse brainstem (Lin et al., 2008), particularly in raphe neurons (Doobay et al., 2007). The functional role of the Ang II or Ang-(1–7) in primary respiratory neurons of pre-Bötzinger Complex in the medulla has not been investigated yet, but solid evidence exists that Ang II or Ang-(1–7) modulate the activity of cardiac neurons neighboring the respiratory neurons in the ventral lateral medulla (de Moura et al., 2010) as well as neurons in the nucleus of the solitary tract (Diz et al., 2002). Several recent studies demonstrate that ACE2/Ang-(1–7)/MasR interacts in the CNS with different neurotransmitter systems, including GABA, dopamine, and norepinephrine (Gironacci et al., 2004; Stragier et al., 2005; Wang et al., 2016). MasR are robustly expressed in GABAergic neurons in the basolateral amygdala (BLA), and ACE2 overexpression increases the spontaneous postsynaptic inhibitory currents in this region (Wang et al., 2016).

A NOVEL HYPOTHESIS: SYNTHESIS OF THE OBVIOUS

Based on the literature reviewed above, we suggest the following hypothesis: In acute respiratory distress syndrome (ARDS) and in severe acute respiratory syndrome (SARS/COVID-19), alterations of the renin-angiotensin-system (RAS) signal a change

of the chemosensitive reflex control of breathing, which results in an increase of the respiratory drive, which becomes independent from alterations of blood gases. Our hypothesis is based on the following key observation: In ARDS and especially in SARS/COVID-19, the RAS is dysregulated and shifted toward the ACE/Ang II/AT1R axis. This dysregulation is expected to stimulate, apart from any potential effect on the lung tissue, chemosensitive neurons in the brainstem and also chemosensitive cells in the carotid body (**Figure 2**), making them more sensitive to changes of CO_2 and O_2 and, thus, shifting their baseline activity and response curves to higher values.

DISCUSSION

Confirmation of this hypothesis requires a joint effort of clinical and basic scientists with broad knowledge in physiology and neurosciences. Experimental approaches should include *in vivo* and ex vivo studies in animal models of ARDS.

What Types of Animal Models Are Available?

In general, so far, only animal models for the "classical" ARDS have been established and used, trying to mimic the uniform pathophysiology of this syndrome, characterized by a marked shunt volume and heavy, hard-to-move lungs. A COVID-19 affliction might – in the early phase – present with nearly normally compliant lungs but a heavily altered ventilation/perfusion (V/Q) ratio and a marked functional shunt volume, leading to severe hypoxia. The classical ARDS models have their clear limitations with regard to their transferability to clinical practice; they are what they are: models. To the best of our knowledge, a model for mimicking low V/Q and functional shunt does not exist and seems difficult to develop (Matute-Bello et al., 2008). Some of the "classical" ARDS models require intravenous application of agents, e.g., oleic acid (Schuster, 1994), and in others, the lung injury is induced by intratracheal application of the toxic agent, e.g., of acid (Imai et al., 2005) or bleomycin (Moore and Hogaboam, 2008). Data about alteration of respiratory control in animal models of acute lung injury and ARDS are limited. In the bleomycin model, alteration of the respiratory drive is described, which is independent of the impairment of oxygen exchange in the lung tissue (Jacono et al., 2006; Hsieh et al., 2020; Litvin et al., 2020). Alteration of Ang II serum levels have yet not been analyzed in the bleomycin model but are confirmed, among others, in the acid-instillation model (Imai et al., 2005; Chen et al., 2013; Zou et al., 2014).

Mouse models for COVID-19 that allow the analysis of breathing regulation are more complicated to develop, not only because the animal experiments are hindered by the need of laboratories with high biosafety levels, but because the spike proteins of SARS-CoV and SARS-CoV2 have a much lower

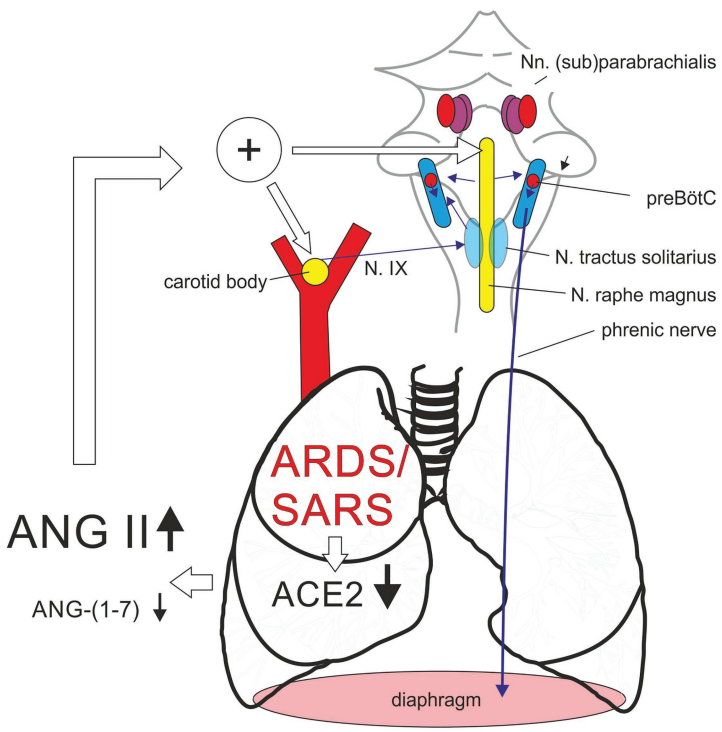


FIGURE 2 | Alterations of renin-angiotensin-system (RAS) in the patient with acute respiratory distress syndrome or coronavirus-induced severe respiratory syndrome (SARS) led to an increase of respiratory chemosensitivity by an Angiotensin II (Ang II) mediated shift of the activity of the chemosensitive cell population in the carotid body and the medullary raphe. ACE2, angiotensin-converting enzyme 2; N. IX, glossopharyngeal nerve; preBötC, pre-Bötzinger complex.

binding affinity to the murine ACE2 than to its human homolog (Lutz et al., 2020). However, transgenic mice have been developed that express the human ACE2 (McCray et al., 2007; Bao et al., 2020; Sun et al., 2020). To our knowledge, no experiments on chemosensitivity have been performed in later mouse models yet.

How to Test Change of Chemosensitivity in ARDS Models?

Based on this hypothesis, it will be necessary to determine how the shift of the RAS toward the ACE/Ang II/AT1R axis influences the target cell population of the chemosensitive reflex. Therefore, experiments in animal models of ARDS and SARS are necessary to establish the cellular basis of alteration of neuronal control of breathing. There is a wide range of experimental tools available that allow addressing chemosensitivity of the respiratory network at different levels. Experiments could be performed in acutely isolated brainstem slices, allowing measurement of the direct response of cells to alteration of CO_2 or O_2 (Gourine et al., 2010; Rajani et al., 2018).

Alteration of chemosensitivity in mice with ARDS can also be tested *in vivo* using whole body plethysmography, where alteration of tidal volume and respiratory rate can be analyzed in animals exposed to different levels of CO_2 or and/or O_2 (Bissonnette and Knopp, 2004; Hsieh et al., 2020). Moreover, the whole respiratory network can be analyzed in an arterially perfused preparation [the working heart brainstem preparation, WHBP (Paton, 1996; Dhingra et al., 2019)], which has the advantage that it allows testing for alterations of the chemosensitivity and respiratory drive that are independent from the injury of the lung since blood gas can be controlled *via* the perfusate.

Alternative Mechanisms of Modulation of Respiratory Drive in ARDS

Ang II might increase respiratory drive *via* activation of carotid body (CB) glomus cells (Allen, 1998; Schultz, 2011, chemosensitive neurons of the raphe Severson et al., 2003 #3721; Richerson, 2004 #4146; Bhandare et al., 2020 #304), and in the relay nucleus of the solitary tract (NTS; Paton and Kasparov, 1999 #14118). However, further experiential effort is necessary to identify ARDS-dependent changes in other areas of the respiratory network, whether RAS may be involved directly or indirectly. This includes retrotrapezoid body (RTN) and the parafacial respiratory group, the pontine parabrachial/Kölliker-Fuse complex (pB/KF) as well as the ventrolateral medulla with BötC, preBötC, and VRG (Li and Guyenet, 1995).

Apart from its action on neurons, Ang II might be involved in alterations of astrocytes-dependent modulation of the respiratory network. Indeed, in many regions of the brain, AT-receptors have been found to be expressed on astrocytes (Sumners et al., 1991; Tallant et al., 1991; Gebke et al., 1998). Moreover, sequencing data indicate MasR-expression in astrocytes at least in older animals (Clarke et al., 2018). Whether the O_2-sensitive astrocyte population in the medulla (Gourine and Funk, 2017; Rajani et al., 2018) or the population of CO_2-sensitive astrocytes in the retrotrapezoid nucleus [RTN; (Gourine et al., 2010)] also expresses AT1R, AT2R, or MasR remains to be investigated.

From the beginning of the 1970s, it has been postulated that lung fibrosis can change breathing by alteration of lung reflexes (Guz and Widdicombe, 1970; Mansoor et al., 1997; Schelegle, 2003). Recently, lung reflex receptors, e.g., J-reflex, head deflation reflex, and Hering-Breuer inflation reflex, were again suggested to contribute to ARDS- and SARS-induced modulation of ventilatory response in patients (de Vries et al., 2020).

Are There any Potential Secondary Effects of Elevated Angiotensin II?

Focus of the research should be extended beyond the direct effects of, e.g., Ang II on the target cells. Since Ang II is involved in the inflammatory response of the body, secondary neuroinflammatory effects that might modulate the neural control of breathing have to be considered as well (Pena-Ortega, 2019). Indeed, the elevated level of pro-inflammatory cytokines in critically ill COVID-19 patients sheds new light on this topic (Herold et al., 2020; Huang et al., 2020; Schett et al., 2020). Many of these mediators have also be found to be elevated in classical ARDS (Tzouvelekis et al., 2005), and their expression is often stimulated by Ang II (Han et al., 1999; Nakamura et al., 2002; Luther et al., 2006; Qi et al., 2011). For IL 6, IL-1β, and TNF-α, stimulatory effects in the carotid body have been demonstrated (Fan et al., 2009; Del Rio et al., 2012), and there is little doubt that these three cytokines can have potentially stimulating effects also on respiratory and chemosensitive neurons in the brainstem (Kawasaki et al., 2008; Pena-Ortega, 2019). Along with this, it has been recently shown that ARDS is associated with a specific modulation of the post-hypoxic frequency decline, a component of the respiratory chemoreflex (Hsieh et al., 2020). Further, it has been previously shown that carotid body chemosensitivity is upregulated even before the presence of severe lung injury pathology (Jacono et al., 2006). Similarly, 2nd-order NTS neurons have also been implicated in mediating a sensory-plasticity after lung injury (Getsy et al., 2019).

CONCLUSION

In summary, imbalance of the renin-angiotensin-system in ARDS and SARS is expected to have substantial impact on the neuronal control of breathing and the chemosensitive reflex of the human body. While our hypothesis awaits experimental confirmation, it might lead to new therapeutic concepts and treatment options for intensive care patients with acute lung injury.

AUTHOR CONTRIBUTIONS

SH and MQ conceptualization, writing – review and editing. SK and KM writing – review and editing. All authors contributed to the article and approved the submitted version.

FUNDING

SH received funding from VolkswagenStiftung and DFG.

REFERENCES

Adamzik, M., Frey, U., Sixt, S., Knemeyer, L., Beiderlinden, M., Peters, J., et al. (2007). ACE I/D but not AGT (−6)A/G polymorphism is a risk factor for mortality in ARDS. *Eur. Respir. J.* 29, 482–488. doi: 10.1183/09031936.00046106

Albashir, A. A. D. (2020). The potential impacts of obesity on COVID-19. *Clin. Med.* 20, e109–e113. doi: 10.7861/clinmed.2020-0239

Allen, A. M. (1998). Angiotensin AT1 receptor-mediated excitation of rat carotid body chemoreceptor afferent activity. *J. Physiol.* 510, 773–781.

Allen, A. M., Paxinos, G., Mckinley, M. J., Chai, S. Y., and Mendelsohn, F. A. (1991). Localization and characterization of angiotensin II receptor binding sites in the human basal ganglia, thalamus, midbrain pons, and cerebellum. *J. Comp. Neurol.* 312, 291–298.

Balibrea, J. L., and Arias-Diaz, J. (2003). Acute respiratory distress syndrome in the septic surgical patient. *World J. Surg.* 27, 1275–1284. doi: 10.1007/s00268-003-7258-3

Bao, L., Deng, W., Huang, B., Gao, H., Liu, J., Ren, L., et al. (2020). The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Nature* 583, 830–833. doi: 10.1038/s41586-020-2312-y

Bhandare, A., van de Wiel, J., Roberts, R., Braren, I., Huckstepp, R., and Dale, N. (2020). Analyzing the brainstem circuits for respiratory chemosensitivity in freely moving mice.

Bissonnette, J. M., and Knopp, S. J. (2004). Hypercapnic ventilatory response in mice lacking the 65 kDa isoform of glutamic acid decarboxylase (GAD65). *Respir. Res.* 5:3. doi: 10.1186/1465-9921-5-3

Cha, S. A., Park, B. M., and Kim, S. H. (2018). Angiotensin-(1-9) ameliorates pulmonary arterial hypertension via angiotensin type II receptor. *Korean J. Physiol. Pharmacol.* 22, 447–456. doi: 10.4196/kjpp.2018.22.4.447

Chandra, A., Chakraborty, U., Pal, J., and Karmakar, P. (2020). Silent hypoxia: a frequently overlooked clinical entity in patients with COVID-19. *BMJ Case Rep.* 13:e237207. doi: 10.1136/bcr-2020-237207

Chau, V. Q., Giustino, G., Mahmood, K., Oliveros, E., Neibart, E., Oloomi, M., et al. (2020). Cardiogenic shock and hyperinflammatory syndrome in young males with COVID-19. *Circ. Heart Fail.* 13:e007485. doi: 10.1161/CIRCHEARTFAILURE.120.007485

Chen, L. N., Yang, X. H., Nissen, D. H., Chen, Y. Y., Wang, L. J., Wang, J. H., et al. (2013). Dysregulated renin-angiotensin system contributes to acute lung injury caused by hind-limb ischemia-reperfusion in mice. *Shock* 40, 420–429. doi: 10.1097/SHK.0b013e3182a6953e

Clarke, L. E., Liddelow, S. A., Chakraborty, C., Munch, A. E., Heiman, M., and Barres, B. A. (2018). Normal aging induces A1-like astrocyte reactivity. *Proc. Natl. Acad. Sci. U. S. A.* 115, E1896–E1905. doi: 10.1073/pnas.1800165115

Couzin-Frankel, J. (2020). The mystery of the pandemic's 'happy hypoxia'. *Science* 368, 455–456. doi: 10.1126/science.368.6490.455

Crotti, S., Bottino, N., Ruggeri, G. M., Spinelli, E., Tubiolo, D., Lissoni, A., et al. (2017). Spontaneous breathing during extracorporeal membrane oxygenation in acute respiratory failure. *Anesthesiology* 126, 678–687. doi: 10.1097/ALN.0000000000001546

Cruces, P., Retamal, J., Hurtado, D. E., Erranz, B., Iturrieta, P., Gonzalez, C., et al. (2020). A physiological approach to understand the role of respiratory effort in the progression of lung injury in SARS-CoV-2 infection. *Crit. Care* 24:494. doi: 10.1186/s13054-020-03197-7

de Moura, M. M., Dos Santos, R. A., Campagnole-Santos, M. J., Todiras, M., Bader, M., Alenina, N., et al. (2010). Altered cardiovascular reflexes responses in conscious angiotensin-(1-7) receptor mas-knockout mice. *Peptides* 31, 1934–1939. doi: 10.1016/j.peptides.2010.06.030

de Vries, H., Endeman, H., van der Hoeven, J., and Heunks, L. (2020). Lung-protective mechanical ventilation in patients with COVID-19. *Neth. J. Crit. Care* 28, 120–124.

Del Rio, R., Moya, E. A., Parga, M. J., Madrid, C., and Iturriaga, R. (2012). Carotid body inflammation and cardiorespiratory alterations in intermittent hypoxia. *Eur. Respir. J.* 39, 1492–1500. doi: 10.1183/09031936.00141511

Dhingra, R. R., Furuya, W. I., Bautista, T. G., Dick, T. E., Galan, R. F., and Dutschmann, M. (2019). Increasing local excitability of brainstem respiratory nuclei reveals a distributed network underlying respiratory motor pattern formation. *Front. Physiol.* 10:887. doi: 10.3389/fphys.2019.00887

Dhont, S., Derom, E., Van Braeckel, E., Depuydt, P., and Lambrecht, B. N. (2020). The pathophysiology of 'happy' hypoxemia in COVID-19. *Respir. Res.* 21:198. doi: 10.1186/s12931-020-01462-5

Diz, D. I., Jessup, J. A., Westwood, B. M., Bosch, S. M., Vinsant, S., Gallagher, P. E., et al. (2002). Angiotensin peptides as neurotransmitters/neuromodulators in the dorsomedial medulla. *Clin. Exp. Pharmacol. Physiol.* 29, 473–482. doi: 10.1046/j.1440-1681.2002.03659.x

Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., et al. (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ. Res.* 87, E1–E9. doi: 10.1161/01.res.87.5.e1

Doobay, M. F., Talman, L. S., Obr, T. D., Tian, X., Davisson, R. L., and Lazartigues, E. (2007). Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin-angiotensin system. *Am. J. Phys. Regul. Integr. Comp. Phys.* 292, R373–R381. doi: 10.1152/ajpregu.00292.2006

Fan, J., Zhang, B., Shu, H. F., Zhang, X. Y., Wang, X., Kuang, F., et al. (2009). Interleukin-6 increases intracellular Ca^{2+} concentration and induces catecholamine secretion in rat carotid body glomus cells. *J. Neurosci. Res.* 87, 2757–2762. doi: 10.1002/jnr.22107

Fanelli, V., and Ranieri, V. M. (2015). Mechanisms and clinical consequences of acute lung injury. *Ann. Am. Thorac. Soc.* 12(Suppl. 1), S3–S8. doi: 10.1513/AnnalsATS.201407-340MG

Fletcher, A. N., Molteni, A., Ponnapureddy, R., Patel, C., Pluym, M., and Poisner, A. M. (2017). The renin inhibitor aliskiren protects rat lungs from the histopathological effects of fat embolism. *J. Trauma Acute Care Surg.* 82, 338–344. doi: 10.1097/TA.0000000000001278

Fung, M. L. (2014). The role of local renin-angiotensin system in arterial chemoreceptors in sleep-breathing disorders. *Front. Physiol.* 5:336. doi: 10.3389/fphys.2014.00336

Gattinoni, L., Chiumello, D., Caironi, P., Busana, M., Romitti, F., Brazzi, L., et al. (2020a). COVID-19 pneumonia: different respiratory treatments for different phenotypes? *Intensive Care Med.* 46, 1099–1102. doi: 10.1007/s00134-020-06033-2

Gattinoni, L., Coppola, S., Cressoni, M., Busana, M., Rossi, S., and Chiumello, D. (2020b). COVID-19 does not Lead to a "typical" acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 201, 1299–1300. doi: 10.1164/rccm.202003-0817LE

Gattinoni, L., Meissner, K., and Marini, J. J. (2020c). The baby lung and the COVID-19 era. *Intensive Care Med.* 46, 1438–1440. doi: 10.1007/s00134-020-06103-5

Gebke, E., Müller, A. R., Jurzak, M., and Gerstberger, R. (1998). Angiotensin II-induced calcium signalling in neurons and astrocytes of rat circumventricular organs. *Neuroscience* 85, 509–520.

Getsy, P. M., Mayer, C. A., Macfarlane, P. M., Jacono, F. J., and Wilson, C. G. (2019). Acute lung injury in neonatal rats causes postsynaptic depression in nucleus tractus solitarii second-order neurons. *Respir. Physiol. Neurobiol.* 269:103250. doi: 10.1016/j.resp.2019.103250

Gironacci, M. M., Valera, M. S., Yujnovsky, I., and Pena, C. (2004). Angiotensin-(1-7) inhibitory mechanism of norepinephrine release in hypertensive rats. *Hypertension* 44, 783–787. doi: 10.1161/01.HYP.0000143850.73831.9d

Gourine, A. V., and Funk, G. D. (2017). On the existence of a central respiratory oxygen sensor. *J. Appl. Physiol.* 123, 1344–1349. doi: 10.1152/japplphysiol.00194.2017

Gourine, A. V., Kasymov, V., Marina, N., Tang, F., Figueiredo, M. F., Lane, S., et al. (2010). Astrocytes control breathing through pH-dependent release of ATP. *Science* 329, 571–575. doi: 10.1126/science.1190721

Guz, A., and Widdicombe, J. (1970). "Pattern of breathing during hypercapnia before and after vagal blockade in man" in *Ciba foundation symposium-breathing: Hering-Breuer centenary symposium*. ed. R. Porter (Wiley Online Library), 41–52.

Hagiwara, S., Iwasaka, H., Matumoto, S., Hidaka, S., and Noguchi, T. (2009). Effects of an angiotensin-converting enzyme inhibitor on the inflammatory response in in vivo and in vitro models. *Crit. Care Med.* 37, 626–633. doi: 10.1097/CCM.0b013e3181958d91

Hamming, I., Timens, W., Bulthuis, M. L., Lely, A. T., Navis, G., and van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203, 631–637. doi: 10.1002/path.1570

Han, Y., Runge, M. S., and Brasier, A. R. (1999). Angiotensin II induces interleukin-6 transcription in vascular smooth muscle cells through pleiotropic activation of nuclear factor-kappa B transcription factors. *Circ. Res.* 84, 695–703. doi: 10.1161/01.res.84.6.695

He, X., Han, B., Mura, M., Xia, S., Wang, S., Ma, T., et al. (2007). Angiotensin-converting enzyme inhibitor captopril prevents oleic acid-induced severe acute lung injury in rats. *Shock* 28, 106–111. doi: 10.1097/SHK.0b013e3180310f3a

Herold, T., Jurinovic, V., Arnreich, C., Lipworth, B. J., Hellmuth, J. C., Bergwelt-Baildon, M. V., et al. (2020). Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *J. Allergy Clin. Immunol.* 46, 128.e4–136.e4. doi: 10.1016/j.jaci.2020.05.008

Hippisley-Cox, J., Young, D., Coupland, C., Channon, K. M., Tan, P. S., Harrison, D. A., et al. (2020). Risk of severe COVID-19 disease with ACE inhibitors and angiotensin receptor blockers: cohort study including 8.3 million people. *Heart* 106, 1503–1511. doi: 10.1136/heartjnl-2020-317393

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271.e278–280.e278. doi: 10.1016/j.cell.2020.02.052

Hsieh, Y. H., Litvin, D. G., Zaylor, A. R., Nethery, D. E., Dick, T. E., and Jacono, F. J. (2020). Brainstem inflammation modulates the ventilatory pattern and its variability after acute lung injury in rodents. *J. Physiol.* 598, 2791–2811. doi: 10.1113/JP279177

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Imai, Y., Kuba, K., and Penninger, J. M. (2008). The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. *Exp. Physiol.* 93, 543–548. doi: 10.1113/expphysiol.2007.040048

Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., et al. (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 436, 112–116. doi: 10.1038/nature03712

Jacono, F. J., Peng, Y. J., Nethery, D., Faress, J. A., Lee, Z., Kern, J. A., et al. (2006). Acute lung injury augments hypoxic ventilatory response in the absence of systemic hypoxemia. *J. Appl. Physiol.* 101, 1795–1802. doi: 10.1152/japplphysiol.00100.2006

Jerng, J. S., Yu, C. J., Wang, H. C., Chen, K. Y., Cheng, S. L., and Yang, P. C. (2006). Polymorphism of the angiotensin-converting enzyme gene affects the outcome of acute respiratory distress syndrome. *Crit. Care Med.* 34, 1001–1006. doi: 10.1097/01.CCM.0000206107.92476.39

Jia, H. (2016). Pulmonary angiotensin-converting enzyme 2 (ACE2) and inflammatory lung disease. *Shock* 46, 239–248. doi: 10.1097/SHK.0000000000000633

Kawasaki, Y., Zhang, L., Cheng, J. K., and Ji, R. R. (2008). Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. *J. Neurosci.* 28, 5189–5194. doi: 10.1523/JNEUROSCI.3338-07.2008

Khan, A., Benthin, C., Zeno, B., Albertson, T. E., Boyd, J., Christie, J. D., et al. (2017). A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit. Care* 21:234. doi: 10.1186/s13054-017-1823-x

Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., et al. (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.* 11, 875–879. doi: 10.1038/nm1267

Kuster, G. M., Pfister, O., Burkard, T., Zhou, Q., Twerenbold, R., Haaf, P., et al. (2020). SARS-CoV2: should inhibitors of the renin-angiotensin system be withdrawn in patients with COVID-19? *Eur. Heart J.* 41, 1801–1803. doi: 10.1093/eurheartj/ehaa235

Leung, P. S., Lam, S. Y., and Fung, M. L. (2000). Chronic hypoxia upregulates the expression and function of AT(1) receptor in rat carotid body. *J. Endocrinol.* 167, 517–524. doi: 10.1677/joe.0.1670517

Li, Y. W., and Guyenet, P. G. (1995). Neuronal excitation by angiotensin II in the rostral ventrolateral medulla of the rat in vitro. *Am. J. Phys.* 268, R272–R277. doi: 10.1152/ajpregu.1995.268.1.R272

Li, Y. W., and Guyenet, P. G. (1996). Angiotensin II decreases a resting K^+ conductance in rat bulbospinal neurons of the C1 area. *Circ. Res.* 78, 274–282. doi: 10.1161/01.res.78.2.274

Li, X., Molina-Molina, M., Abdul-Hafez, A., Uhal, V., Xaubet, A., and Uhal, B. D. (2008). Angiotensin converting enzyme-2 is protective but downregulated in human and experimental lung fibrosis. *Am. J. Phys. Lung Cell. Mol. Phys.* 295, L178–L185. doi: 10.1152/ajplung.00009.2008

Li, W. H., Moore, M. J., Vasilieva, N., Sui, J. H., Wong, S. K., Berne, M. A., et al. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 426, 450–454. doi: 10.1038/nature02145

Li, T., Yin, P. -F., Li, A., Shen, M. R., and Yao, Y. -X. (2020). Acute respiratory distress syndrome treated with awake extracorporeal membrane oxygenation in a patient with COVID-19 pneumonia. *J. Cardiothorac. Vasc. Anesth.* doi: 10.1053/j.jvca.2020.11.017 [Epub ahead of print]

Lin, Z., Chen, Y., Zhang, W., Chen, A. F., Lin, S., and Morris, M. (2008). RNA interference shows interactions between mouse brainstem angiotensin AT1 receptors and angiotensin-converting enzyme 2. *Exp. Physiol.* 93, 676–684. doi: 10.1113/expphysiol.2007.041657

Litvin, D. G., Denstaedt, S. J., Borkowski, L. F., Nichols, N. L., Dick, T. E., Smith, C. B., et al. (2020). Peripheral-to-central immune communication at the area postrema glial-barrier following bleomycin-induced sterile lung injury in adult rats. *Brain Behav. Immun.* 87, 610–633. doi: 10.1016/j.bbi.2020.02.006

Liu, Y., Yang, Y., Zhang, C., Huang, F., Wang, F., Yuan, J., et al. (2020). Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci. China Life Sci.* 63, 364–374. doi: 10.1007/s11427-020-1643-8

Luther, J. M., Gainer, J. V., Murphey, L. J., Yu, C., Vaughan, D. E., Morrow, J. D., et al. (2006). Angiotensin II induces interleukin-6 in humans through a mineralocorticoid receptor–dependent mechanism. *Hypertension* 48, 1050–1057. doi: 10.1161/01.HYP.0000248135.97380.76

Lutz, C., Maher, L., Lee, C., and Kang, W. (2020). COVID-19 preclinical models: human angiotensin-converting enzyme 2 transgenic mice. *Hum. Genomics* 14:20. doi: 10.1186/s40246-020-00272-6

Magalhães, G. S., Campagnole-Santos, M. J., and da Glória Rodrigues-Machado, M. (2019). "Lung" in *Angiotensin-(1-7)*. ed. R. Santos (Cham: Springer), 131–152.

Mansoor, J. K., Hyde, D. M., and Schelegle, E. S. (1997). Contribution of vagal afferents to breathing pattern in rats with lung fibrosis. *Respir. Physiol.* 108, 45–61. doi: 10.1016/s0034-5687(96)02505-4

Marini, J. J., and Gattinoni, L. (2020). Management of COVID-19 respiratory distress. *JAMA* 323, 2329–2330. doi: 10.1001/jama.2020.6825

Marshall, R. P., Gohlke, P., Chambers, R. C., Howell, D. C., Bottoms, S. E., Unger, T., et al. (2004). Angiotensin II and the fibroproliferative response to acute lung injury. *Am. J. Phys. Lung Cell. Mol. Phys.* 286, L156–L164. doi: 10.1152/ajplung.00313.2002

Marshall, R. P., Webb, S., Bellingan, G. J., Montgomery, H. E., Chaudhari, B., Mcanulty, R. J., et al. (2002). Angiotensin converting enzyme insertion / deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 166, 646–650. doi: 10.1164/rccm.2108086

Matute-Bello, G., Frevert, C. W., and Martin, T. R. (2008). Animal models of acute lung injury. *Am. J. Phys. Lung Cell. Mol. Phys.* 295, L379–L399. doi: 10.1152/ajplung.00010.2008

McCray, P. B. Jr., Pewe, L., Wohlford-Lenane, C., Hickey, M., Manzel, L., Shi, L., et al. (2007). Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. *J. Virol.* 81, 813–821. doi: 10.1128/JVI.02012-06

McFadyen, J. D., Stevens, H., and Peter, K. (2020). The emerging threat of (micro)thrombosis in COVID-19 and its therapeutic implications. *Circ. Res.* 127, 571–587. doi: 10.1161/CIRCRESAHA.120.317447

Merrill, J. T., Erkan, D., Winakur, J., and James, J. A. (2020). Emerging evidence of a COVID-19 thrombotic syndrome has treatment implications. *Nat. Rev. Rheumatol.* 16, 581–589. doi: 10.1038/s41584-020-0474-5

Moore, B. B., and Hogaboam, C. M. (2008). Murine models of pulmonary fibrosis. *Am. J. Phys. Lung Cell. Mol. Phys.* 294, L152–L160. doi: 10.1152/ajplung.00313.2007

Nahmod, V. E., Finkielman, S., Benarroch, E. E., and Pirola, C. J. (1978). Angiotensin regulates release and synthesis of serotonin in brain. *Science* 202, 1091–1093. doi: 10.1126/science.152460

Nakamura, A., Johns, E. J., Imaizumi, A., Niimi, R., Yanagawa, Y., and Kohsaka, T. (2002). Role of angiotensin II-induced cAMP in mesangial TNF-alpha production. *Cytokine* 19, 47–51. doi: 10.1006/cyto.2002.1040

Ottestad, W., Seim, M., and Maehlen, J. O. (2020). COVID-19 with silent hypoxemia. *Tidsskr. Nor. Laegeforen.* 140. doi: 10.4045/tidsskr.20.0299

Paton, J. F. R. (1996). A working heart-brainstem preparation of the mouse. *J. Neurosci. Methods* 65, 63–68. doi: 10.1016/0165-0270(95)00147-6

Paton, J. F. R., and Kasparov, S. (1999). Differential effects of angiotensin II on cardiorespiratory reflexes mediated by nucleus tractus solitarii a microinjection study in the rat. *J. Physiol.* 521, 213–225. doi: 10.1111/j.1469-7793.1999.00213.x

Pena-Ortega, F. (2019). Clinical and experimental aspects of breathing modulation by inflammation. *Auton. Neurosci.* 216, 72–86. doi: 10.1016/j.autneu.2018.11.002

Potter, E. K., and Mccloskey, D. I. (1979). Respiratory stimulatiion by angiotensin II. *Respir. Physiol.* 36, 367–373. doi: 10.1016/0034-5687(79)90048-3

Qi, G., Jia, L., Li, Y., Bian, Y., Cheng, J., Li, H., et al. (2011). Angiotensin II infusion-induced inflammation, monocytic fibroblast precursor infiltration, and cardiac fibrosis are pressure dependent. *Cardiovasc. Toxicol.* 11, 157–167. doi: 10.1007/s12012-011-9109-z

Raiden, S., Nahmod, K., Nahmod, V., Semeniuk, G., Pereira, Y., Alvarez, C., et al. (2002). Nonpeptide antagonists of AT1 receptor for angiotensin II delay the onset of acute respiratory distress syndrome. *J. Pharmacol. Exp. Ther.* 303, 45–51. doi: 10.1124/jpet.102.037382

Rajani, V., Zhang, Y., Jalubula, V., Rancic, V., Sheikhbahaei, S., Zwicker, J. D., et al. (2018). Release of ATP by pre-Botzinger complex astrocytes contributes to the hypoxic ventilatory response via a Ca^{2+}-dependent P2Y1 receptor mechanism. *J. Physiol.* 596, 3245–3269. doi: 10.1113/JP274727

Ranieri, V. M., Rubenfeld, G. D., Thompson, B. T., Ferguson, N. D., Caldwell, E., Fan, E., et al. (2012). ARDS definition task force. Acute respiratory distress syndrome: the Berlin definition. *JAMA* 307, 2526–2533. doi: 10.1001/jama.2012.5669

Reddy, R., Asante, I., Liu, S., Parikh, P., Liebler, J., Borok, Z., et al. (2019). Circulating angiotensin peptides levels in acute respiratory distress syndrome correlate with clinical outcomes: a pilot study. *PLoS One* 14:e0213096. doi: 10.1371/journal.pone.0213096

Reynolds, H. R., Adhikari, S., Pulgarin, C., Troxel, A. B., Iturrate, E., Johnson, S. B., et al. (2020). Renin-angiotensin-aldosterone system inhibitors and risk of Covid-19. *N. Engl. J. Med.* 382, 2441–2448. doi: 10.1056/NEJMoa2008975

Richerson, G. B. (2004). Serotonergic neurons as carbon dioxide sensors that maintain pH homeostasis. *Nat. Rev. Neurosci.* 5, 449–461. doi: 10.1038/nrn1409

Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P., and Soubrier, F. (1990). An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J. Clin. Invest.* 86, 1343–1346. doi: 10.1172/JCI114844

Schelegle, E. S. (2003). Functional morphology and physiology of slowly adapting pulmonary stretch receptors. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* 270, 11–16. doi: 10.1002/ar.a.10004

Schett, G., Sticherling, M., and Neurath, M. F. (2020). COVID-19: risk for cytokine targeting in chronic inflammatory diseases? *Nat. Rev. Immunol.* 20, 271–272. doi: 10.1038/s41577-020-0312-7

Schultz, H. D. (2011). Angiotensin and carotid body chemoreception in heart failure. *Curr. Opin. Pharmacol.* 11, 144–149. doi: 10.1016/j.coph.2010.12.004

Schuster, D. P. (1994). ARDS: clinical lessons from the oleic acid model of acute lung injury. *Am. J. Respir. Crit. Care Med.* 149, 245–260. doi: 10.1164/ajrccm.149.1.8111590

Severson, C. A., Wang, W., Pieribone, V. A., Dohle, C. I., and Richerson, G. B. (2003). Midbrain serotonergic neurons are central pH chemoreceptors. *Nat. Neurosci.* 6, 1139–1140. doi: 10.1038/nn1130

Shen, L., Mo, H., Cai, L., Kong, T., Zheng, W., Ye, J., et al. (2009). Losartan prevents sepsis-induced acute lung injury and decreases activation of nuclear factor kappaB and mitogen-activated protein kinases. *Shock* 31, 500–506. doi: 10.1097/SHK.0b013e318189017a

Silhol, F., Sarlon, G., Deharo, J. C., and Vaisse, B. (2020). Downregulation of ACE2 induces overstimulation of the renin-angiotensin system in COVID-19: should we block the renin-angiotensin system? *Hypertens. Res.* 43, 854–856. doi: 10.1038/s41440-020-0476-3

Smit, F., Oelofse, A., Linegar, A., Botes, L., and Turton, E. (2020). Supplemental oxygen therapy in COVID-19. *SA Heart J.* 17, 324–328.

Soliz, J., Schneider-Gasser, E. M., Arias-Reyes, C., Aliaga-Raduan, F., Poma-Machicao, L., Zubieta-Calleja, G., et al. (2020). Coping with hypoxemia: could erythropoietin (EPO) be an adjuvant treatment of COVID-19? *Respir. Physiol. Neurobiol.* 279:103476. doi: 10.1016/j.resp.2020.103476

Spinelli, E., Mauri, T., Beitler, J. R., Pesenti, A., and Brodie, D. (2020). Respiratory drive in the acute respiratory distress syndrome: pathophysiology, monitoring, and therapeutic interventions. *Intensive Care Med.* 46, 606–618. doi: 10.1007/s00134-020-05942-6

Stragier, B., Hristova, I., Sarre, S., Ebinger, G., and Michotte, Y. (2005). In vivo characterization of the angiotensin-(1-7)-induced dopamine and gamma-aminobutyric acid release in the striatum of the rat. *Eur. J. Neurosci.* 22, 658–664. doi: 10.1111/j.1460-9568.2005.04188.x

Sumners, C., Tang, W., Zelezna, B., and Raizada, M. K. (1991). Angiotensin II receptor subtypes are coupled with distinct signal-transduction mechanisms in neurons and astrocytes from rat brain. *Proc. Natl. Acad. Sci. U. S. A.* 88, 7567–7571. doi: 10.1073/pnas.88.17.7567

Sun, S. -H., Chen, Q., Gu, H. -J., Yang, G., Wang, Y. -X., Huang, X. -Y., et al. (2020). A mouse model of SARS-CoV-2 infection and pathogenesis. *Cell Host Microbe* 28, 124–133.e4. doi: 10.1016/j.chom.2020.05.020

Tallant, E. A., Jaiswal, N., Diz, D. I., and Ferrario, C. M. (1991). Human astrocytes contain two distinct angiotensin receptor subtypes. *Hypertension* 18, 32–39. doi: 10.1161/01.hyp.18.1.32

Teuwen, L. A., Geldhof, V., Pasut, A., and Carmeliet, P. (2020). COVID-19: the vasculature unleashed. *Nat. Rev. Immunol.* 20, 389–391. doi: 10.1038/s41577-020-0343-0

Tipnis, S. R., Hooper, N. M., Hyde, R., Karran, E., Christie, G., and Turner, A. J. (2000). A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* 275, 33238–33243. doi: 10.1074/jbc.M002615200

Tsantes, A. E., Kopterides, P., Bonovas, S., Bagos, P., Antonakos, G., Nikolopoulos, G. K., et al. (2013). Effect of angiotensin converting enzyme gene I/D polymorphism and its expression on clinical outcome in acute respiratory distress syndrome. *Minerva Anestesiol.* 79, 861–870.

Tzouvelekis, A., Pneumatikos, I., and Bouros, D. (2005). Serum biomarkers in acute respiratory distress syndrome an ailing prognosticator. *Respir. Res.* 6:62. doi: 10.1186/1465-9921-6-62

Wang, D., Chai, X. Q., Magnussen, C. G., Zosky, G. R., Shu, S. H., Wei, X., et al. (2019). Renin-angiotensin-system, a potential pharmacological candidate, in acute respiratory distress syndrome during mechanical ventilation. *Pulm. Pharmacol. Ther.* 58:101833. doi: 10.1016/j.pupt.2019.101833

Wang, L., De Kloet, A. D., Pati, D., Hiller, H., Smith, J. A., Pioquinto, D. J., et al. (2016). Increasing brain angiotensin converting enzyme 2 activity decreases anxiety-like behavior in male mice by activating central mas receptors. *Neuropharmacology* 105, 114–123. doi: 10.1016/j.neuropharm.2015.12.026

Wiberg-Jorgensen, F., Klausen, N. O., Hald, A., Qvist, J., Giese, J., and Damkjaer Nielsen, M. (1983). Pulmonary angiotensin II production in respiratory failure. *Clin. Physiol.* 3, 59–67. doi: 10.1111/j.1475-097x.1983.tb00699.x

Wosten-van Asperen, R. M., Lutter, R., Specht, P. A., Moll, G. N., van Woensel, J. B., van der Loos, C. M., et al. (2011). Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1-7) or an angiotensin II receptor antagonist. *J. Pathol.* 225, 618–627. doi: 10.1002/path.2987

Wu, Z., Hu, R., Zhang, C., Ren, W., Yu, A., and Zhou, X. (2020). Elevation of plasma angiotensin II level is a potential pathogenesis for the critically ill COVID-19 patients. *Crit. Care* 24:290. doi: 10.1186/s13054-020-03015-0

Zambelli, V., Grassi, A., and Bellani, G. (2012). "Role of the renin-angiotensin system in ARDS" in *Annual update in intensive care and emergency medicine 2012*. ed. J.-L. Vincent (Berlin, Heidelberg: Springer)

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., et al. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.* 382, 727–733. doi: 10.1056/NEJMoa2001017

Zou, Z., Yan, Y., Shu, Y., Gao, R., Sun, Y., Li, X., et al. (2014). Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. *Nat. Commun.* 5:3594. doi: 10.1038/ncomms4594

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 26 January 2021
doi: 10.3389/fphys.2020.625110

COVID 19-Induced Smell and Taste Impairments: Putative Impact on Physiology

*Nicolas Meunier[1], Loïc Briand[2], Agnès Jacquin-Piques[2,3], Laurent Brondel[2] and Luc Pénicaud[4]**

[1] Université Paris-Saclay, INRAE, UVSQ, VIM, Jouy-en-Josas, France, [2] Centre des Sciences du Goût et de l'Alimentation, AgroSup Dijon, CNRS UMR6265, INRAE UMR 1324, Université de Bourgogne Franche Comté, Dijon, France, [3] Department of Clinical Neurophysiology, University Hospital, Dijon, France, [4] STROMALab, Université de Toulouse, CNRS ERL 5311, Inserm U1031, Université Paul Sabatier (UPS), Toulouse, France

Smell and taste impairments are recognized as common symptoms in COVID 19 patients even in an asymptomatic phase. Indeed, depending on the country, in up to 85–90% of cases anosmia and dysgeusia are reported. We will review briefly the main mechanisms involved in the physiology of olfaction and taste focusing on receptors and transduction as well as the main neuroanatomical pathways. Then we will examine the current evidences, even if still fragmented and unsystematic, explaining the disturbances and mode of action of the virus at the level of the nasal and oral cavities. We will focus on its impact on the peripheral and central nervous system. Finally, considering the role of smell and taste in numerous physiological functions, especially in ingestive behavior, we will discuss the consequences on the physiology of the patients as well as management regarding food intake.

Keywords: COVID 19, taste, smell, feeding behavior, physiopathology

OPEN ACCESS

Edited by:
Georges Leftheriotis, Université Côte d'Azur, France

Reviewed by:
Christopher von Bartheld, University of Nevada, Reno, United States
Kathleen S. Curtis, Oklahoma State University Center for Health Sciences, United States

****Correspondence:***
Luc Pénicaud
luc.penicaud@inserm.fr

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *02 November 2020*
Accepted: *31 December 2020*
Published: *26 January 2021*

Citation:
Meunier N, Briand L, Jacquin-Piques A, Brondel L and Pénicaud L (2021) COVID 19-Induced Smell and Taste Impairments: Putative Impact on Physiology. Front. Physiol. 11:625110. doi: 10.3389/fphys.2020.625110

INTRODUCTION

In the list of clinical symptoms of COVID-19, a sudden loss of sense of smell and taste has been identified (Mehraeen et al., 2020). This is now recognized as a "significant symptom" that can be found even in the absence of the "usual symptoms" such as fever, cough, respiratory failure. While reports were at first anecdotal and generally without quantitative measurements, a recent study on around 4,000 participants from more than 40 countries confirms that COVID-19 broadly impacts chemosensory function across multiple sensory modalities (Parma et al., 2020). A major reduction in smell, independently of nasal obstruction, and in taste was reported without significant differences between participants tested in laboratory or by clinical assessment via a multi-lingual questionnaire (von Bartheld et al., 2020).

In this review we will describe (i) the main mechanism and neurological pathways underlying olfaction and taste, (ii) the current hypothesis to explain the pathophysiology of anosmia and ageusia, and (iii) the physiological consequences these defects can have, with a focus on feeding behavior.

PHYSIOLOGY OF OLFACTION AND TASTE

Olfaction, taste and chemesthesis are the three separate modalities involved in food flavor perception. Olfaction is involved in the detection of volatile chemical compounds present in the

environment or in the oral cavity (by retronasal olfaction), whereas taste (gustation) is involved in the chemical detection of soluble compounds by taste detectors present in taste buds. Chemesthesis, also referred as trigeminal chemosensation, is the chemical sense allowing the detection of another class of taste-related compounds, producing sensations of irritation pungency, burning, tingling or coolness, which can be part of flavor perception (Roper, 2014).

Odorant molecules are detected by a complex self-regenerating olfactory epithelium (OE) located in the superior parts of the nasal cavity below the cribriform plate (**Figure 1**). The OE is composed of several cell types including millions of olfactory sensory neurons (OSNs), in addition to microvillar, sustentacular cells, and basal cells, which are multipotent stem cells (Mombaerts, 2004). OSNs are bipolar neurons extending dendrites over the mucosa surface with axons passing through the cribriform plate to form synapses within glomeruli in the olfactory bulbs. Importantly, the OE is rich in basal stem cells, allowing OSNs to undergo continuous turnover during the life (Kondo et al., 2010). Odorant detection is mediated by a large multigene family that codes for olfactory receptors (ORs). ORs are G protein-coupled receptors (GPCRs) expressed within the membrane of OSN dendrites (Malnic et al., 2004). Myriads of chemically diverse odorants are discriminated in a combinatorial manner in which, one odorant activates a combination of ORs and one OR recognizes multiple odorants (Duchamp-Viret et al., 1999; Malnic et al., 1999). The main components of the canonical signal transduction pathway have been identified. The odorant-bound OR activates the olfactory specific G-protein α subunit, $G\alpha_{olf}$, which in turn dissociates from Gβγ dimer and activates type III adenylyl cyclase (ACIII). ACIII activation leads to an increased production of cAMP causing the opening of a cyclic nucleotide-gated ion channel (CNG) resulting in neuron depolarization. OSNs project axons to the olfactory bulb located in the brain, where the axons synapse with bulb neurons (mitral and tufted cells). The olfactory information is then transmitted toward a great number of higher brain regions including at first piriform cortex, amygdala, olfactory tubercle, and entorhinal cortex; then to other regions such as orbitofrontal cortex, hypothalamus, thalamus, and hippocampus (Simon et al., 2006; Diodato et al., 2016).

OE is covered by a thin layer of mucus secreted in the olfactory mucosa by the Bowman's glands (Getchell et al., 1984). The mucus contains large concentrations of odorant-binding proteins (OBPs). OBPs are small soluble proteins secreted in the nasal mucus that reversibly bind odorant molecules (Briand et al., 2002). While their physiological function is not fully understood, they are good candidates for carrying odorants, through the nasal mucus toward the olfactory receptors. The OE contains also many xenobiotic metabolizing enzymes (XMEs). XMEs constitute a large family of enzymes [including Glutathione-S-transferases (GSTs), UDP glucuronosyltransferase (UGT) and cytochrome P_{450} (CYP450)] that are highly expressed in the olfactory epithelium (Heydel et al., 2013). Although their functions in olfaction are still poorly understood, these enzymes are supposed to be involved in odorant transformation, degradation and/or olfactory signal termination (Schwartz et al., 2020).

The sense of taste is essential for the evaluation of the food quality in the oral cavity. It detects nutritive molecules such as carbohydrates or amino acids, electrolytes such as sodium or protons and potentially toxic molecules, which should be avoided (Briand and Salles, 2016). The gustatory system allows perceiving five basic taste qualities, sweet, salty, sour, bitter, and umami (the taste of some amino acids such as L-glutamate and 5'-ribonucleotides). In addition to these five fundamental taste qualities, a number of other taste sensations including fat taste (Laugerette et al., 2005; Mouillot et al., 2019), kokumi (mouthfulness in Japanese) taste (Maruyama et al., 2012) and calcium taste (Behrens et al., 2011; Tordoff et al., 2012) are still a matter of debate. Tasting substances are detected by 2,000–5,000 taste buds, which are located primarily on the tongue, soft palate, and epiglottis in mammals (Briand and Salles, 2016). Taste buds contain specialized taste receptor-cells (TRCs) expressing specific taste receptors, which are stimulated by sapid molecules dissolved in saliva (Behrens et al., 2011). Like OSNs, TRCs are able to undergo continuous renewal throughout the life course (Barlow and Klein, 2015).

The detection of the sweet, bitter, and umami molecules is mediated by G-protein coupled receptors (GPCRs). The sweet taste receptor is composed of two subunits, TAS1R2 (taste receptor type 1, member 2) and TAS1R3 (taste receptor type 1, member 3). These subunits assemble to form a single sweet taste receptor (Nelson et al., 2001) able to detect all the chemically diverse sweet-taste-eliciting chemicals. The bitter tasting compounds are detected in humans by a set of 25 different taste receptors (TAS2Rs) (Meyerhof et al., 2010). Whereas, some bitter receptors respond to only a few bitter compounds, other TAS2Rs are broadly tuned bitter receptors. The umami receptor is a heterodimer composed of TAS1R1 (taste receptor type 1, member 1) and TAS1R3, that assemble to detect the umami tastants (Nelson et al., 2002). The detection of sweet, umami and bitter molecules involves a common transduction mechanism. The main components of this signal cascade have been identified (Iwata et al., 2014). The binding of the tasting compounds to the receptors results in the dissociation of the heterotrimeric G protein (α-gustducin, Gβ3, and Gγ13). The release of the Gβγ protein induces an increase in phospholipase C-β2 (PLC-β2) activity. Activation of PLC-β2 results in the inositol 1,4,5-triphosphate (IP_3) receptor, type 3-mediated release of calcium from intracellular stores and the gating of a transient receptor potential ion channel, TRPMP5 (Behrens et al., 2018). The epithelial Na^+ channels (ENaCs) have been proposed to be the sodium receptor (Chandrashekar et al., 2010), whereas, the proton channel Otopetrin-1 has been recently demonstrated to be the sour taste sensor (Tu et al., 2018).

Taste buds are innervated by three nerves, chorda tympani nerve (a branch of the facial nerve CN-VII), the glossopharyngeal (CN-IX) and vagus nerve (CN-X), conveying taste information to the nucleus tractus solitarius (NTS) within the central nervous system. From the NTS, the gustatory information is transmitted to numerous regions including the thalamus, for relay to the

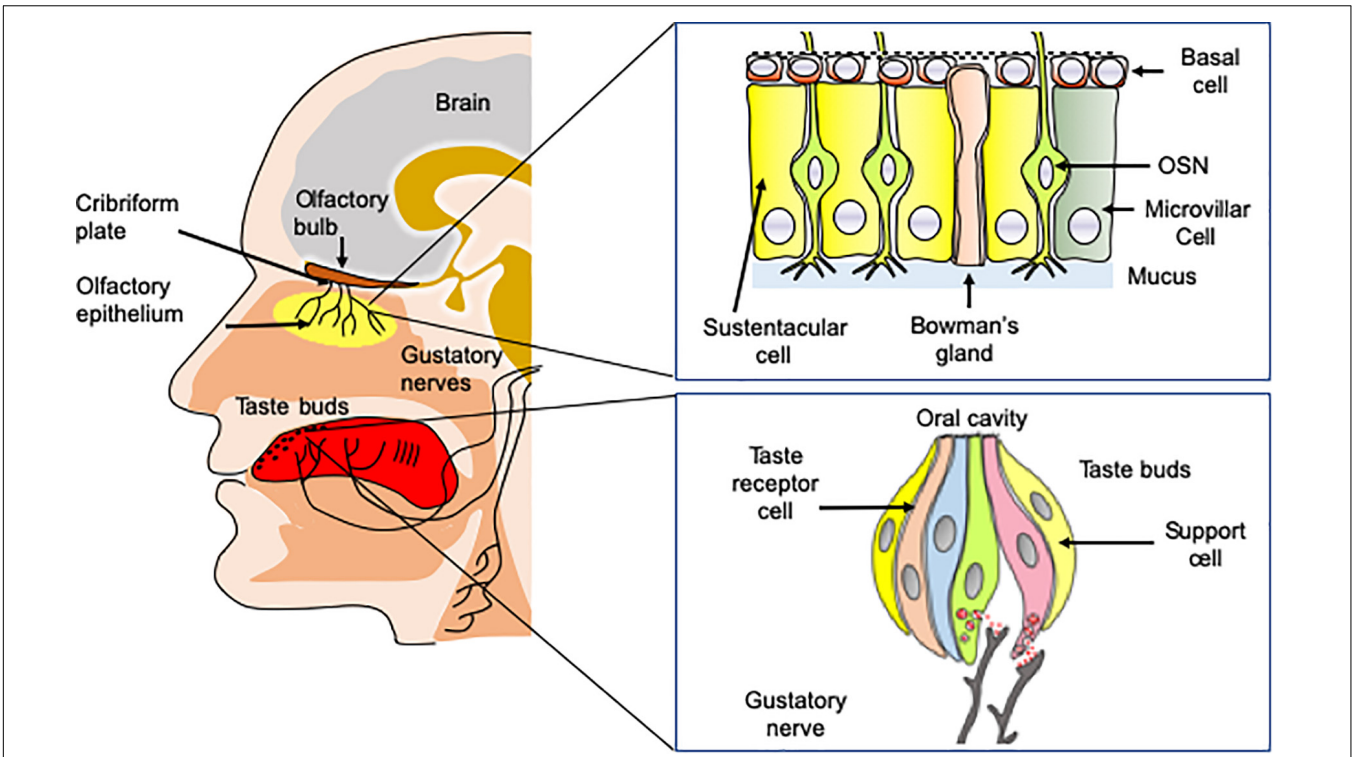


FIGURE 1 | Anatomy of taste and olfaction. Volatile odorant molecules are detected by olfactory sensory neurons (OSNs) located in the olfactory epithelium. OSNs are neurons extending ciliated dendrites in the periphery and with axons forming synapses with glomeruli in the olfactory bulbs situated in the brain. Taste buds located on the tongue are involved in the detection of soluble tasting compounds. The detection is achieved by taste receptor cells located in taste buds. Taste information is transmitted to the brain by three nerves, including chorda tympani, the glossopharyngeal, and the vagus nerves.

primary gustatory cortex located in the somatosensory cortex (Galindo et al., 2012).

The capacity of trigeminal nerve endings located in the nasal and oral cavity to detect the pungent or sharp feel, the coolness, the tingle or the irritation produced by different foods or beverages is called chemesthesis or trigeminal sensitivity (Bryant and Silver, 2000; Viana, 2011). They are detected by transient receptor potential (TRPs) channels, which are present on primary sensory neurons. The information is relayed to the brainstem via trigeminal ganglion sensory neurons (Roper, 2014). Chemesthetic stimuli are transduced by terminals of unmyelinated fibers traveling in trigeminal nerves (V) or by isolated chemosensory cells innervated by afferent axons traveling in these nerves, and possibly by epithelial keratinocytes, as discussed below.

It is then important to review what are the main mechanism by which SARS-COV-2 can affect smell and taste and what are the putative cells infected in these sensory systems.

SARS-CoV-2 AND ANOSMIA: CELLULAR TROPISM IN OLFACTORY EPITHELIUM

Cellular Expression of the Virus Receptor

ACE2 (angiotensin-converting enzyme 2) was characterized as the main entrance receptor for SARS-CoV-2 (Letko et al., 2020) interacting with its spike proteins. The spike protein allows the entrance into the host cell via a fusion domain (Delmas and Laude, 1990; Matsuyama et al., 2010). This fusion domain is uncovered after maturation of the spike protein by both ACE2 and the transmembrane serine protease 2 (TMPRSS2). These proteins mainly direct the cellular sensitivity to SARS-CoV-2.

Both proteins are mainly expressed in the upper part of the respiratory tract (Hou et al., 2020) and the highest density of these proteins is found in the olfactory epithelium. Sustentacular cells express most of ACE2 and TMPRSS2 and these proteins are absent from OSN (Bilinska et al., 2020; Fodoulian et al., 2020). Both are also expressed to a lesser extent in Bowman's gland, microvillar cells and basal stem cells (Brann et al., 2020). Based on this expression profile, sustentacular cells seem to be the main target of the SARS-CoV-2 in the olfactory epithelium.

Interestingly, chemical disorders associated with COVID-19 seem to be linked to the ethnicity. A recent review reporting on nearly 40,000 patients across 104 studies found that anosmia (and ageusia) is more prevalent in Caucasians than Asians (54.8 vs. 17.7%, respectively) (von Bartheld et al., 2020). Such differences in chemical disorder susceptibility do not seem to be due to underreporting, but may be explained by virus strain differences among SARS-CoV-2 (D614G mutation) and/or ethnic variation in the frequencies of ACE2 and/or TMPRSS2 sequences giving more affinity of SARS-CoV-2 to Caucasians (Butowt et al., 2020).

Cellular Impact: *in vivo* Evidence of SARS-CoV-2 in the Olfactory System

Mouse

The first *in vivo* data on the cellular target of the SARS-CoV-2 came from earlier studies of SARS-CoV-1 impact on the central nervous system. These studies are interesting because SARS-CoV-1 and 2 share the same receptor and SARS-CoV-1 has been shown to be neurotropic in studies using ACE2 humanized mice (Netland et al., 2008), thereby raising the possibility that SARS-CoV-2 could infect OSNs. Such infection would open a way for SARS-CoV-2 to enter the brain through the "olfactory pathway" (Bryche et al., 2020a; Forrester et al., 2018) and would explain the prevalence of encephalopathies observed in patients with COVID-19 (Azizi and Azizi, 2020). However, many cells of these humanized mice ectopically express ACE2 as it is under the control of keratin 18 (K18) a promoter of all epithelial cells. SARS-CoV-1 may thus infect OSNs which physiologically do not express ACE2 and the observation of presence of the virus in the brain may not be relevant for a more physiological model. Thus, the mouse—usually favored due to all the different strains and genetic tools available—cannot be directly used to understand the cellular basis of SARS-CoV-2 induced anosmia. Better mouse models are in development to implement a humanized ACE2 with a physiological expression profile (Butowt and von Bartheld, 2020; Sun et al., 2020). Recent studies using this model demonstrates that sustentacular cells and Bowman's gland cells in the olfactory epithelium are the major targets of SARS-CoV-2 before the invasion into olfactory sensory neurons (Ye et al., 2020; Zheng et al., 2020). Nonetheless other animal models have proved to be relevant to unravel the cellular mechanism behind COVID-19 related anosmia.

Golden Syrian Hamsters

Golden Syrian hamsters have been successfully used as a model of SARS-CoV-1 infection (Roberts et al., 2005). Indeed, the expression profile and sequences of ACE2 are very similar in hamsters and humans (Luan et al., 2020). The first study on SARS-CoV-2 impact on hamsters did not focus on anosmia, but provided some information on SARS-CoV-2 presence in the nasal cavity. The authors found that the virus was mainly infecting the olfactory epithelium in the nasal cavity and their results suggested that olfactory sensory neurons may be infected (Sia et al., 2020). Using the same animal model, we published shortly thereafter a study specifically focused on the impact of the SARS-CoV-2 in the nasal cavity (Bryche et al., 2020b). Using confocal double label immunostaining, we observed a massive infection of sustentacular cells by SARS-CoV-2 as early as 2 days post-infection. This infection was accompanied by immune cell infiltration and a global desquamation of the OE. At 2 days post-infection, the lumen of the nasal cavity was filled with cellular aggregates containing infected sustentacular cells, olfactory neurons and immune cells. At 4 days post-infection, the number of infected sustentacular cells was greatly reduced while the olfactory epithelial thickness was reduced up to 80%. Furthermore, the remaining OSNs had mostly lost their cilia involved in odor detection. Seven days post-infection, the virus was almost completely absent from the nasal cavity and we observed a gradual recovery of the olfactory epithelial thickness which reached about 50% of that of the control 14 days after infection. This recovery was also observed for OSN cilia. While we did not measure olfactory based behavior in our study, the massive loss of OSN dendrites undoubtedly had an important impact on odor detection efficiency and could explain most of the observed anosmia symptom if similar cellular events occur in humans. The recovery kinetic is also consistent with the observed recovery of anosmia in COVID-19 patients. Indeed, most patients suffering from anosmia recover relatively fast (∼10 days) (Dell'Era et al., 2020; Meini et al., 2020; von Bartheld et al., 2020), which is compatible with the observed partial recovery of the olfactory epithelium in hamsters 14 days post-infection. These results were later confirmed by another group (Zhang et al., 2020); using a much higher virus load during infection (10^5 vs. 5.10^3 pfu in our study). This group looked carefully for a potential infection of OSN. They found that some mature and immature OSN can be infected by SARS-CoV-2 but the study presents only a few images and this infection may be exceptionally rare compared to the occurrence of sustentacular cells infection. The infection of immature neurons could impair regeneration. Similar to other studies (Bryche et al., 2020b; Sia et al., 2020), Zhang et al. (2020) did not find any presence of the virus in the olfactory bulb indicating that if infection of OSN did occur, it did not lead to a detectable presence of the virus in the brain. Thus, so far, the possibility that SARS-CoV-2 could enter the brain through the "olfactory pathway" remains to be demonstrated.

Other Animal Models and Human Biopsies

Ferrets are also classically used as a model for respiratory viruses, especially influenza (Belser et al., 2020). The first study on ferrets infected with SARS-CoV-2 did not specifically focus on the olfactory epithelium; however, when they observed the presence of the virus in the nasal cavity, they found only infection of respiratory epithelial cells (Ryan et al., 2020). This result was confirmed in a broader study including fruit bats, pigs and chickens (Schlottau et al., 2020). While both pigs and chicken were resistant to SARS-CoV-2 infection, the fruit bat was susceptible but only few respiratory cells were infected by the virus. The authors observed, however, cellular debris in the lumen of the nasal cavity for both fruit bats and ferrets similarly to reports with infected golden Syrian hamsters.

Data from human biopsies are scarce, and they do not provide a link between the cellular tropism of SARS-CoV-2 to the observed anosmia. Some studies explored olfactory epithelium obtained from autopsied patients with COVID-19 patients. Results are rather controversial so far. A study performed on four samples did not find the virus in the olfactory epithelium by immunohistochemistry (Kantonen et al., 2020). A study based on 33 samples from autopsied patients explored specifically the presence of the virus by RT-qPCR (Meinhardt et al., 2020). They observed the presence of the virus in the olfactory epithelium in 20 patients (∼60%) and in the olfactory bulb in 3 (∼10%). While

the authors conclude that SARS-CoV-2 must thus infect OSN allowing it to enter the brain through olfactory bulb invasion, the study does not present any evidence of the presence of infected neurons by immunohistochemistry in the olfactory bulb. Another study focused on biopsies from olfactory epithelium of living COVID-19 positive patients (Chung et al., 2020). In this work, the authors only observed the presence of a few SARS-CoV-2 infected macrophages in the olfactory epithelium but no other cells were found SARS-CoV-2 positive. However, the delay between biopsies and SARS-CoV-2 infection detection was not presented in this study. As biopsies were harvested from patients already suffering from anosmia, it could be that they were performed several days after the onset of the COVID-19 infection and the virus could then be already mostly eliminated from the nasal cavity if a similar kinetic of virus clearance from the nasal cavity occurs in hamsters and humans. If so, earlier human biopsies could be very informative as the virus would be present and impact the olfactory epithelium mostly during the first 4 days following infection. Thus, so far more studies are required to evaluate to which extent the impact of SARS-CoV-2 on the olfactory epithelium differs from the model based on the hamster study. The fact that very few studies observed the presence of SARS-CoV-2 in human OSNs indicates that it may be a rare occurrence (Ellul et al., 2020; Matschke et al., 2020). In any case, it must be noted that the very rapid recovery of smell usually described in both humans and rodents may not be consistent with the timing of olfactory neuron regeneration (which is thought to take 10 or more days; Kondo et al., 2010; Liberia et al., 2019). However, as the onset of infection is very difficult to assess in humans, further studies are required to understand these events.

MODELS TO EXPLAIN COVID-19 RELATED ANOSMIA

Overall, most data indicate that the main targets of SARS-CoV-2 in the olfactory epithelium are sustentacular cells. Following their infection, most of the olfactory epithelium seems to be lost by desquamation as indicated by the presence of cellular debris in the lumen of the nasal cavity from numerous studies. This desquamation will remove part of the OSN population but could be accompanied by a loss of the dendrite layer of OSN where olfactory transduction occurs. These two consequences of the SARS-CoV-2 infection could explain the anosmia observed in COVID-19 patient. Subsequently two different scenarios could occur according to the physiological state of the infected individuals as well as the initial virus load. However, in healthy individuals the recovery would be fast due to the basal cells regenerating the olfactory epithelium. This recovery may be impaired by several factors:

- *Individuals characteristics*. Indeed, aged and/or overweight individuals are much more susceptible to COVID-19 (Simonnet et al., 2020). The olfactory epithelium integrity declines with age (Doty and Kamath, 2014) and overweight individual often present an increased basal inflammation state in their tissue (Ellulu et al., 2017) which could also impair regeneration (Chen et al., 2019; Sultan et al., 2011). Infection by SARS-CoV-2 of olfactory epithelium already in an inflammation state may facilitate the virus infection efficiency as its receptor ACE2 is overexpressed during inflammation (Ziegler et al., 2020).
- *Initial virus load*. OSN seems to be infected only with higher virus loads. If this infection reaches a certain threshold, it could begin to affect immature OSNs which will impact the regeneration of the olfactory epithelium.
- *Invasion of the respiratory epithelium*. Part of the olfactory epithelium can be replaced by respiratory epithelium as usually observed in post viral olfactory disorders (Doty and Kamath, 2014). It would diminish the recovery from anosmia.

This model is summarized in **Figure 2**. Many questions remain to elucidate the mechanism behind this desquamation.

Is it simply due to the destruction of sustentacular cells following SARS-CoV-2 infection? Indeed, these cells are essential to maintain the integrity of the olfactory epithelium and are tightly enwrapped around olfactory sensory neurons (Liang, 2020). Their disappearance from the olfactory epithelium will certainly impact the olfactory sensory neurons integrity; at least the dendrite layer, if not the cell body as well.

What is the role of the immune cells infiltrating the olfactory epithelium following infection? Are they actively involved in the desquamation of the olfactory epithelium or do they invade the olfactory epithelium following chemo-attractive signals after sustentacular infection and destruction? Indeed, as expected, inflammatory signals are increased in the olfactory epithelium following SARS-CoV-2 infection (Lee et al., 2020).

SARS-CoV-2 AND AGEUSIA: CELLULAR TROPISM IN TASTE BUDS

Unlike anosmia, COVID-induced ageusia has drawn much less interest in the scientific community, probably because to date, infection of the taste buds has been mostly overlooked. One study on the rabies virus impact in dogs found that taste buds were infected (Shiwa et al., 2018). The virus may reach the taste buds by retrograde transport from the infected brain. Thus, unlike anosmia which could be linked to a potential invasion of the brain through the olfactory nerve, an infection of taste buds which do not contain neurons may not be threatening for the infected individual.

Nevertheless, understanding how SARS-CoV-2 could impact gustation as frequently as olfaction may reveal unsuspected virus-host interactions. ACE2 was suspected to be expressed mainly outside the taste buds (Cooper et al., 2020). This was confirmed by a comprehensive study of the ACE2 expression profile in mice tongue showing that ACE2 is mainly expressed in epithelial cells outside of taste papillae which contain the taste buds (Wang et al., 2020). According to this study, taste buds are thus very unlikely

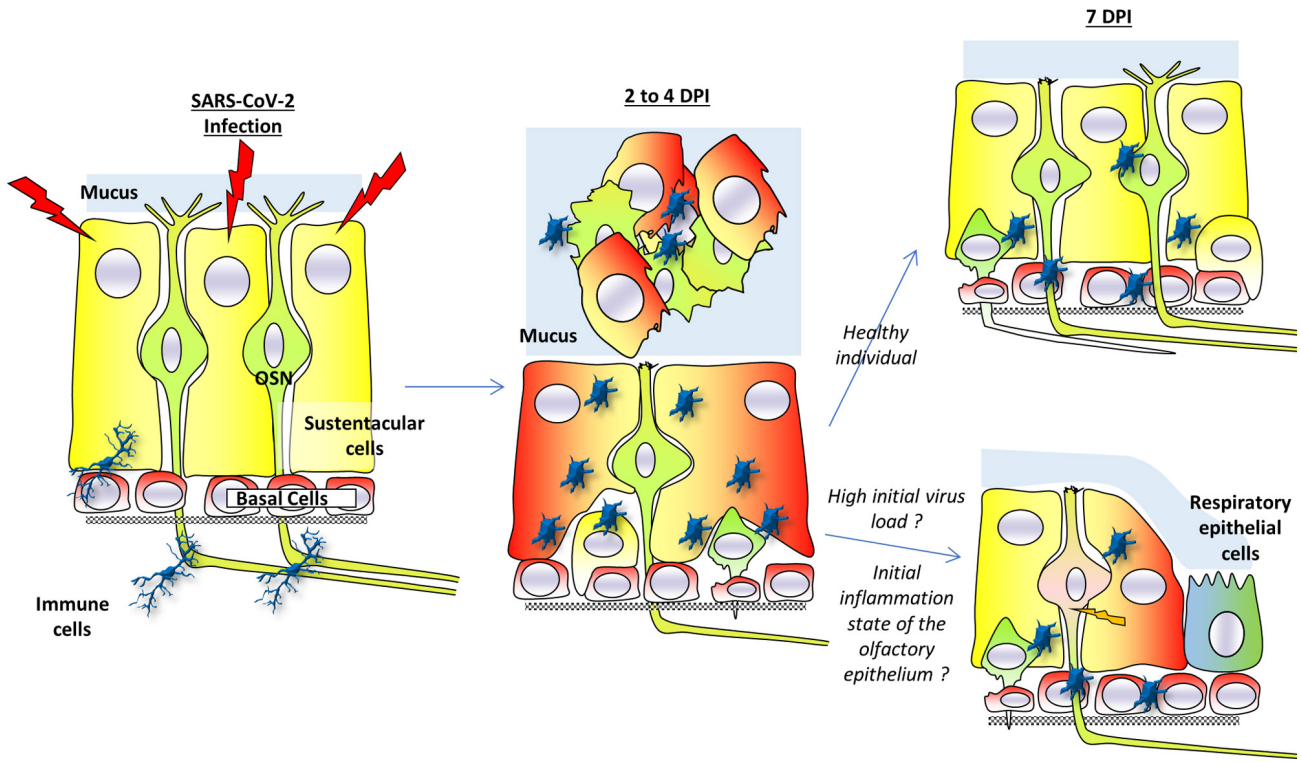


FIGURE 2 | A model for anosmia based on hamsters studies. Sustentacular cells are the main target of SARS-CoV-2 infection. Two to four days post-infection (dpi), the virus is present mainly in these cells. The massive infection of sustentacular cells is followed by a desquamation of the olfactory epithelium which is simultaneously invaded by activated immunity cells. The desquamation is visible through a reduced thickness of the olfactory epithelium and presence of cellular debris in the lumen of the nasal cavity. The cellular aggregates contain sustentacular cells, activated immune cells as well as olfactory neurons. In the non-desquamated zone, the olfactory neurons are losing their dendrite. Seven days post-infection, the olfactory epithelium is already regenerating due to basal cells in healthy individuals and injured olfactory sensory neurons could recover their dendrite layer. If initial virus load is high or in overweight/aged individuals whose initial integrity of the olfactory epithelium may already be impaired, the regeneration of the olfactory epithelium may be less efficient. This could lead to secondary infection of olfactory neurons as well as a slower recovery of the olfactory epithelium function. Part of the olfactory epithelium could also be replaced by respiratory epithelium following the massive inflammation.

to be directly impacted by the SARS-CoV-2 which may instead infect cells distant from taste buds.

MODELS TO EXPLAIN COVID-19 RELATED AGEUSIA

In order to improve our understanding of the cellular basis of ageusia, studies on the oral cavity impact of SARS-CoV-2 in model animals are required. In their absence, only a hypothetical scenario based on other pathological ageusia can be drawn.

One explanation of the SARS-CoV-2 induced ageusia could be that taste nerves are damaged following central nervous infection by SARS-CoV-2. It seems unlikely as a recent study performed in human indicates that impairments of chemical senses are correlated with low severity in COVID-19 patients excluding encephalitis (Nouchi et al., 2020) and the prevalence of central nervous damage by SARS-CoV-2 remains limited (Matschke et al., 2020).

The taste buds have a fast turnover as they are renewed within approximately 10 days (Beidler and Smallman, 1965). Another explanation could thus be that following infection of epithelial cells in the tongue, inflammatory cytokines could reach the taste buds impairing their renewal. Indeed, Toll-like receptors (TLR) and interferon (IFN) receptors are highly expressed in taste buds and their activation may limit taste cell regeneration (Wang et al., 2007, 2009). Thus, ageusia could be the result of impaired renewal of taste buds following the cytokines storm induced by SARS-CoV-2 in distant cells. The cytokine storm could also make taste buds cells permissive to SARS-CoV-2. Indeed, ACE2 has been shown to be overexpressed in the presence of IFN (Ziegler et al., 2020). Thus, a distant production of IFN from infected keratinocytes could lead to ACE2 expression in taste bud cells which could in turn be infected by SARS-CoV-2. A last explanation could be that taste nerves are damaged following central nervous infection by SARS-CoV-2. However, this seems unlikely, because the prevalence of central nervous system damage by SARS-CoV-2 remains limited (Matschke et al., 2020) while the prevalence of ageusia is high. Furthermore, COVID-19 patients suffering from impairments of chemical senses develop low severity symptoms excluding encephalitis (Nouchi et al., 2020).

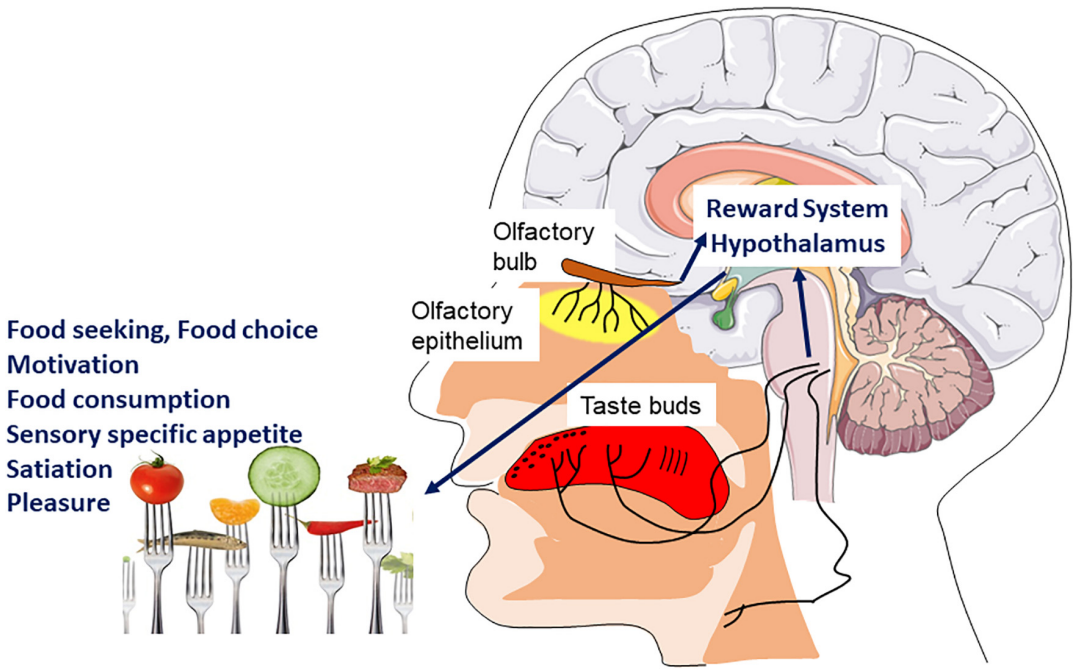


FIGURE 3 | Hedonic sensory signal and food intake. Signals coming from the chemosensory systems (olfaction, taste, and chemesthesis) directly inform the brain of the sensory quality of food. The main brain areas involved are those of the reward system as well as hypothalamic nuclei. These will be involved in different components of feeding behavior such as motivation and pleasure to eat but also sensory specific appetite and satiation.

IMPACT OF ANOSMIA AND AGEUSIA IN COVID-19 ON FEEDING BEHAVIOR

Role of Flavor in Eating Behavior in Physiological Conditions

Smell and taste make an important contribution to the general appetite, food choice, the onset of satiation, thereby participating in the control of energy intake allows an organism to connect the structural and chemical properties of foods to palatability and the foods' underlying nutritional value (**Figure 3**). Therefore, the sensory perception resulting from taste, odor and texture of the food, that is, its flavor, allows us to decide to ingest the food or not (Ventura and Worobey, 2013).

Olfaction, despite being perceptually intertwined with taste to produce the food flavor, has different independent physiological mechanisms, neural circuits and effects on food selection and intake (McCrickerd and Forde, 2016). While taste is based on a small class of receptors to detect a few important chemicals of the food once it is in the mouth, olfaction uses many receptors to detect thousands of different smells before and during intake in order to identify a wide variety of foods. Food odor has an important impact on general appetite: it can influence the quality and quantity of food chosen (Fedoroff et al., 1997; Ferriday and Brunstrom, 2008) and stimulate appetite, even in the absence of hunger (Lowe et al., 2009). It has also been suggested that there is a quality-specific effect of each odor that influences food choices (Gaillet-Torrent et al., 2013). Food odors also seem to stimulate sensory specific appetite (Gaillet-Torrent et al., 2014), motivate spontaneous consumption behaviors and help to distinguish different food sources. Some studies have observed that food odors could enhance the onset of satiation and reduce food intake (Ramaekers et al., 2014). Perceived odor intensity during food intake influences the quantity of eaten food (de Wijk et al., 2012): if food odor is perceived more intense, food consumption will be reduced. Food aromas are also signals associated with both food's availability and pleasure. Consequently, food aromas under fasting conditions and in obesity induce activation of several regions implicated in the reward system according to fMRI's (functional magnetic resonance imaging) studies (Bragulat et al., 2010; Eiler et al., 2012) and in contrast, decrease their activation in anorexia nervosa (Jiang et al., 2010).

Food taste plays an important role in the control of food intake and taste intensity may stimulate satiation (Bolhuis et al., 2012). In fact, when food enters directly the stomach without being processed by the taste receptors, satiation and reward values are lower (Wijlens et al., 2012; Spetter et al., 2014). Taste is commonly referred to as the body's "nutritional gatekeeper" of food intake (Feeney et al., 2011). Indeed, the sense of taste is an important factor in food seeking behaviors and dietary intake. Each taste quality has been associated with specific nutrients: sweet taste to identify sources of carbohydrates, sour for the presence of vitamins, salty for the presence of electrolytes and umami for source of proteins (Tucker and Mattes, 2012). In contrast, the bitter taste prevents the ingestion of toxic or spoiled substances (Tucker and Mattes, 2012). All basic tastes, combined with food odors to form flavor, influence food intake and satiation. Numerous studies

reported the effect of other taste modalities on the stimulation on food intake. For example, salt enhances palatability and can motivate food intake and then lead to satiation (Bolhuis et al., 2012). Umami is also known to stimulate palatability, appetite, the desire to eat, and therefore food intake (Simpson and Raubenheimer, 2005; Hermanussen et al., 2006). Sweetness contributes to the palatability of food and enhances food intake by increasing its acceptance, especially in children (de Graaf et al., 1993; Mennella and Bobowski, 2015). To illustrate the role of sweet taste, a systematic review reports that in healthy subjects, a strong hedonic preference for sweetness increases the energy intake from sweet foods, especially in subjects with sweet lovers' phenotypes (Tan and Tucker, 2019). Conversely, a high sensitivity to sweetness (low detection and recognition thresholds) is associated with a low consumption of carbohydrate-rich foods associated with a higher intake of non-sweet foods and dietary protein (Han et al., 2017). Similarly, a strong perception of the intensity of sweetness decreases total energy intake and the consumption of carbohydrate-rich foods (Jayasinghe et al., 2017). These observations indicate that inter-individual differences in sweetness perception (sensitivity and intensity) seem to have a weak and even opposing influence on carbohydrate intakes, in contrast to the sweet induced pleasure which has a great influence on consumption (Tan and Tucker, 2019).

The multisensory properties of food stimuli are transmitted to the brain through specialized taste, olfactory and somatosensory pathways that converge on several central nervous system centers involved in homeostatic and hedonic control of food intake. Hedonic factors that participate in the control of eating behavior by four classic mechanisms (conditioned satiety, food reward system, sensory specific satiety, and alliesthesia) are directly linked to taste and olfaction, and reinforce the flavor pleasantness of food.

Role of Flavor in Eating Behavior in Pathophysiological Conditions

Taste and smell dysfunctions are common clinical problems associated with disease processes but are often neglected (Henkin et al., 2013). Yet deficits in taste and olfactory chemical senses have a severe impact on the pleasure from foods and represent risk factors for nutritional deficiencies. Causes of smell, taste, and oral somatosensory disorders that affect intakes are numerous: aging, chronic nasal-sinus disease, upper respiratory tract infection, pathologies of the middle ear, head trauma, neurodegenerative disorders, obesity, liver and kidney diseases, cancer, environmental chronic exposures, medications, oral health, surgical interventions, infections and nutritional intervention for chemosensory disorders (for reviews see Schiffman, 1997, 2018; Brondel et al., 2016; Duffy, 2020).

Several studies observed that the coronavirus causing COVID-19 is responsible for smell and taste dysfunctions (Lechien et al., 2020; Parma et al., 2020; von Bartheld et al., 2020). Some studies have reported that 11% of COVID-19 patients with smell loss have chronic deficits, with a chemosensory dysfunction that persists beyond 4 weeks after onset (Boscolo-Rizzo et al., 2020). Accordingly, the current number of such patients worldwide can be estimated. This unprecedent magnitude of the number of cases emphasizes the importance of understanding the clinical consequences of loss of smell/taste.

It was suggested that SARS-CoV-2 is a neurotropic and neuro-invasive virus, by infecting peripheral neurons and then by spreading into the central nervous system like other neuroinvasive viruses (Koyuncu et al., 2013). Concerning the smell functions, the virus may invade the olfactory nerves and the olfactory bulb, causing, on the one hand, olfactory epithelium desquamation and olfactory bulb atrophy, and on the other hand olfactory bulb inflammation (Cooper et al., 2020). Concerning the taste functions, the viral infection and inflammatory response may lead to disruption of saliva composition, taste transduction and impair the continuous renewal of taste buds. Some investigators proposed that the coronavirus causing COVID-19 could also target cells of the central nervous system (Baig et al., 2020). It has also been observed that another coronavirus (SARS-CoV) might enter the central nervous system through the olfactory bulb to spread to some brain areas which are particularly vulnerable to this virus family: piriform and infralimbic cortices, ventral pallidum and lateral preoptic regions in the basal ganglia, and dorsal raphe nuclei in the midbrain (Netland et al., 2008). But as already mentioned a limitation of this study is that it was performed on humanized mice expressing ACE-2 in every epithelial cell.

The influence of hypogeusia (dysgeusia) and/or hyposmia (dysosmia) during COVID-19 on food/energy intake or food preferences has not yet been reported. At the most, literature discusses changes in feeding behavior during the lockdown period, without direct relationship with COVID-19, but in the context of sudden lifestyle changes (Di Renzo et al., 2020; Rodriguez-Perez et al., 2020).

To demonstrate the putative impact of smell and taste disorders in COVID-19 on feeding behavior, we can consider examples of other well-known pathological situations causing the same sensory perturbances. For example, taste and smell alterations resulting from cancers and chemotherapy can reduce appetite and contribute to poor nutritional status (Brisbois et al., 2006; Cohen et al., 2016). In the same way, olfactory dysfunctions in Parkinson disease can lead to changes in feeding behavior (Landis et al., 2009).

Taking into account the potential neurological damage caused by the COVID-19 infection, it is understandable that this virus could have a strong impact on feeding behavior, mediated by taste and smell dysfunctions, and possibly the spreading of the virus to brain regions implicated in hedonic controls of food intake. We can hypothesize that this viral infection, depending on the severity of symptoms, could alter alimentary consumption and nutritional status as in the above-cited pathologies. Indeed, decreases of taste and/or smell may alter the hedonic response associated with the sensory sensations and, therefore, the response to the sensory experience of eating (McCrickerd and Forde, 2016). Putative mechanisms could be a decrease in conditioned satiety (misperception of the aliment before intake), a decrease in the reward system (i.e., low liking and wanting for foods during ingestion) and an early sensory

specific satiety (premature termination of the consumed food) (Pénicaud et al., 2016).

The consequence of a decrease in energy intake would then be weight loss (associated with other nutritional imbalances). Studies conducted on animals indicated that SARS-CoV-2 causes weight loss associated with an increase in inflammatory cytokines (Bao et al., 2020). In humans, COVID-19 causes anorexia, weight loss and low albumin levels. The variation between infected individuals is immense; some subjects are asymptomatic or with minimal symptoms, while others develop a severe or even fatal course of the disease. Many factors have been identified in weight loss and sarcopenia/cachexia (Morley et al., 2020). Furthermore, many confounding factors (independent of the increase in energy expenditure related to inflammatory phenomena) may interfere with weight changes linked to taste and smell dysfunctions and food intake reduction, and may be related to change in food and physical activity, sleeping habits, anxiety and depression (Almandoz et al., 2020; Fernandez-Rio et al., 2020; Gualtieri et al., 2020; Ramachandran and Gill, 2020; Zachary et al., 2020). Thus, in absence of studies investigating the direct effect of smell and taste dysfunctions on food intake and preference, it is difficult to quantify precisely their effect in humans. Indeed all these considerations are primarily relevant for the fraction of COVID cases with chronic, not acute loss of smell and taste.

CONCLUSION

The relationships between the chemical senses and physiological regulation of food intake are well-known and documented. Even in the absence of relevant studies on the effect of taste and smell alterations on food consumption during COVID-19, special attention should be paid during this period to high-risk individuals with food sensory disturbances, i.e., those with co-morbidities (cardiac, hepatic, and renal), sarcopenia, diabetes, hypertension, smoking, eating disorders, and malnutrition, as well as the elderly. It is crucial to prevent a decrease in food intake during COVID-19 pandemic (Fernandez-Aranda et al., 2020; Pallanti, 2020).

AUTHOR CONTRIBUTIONS

NM, LoB, AJ-P, LaB, and LP discussed the concepts, wrote parts, and reviewed the entire manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from the Conseil Régional Bourgogne, Franche-Comté (PARI grant) and the FEDER (European Funding for Regional Economical Development).

ACKNOWLEDGMENTS

We deeply thanks the two reviewers for their comments and suggestions that greatly help us to ameliorate the manuscript.

REFERENCES

Almandoz, J. P., Xie, L., Schellinger, J. N., Mathew, M. S., Gazda, C., Ofori, A., et al. (2020). Impact of COVID-19 stay-at-home orders on weight-related behaviours among patients with obesity. *Clin. Obes.* 10:e12386.

Azizi, S. A., and Azizi, S. A. (2020). Neurological injuries in COVID-19 patients: direct viral invasion or a bystander injury after infection of epithelial/endothelial cells. *J. Neurovirol.* 26, 631–641. doi: 10.1007/s13365-020-00903-7

Baig, A. M., Khaleeq, A., Ali, U., and Syeda, H. (2020). Evidence of the COVID-19 virus targeting the CNS: tissue distribution, host-virus interaction, and proposed neurotropic mechanisms. *ACS Chem. Neurosci.* 11, 995–998. doi: 10.1021/acschemneuro.0c00122

Bao, L., Deng, W., Huang, B., Gao, H., Liu, J., Ren, L., et al. (2020). The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Nature* 583, 830–833.

Barlow, L. A., and Klein, O. D. (2015). Developing, and regenerating a sense of taste. *Curr. Top. Dev. Biol.* 111, 401–419. doi: 10.1016/bs.ctdb.2014.11.012

Behrens, M., Briand, L., de March, C. A., Matsunami, H., Yamashita, A., Meyerhof, W., et al. (2018). Structure-function relationships of olfactory and taste receptors. *Chem. Senses* 43, 81–87. doi: 10.1093/chemse/bjx083

Behrens, M., Meyerhof, W., Hellfritsch, C., and Hofmann, T. (2011). Sweet and umami taste: natural products, their chemosensory targets, and beyond. *Angew Chem. Int.* 50, 2220–2242. doi: 10.1002/anie.201002094

Beidler, L. M., and Smallman, R. L. (1965). Renewal of cells within taste buds. *J. Cell Biol.* 27, 263–272. doi: 10.1083/jcb.27.2.263

Belser, J. A., Eckert, A. M., Huynh, T., Gary, J. M., Ritter, J. M., Tumpey, T. M., et al. (2020). A guide for the use of the ferret model for influenza virus infection. *Am. J. Pathol.* 190, 11–24. doi: 10.1016/j.ajpath.2019.09.017

Bilinska, K., Jakubowska, P., Von Bartheld, C. S., and Butowt, R. (2020). Expression of the SARS-CoV-2 entry proteins, ACE2 and TMPRSS2, in cells of the olfactory epithelium: identification of cell types and trends with age. *ACS Chem. Neurosci.* 11, 1555–1562. doi: 10.1021/acschemneuro.0c00210

Bolhuis, D. P., Lakemond, C. M., de Wijk, R. A., Luning, P. A., and de Graaf, C. (2012). Effect of salt intensity in soup on ad libitum intake and on subsequent food choice. *Appetite* 58, 48–55. doi: 10.1016/j.appet.2011.09.001

Boscolo-Rizzo, P., Borsetto, D., Fabbris, C., Spinato, G., Frezza, D., Menegaldo, A., et al. (2020). Evaluation of altered sense of smell or taste in patients with mild symptomatic COVID-19. *JAMA Otolaryngol Head Neck Surg.* 146, 729–732. doi: 10.1001/jamaoto.2020.1379

Bragulat, V., Dzemidzic, M., Bruno, C., Cox, C. A., Talavage, T., Considine, R. V., et al. (2010). Food-related odor probes of brain reward circuits during hunger: a pilot fMRI study. *Obesity* 18, 1566–1571. doi: 10.1038/oby.2010.57

Brann, D. H., Tsukahara, T., Weinreb, C., Lipovsek, M., van den Berge, K., Gong, B., et al. (2020). Non-neuronal expression of SARS-COV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19 associated anosmia. *Sci. Adv.* 6:eabc5801. doi: 10.1126/sciadv.abc5801

Briand, L., Eloit, C., Nespoulous, C., Bézirard, V., Huet, J. C., Henry, C., et al. (2002). Evidence of an odorant-binding protein in the human olfactory mucus: location, structural characterization, and odorant-binding properties. *Biochemistry* 41, 7241–7252. doi: 10.1021/bi015916c

Briand, L., and Salles, C. (2016). "Taste perception and integration," in *Flavor: From Food to Behaviors*, eds P. Etiévant, E. Guichard, C. Salles, and A. Voilley (Cambridge: Elservier Ltd.), 101–119. doi: 10.1016/b978-0-08-100295-7.00004-9

Brisbois, T. D., Hutton, J. L., Baracos, V. E., and Wismer, W. V. (2006). Taste and smell abnormalities as an independent cause of failure of food intake in patients with advanced cancer–an argument for the application of sensory science. *J. Palliat. Care* 2, 111–114. doi: 10.1177/082585970602200208

Brondel, L., Brindisi, M., Jacquin-Piques, A., Mouillot, T., and Pénicaud, L. (2016). "Taste disorders in disease," in *Flavor: From Food to Behaviors*, eds P.

Etiévant, E. Guichard, C. Salles, and A. Voilley (Cambridge: Elservier Ltd.), 337–361.

Bryant, B. P., and Silver, W. L. (2000). "Chemesthesis: the common chemical sense," in *The Neurobiology of Taste and Smell*, eds T. E. Finger and W. L. Silver (New York, NY: Wiley-Liss), 73–100.

Bryche, B., Fretaud, M., Saint-Albin Deliot, A., Galloux, M., Sedano, L., Langevin, C., et al. (2020a). Respiratory syncytial virus tropism for olfactory sensory neurons in mice. *J. Neurochem.* 155, 137–153. doi: 10.1111/jnc.14936

Bryche, B., St Albin, A., Murri, S., Lacôte, S., Pulido, C., Ar Gouilh, M., et al. (2020b). Massive transient damage of the olfactory epithelium associated with infection of sustentacular cells by SARS-CoV-2 in golden Syrian hamsters. *Brain Behav. Immun.* 89, 579–586. doi: 10.1016/j.bbi.2020.06.032

Butowt, R., Bilinska, K., and Von Bartheld, C. S. (2020). Chemosensory dysfunction in COVID-19: integration of genetic and epidemiological data points to D614G spike protein variant as a contributing factor. *ACS Chem. Neurosci.* 11, 3180–3184. doi: 10.1021/acschemneuro.0c00596

Butowt, R., and von Bartheld, C. S. (2020). Anosmia in COVID-19: underlying mechanisms and assessment of an olfactory route to brain infection. *Neuroscientist* doi: 10.1177/1073858420956905 Online ahead of print.

Chandrashekar, J., Kuhn, C., Oka, Y., Yarmolinsky, D. A., Hummler, E., Ryba, N. J., et al. (2010). The cells and peripheral representation of sodium taste in mice. *Nature* 464, 297–301. doi: 10.1038/nature08783

Chen, M., Reed, R. R., and Lane, A. P. (2019). Chronic inflammation directs an olfactory stem cell functional switch from neuroregeneration to immune defense. *Cell Stem Cell.* 25, 501–513. doi: 10.1016/j.stem.2019.08.011

Chung, T. W., Sridhar, S., Zhang, A. J., Chan, K. H., Li, H. L., Wong, F. K., et al. (2020). Olfactory dysfunction in coronavirus disease 2019 patients: observational cohort study and systematic review. *Open Forum Infect. Dis.* 7:ofaa199.

Cohen, J., Wakefield, C. E., and Laing, D. G. (2016). Smell and taste disorders resulting from cancer and chemotherapy. *Cur. Pharmaceutical Design.* 22, 2253–2263. doi: 10.2174/1381612822666160216150812

Cooper, K. W., Brann, D. H., Farruggia, M. C., Bhutani, S., Pellegrino, R., Tsukahara, T., et al. (2020). COVID-19 and the chemical senses: supporting players take center stage. *Neuron* 107, 219–233. doi: 10.1016/j.neuron.2020.06.032

de Graaf, C., Schreurs, A., and Blauw, Y. H. (1993). Short-term effects of different amounts of sweet and nonsweet carbohydrates on satiety and energy intake. *Physiol. Behav.* 54, 833–843. doi: 10.1016/0031-9384(93)90290-v

de Wijk, R., Polet, I., Boek, W., Coenraad, S., and Bult, J. (2012). Food aroma affects bite size. *Flavour* 1:3. doi: 10.1186/2044-7248-1-3

Dell'Era, V., Farri, F., Garzaro, G., Gatto, M., Aluffi Valletti, P., and Garzaro, M. (2020). Smell and taste disorders during COVID-19 outbreak: cross-sectional study on 355 patients. *Head Neck* 42, 1591–1596. doi: 10.1002/hed.26288

Delmas, B., and Laude, H. (1990). Assembly of coronavirus spike protein into trimers and its role in epitope expression. *J. Virol.* 64, 5367–5375. doi: 10.1128/jvi.64.11.5367-5375.1990

Di Renzo, L., Gualtieri, P., Pivari, F., Soldati, L., Attina, A., Cinelli, G., et al. (2020). Eating habits and lifestyle changes during COVID-19 lockdown: an Italian survey. *J. Trans. Med.* 18:229.

Diodato, A., Ruinart, de Brimont, M., Yim, Y. S., Derian, N., Perrin, S., et al. (2016). Molecular signatures of neural connectivity in the olfactory cortex. *Nat. Commun.* 7:12238.

Doty, R. L., and Kamath, V. (2014). The influences of age on olfaction: a review. *Front. Psychol.* 5:20. doi: 10.3389/fpsyg.2014.00020

Duchamp-Viret, P., Chaput, M. A., and Duchamp, A. (1999). Odor response properties of rat olfactory receptor neurons. *Science* 284, 2171–2174. doi: 10.1126/science.284.5423.2171

Duffy, V. (2020). "Causes of smell, taste, and oral somatosensory disorders affecting eating and drinking," in *Handbook of Eating and Drinking*, ed. H. Meiselman (Berlin: Springer).

Eiler, W. J., Dzemidzic, M., Case, K. R., Considine, R. V., and Kareken, D. A. (2012). Correlation between ventromedial prefrontal cortex activation to food aromas and cue-driven eating: an fMRI study. *Chemosensory Percept.* 5, 27–36. doi: 10.1007/s12078-011-9112-6

Ellul, M. A., Benjamin, L., Singh, B., Lant, S., Michael, B. D., Easton, A., et al. (2020). Neurological associations of COVID-19. *Lancet Neurol.* 19, 767–783.

Ellulu, M. S., Patimah, I., Khaza'ai, H., Rahmat, A., and Abed, Y. (2017). Obesity and inflammation: the linking mechanism and the complications. *Arch. Med. Sci.* 13, 851–863. doi: 10.5114/aoms.2016.58928

Fedoroff, I. C., Polivy, J., and Herman, C. P. (1997). The effect of pre-exposure to food cues on the eating behavior of restrained and unrestrained eaters. *Appetite* 28, 33–47. doi: 10.1006/appe.1996.0057

Feeney, E., O'Brien, S., Scannell, A., Markey, A., and Gibney, E. R. (2011). Genetic variation in taste perception: does it have a role in healthy eating? *Proc. Nutr. Soc.* 70, 135–143. doi: 10.1017/s0029665110003976

Fernandez-Aranda, F., Munguia, L., Mestre-Bach, G., Steward, T., Extandi, M., Baenas, I., et al. (2020). COVID isolation eating scale (CIES) : analysis of the impact of confinement in eating disorders and obesity-A collaborative international study. *Eur. Eat. Disord. Rev.* 28, 871–883. doi: 10.1002/erv.2784

Fernandez-Rio, J., Cecchini, J. A., Mendez-Gimenez, A., and Carriedo, A. (2020). Weight changes during the COVID-19 home confinement. effects on psychosocial variables. *Obes. Res. Clin. Pract.* 14, 383–385. doi: 10.1016/j.orcp.2020.07.006

Ferriday, D., and Brunstrom, J. M. (2008). How does food-cue exposure lead to larger meal sizes? *British J. Nutr.* 100, 1325–1332. doi: 10.1017/s0007114508978296

Fodoulian, L., Tuberosa, J., Rossier, D., Boillat, M., Kan, C., Pauli, V., et al. (2020). SARS-CoV-2 receptor and entry genes are expressed by sustentacular cells in the human olfactory neuroepithelium. *iScience* 23:101839. doi: 10.1016/j.isci.2020.101839

Forrester, J. V., McMenamin, P. G., and Dando, S. J. (2018). CNS infection and immune privilege. *Nat. Rev. Neurosci.* 19, 655–671. doi: 10.1038/s41583-018-0070-8

Gaillet-Torrent, M., Sulmont-Rossé, C., Issanchou, S., Chabanet, C., and Chambaron, S. (2013). Priming effects of an olfactory food cue on subsequent food-related behaviour. *Food Qual. Pref.* 30, 274–281. doi: 10.1016/j.foodqual.2013.06.008

Gaillet-Torrent, M., Sulmont-Rossé, C., Issanchou, S., Chabanet, C., and Chambaron, S. (2014). Impact of a non-attentively perceived odour on subsequent food choices. *Appetite* 76, 17–22. doi: 10.1016/j.appet.2014.01.009

Galindo, M. M., Schneider, N. Y., Stähler, F., Töle, J., and Meyerhof, W. (2012). Taste preferences. *Prog. Mol. Biol. Transl. Sci.* 108, 383–426.

Getchell, T. V., Margolis, F. L., and Getchell, M. L. (1984). Perireceptor and receptor events in vertebrate olfaction. *Prog. Neurobiol.* 23, 317–345. doi: 10.1016/0301-0082(84)90008-x

Gualtieri, P., Falcone, C., Romano, L., Macheda, S., Correale, P., Arciello, P., et al. (2020). Body composition findings by computed tomography in SARS-CoV-2 patients: increased risk of muscle wasting in obesity. *Int. J. Mol. Sci.* 21:4670. doi: 10.3390/ijms21134670

Han, P., Keast, R. S. J., and Roura, E. (2017). Salivary leptin and TAS1R2/TAS1R3 polymorphisms are related to sweet taste sensitivity and carbohydrate intake from a buffet meal in healthy young adults. *Br. J. Nutr.* 118, 763–770. doi: 10.1017/s0007114517002872

Henkin, R. I., Levy, L. M., and Fordyce, A. (2013). Taste and smell function in chronic disease: a review of clinical and biochemical evaluations of taste and smell dysfunction in over 5000 patients at the taste and smell clinic in Washington. DC. *Am. J. Otolaryngol.* 34, 477–489.

Hermanussen, M., Garcia, A. P., Sunder, M., Voigt, M., Salazar, V., and Tresguerres, J. A. (2006). Obesity, voracity, and short stature: the impact of glutamate on the regulation of appetite. *Europ. J. Clin. Nutr.* 60, 25–31. doi: 10.1038/sj.ejcn.1602263

Heydel, J. M., Coelho, A., Thiebaud, N., Legendre, A., Le Bon, A. M., Faure, P., et al. (2013). Odorant-binding proteins and xenobiotic metabolizing enzymes: implications in olfactory perireceptor events. *Anat Rec (Hoboken)* 296, 1333–1345. doi: 10.1002/ar.22735

Hou, Y. J., Okuda, K., Edwards, C. E., Martinez, D. R., Asakura, T., Dinnon, K. H. III, et al. (2020). SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. *Cell* 182, 429–446.

Iwata, S., Yoshida, R., and Ninomiya, Y. (2014). Taste transductions in taste receptor cells: basic tastes and moreover. *Curr. Pharm. Des.* 20, 2684–2692. doi: 10.2174/13816128113199990575

Jayasinghe, S. N., Kruger, R., Walsh, D. C. I., Cao, G., Rivers, S., Richter, M., et al. (2017). Is sweet taste perception associated with sweet food liking and intake? *Nutrients* 9:750. doi: 10.3390/nu9070750

Jiang, T., Soussignan, R., Rigaud, D., and Schaal, B. (2010). Pleasure for visual and olfactory stimuli evoking energy-dense foods is decreased in anorexia nervosa. *Psychiatry Res.* 18, 42–47. doi: 10.1016/j.psychres.2010.04.041

Kantonen, J., Mahzabin, S., Mäyränpää, M. I., Tynninen, O., Paetau, A., Andersson, N., et al. (2020). Neuropathologic features of four autopsied COVID-19 patients. *Brain Pathol.* 30, 1012–1016. doi: 10.1111/bpa.12889

Kondo, K., Suzukawa, K., Sakamoto, T., Watanabe, K., Kanaya, K., Ushio, M., et al. (2010). Age-related changes in cell dynamics of the postnatal mouse olfactory neuroepithelium: cell proliferation, neuronal differentiation, and cell death. *J. Comp. Neurol.* 518, 1962–1975. doi: 10.1002/cne.22316

Koyuncu, O. O., Hogue, I. B., and Enquist, L. W. (2013). Virus infections in the nervous system. *Cell Host Microbe* 13, 379–393.

Landis, B. N., Cao Van, H., Guinand, N., Horvath, J., Haehner, A., Savva, E., et al. (2009). Retronasal olfactory function in Parkinson's disease. *Laryngoscope* 119, 2280–2283.

Laugerette, F., Passilly-Degrace, P., Patris, B., Niot, I., Febbraio, M., Montmayeur, J. P., et al. (2005). CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. *J. Clin. Invest.* 115, 3177–3184. doi: 10.1172/jci25299

Lechien, J. R., Chiesa-Estomba, C. M., De Siati, D. R., Horoi, M., Le Bon, S. D., Rodriguez, A., et al. (2020). Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur. Arch. Oto-rhino-laryngol.* 277, 2251–2261.

Lee, A. C., Zhang, A. J., Chan, J. F., Li, C., Fan, Z., Liu, F., et al. (2020). Oral SARS-CoV-2 inoculation establishes subclinical respiratory infection with virus shedding in golden syrian hamsters. *Cell Rep. Med.* 1:100121. doi: 10.1016/j.xcrm.2020.100121

Letko, M., Marz, A., and Munster, V. (2020). Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat. Microbiol.* 5, 562–569. doi: 10.1038/s41564-020-0688-y

Liang, F. (2020). Sustentacular cell enwrapment of olfactory receptor neuronal dendrites: an update. *Genes (Basel)* 11:493. doi: 10.3390/genes11050493

Liberia, T., Martin-Lopez, E., Meller, S. J., and Greer, C. A. (2019). Sequential maturation of olfactory sesory neurons in the mature olfactory epithelium. *eNeuro* 6:ENEURO.0266-19.2019. doi: 10.1523/ENEURO.0266-19.2019

Lowe, M. R., Butryn, M. L., Didie, E. R., Annunziato, R. A., Thomas, J. G., Crerand, C. E., et al. (2009). The power of food scale. a new measure of the psychological influence of the food environment. *Appetite* 53, 114–118. doi: 10.1016/j.appet.2009.05.016

Luan, J., Lu, Y., Jin, X., and Zhang, L. (2020). Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. *Biochem. Biophys. Res. Commun.* 526, 165–169. doi: 10.1016/j.bbrc.2020.03.047

Malnic, B., Godfrey, P. A., and Buck, L. B. (2004). The human olfactory receptor gene family. *Proc. Natl. Acad. Sci. U S A.* 101, 2584–2589.

Malnic, B., Hirono, J., Sato, T., and Buck, L. B. (1999). Combinatorial receptor codes for odors. *Cell* 96, 713–723. doi: 10.1016/s0092-8674(00)80581-4

Maruyama, Y., Yasuda, R., Kuroda, M., and Eto, Y. (2012). Kokumi substances, enhancers of basic tastes, induce responses in calcium-sensing receptor expressing taste cells. *PLoS One* 7:e34489. doi: 10.1371/journal.pone.0034489

Matschke, J., Lütgehetmann, M., Hagel, C., Sperhake, J. P., Schröder, A. S., Edler, C., et al. (2020). Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. *Lancet Neurol.* 19, 919–929. doi: 10.1016/s1474-4422(20)30308-2

Matsuyama, S., Nagata, N., Shirato, K., Kawase, M., Takeda, M., and Taguchi, F. (2010). Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2. *J. Virol.* 84, 12658–12664. doi: 10.1128/jvi.01542-10

McCrickerd, K., and Forde, C. G. (2016). Sensory influences on food intake control: moving beyond palatability. *Obesity Rev.* 17, 18–29. doi: 10.1111/obr.12340

Mehraeen, E., Behnezhad, F., Salehi, M. A., Noori, T., Harandi, H., and SeyedAlinaghi, S. (2020). Olfactory and gustatory dysfunctions due to coronavirus disease (COVID-19): a review of current evidence. *Eur. Arch. Otorhinolaryngol.* 17, 1–6.

Meinhardt, J., Radke, J., Dittmayer, C., Mothes, R., Franz, J., Laueet, M., et al. (2020). Olfactory transmucosal SARS-COV-2 invasion as port of central nervous system entry in COVID-19 patients. *Nat. Neurosci.* doi: 10.1038/s41593-020-00758-755 Online ahead of print.

Meini, S., Suardi, L. R., Busoni, M., Roberts, A. T., and Fortini, A. (2020). Olfactory and gustatory dysfunctions in 100 patients hospitalized for COVID-19: sex differences and recovery time in real-life. *Eur. Arch. Otorhinolaryngol.* 4, 1–5.

Mennella, J. A., and Bobowski, N. K. (2015). The sweetness and bitterness of childhood: Insights from basic research on taste preferences. *Physiol. Behav.* 152, 502–507. doi: 10.1016/j.physbeh.2015.05.015

Meyerhof, W., Batram, C., Kuhn, C., Brockhoff, A., Chudoba, E., Bufe, B., et al. (2010). The molecular receptive ranges of human TAS2R bitter taste receptors. *Chem. Senses* 35, 157–170. doi: 10.1093/chemse/bjp092

Mombaerts, P. (2004). Genes and ligands for odorant, vomeronasal and taste receptors. *Nat. Rev. Neurosci.* 5, 263–278. doi: 10.1038/nrn1365

Morley, J. E., Kalantar-Zadeh, K., and Anker, S. D. (2020). COVID-19: a major cause of cachexia and sarcopenia? *J. Cachexia Sarcopenia Muscle* 1, 863–865. doi: 10.1002/jcsm.12589

Mouillot, T., Szleper, E., Vagne, G., Barthet, S., Litime, D., Brindisi, M. C., et al. (2019). Cerebral gustatory activation in response to free fatty acids using gustatory evoked potentials in humans. *J. Lipid. Res.* 60, 661–670. doi: 10.1194/jlr.m086587

Nelson, G., Chandrashekar, J., Hoon, M. A., Feng, L., Zhao, G., Ryba, N. J., et al. (2002). An amino-acid taste receptor. *Nature* 416, 199–202.

Nelson, G., Hoon, M. A., Chandrashekar, J., Zhang, Y., Ryba, N. J., and Zuker, C. S. (2001). Mammalian sweet taste receptors. *Cell* 106, 381–390. doi: 10.1016/s0092-8674(01)00451-2

Netland, J., Meyerholz, D. K., Moore, S., Cassell, M., and Perlman, S. (2008). Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. *J. Virol.* 82, 7264–7275. doi: 10.1128/jvi.00737-08

Nouchi, A., Chastang, J., Miyara, M., Lejeune, J., Soares, A., Ibanez, G., et al. (2020). Prevalence of hyposmia and hypogeusia in 390 COVID-19 hospitalized patients and outpatients: a cross-sectional study. *Eur. J. Clin. Microbiol. Infect. Dis.* 8, 1–7.

Pallanti, S. (2020). Importance of SARs-COV-2 anosmia: from phenomology to neurobiology. *Compr. Psychiatry* 100:152184. doi: 10.1016/j.comppsych.2020.152184

Parma, V., Ohla, K., Veldhuizen, M. G., Niv, M. Y., Kelly, C. E., Bakke, A. J., et al. (2020). More than smell - COVID-19 is associated with severe impairment of smell, taste, and chemesthesis. *Chem. Senses* 9, 609–622.

Pénicaud, L., Valentin, D., and Brondel, L. (2016). "Mechanisms involved in the control of feeding behaviour in relation to food flavor", in *Flavor: From Food to Behaviors*, eds P. Etiévant, E. Guichard, C. Salles, and A. Voilley (Cambridge: Elsevier Ltd), 101–119.

Ramachandran, D., and Gill, T. (2020). Impact of COVID-19 lockdown on self-managed weight loss journeys. *Obes. Res. Clin. Pract.* 14, 386–387. doi: 10.1016/j.orcp.2020.08.001

Ramaekers, M. G., Luning, P. A., Ruijschop, R. M., Lakemond, C. M., Bult, J. H., Gort, G., et al. (2014). Aroma exposure time and aroma concentration in relation to satiation. *Br. J. Nutr.* 111, 554–562. doi: 10.1017/s0007114513002729

Roberts, A., Vogel, L., Guarner, J., Hayes, N., Murphy, B., Zaki, S., et al. (2005). Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters. *J. Virol.* 79, 503–511. doi: 10.1128/jvi.79.1.503-511.2005

Rodriguez-Perez, C., Molina-Montes, E., Verardo, V., Artacho, R., Garcia-Villanova, B., Guerra-Hernandez, E. J., et al. (2020). Changes in dietary behaviours during the COVID-19 outbreak confinement in the Spanish COVIDiet study. *Nutrients* 12:1730. doi: 10.3390/nu12061730

Roper, S. D. (2014). TRPs in taste and chemesthesis. *Handb. Exp. Pharmacol.* 223, 827–871. doi: 10.1007/978-3-319-05161-1_5

Ryan, K. A., Bewley, K. R., Fotherinngham, S. S., Brown, P., Hall, Y., Marriott, A. C., et al. (2020). Dsoe-dependent response to infection with SARS-CoV-2 in the ferret model: evidence of protection ot re-challenge. *bioRxiv* [preprint] doi: 10.1101/2020.05.29.123810

Schiffman, S. S. (1997). Taste and smell losses in normal aging and disease. *JAMA* 278, 1357–1362. doi: 10.1001/jama.1997.03550160077042

Schiffman, S. S. (2018). Influence of medications on taste and smell. *World J. Otorhinolaryngol. Head Neck Surg.* 4, 84–91. doi: 10.1016/j.wjorl.2018.02.005

Schlottau, K., Rissmann, M., Graaf, A., Schön, J., Sehl, J., Wylezich, C., et al. (2020). SARS-CoV-2 in fruit bats, ferrets, pigs, and chickens: an experimental transmission study. *Lancet Microbe* 1, e218–e225.

Schwartz, M., Menetrier, F., Heydel, J. M., Chavanne, E., Faure, P., Labrousse, M., et al. (2020). Interactions between odorants and glutathione transferases in the human olfactory cleft. *Chem. Senses* 45, 645–654. doi: 10.1093/chemse/bjaa055

Shiwa, N., Kimitsuki, K., Manalo, D. L., Inoue, S., and Park, C. H. (2018). A pathological study of the tongues of rabid dogs in the Philippines. *Arch. Virol.* 163, 1615–1621. doi: 10.1007/s00705-018-3785-y

Sia, S. F., Yan, L. M., Chin, A. W. H., Fung, K., Choy, K. T., Wong, A. Y. L., et al. (2020). Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature* 583, 834–838. doi: 10.1038/s41586-020-2342-5

Simon, S. A., de Araujo, I. E., Gutierrez, R., and Nicolelis, M. A. (2006). The neural mechanisms of gustation: a distributed processing code. *Nat. Rev. Neurosci.* 7, 890–901. doi: 10.1038/nrn2006

Simonnet, A., Chetboun, M., Poissy, J., Raverdy, V., Noulette, J., Duhamel, A., et al. (2020). LICORN and the Lille COVID-19 and obesity study group. high prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-COV-2) requiring invasive mechanical ventilation. *Obesity* 28, 1195–1199. doi: 10.1002/oby.22831

Simpson, S. J., and Raubenheimer, D. (2005). Obesity: the protein leverage hypothesis. *Obesity Rev.* 6, 133–142. doi: 10.1111/j.1467-789x.2005.00178.x

Spetter, M. S., Mars, M., Viergever, M. A., de Graaf, C., and Smeets, P. A. (2014). Taste matters - effects of bypassing oral stimulation on hormone and appetite responses. *Physiol. Behav.* 137, 9–17. doi: 10.1016/j.physbeh.2014.06.021

Sultan, B., May, L. A., and Lane, A. P. (2011). The role of TNF-α in inflammatory olfactory loss. *Laryngoscope* 121, 2481–2486. doi: 10.1002/lary.22190

Sun, S. H., Chen, Q., Gu, H. J., Yang, G., Wang, Y. X., Huang, X. Y., et al. (2020). A mouse model of SARS-CoV-2 infection and pathogenesis. *Cell Host Microbe* 28, 124–133.

Tan, S. Y., and Tucker, R. M. (2019). Sweet taste as a predictor of dietary intake: a systematic review. *Nutrients* 11:94. doi: 10.3390/nu11010094

Tordoff, M. G., Alarcón, L. K., Valmeki, S., and Jiang, P. (2012). T1R3: a human calcium taste receptor. *Sci. Rep.* 2:496. doi: 10.1038/srep00496

Tu, Y. H., Cooper, A. J., Teng, B., Chang, R. B., Artiga, D. J., Turner, H. N., et al. (2018). An evolutionarily conserved gene family encodes proton-selective ion channels. *Science* 359, 1047–1050. doi: 10.1126/science.aao3264

Tucker, R. M., and Mattes, R. D. (2012). Are free fatty acids effective taste stimuli in humans? *J. Food Sci.* 77, S148–S151.

Ventura, A. K., and Worobey, J. (2013). Early influences on the development of food preferences. *Curr. Biol.* 23, R401–R408.

Viana, F. (2011). Chemosensory properties of the trigeminal system. *ACS Chem. Neurosci.* 2, 38–50. doi: 10.1021/cn100102c

von Bartheld, C. S., Hagen, M. M., and Butowt, R. (2020). Prevalence of chemosensory dysfunction in COVID-19 patients: a systematic review and meta-analysis reveals significant ethnic differences. *ACS Chem. Neurosci.* 11, 2944–2961. doi: 10.1021/acschemneuro.0c00460

Wang, H., Zhou, M., Brand, J., and Huang, L. (2007). Inflammation activates the interferon signaling pathways in taste bud cells. *J. Neurosci.* 27, 10703–10713. doi: 10.1523/jneurosci.3102-07.2007

Wang, H., Zhou, M., Brand, J., and Huang, L. (2009). Inflammation and taste disorders: mechanisms in taste buds. *Ann. N. Y. Acad. Sci.* 1170, 596–603. doi: 10.1111/j.1749-6632.2009.04480.x

Wang, Z., Zhou, J., Marshall, B., Rekaya, R., Ye, K., and Liu, H. X. (2020). SARS-CoV-2 Receptor ACE2 is enriched in a subpopulation of mouse tongue epithelial cells in nongustatory papillae but not in taste buds or embryonic oral epithelium. *ACS Pharmacol. Transl. Sci.* 3, 749–758. doi: 10.1021/acsptsci.0c00062

Wijlens, A. G., Erkner, A., Alexander, E., Mars, M., Smeets, P. A., and de Graaf, C. (2012). Effects of oral and gastric stimulation on appetite and energy intake. *Obesity* 20, 2226–2232. doi: 10.1038/oby.2012.131

Ye, Q., Zhou, J., Yang, G., Li, R. T., He, Q., Zhang, Y., et al. (2020). SARS-CoV-2 infection causes transient olfactory dysfunction in mice. *BioRxiv* [preprint] doi: 10.1101/2020.11.10.376673

Zachary, Z., Brianna, F., Brianna, L., Garrett, P., Jade, W., Alyssa, D., et al. (2020). Self-quarantine and weight gain related risk factors during the COVID-19 pandemic. *Obes. Res. Clin. Pract.* 14, 210–216. doi: 10.1016/j.orcp.2020.05.004

Zhang, A. J., Lee, A. C., Chu, H., Chan, J. F., Fan, Z., Li, C., et al. (2020). SARS-CoV-2 infects and damages the mature and immature olfactory sensory neurons of hamsters. *Clin. Infect. Dis.* doi: 10.1093/cid/ciaa995 Online ahead of print.

Zheng, J., Wong, L. R., Li, K., Verma, A. K., Ortiz, M., Wohlford-Lenane, C., et al. (2020). COVID-19 treatments and pathogenesis including anosmia in K18-haCE2 mice. *Nature* doi: 10.1038/41586-020-2943-z Online ahead of print.

Ziegler, C. G. K., Allon, S. J., Nyquist, S. K., Mbano, I. M., Miao, V. N., Tzouanas, C. N., et al. (2020). SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. *Cell* 181, 1016–1035. doi: 10.1016/j.cell.2020.04.035

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 28 January 2021
doi: 10.3389/fphys.2021.593223

Pathogenesis of Multiple Organ Injury in COVID-19 and Potential Therapeutic Strategies

Miquéias Lopes-Pacheco[1], Pedro Leme Silva[2,3,4,5,6,7], Fernanda Ferreira Cruz[2,3,4,5,6,7], Denise Battaglini[8], Chiara Robba[8], Paolo Pelosi[8,9], Marcelo Marcos Morales[3,4,5,7,10], Celso Caruso Neves[3,4,6,7,11] and Patricia Rieken Macedo Rocco[2,3,4,5,6,7]**

[1] Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal, [2] Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [3] National Institute of Science and Technology for Regenerative Medicine, Rio de Janeiro, Brazil, [4] Rio de Janeiro Innovation Network in Nanosystems for Health-NanoSAÚDE/FAPERJ, Rio de Janeiro, Brazil, [5] COVID-19 Virus Network, Ministry of Science, Technology and Innovation, Brasília, Brazil, [6] COVID-19 Virus Network, Brazilian Council for Scientific and Technological Development, Brasília, Brazil, [7] COVID-19 Virus Network, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ, Rio de Janeiro, Brazil, [8] Anesthesia and Intensive Care, San Martino Policlinico Hospital, IRCCS for Oncology and Neuroscience, Genoa, Italy, [9] Department of Surgical Sciences and Integrated Diagnostic, University of Genoa, Genoa, Italy, [10] Laboratory of Cellular and Molecular Physiology, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [11] Laboratory of Biochemistry and Cell Signaling, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

OPEN ACCESS

Edited by:
Ravi Nistala,
University of Missouri, United States

Reviewed by:
Massimo Volpe,
Sapienza University of Rome, Italy
Savneet Kaur,
The Institute of Liver and Biliary Sciences (ILBS), India

***Correspondence:**
Miquéias Lopes-Pacheco
mlopes0811@gmail.com
Patricia Rieken Macedo Rocco
prmrocco@gmail.com

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *11 August 2020*
Accepted: *08 January 2021*
Published: *28 January 2021*

Citation:
Lopes-Pacheco M, Silva PL, Cruz FF, Battaglini D, Robba C, Pelosi P, Morales MM, Caruso Neves C and Rocco PRM (2021) Pathogenesis of Multiple Organ Injury in COVID-19 and Potential Therapeutic Strategies. Front. Physiol. 12:593223. doi: 10.3389/fphys.2021.593223

Severe acute respiratory disease coronavirus 2 (SARS-CoV-2, formerly 2019-nCoV) is a novel coronavirus that has rapidly disseminated worldwide, causing the coronavirus disease 2019 (COVID-19) pandemic. As of January 6th, 2021, there were over 86 million global confirmed cases, and the disease has claimed over 1.87 million lives (a ∼2.2% case fatality rate). SARS-CoV-2 is able to infect human cells by binding its spike (S) protein to angiotensin-conversing enzyme 2 (ACE2), which is expressed abundantly in several cell types and tissues. ACE2 has extensive biological activities as a component of the renin-angiotensin-aldosterone system (RAAS) and plays a pivotal role as counter-regulator of angiotensin II (Ang II) activity by converting the latter to Ang (1-7). Virion binding to ACE2 for host cell entry leads to internalization of both via endocytosis, as well as activation of ADAM17/TACE, resulting in downregulation of ACE2 and loss of its protective actions in the lungs and other organs. Although COVID-19 was initially described as a purely respiratory disease, it is now known that infected individuals can rapidly progress to a multiple organ dysfunction syndrome. In fact, all human structures that express ACE2 are susceptible to SARS-CoV-2 infection and/or to the downstream effects of reduced ACE2 levels, namely systemic inflammation and injury. In this review, we aim to summarize the major features of SARS-CoV-2 biology and the current understanding of COVID-19 pathogenesis, as well as its clinical repercussions in the lung, heart, kidney, bowel, liver, and brain. We also highlight potential therapeutic targets and current global efforts to identify safe and effective therapies against this life-threatening condition.

Keywords: ACE2, coronavirus, lung, multiple organ dysfunction, pathophysiology, SARS-CoV-2, therapy, viral infection

INTRODUCTION

In late 2019, a cluster of infections by a novel coronavirus – the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, formerly 2019-nCoV) – was epidemiologically linked to a large seafood market in Wuhan, China (Wang C. et al., 2020; Zhou P. et al., 2020). This outbreak has rapidly disseminated domestically and internationally, becoming a global pandemic[1]. SARS-CoV-2 has demonstrated to be much more infectious than SARS-CoV and Middle East Respiratory Syndrome coronavirus (MERS-CoV), which were responsible for two previous large-scale outbreaks (**Table 1**). As of January 6th, 2020, there were over 86 million global confirmed cases of coronavirus disease 2019 (COVID-19) and over 1.87 million fatalities[2][3].

Human coronaviruses usually attach to cell-surface ectopeptidases, such as dipeptidyl peptidase 4 (DPP4), aminopeptidase N, and angiotensin-conversing enzyme 2 (ACE2), for host cell entry (Fehr and Perlman, 2015; Cui et al., 2019). Both SARS-CoV and SARS-CoV-2 were found to use ACE2, which has extensive biological activities (Santos et al., 2018), as their key cell entry receptor (Hoffmann et al., 2020a; Walls et al., 2020; Zhou P. et al., 2020). The distribution of ACE2 in the host organism appears to be an important factor associated with organ injury (**Figure 1**). There is also evidence that individual variation in expression and/or polymorphisms in ACE2 may influence susceptibility to SARS-CoV-2 infection and COVID-19 phenotype (Calcagnile et al., 2020; Cao et al., 2020a; Chen J. et al., 2020; Devaux et al., 2020; Hou et al., 2020; Hussain et al., 2020; Leung et al., 2020).

The clinical spectrum of COVID-19 is very heterogeneous (Williamson et al., 2020). Many individuals infected with SARS-CoV-2 are asymptomatic or develop a mild illness with non-specific symptoms, such as fever, fatigue, dry cough, and headache (Huang C. et al., 2020; Sakurai et al., 2020; Wang C. et al., 2020). However, ∼20% of individuals require hospitalization, and ∼25% of these (∼5% of all cases) experience a rapid progression of their symptoms to severe pneumonia/acute respiratory distress syndrome (ARDS), requiring invasive mechanical ventilation (Guan et al., 2020; Huang C. et al., 2020; Wang D. et al., 2020). Individuals with a more severe phenotype usually have a high viral load and long virus-shedding period (He et al., 2020; Liu et al., 2020b). Some risk factors for the development of severe COVID-19 and poor prognosis include advanced age and presence of certain comorbidities, such as chronic obstructive pulmonary disease, coronary heart disease, diabetes mellitus, and hypertension (Guan et al., 2020; Leung et al., 2020; Wu and McGoogan, 2020; Williamson et al., 2020; Zhou F. et al., 2020), although the latter remains controversial (Iaccarino et al., 2020). Multivariable parameters such as higher sequential organ failure assessment score and D-dimer >1 μg/mL on hospital admission have also been associated with higher risk of fatality (Chen N. et al., 2020; Guan et al., 2020; Tang et al., 2020; Zhou F. et al., 2020).

Although SARS-CoV-2 infection was initially described as causing severe respiratory disease, it is now known that infected individuals can rapidly progress to a multiple organ dysfunction syndrome (MODS); therefore, multi-target therapeutic approaches are warranted, and a wide range of distinct therapeutic protocols have been investigated (Gupta et al., 2020; Li H. et al., 2020b; Robba et al., 2020a,b; Zhang et al., 2020b). In this review, we summarize major features of SARS-CoV-2 biology and the current understanding of COVID-19 pathogenesis, as well as its clinical repercussions in the lung, heart, kidney, bowel, liver, and brain. We also shed light on potential therapeutic targets and the current global efforts to identify effective therapies against this devastating condition.

STRUCTURE OF SARS-CoV-2 AND HOST CELL INFECTION

The SARS-CoV-2 Genome

Coronaviruses are named for the crown-shaped spikes on their outer surface. The novel coronavirus (SARS-CoV-2) is an enveloped, 29.9 kb-long, positive-sense, single-stranded RNA virus belonging to the β-coronavirus genus (**Figure 2A**). The open-reading frames (ORFs) 1a and 1b represent ∼70% of the complete viral genome and possess several conserved non-structural protein sequences (Chan et al., 2020; Kim et al., 2020; Wu A. et al., 2020). A frameshift between these ORFs encodes two polypeptides (1a and 1ab) that are processed by viral proteases to produce non-structural proteins, which are involved in viral replication and suppression of host innate immune defenses (Chan et al., 2020; Wu A. et al., 2020). Among all known coronavirus sequences, SARS-CoV-2 shares the highest genetic similarity with the bat coronavirus RATG13 (∼96%) and the Malayan pangolin coronavirus (∼91%), although it also has considerable genetic similarity with the human coronaviruses SARS-CoV (∼79%) and MERS-CoV (∼50%) (Andersen et al., 2020; Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020; Lam et al., 2020; Zhang T. et al., 2020).

The SARS-CoV-2 Structure

The structure of SARS-CoV-2 is composed of four main structural proteins: the spike, envelope, and membrane glycoproteins, and the nucleocapsid protein (**Figure 2B**). The nucleocapsid is a phosphorylated protein which consists of the structure that directly binds to viral RNA and plays multiple critical roles during the viral life cycle. The envelope glycoprotein is a small, integral membrane structure involved in the maturation and pathogenesis of coronaviruses. The membrane glycoprotein is the most abundant component of the virus structure and plays a central role in viral assembly by interconnecting with all main structural proteins of the viral particle. This protein also delineates the shape of the viral envelope (Fehr and Perlman, 2015; Cui et al., 2019).

The spike glycoprotein is a transmembrane structure present in the outer surface of the viral particle (**Figure 2C**). It has

[1]WHO Director-General's opening remarks at the media briefing on COVID-19 – 11 March 2020: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020

[2]WHO Coronavirus Disease (COVID-19) Dashboard: https://covid19.who.int/

[3]Johns Hopkins University & Medicine – Coronavirus Resource Center: https://coronavirus.jhu.edu/map.html

TABLE 1 | Main features of SARS, MERS, and COVID-19.

Disease/Outbreak	SARS (2002)	MERS (2012)	COVID-19 (2019)
Virology			
Etiological agent	SARS-CoV	MERS-CoV	SARS-CoV-2
Genome size (kb)	30.1	27.9	29.9
Natural reservoir	Bats	Bats	Bats
Intermediate host	Civet cats/Raccoon dogs	Camels	Pangolins (?)
Attachment receptor	ACE2	DPP4	ACE2
Spike protein priming	TMPRSS2	–	TMPRSS2
Basic reproduction number (R_0)	2.2-3.7	0.3-1.3	2.2-6.4
Epidemiology			
First cases	Guangdong, China	Jeddah, Saudi Arabia	Wuhan, China
Main route of transmission	Airborne	Airborne	Airborne
Incubation period (days after infection)	2-10	2-15	2-14
Peak viral load (days after symptom onset)	∼10	7-10	3-7
Hospitalization rate	Most cases	Most cases	∼20%
Cases	8,096	2,494	> 86,000,000*
Deaths (case fatality rate)	744 (9.2%)	858 (34%)	>1,870,000 (∼2.2%)*
Clinical features and management			
Common symptoms	Fever, dry cough, dyspnea, shortness of breath, fatigue	Fever, dry cough, dyspnea, shortness of breath, fatigue	Fever, dry cough, dyspnea, shortness of breath, fatigue
Common laboratory findings	Abnormalities in coagulation and blood cell counts, cytokine storm, increased levels of transaminases and C-reactive protein	Abnormalities in coagulation and blood cell counts, cytokine storm, increased levels of transaminases and C-reactive protein	Abnormalities in coagulation and blood cell counts, cytokine storm, increased levels of transaminases and C-reactive protein
Chest computed tomography findings	Multiple, focal, ground-glass opacities, atelectasis or bilateral patchy consolidations in lungs	Multiple, focal, ground-glass opacities, atelectasis or bilateral patchy consolidations in lungs	Multiple, focal, ground-glass opacities, atelectasis or bilateral patchy consolidations in lungs
Complications	ARDS, renal failure, sepsis or septic shock	ARDS, renal failure, sepsis or septic shock	ARDS, renal failure, sepsis or septic shock
Therapeutic approach	Early intensive care and supportive monitoring	Early intensive care and supportive monitoring	Early intensive care and supportive monitoring
Vaccine or specific antiviral available	No	No	Multiple vaccines are receiving interim or conditional approval for emergency use in different countries (refer to **Table 2**)

**As of January 6th, 2021.*

two subunits (S1 and S2) that are cleaved by the host cell proteases. Interestingly, the furin protease-like cleavage spot is present in SARS-CoV-2, MERS-CoV, and human coronavirus OC43, but absent in SARS-CoV (Andersen et al., 2020; Coutard et al., 2020; Wrapp et al., 2020). The S1 subunit consists of an N-terminal domain and a receptor-binding domain (RBD), which determine host range and cellular tropism of the virus. This subunit is released during the fusion process, thus inducing a conformational change in the S2 subunit, which is the viral membrane-anchored fraction and consists of a hydrophobic fusion peptide and two heptad repeated domains (Cui et al., 2019; Wu A. et al., 2020).

The Host Cell Receptor

ACE2 is a zinc-dependent carboxypeptidase and component of the renin-angiotensin-aldosterone system (RAAS), a complex network that plays a pivotal role in maintaining fluid and electrolyte homeostasis, thus affecting function of multiple organs (**Figure 3**). Briefly, the protease renin cleaves angiotensinogen to angiotensin I (Ang I), which is subsequently cleaved to Ang II by ACE. Ang II can bind to angiotensin type 1 receptors (AT1-R) and induce vasoconstriction as well as pro-inflammatory, oxidant, and fibrotic responses (Forrester et al., 2018; Danser et al., 2020). On the other hand, ACE2 can convert Ang II to Ang (1-7), which binds to the Mas receptor and counteracts the aforementioned actions mediated by Ang II/AT1-R. ACE2 can also convert Ang I to Ang (1-9), which is subsequently cleaved to Ang (1-7) by ACE. However, the former pathway is more common due to higher affinity between ACE and Ang I (Santos et al., 2018; Gheblawi et al., 2020).

SARS-CoV-2 Infection and Replication

SARS-CoV-2 RBD attaches to ACE2 for host cell entry (**Figure 4**), a proteolytic process that is facilitated by TMPRSS2 priming and involves cathepsin L (Hoffmann et al., 2020a; Ou et al., 2020; Wrapp et al., 2020; Zhou P. et al., 2020). This interaction

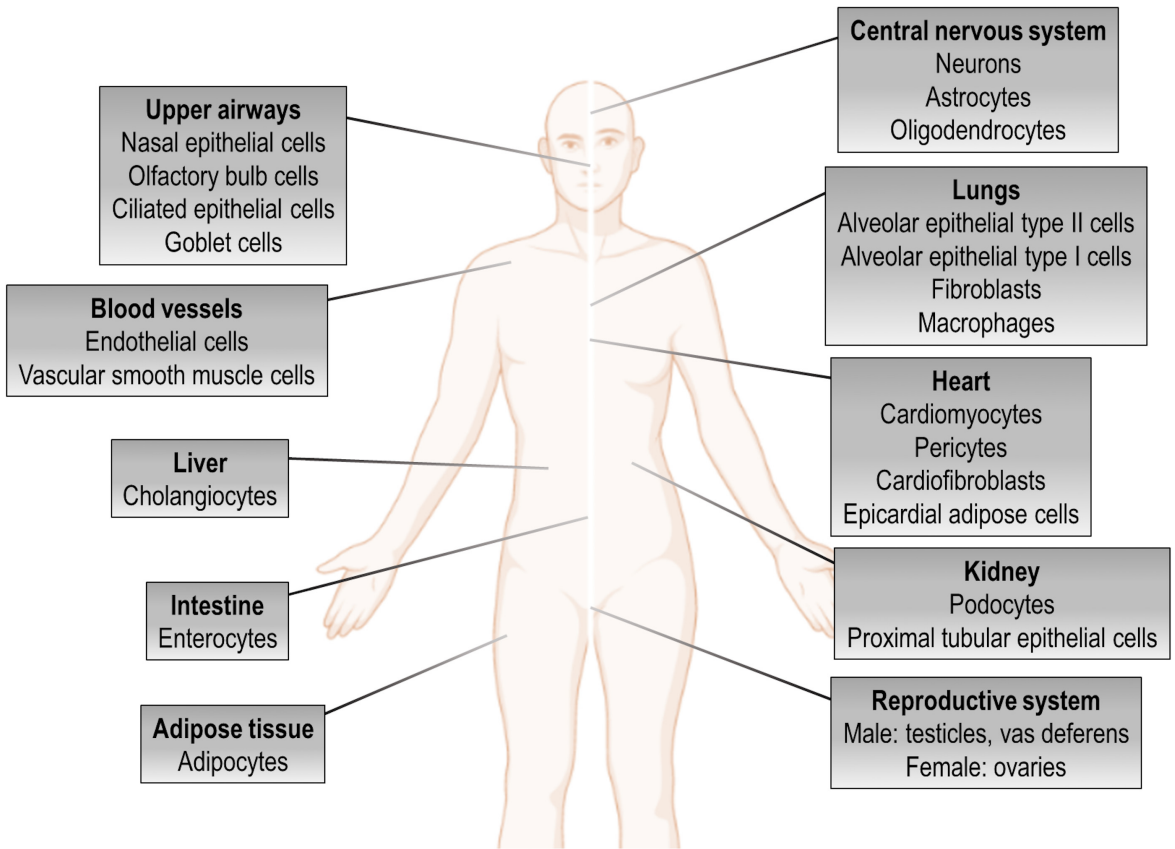


FIGURE 1 | ACE2 expression in the human body. Tissue distribution of angiotensin-converting enzyme 2 (ACE2) expression and potential targets for direct cytotoxicity induced by SARS-CoV-2 infection.

then triggers endocytosis of ACE2 together with the SARS-CoV-2 virion and fusion of the viral membrane and host cell. Interestingly, SARS-CoV-2 not only reduces surface tissue expression of ACE2, but also inhibits its messenger RNA expression after infection (Kuba et al., 2005; Walls et al., 2020). Virus-host receptor interactions also activate ADAM17/TACE, a disintegrin and metalloprotease component, which leads to cleavage and shedding of ACE2 from the cell surface and local production of hyaluronan (Haga et al., 2010; Xu et al., 2017). Concomitantly, the viral spike protein is exposed to endosomal cysteine proteases that lead to its cleavage at two different sites: the first removes the S1 subunit, while the second occurs within the S2 subunit and results in exposure of the fusion peptide (Wan et al., 2020; Walls et al., 2020). Neutrophil elastase was recently found to exert an important role in SARS-CoV-2 infection, as it possesses a cleavage site near the S1-S2 subunits (Bhattacharyya et al., 2020). The viral package is released into the host cytoplasm, where it usurps the cellular machinery to produce new viral particles. As SARS-CoV-2 is a single-stranded RNA virus, its own genetic material serves as messenger RNA, thus driving the synthesis of viral proteins by host cell ribosomes. The uncoated viral RNA encodes the 1a and 1ab polypeptides, which are processed into non-structural proteins. These form a complex with viral genomic RNA to continuously synthesize subgenomic products that encode structural proteins (Jiang et al., 2020). TMPRSS2 also appears to participate in the SARS-CoV-2 replication process, although the mechanisms are unclear (Matsuyama et al., 2020). In fact, protein-protein mapping has identified 332 high-confidence SARS-CoV-2/human protein-protein interactions involved with the virus life cycle (Gordon D.E. et al., 2020). Once all viral components are produced and assembled in the endoplasmic reticulum–Golgi intermediate compartment (ERGIC), the new viruses are released via exocytosis into the extracellular compartment (Jiang et al., 2020).

PATHOGENESIS AND MULTIPLE ORGAN INJURY IN COVID-19

Many features of virus-host interactions involving SARS-CoV-2 remain unknown, but several of these have been demonstrated to recapitulate the infection process of other human coronaviruses. COVID-19 development and progression consist of five major pathological mechanisms (**Figure 5**): (1) direct virus-induced cytotoxicity in ACE2-expressing cells; (2) dysregulation of the RAAS as a result of virus-mediated ACE2 downregulation; (3) dysregulation of immune responses; (4) endothelial cell

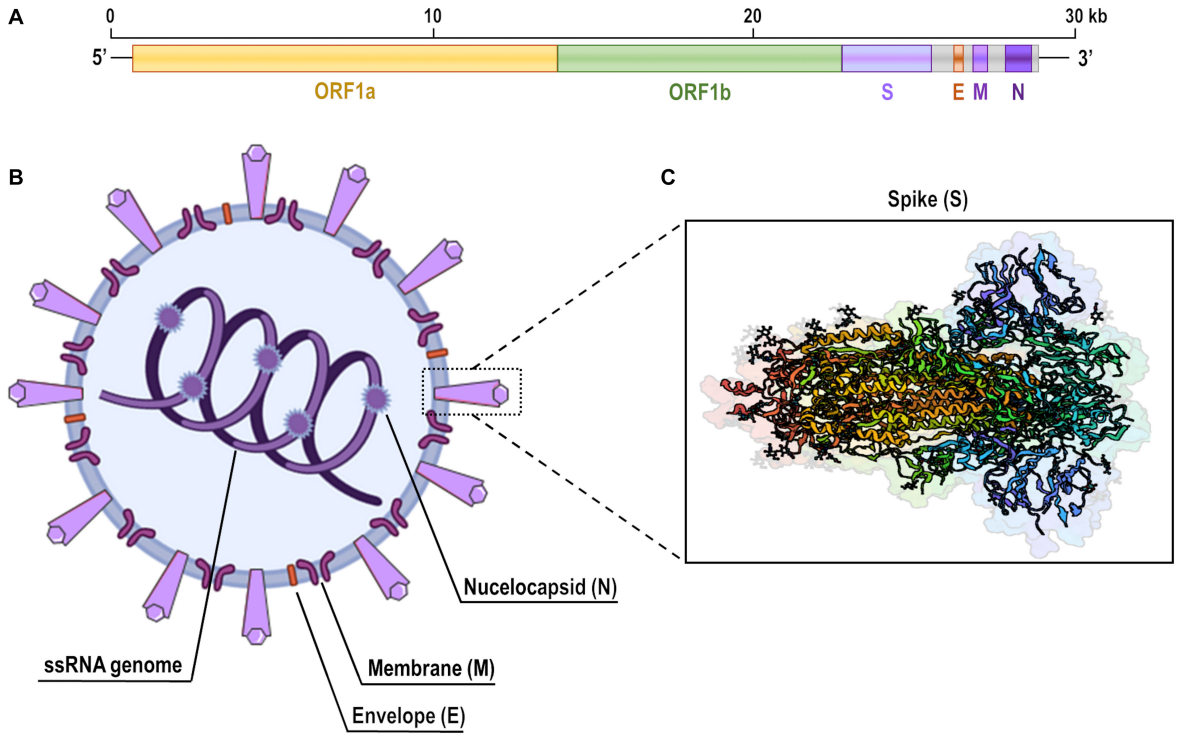


FIGURE 2 | Genome and structure of SARS-CoV-2. **(A)** Full-length single-strand (ss)RNA genome of SARS-CoV-2 demonstrating open-reading frames (ORFs) 1a and 1b, which encode non-structural proteins, and the position of genes that encode main structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N). **(B)** Schematic representation of a SARS-CoV-2 viral particle. **(C)** Ribbon diagram of the open conformation of SARS-CoV-2 spike protein. This structure has been deposited on the Protein Data Bank under accession number 6VYB (SARS-CoV-2 spike ectodomain structure).

injury and thrombo-inflammation; and (5) tissue fibrosis (Gupta et al., 2020).

The lungs possess several features that facilitate their role as an initial reservoir for viral replication and human-to-human transmission. Lung tissue has a large surface area, making it highly susceptible to inhaled viruses. It is also highly vascularized, which allows rapid dissemination of viral particles to other organs. Furthermore, ACE2 is expressed in several pulmonary cell types (Hamming et al., 2004), with alveolar epithelial type II cells being the major ACE2-expressing cells (Zhao Y. et al., 2020). Gene ontology enrichment analysis has demonstrated that alveolar epithelial type II cells express multiple viral life cycle-associated functional genes, including those related to virus internalization, genome replication, assembly, and transmission (Zhao Y. et al., 2020).

The production and exocytosis of new viral particles can induce the host cell to undergo pyroptosis and release damage-associated molecular pattern signals. In lung tissue, these are recognized by adjacent epithelial and endothelial cells that are primed to activate certain transcription factors, such as nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF3), to produce and secrete pro-inflammatory mediators (Li G. et al., 2020). Concomitantly, secretion of type I interferons occurs and induces antiviral actions by multiple mechanisms. In an immunocompetent response, these initial inflammatory signals are recognized by antigen-presenting cells—such as resident macrophages and dendritic cells—that present the foreign antigen to $CD4^+$ T-cells, which then prime other $CD4^+$ T-cells, $CD8^+$ T-cells, and B-cells (Channappanavar et al., 2014). $CD8^+$ T-cells are cytotoxic and directly kill virus-infected cells, whereas B-cells produce neutralizing antibodies against the nucleocapsid and spike protein. Phagocytes remove apoptotic cells and neutralized viruses, resulting in well-coordinated clearance of infection with recovery and minimal lung tissue injury (Channappanavar et al., 2014; Li G. et al., 2020). On the other hand, during a faulty immune response occurs an intense inflammation with inefficient virus clearance, as well as overactivation of innate immunity with overproduction of pro-inflammatory cytokines and chemokines, including interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and others. This uncontrolled inflammatory process leads to alveolar-capillary barrier disruption; viral particles, along with the emerging cytokine storm, can then circulate to other organs, resulting in multiple organ dysfunction (Zhang et al., 2020b). Neutrophil extravasation into the alveolar space, hyaline membrane formation, and acute capillaritis are major features observed in histopathological analysis of lung cells in cases of severe COVID-19 (Barnes et al., 2020; Fox et al., 2020; Tian S. et al., 2020; BR236). The thromboembolic events observed in severe COVID-19 cases may also be associated with the inflammatory response triggered by the host-virus interaction, including activation of the coagulation cascade, formation of

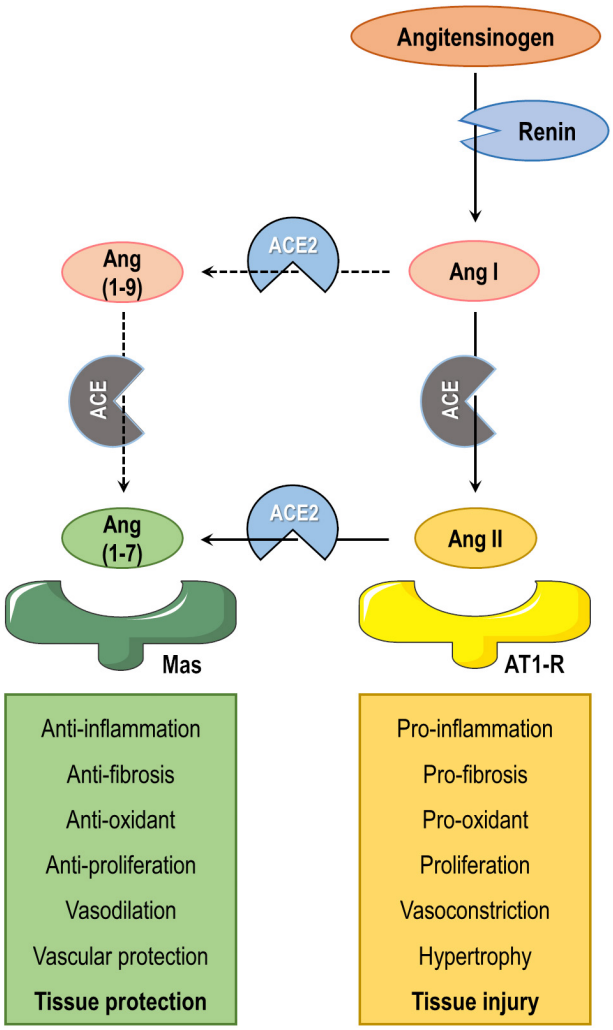


FIGURE 3 | Simplified diagram of the renin-angiotensin-aldosterone system. Angiotensinogen is cleaved by renin to angiotensin I (Ang I). Ang I is then converted by angiotensin-converting enzyme (ACE) to Ang II, which interacts with the AT1 receptor (AT1-R), or is cleaved by ACE2 to Ang (1-7), which interacts with Mas receptor. The ACE2/Ang (1-7)/Mas axis exerts counter-regulatory effects to the ACE/Ang II/AT1-R axis in multiple organs. ACE2 can also convert Ang I to Ang (1-9), which is then cleaved to Ang (1-7) by ACE. However, ACE2 converts Ang II to Ang (1-7) with higher efficiency than it converts Ang I to Ang (1-9).

neutrophil extracellular traps (NETs), and leakage of fluid in the subendothelial compartment (Barnes et al., 2020; Helms et al., 2020; Tang et al., 2020; Varga et al., 2020).

The heterogeneous clinical manifestations in COVID-19 may be attributed to individual variation in expression not only of ACE2 and TMPRSS2 receptors, but also of genes related to inflammation and immune responses (Elhabyan et al., 2020; Ellinghaus et al., 2020; Russo et al., 2020; Zhao J. et al., 2020). Accordingly, the excessive acute inflammatory responses seen in individuals with severe COVID-19 may lead to MODS and death (Li H. et al., 2020b; Wang D. et al., 2020). Compared to healthy individuals, those infected with SARS-CoV-2 have demonstrated increased serum concentrations of many pro-inflammatory mediators, including IL-1β, IL-2, IL-6, IL-7, IL-8, interferon (IFN)-γ-induced protein 10 (IP-10), granulocyte colony-stimulating factor (G-CFS), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein (MIP)-1α, platelet-derived growth factor (PDGF), TNF-α, vascular endothelial growth factor (VEGF), and others (Ackermann et al., 2020; Chen N. et al., 2020; Huang C. et al., 2020; Liu J. et al., 2020; Zhang et al., 2020b). This cytokine storm is even more evident in individuals admitted to the intensive care unit (ICU), as demonstrated by greater increases in serum concentration of several of these pro-inflammatory mediators, which suggests a correlation with disease severity (Chen N. et al., 2020; Huang C. et al., 2020; Liu J. et al., 2020; Zhang et al., 2020b). Aberrant pathogenic $CD4^+$ T-cells co-expressing IFN-γ and G-CFS have also been observed in severe COVID-19 (Zhou Y. et al., 2020). A high neutrophil-to-lymphocyte ratio has also been reported as a risk factor for mortality in hospitalized individuals with COVID-19 (Liu et al., 2020a). Nevertheless, individuals with severe COVID-19 may also present with lymphopenia, characterized by a significant reduction in peripheral $CD4^+$ T-cell and $CD8^+$ T-cell counts. Such countervailing effects may prevent complete clearance of SARS-CoV-2 or lead to secondary infections by opportunistic pathogens, thus resulting in a sepsis-like state (Guan et al., 2020; Liu J. et al., 2020; Zhou F. et al., 2020). Below, we summarize the main features of SARS-CoV-2 infection and its potential repercussions on multiple organs (**Figure 6**).

Respiratory System

The lung is undoubtedly the organ most vulnerable to, and most affected by, SARS-CoV-2 infection. Accordingly, severe pneumonia is the most common and serious clinical manifestation observed in severe COVID-19 cases (Huang C. et al., 2020; Zhou Y. et al., 2020). As ACE2 is expressed in various cell types along the respiratory tract, SARS-CoV-2 may either enter through mucosal membranes in the upper respiratory tract or directly infect bronchial and alveolar epithelial cells in the lower respiratory tract (Sungnak et al., 2020; Wölfel et al., 2020). Interestingly, nasal expression of ACE2 was found to be lower in children compared to adults, which might partially explain age-related differences in the risk of developing COVID-19 (Bunyavanich et al., 2020; Sharif-Askari et al., 2020). SARS-CoV-2 RNA has also been detected in the sputum of infected individuals before the onset of clinical symptoms (Zhang W. et al., 2020), and in some cases, even 2 weeks after recovery (Lauer et al., 2020; Wölfel et al., 2020). Current and former smokers, as well as individuals with chronic obstructive pulmonary disease, overexpress ACE2 in airway cells compared to non-smokers and healthy individuals, which may explain at least partly the increased risk of severe COVID-19 in these individuals (Cai, 2020; Leung et al., 2020; Sharif-Askari et al., 2020).

ACE2 acts as a double-edged sword in both SARS-CoV and SARS-CoV-2 infection; it not only serves as the functional receptor for virus entry into host cells, but also has a pivotal role in protecting lung tissue from injury by counter-regulating the vasoconstrictive, pro-inflammatory, and pro-fibrotic effects of Ang II on pulmonary vascular and

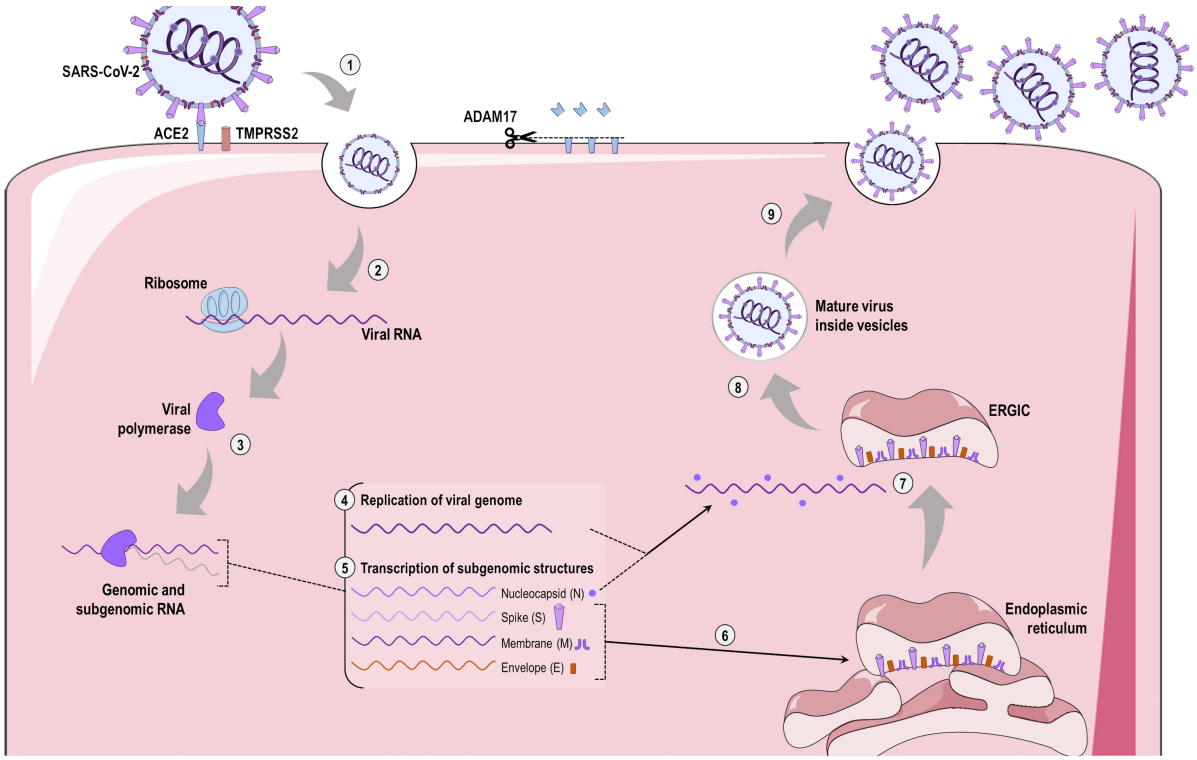


FIGURE 4 | SARS-CoV-2 infection and replication. **(1)** SARS-CoV-2 spike protein is primed by the type II transmembrane serine protease (TMPRSS2) and interacts with the angiotensin-converting enzyme 2 (ACE2) expressed on the surface of host cells, allowing binding and entry of the virus via clathrin-dependent endocytosis. Concomitantly, ADAM17 is activated and leads to cleavage of ACE2 in its soluble form. **(2)** The SARS-CoV-2 virion uses host ribosomes to produce viral RNA-dependent RNA polymerase **(3)**. This viral polymerase then initiates replication of the SARS-CoV-2 genome **(4)** and transcription of subgenomic structures **(5)**. The spike, membrane, and envelope subgenomic transcripts use the endoplasmic reticulum for translation of these viral structural proteins **(6)**, while the viral genome joins the nucleocapsid. **(7)** All viral structures are exported to the endoplasmic-reticulum-Golgi intermediated compartment (ERGIC), where new viral particles are assembled. **(8)** After the formation of mature virions, these are exported inside Golgi vesicles to the extracellular compartment via exocytosis. **(9)** These new viruses are now able to infect adjacent ACE2-expressing cells or enter the bloodstream and infect other tissues.

epithelial cells (Kuba et al., 2005; Uhal et al., 2011). Virus-receptor interactions lead to virion-ACE2 internalization (Wang et al., 2008) or cleavage and shedding of ACE2 (Haga et al., 2010; Xu et al., 2017), thus reducing its expression while promoting accumulation of Ang II, production of TNF-α and IL-6 receptor, and activation of macrophages to a pro-inflammatory state (Jia et al., 2009; Glowacha et al., 2010; Haga et al., 2010; Gheblawi et al., 2020). Furthermore, the virus nucleocapsid protein may interact with Smad3 to prevent apoptosis of infected host cells, while promoting transforming growth factor (TGF)-β-mediated tissue fibrosis (Zhao et al., 2008). As alveolar epithelial type II cells are self-renewing and express high levels of ACE2, they may be continuously targeted for viral entry and replication, which induces a vicious cycle of tissue injury and repair that may ultimately result in replacement of gas-exchange areas to non-functional fibrotic tissue. Lung stem/progenitor cells also express ACE2, and active virus replication in these cells may impair lung tissue repair (Ling et al., 2006).

In addition to the pivotal role of ACE2 in COVID-19 pathogenesis, a genome-wide association analysis identified other host genetic factors that may contribute to the development of COVID-19–induced respiratory failure. Although there were no single nucleotide polymorphism (SNP) associations in the human leukocyte antigen (HLA) complex, SNPs in a cluster of six genes on chromosome 3p21.31 (*SLC6A20, ZTFL1, CCR9, FYCO1, CXCR6, and XCR1*) were found to be potentially influential (Ellinghaus et al., 2020). Furthermore, ABO blood group has been suggested to be involved in susceptibility to SARS (Cheng et al., 2005a) and COVID-19 (Ellinghaus et al., 2020; Zhao J. et al., 2020; Zietz and Tatonetti, 2020).

Lung histopathological findings have demonstrated considerable similarities among ARDS, SARS, and COVID-19 (Tian S. et al., 2020; Xu Z. et al., 2020; Zhang H. et al., 2020). These consist of an increased neutrophil and mononuclear cell count, diffuse alveolar injury with proteinaceous alveolar exudates, and hyperplasia of epithelial type II cells. In more severe lung injury, thickened alveolar septa, hyaline membrane formation, and thrombus formation have been observed, as well as consolidation with fibroblast proliferation and fibrosis. Multinucleated giant cells in alveoli have also been found in some cases (Tian S. et al., 2020; Xu Z. et al., 2020; Zhang H. et al., 2020). An increased rate of deep venous thrombosis and pulmonary embolism was found in COVID-19 cases admitted to the ICU, despite prophylactic use of anticoagulation agents

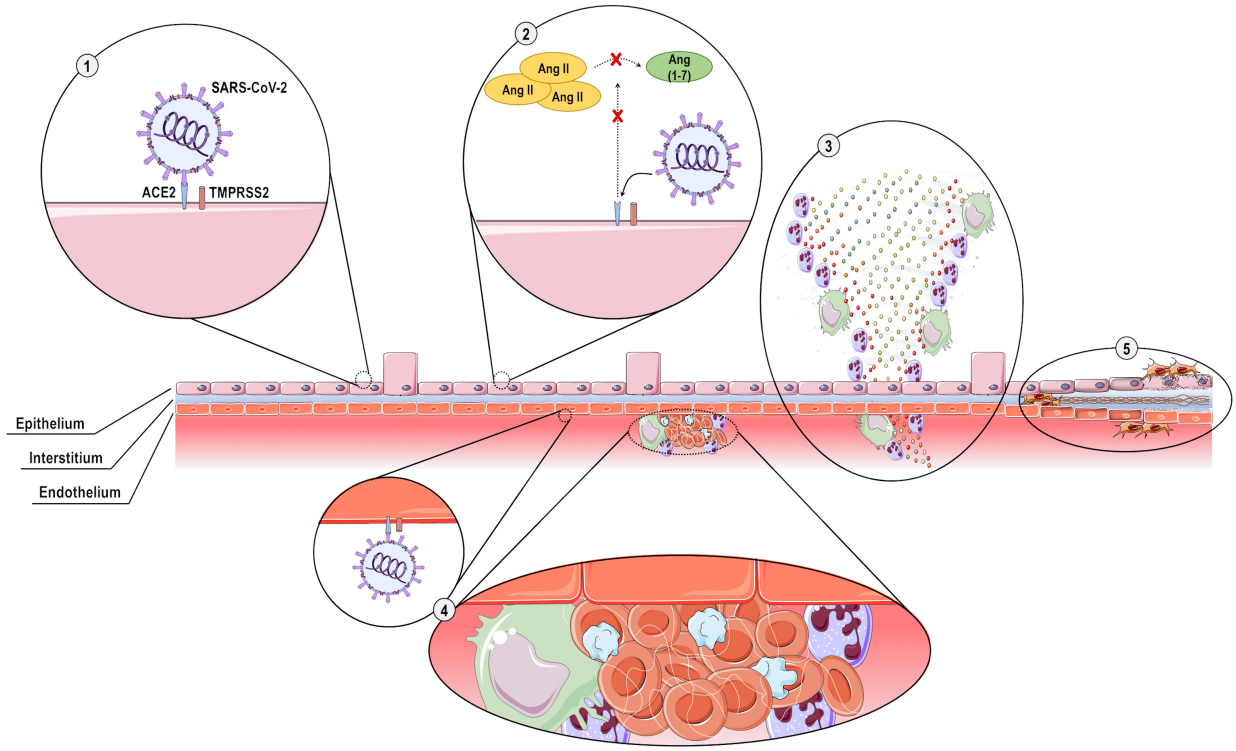


FIGURE 5 | Pathogenesis of COVID-19. Five main pathological mechanisms are implicated in COVID-19 development and progression: **(1)** SARS-CoV-2 spike protein is primed by the type II transmembrane serine protease (TMPRSS2) and interacts with angiotensin-converting enzyme 2 (ACE2) expressed on the cell surface of epithelial cells, inducing direct cytotoxicity; **(2)** The interaction of SARS-CoV-2 with ACE2 causes downregulation of ACE2 expression, thus preventing cleavage of angiotensin II (Ang II) to Ang (1-7) and resulting in dysregulation of the renin-angiotensin-aldosterone system; **(3)** excessive acute inflammatory responses are elicited, with overproduction of pro-inflammatory cytokines and chemokines; **(4)** SARS-CoV-2 exerts direct cytotoxic effects on endothelial cells, leading to recruitment of pro-inflammatory cells and activation of the coagulation cascade, with intravascular thrombus formation; and **(5)** extensive tissue destruction with interstitial thickening, fibroblast proliferation, and fibrosis.

(Helms et al., 2020; Klok et al., 2020; Lodigiani et al., 2020); this can rapidly worsen lung function and lead to respiratory failure.

Based on chest computed tomography findings, COVID-19–induced lung impairment can be grouped into three main phenotypes: (1) multiple, focal, potently overperfused ground-glass opacities; (2) inhomogeneously dispersed atelectasis; and (3) typical moderate-to-severe ARDS, with alveolar edema and low compliance (Robba et al., 2020a). As these phenotypes may be related to different pathological mechanisms and disease progression, personalized mechanical ventilation approaches should be implemented in order to allow more efficient clinical recovery for each individual.

Circulatory System

Coagulation dysfunction has been found in a high proportion of COVID-19 cases, as evidenced by increasing D-dimer levels and prolonged prothrombin time, as well as overt thrombotic manifestations (Guan et al., 2020; Helms et al., 2020; Huang C. et al., 2020; Tang et al., 2020; Wang D. et al., 2020). SARS-CoV-2 RNA has been detected in blood specimens of individuals with COVID-19 (Huang C. et al., 2020; Wölfel et al., 2020); as ACE2 is highly expressed in smooth muscle as well as arterial and venous endothelium in virtually all organs, these tissues can be directly targeted by SARS-CoV-2, with induction of endothelial dysfunction (Hamming et al., 2004; Ackermann et al., 2020; Varga et al., 2020). COVID-19–associated hypoxia resulting from pulmonary dysfunction leads to reduced blood flow and vasoconstriction, which also contribute to endothelial dysfunction (Helms et al., 2020; Levi et al., 2020; Varga et al., 2020). Furthermore, the excessive production of pro-inflammatory mediators leads to an imbalance between the amount of pro- vs. anti-coagulant factors and induction of platelet aggregation (Tang et al., 2020; Zuo et al., 2020). An increase in levels of thrombin, tissue factor V and VIII, and fibrinogen, alongside NET formation, results in a hypercoagulable state with increased risk of systemic macro- and micro-thrombosis (Barnes et al., 2020; Levi et al., 2020; Tang et al., 2020; Zuo et al., 2020). Indeed, fibrinous exudates and thrombi have been observed in histopathological specimens obtained from individuals with COVID-19 (Ackermann et al., 2020; Tian S. et al., 2020; Zhang H. et al., 2020). Therefore, laboratory monitoring of D-dimer levels, fibrinogen, platelet count and prothrombin time is crucial in all hospitalized and severe cases of COVID-19.

An increased incidence of cardiovascular complications has been observed in severe COVID-19, with a high incidence

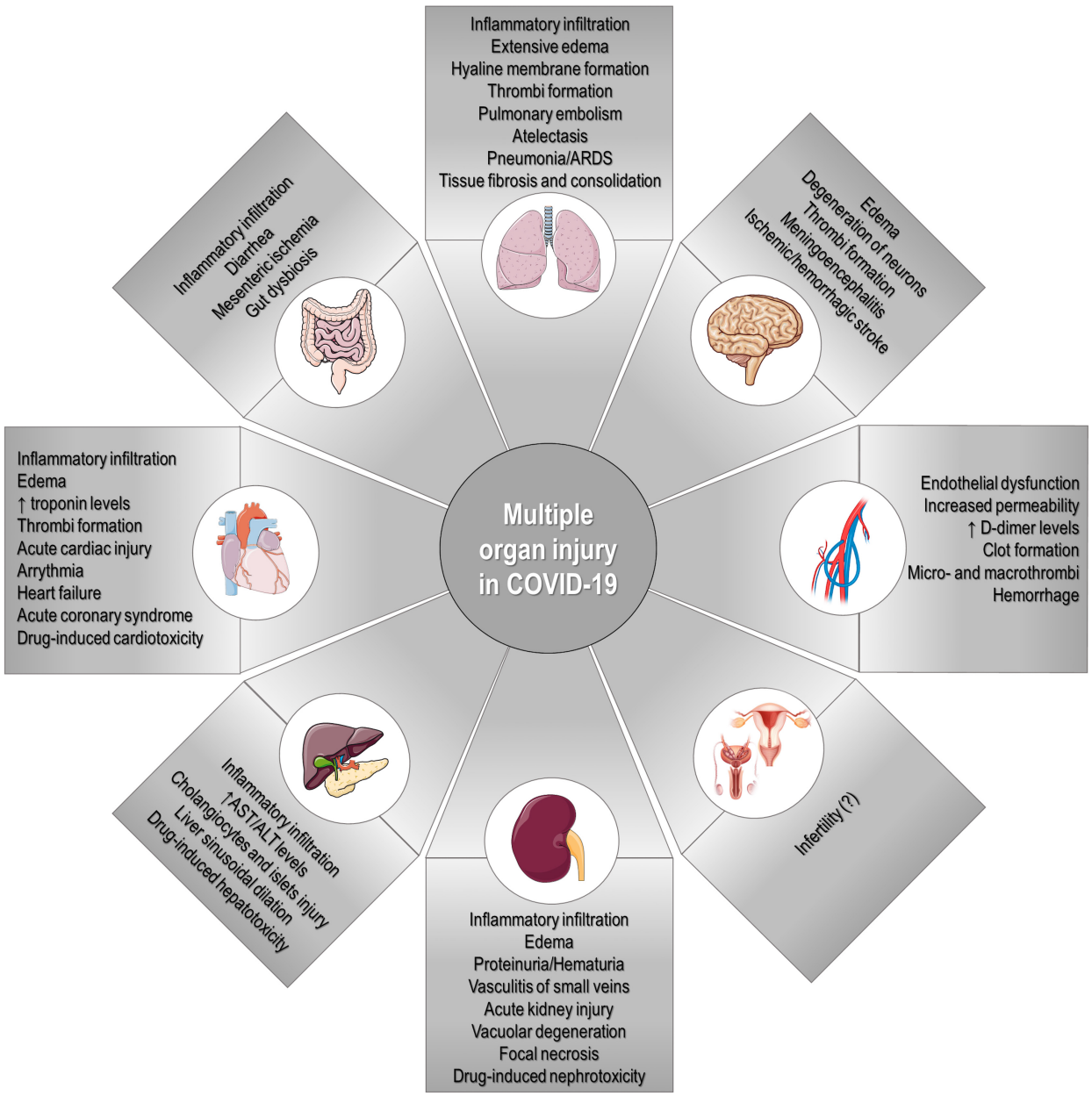


FIGURE 6 | Multiple organ injury in COVID-19. SARS-CoV-2 infection can potentially cause multiple clinical manifestations and affect several organs, including the lungs, heart, blood vessels, kidney, intestine, liver, brain, and reproductive system.

of clinical symptoms of heart disease (palpitations and chest tightness), elevated cardiac biomarker levels tests (especially cardiac troponin I or T), and abnormalities in electro- or echocardiography (Huang C. et al., 2020; Shi et al., 2020; Wang D. et al., 2020). In autopsy series of COVID-19 cases, most patients were found to have cardiomegaly, right ventricular dilatation (Fox et al., 2020), and mild fibrosis (Tian S. et al., 2020). The heart may be susceptible to direct SARS-CoV-2–mediated cytotoxicity, as ACE2 is highly expressed in cardiomyocytes (Hamming et al., 2004; Varga et al., 2020). Nevertheless, single-cell RNA sequencing has demonstrated that pericytes express higher levels of ACE2 than cardiomyocytes, and thus may also be targeted by SARS-CoV-2, leading to capillary endothelial dysfunction upon viral infection (Chen L. et al., 2020; Robba et al., 2020b). Heterozygosis for loss of ACE2 activity is believed to be sufficient to increase the risk of heart disease (Wang et al., 2014). The role of circulating soluble ACE2 is not well understood, but its levels are significantly increased in the presence of cardiovascular dysfunction and/or SARS-CoV-2 infection, and may be used as a biomarker (Úri et al., 2016; Xu et al., 2017; Sama et al., 2020; Wang K. et al., 2020). Furthermore, cytokine storm, compounded by a hypoxic state resulting from pulmonary

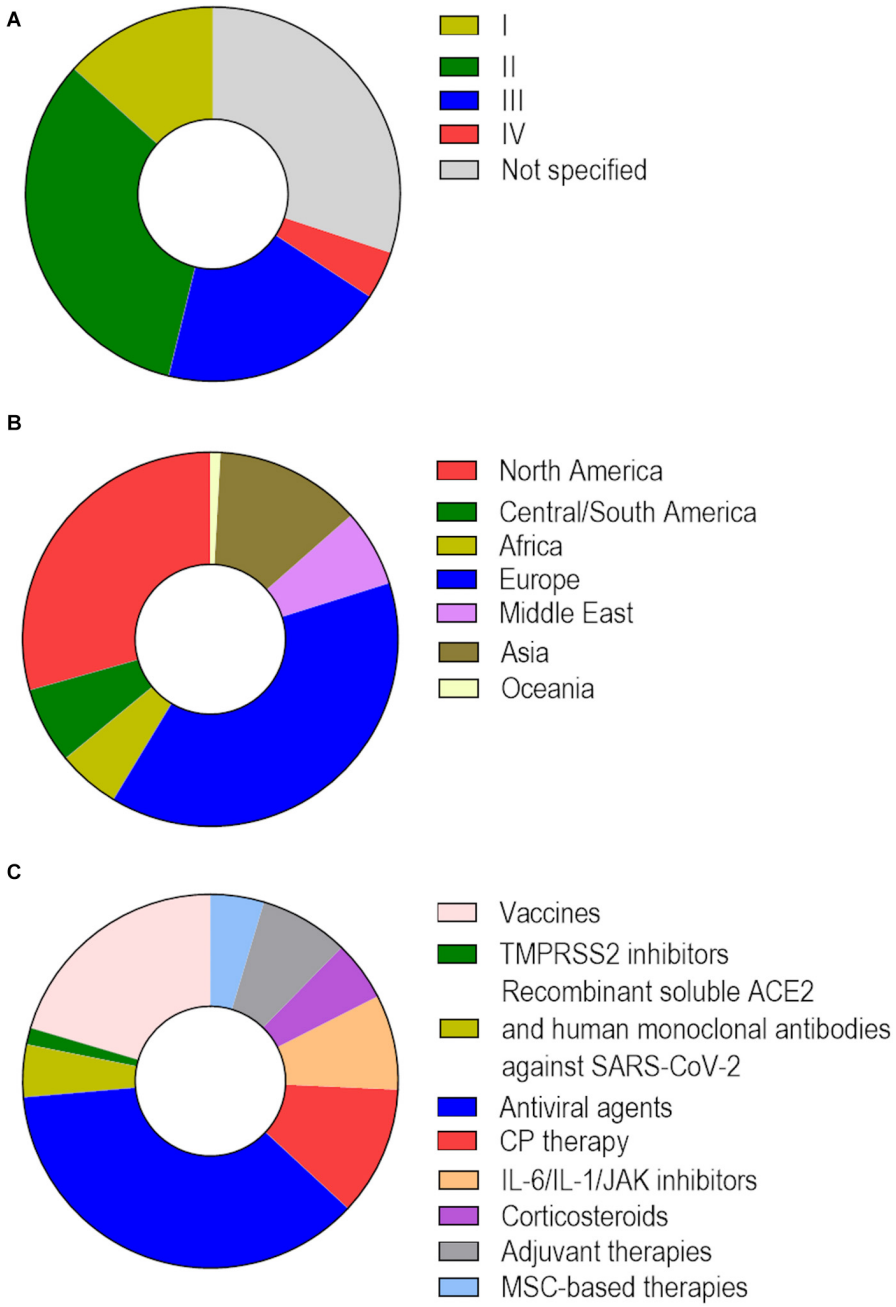


FIGURE 7 | Clinical trials of COVID-19 therapeutics registered on the U.S. National Institutes of Health Clinical Trials Platform. As of December 7th, 2020, there were 4,094 registered clinical trials to evaluate the safety and efficacy of therapeutic approaches for COVID-19. **(A)** Distribution based on study phase: I (13.3%), II (32.9%), III (19.6%), and IV (4.1%). **(B)** Distribution based on region: North America (28.9%), Central/South America (6.5%), Africa (5.2%), Europe (37.9%), Middle East (6.5%), Asia (12.5%), Oceania (0.9%). **(C)** Distribution of 1,520 clinical trials based on therapeutic approaches under investigation: vaccines (20.4%), TMPRSS2 inhibitors (1.4%), recombinant soluble ACE2 and human monoclonal antibodies against SARS-CoV-2 (4.5%), agents with known or purported antiviral activity (ribavirin, favipiravir, remdesivir, lopinavir, umifenovir, ivermectin, nitazoxanide, chloroquine/hydroxychloroquine) (36.7%), convalescent plasma (CP) therapy (11.2%), IL-6/IL-1/JAK inhibitors (8.2%), corticosteroids (5.2%), adjuvant therapies (low-molecular-weight heparin, bevacizumab, recombinant human DNase) (7.7%), and mesenchymal stromal cell (MSC)-based therapies (4.6%).

dysfunction, may lead to direct myocardial injury. Early acute myocardial injury has been associated with a higher risk of in-hospital mortality in COVID-19 (Ni et al., 2020). Pre-existing cardiovascular diseases have also been associated with a worse prognosis, as COVID-19 may aggravate cardiac tissue injury and dysfunction (Chen N. et al., 2020; Wu and McGoogan, 2020; Zhou F. et al., 2020). Monitoring of cardiac function and standard biomarkers is recommended.

Urogenital System

A balance between the effects of ACE2/Ang (1-7) and Ang II is tightly regulated to maintain normal kidney function. As ACE2 is expressed in kidney tissue, mainly in the brush border of the proximal cells, these cells are susceptible to direct injury caused by SARS-CoV-2 infection (Hamming et al., 2004; Fan C. et al., 2020). Laboratory tests have frequently revealed evidence of kidney dysfunction in individuals with COVID-19, including proteinuria, hematuria, and elevated levels of serum creatinine and blood urea nitrogen (Cheng et al., 2020; Hirsch et al., 2020; Li Z. et al., 2020). Renal abnormalities suggestive of inflammation and edema have also been observed on computed tomography (Li Z. et al., 2020). COVID-19–induced acute kidney injury has been associated with a markedly elevated risk of in-hospital mortality—approximately 5 times higher than that of COVID-19 patients without acute kidney injury (Chen N. et al., 2020; Cheng et al., 2020; Guan et al., 2020; Li Z. et al., 2020; Wang D. et al., 2020). A substantial number of individuals who develop COVID-19–induced acute kidney injury also require dialysis (Hirsch et al., 2020). SARS-CoV-2 viral antigens have been found in urine and in kidney tissue, mainly tubular epithelial cells and podocytes, at autopsy (Diao et al., 2020; Pan X. et al., 2020; Su et al., 2020); histopathological studies showed diffuse tubular injury with loss of brush border integrity, vacuolar degeneration, and necrosis (Su et al., 2020). Intraluminal erythrocyte aggregation in capillaries, without the presence of fibrinoid material, has also been reported (Su et al., 2020). The potential for direct viral damage to the kidney notwithstanding, inflammation and antiviral agents can also induce nephrotoxicity; therefore, kidney biomarkers and fluid and electrolyte status should be closely monitored to prevent kidney injury.

Despite a similar prevalence across genders, a higher case fatality rate has been observed in men with COVID-19, regardless of age (Jin et al., 2020). It is worth noting that testicular and vas deferens cells have a much higher expression of ACE2 than ovaries (Fan C. et al., 2020; Shastri et al., 2020; Wang and Xu, 2020), which could partially explain differences in disease severity and, consequently, fatality. Furthermore, sex-chromosome genes and sex hormones may contribute to the differential regulation of immunological responses between genders (Bukowska et al., 2017; Taneja, 2018). A high density of immune-related genes is located on the X chromosome, as is ACE2. This functional mosaic could influence innate and adaptive immune responses or disease severity (Taneja, 2018). In this context, women have been found to mount a more robust T-cell activation response than men during SARS-CoV-2 infection, which may contribute to a more efficient virus clearance (Takahashi et al., 2020).

Gastrointestinal System

A considerable proportion of patients with COVID-19 present with abdominal pain, nausea, diarrhea, and vomiting, suggesting that SARS-CoV-2 infection may cause gastrointestinal dysfunction (Guan et al., 2020; Huang C. et al., 2020; Robba et al., 2020b; Wang D. et al., 2020; Zhou F. et al., 2020). Indeed, ACE2 is abundantly expressed in the luminal surface of intestinal epithelial cells (Hamming et al., 2004), where it acts as a co-receptor for amino acid uptake (Hashimoto et al., 2012). SARS-CoV-2 nucleocapsid protein has been found along multiple gastrointestinal structures (Lamers et al., 2020; Xiao et al., 2020), as well as in stool specimens (Holshue et al., 2020; Wölfel et al., 2020; Zhang et al., 2020a), which suggests that oral-fecal transmission might occur. Diffuse endothelial inflammation of the small intestine and mesenteric ischemia were observed in histopathological examination of COVID-19 cases (Varga et al., 2020). Although gastrointestinal symptoms have not been associated with increased risk of mortality, they appear to correlate with a longer duration of illness (Mao R. et al., 2020; Pan L. et al., 2020). Furthermore, altered liver function tests have been observed in several individuals with COVID-19, including elevated levels of transaminases (aspartate aminotransferase and alanine aminotransferase), total bilirubin (Guan et al., 2020; Huang C. et al., 2020; Wang D. et al., 2020), and γ-glutamyl transferase (Fan Z. et al., 2020; Zhang et al., 2020a). At autopsy, mild lobular infiltration by lymphocytes, central sinusoidal dilation, and patchy necrosis of the liver were reported in a case series of COVID-19 (Tian S. et al., 2020; Xu Z. et al., 2020). Kupffer cell proliferation and chronic hepatic congestion were also observed in another case series (Lax et al., 2020). As cholangiocytes express ACE2, they are susceptible to direct virus-induced cytotoxicity (Chai et al., 2020). However, it remains unclear whether liver injury in COVID-19 is due to SARS-CoV-2 infection, systemic inflammation, drug-related, or multifactorial. As most drugs under investigation as therapies for COVID-19 are metabolized in the liver, its function should be monitored periodically.

Nervous System

Clinical manifestations consistent with neurological dysfunction, such as headache, confusion, and sudden loss of olfaction and gustation, as well as visual impairment, have been reported with increasing frequency in cases of COVID-19 (Butowt and Bilinska, 2020; Chen N. et al., 2020; Mao L. et al., 2020; Robba et al., 2020b; Wu P. et al., 2020). As ACE2 is expressed in olfactory bulb cells, neurons, astrocytes, and oligodendrocytes, the virus may rapidly disseminate through important brain areas once the olfactory epithelium is infected (Chen R. et al., 2020). SARS-CoV-2 may also infect the cerebral vascular endothelium and cross the blood–brain barrier via infected leukocytes, thus migrating into the central nervous system (Li Y. et al., 2020). Although no evidence of SARS-CoV-2 infection was observed in a first report of brain autopsies (Solomon et al., 2020), the presence of SARS-CoV-2 RNA in cerebrospinal fluid has been reported in patients with meningoencephalitis, suggesting that neurological manifestations might be due to direct invasion by SARS-CoV-2 (Huang Y.H. et al., 2020; Moriguchi et al., 2020). An autopsy series also reported cerebral edema and partial neuronal degeneration in individuals with COVID-19 (Xu Z. et al., 2020). Further evidence of SARS-CoV-2 neurotropism has been observed in other autopsies of COVID-19 cases, with such manifestations as lymphocytic encephalitis, meningitis, and massive intracranial hemorrhage (Von Weyhern et al., 2020). Furthermore, SARS-CoV-2 infection of the brainstem may be

at least partially responsible for respiratory and cardiovascular failure (Li Y. et al., 2020).

POTENTIAL THERAPEUTIC APPROACHES

The identification of similarities among human coronaviruses has provided some clues for the development of effective therapies (**Table 1**), especially for specific vaccines or effective antivirals against SARS-CoV-2. Several therapeutic approaches are under experimental and clinical investigation for COVID-19, but most are existing drugs approved for other disease indications. At least 66 human proteins or host factors can be targeted by clinically approved drugs in order to potentially inhibit SARS-CoV-2/host cell interactions (Gordon D.E. et al., 2020). Drug repurposing offers an advantage, as these drugs are already available in the clinic and have undergone extensive toxicological studies before marketing approval (Pushpakom et al., 2019; Battaglini et al., 2020; Lopes-Pacheco, 2020; Rocco et al., 2020). Therefore, the time frame to acquire an indication for COVID-19 may be reduced if safety and efficacy are demonstrated in late-stage clinical trials. Nevertheless, as COVID-19 is a multifaceted disease (Li H. et al., 2020b; Zhang B. et al., 2020b), multi-target therapeutic approaches are required to reduce or even prevent SARS-CoV-2 infection and its downstream effects—namely, systemic inflammation and multiple organ injury. To date, over 4,000 clinical trials of COVID-19 therapies have been registered in the U.S. National Institutes of Health Clinical Trials Platform[4] (**Figure 7**). In this section, we collected a wide number of therapeutics under investigations and some that, despite being extensively evaluated in the clinic, demonstrated no efficacy against COVID-19.

Vaccine Development

The best long-term strategy to stop SARS-CoV-2 transmission and prevent new infections is the development of an effective vaccine. There are many efforts in progress from both the scientific community and the pharmaceutical industry to develop vaccines against SARS-CoV-2; the current pipeline has over 200 candidates, of which 45 are in clinical trials and five have received interim or conditional approval for emergency use in different countries. These include the traditional inactivated and attenuated vaccines, as well as novel DNA- and RNA-based vaccines. Major advantages and limitations of each vaccine type have been discussed in detail elsewhere (Badgujar et al., 2020; Rawat et al., 2020). Among these, five have demonstrated promising results in preliminary phase III trials (**Table 2**). AZD1222 (formerly ChAdOx1-S, from Oxford/AstraZeneca) is a non-replicating viral vector vaccine comprising the SARS-CoV-2 spike protein that has demonstrated protective effects in rhesus macaques after a single dose (Van Doremalen et al., 2020), and 62–90% efficacy when given in two-dose regimen in humans (Voysey et al., 2020). Vaccines developed by Moderna and Pfizer/BioNTech (mRNA-based vaccines) (Jackson et al., 2020; Polack et al., 2020), Gamaleya (recombinant viral vector-based vaccine) (Logunov et al., 2020), and Sinovac (inactivated viral-based vaccine) (Zhang Y. et al., 2020) were also reported to have high efficacy. The importance of vaccine development notwithstanding, alternative therapeutic approaches are being concomitantly investigated to identify effective therapies against COVID-19 with the necessary urgency.

TABLE 2 | Vaccine candidates with promising efficacy in phase III trials.

Company	Type	Doses required	Efficacy reported in preliminary or interim analyses of phase III trials
Gamaleya	Two different adenoviral vectors (Ad26 and Ad5)	2×	∼90%
Moderna	mRNA-based (mRNA-1273)	2×	∼94%
Oxford/ AstraZeneca	Adenovirus-based (ChAdOx1)	2×	62-90%
Pfizer/BioNTech	mRNA-based (BNT162b2)	2×	∼95%
Sinovac	Inactivated viral-based	2×	50–80%

Therapeutic Approaches Aimed at Preventing Cell Entry and Replication of SARS-CoV-2

TMPRSS2 Inhibitors

Camostat mesilate (Foipan^{TM}) is a serine protease inhibitor clinically approved for squamous cell carcinoma and chronic pancreatitis in Japan. It significantly reduces viral load of SARS-CoV, human coronavirus NL63, and SARS-CoV-2 *in vitro* by partially blocking TMPRSS2 activity (Kawase et al., 2012; Hoffmann et al., 2020a). Nafamostat mesilate (Buipel^{TM}) is another serine protease inhibitor used in Japan that has been demonstrated to inhibit MERS-CoV and SARS-CoV-2 entry into host cells by targeting TMPRSS2 (Hoffmann et al., 2020b; Yamamoto et al., 2020). Nafamostat was even more effective at inhibiting SARS-CoV-2 infection *in vitro* than camostat (Yamamoto et al., 2016, 2020; Hoffmann et al., 2020b). Furthermore, nafamostat is approved for disseminated intravascular coagulation in Japan due to its anticoagulant properties, which is an additional advantage for the treatment of COVID-19, given the high prevalence of coagulation disturbances described above (Tang et al., 2020; Zhou F. et al., 2020).

Cathepsin Inhibitors

In vitro studies demonstrated that E64d, a non-selective cysteinyl cathepsin inhibitor, was able to limit both SARS-CoV and SARS-CoV-2 infection in human epithelial cells, while the combination of E64d with a TMPRSS2 inhibitor completely abrogated viral entry (Hoffmann et al., 2020a,b). The investigational compound K11777 and three of its analogs demonstrated strong antiviral activity against SARS-CoV pseudotypes *in vitro* (Zhou et al., 2015). Oxocarbazate was also effective at inhibiting SARS-CoV and Ebola virus entry into cells (Shah et al., 2010).

[4]http://clinicaltrials.gov

These agents, all cathepsin inhibitors, have potential therapeutic utility in COVID-19.

RAAS Modulators

Some concerns have been raised regarding the long-term use of ACE inhibitors or angiotensin receptor blockers (e.g., captopril, losartan) for individuals with pre-existing cardiovascular diseases during the COVID-19 pandemic, as these drugs might upregulate ACE2 and could theoretically enhance susceptibility to SARS-CoV-2 infection and COVID-19 severity. However, multiple studies have found no correlation between use of RAAS inhibitors and likelihood of testing positive for SARS-CoV-2 infection, nor with COVID-19 severity in those infected (Iaccarino et al., 2020; Khera et al., 2020; Mancia et al., 2020; Reynolds et al., 2020). In fact, in a retrospective study, a reduction in COVID-19–related mortality was observed in hospitalized individuals with hypertension who had been treated with ACE inhibitors or angiotensin receptor blockers compared to those not using any of these drugs (Zhang P. et al., 2020). Furthermore, abrupt discontinuation of RAAS inhibitors in individuals with cardiovascular disease and on long-term therapy is not recommended, as it may cause clinical decompensation (Danser et al., 2020). Based on the protective role of ACE2 as a counter-regulator of Ang II/AT1-R effects, therapeutic approaches that restore the balance between ACE and ACE2 would be ideal to mitigate COVID-19–induced multiple organ injury in individuals without pre-existing medical conditions, preferably in combination with an effective antiviral agent.

Recombinant Soluble ACE2 and Human Anti-SARS-CoV-2 Monoclonal Antibodies

It has been proposed that a recombinant soluble form of ACE2 (rhACE2), administered exogenously, may competitively bind to the SARS-CoV-2 RBD and thus prevent its interaction with native ACE2 for host-cell entry. Such an approach not only would neutralize the virus but also preserve ACE2 activity, thus protecting lung and cardiovascular tissues from injury (Monteil et al., 2020). In a pilot study, one rhACE2 formulation (known as GSK2586881) demonstrated no safety concerns and was able to decrease levels of Ang II with a trend toward decreasing IL-6 levels in serum (Kahn et al., 2017). This rhACE2 also inhibited SARS-CoV-2 infection in both engineered human blood vessels and kidney organoids *in vitro* (Monteil et al., 2020). A clinical trial is ongoing to evaluate the effects of rhACE2 in individuals with COVID-19 (NCT04335136). Alternatively, CR3022 is a SARS-CoV-specific human monoclonal antibody that was able to bind to SARS-CoV-2 RBD and neutralize viral actions. CR3022 may be a promising candidate to prevent or significantly reduce SARS-CoV-2 infection (Tian X. et al., 2020). A monoclonal antibody obtained from B-cells of individuals recently recovered from COVID-19 has also demonstrated ability to inhibit SARS-CoV-2 RBD and ACE2 interactions (Chen X. et al., 2020).

Antiviral Drugs

An ideal antiviral agent should target factors that are highly conserved among coronaviruses and essential for viral pathogenesis. A number of antivirals under investigation for COVID-19 are nucleoside analogs used for other viruses. These molecules are incorporated into nascent DNA/RNA chains during viral replication, and lead to premature termination of nucleic acid synthesis or insertion of mutations in the viral genome that prevent subsequent viral replication. Ribavirin is a guanosine analog used for the treatment of hepatitis C virus, respiratory syncytial virus, and certain viral hemorrhagic fevers. A recent *in vitro* study demonstrated that high-dose ribavirin can reduce SARS-CoV-2 infection (Wang M. et al., 2020). It was also tested with and without IFN-α in individuals infected with SARS-CoV and MERS-CoV, but whether it has any therapeutic benefit remains controversial (Zhao et al., 2003; Arabi et al., 2020). Severe adverse effects were also reported, including hepatotoxicity and hemolysis.

Favipiravir (Avigan^{TM}) is another guanosine analog that selectively inhibits the influenza viral RNA-dependent RNA polymerase (RdRp). It has been approved against influenza virus but also demonstrated antiviral activity against Ebola virus, yellow fever virus, and other viruses in experimental models (Furuta et al., 2017). As with ribavirin, a high dose of favipiravir was able to reduce SARS-CoV-2 infection *in vitro* (Wang M. et al., 2020). In an early clinical study, favipiravir demonstrated better clinical outcomes compared to umifenovir (see below), with a significant decrease in fever and cough in individuals with COVID-19 (Chen N. et al., 2020). In a similar study, favipiravir therapy was associated with a shorter time to viral clearance and higher improvement rate in chest imaging of individuals with COVID-19 (Cai et al., 2020), resulting in extension of its approved indications by the National Medical Products Administration of China.

Remdesivir (Veklury^{TM}) is an adenosine analog with a broad spectrum of antiviral activity against several RNA viruses. It blocks RdRp, thus preventing an early step of viral replication (Gordon C.J. et al., 2020; Sheahan et al., 2020). Remdesivir has been shown to inhibit viral replication of Ebola virus, MERS-CoV, and SARS-CoV-2 in experimental studies, with higher efficacy compared to other antiviral agents (Gordon C.J. et al., 2020; Sheahan et al., 2020; Wang M. et al., 2020). Furthermore, it demonstrated a favorable benefit-risk profile compared to placebo in early-stage clinical study in individuals with severe COVID-19 (Davies et al., 2020). In a compassionate-use setting, remdesivir was used in hospitalized individuals with severe COVID-19 and 68% of the cohort demonstrated an improvement in oxygen-support class and 25% were discharged during 18 days follow-up (Grein et al., 2020). In phase III multicenter randomized clinical trials, remdesivir appeared to shorten the recovery time of some patients, although therapeutic benefits were not clearly demonstrated in terms of reducing fatality rate of individuals with severe COVID-19 (Beigel et al., 2020; Wang et al., 2020b). Despite its inefficiency in improving survival (Dyer, 2020), final reports of the multicenter clinical trial indicated that remdesivir might shorten the time to recovery of hospitalized adults with COVID-19 and reduce lower respiratory tract infection, which culminated in its FDA approval.

Besides nucleoside analogs, antiviral agents may block viral replication through distinct mechanisms, including inhibition of endosomal acidification and inhibition of proteases

essential for intracellular assembly. The fixed-dose combination lopinavir/ritonavir (Kaletra^{TM}) is an FDA-approved therapy for human immunodeficiency virus (HIV). Lopinavir is an inhibitor of HIV-1 protease, while ritonavir prevents the rapid metabolism of lopinavir by inhibiting CYP3A isoenzymes. This combination has demonstrated antiviral activity against SARS-CoV and MERS-CoV, reducing viral load in experimental studies (Chu et al., 2004; Chan et al., 2015), and was associated with modest clinical improvement of MERS in a case report (Kim et al., 2016). Furthermore, a milder disease course was observed in individuals infected with SARS-CoV who received lopinavir/ritonavir (Chu et al., 2004). Initially, lopinavir/ritonavir appeared to accelerate the recovery process from COVID-19, shortening ICU length of stay compared to standard therapy alone; however, it has since proven unable to reduce viral load or mortality in adults hospitalized with severe COVID-19 (Cao et al., 2020; Osborne et al., 2020). Darunavir is a second-generation protease inhibitor used in combination with ritonavir or cobicistat for HIV therapy. It was also considered as a potential therapeutic alternative in COVID-19; however, darunavir demonstrated no antiviral activity against SARS-CoV-2 *in vitro* (De Meyer et al., 2020).

Umifenovir (Arbidol^{TM}) is a dual-acting antiviral/host-targeting agent approved for treatment of influenza in Russia and China. It inhibits membrane fusion of viral envelope and host cell by preventing clathrin-mediated endocytosis and has been demonstrated to prevent *in vitro* infection with several common pathogenic viruses (Pécheur et al., 2016). In an early clinical study of COVID-19, umifenovir therapy was associated with a trend toward reductions in viral load and mortality rate, albeit not statistically significant (Wang Z. et al., 2020). In a retrospective study, most umifenovir-treated individuals demonstrated SARS-CoV-2 negativity and improvement in chest imaging after 14 days of therapy (Deng et al., 2020). However, there is no additional evidence to support that umifenovir may improve clinical outcomes of individuals with COVID-19.

Two investigational antiviral drug candidates (11a and 11b) have been developed by structure-based design methods to target 3CL protease (M^{pro}), the main SARS-CoV-2 protease. Both demonstrated good pharmacokinetic properties and low toxicity and were able to significantly reduce viral load *in vitro* (Dai et al., 2020). Further development is ongoing.

Despite some promising results in experimental and early clinical studies, large-scale randomized trials are still in progress and their results on safety and efficacy will provide a better guidance for the potential use of these drugs in the treatment of COVID-19.

Other Anti-infectives

Ivermectin and nitazoxanide are two clinically approved antiparasitic agents that have demonstrated significant antiviral activity against SARS-CoV-2 infection *in vitro* (Caly et al., 2020; Rocco et al., 2020; Wang Z. et al., 2020). A recent study (Rocco et al., 2020) confirmed that early administration of nitazoxanide (1-3 days after onset of symptoms) reduced the viral load in individuals with mild COVID-19 with a good safety profile, even though more studies are required to evaluate its efficacy in individuals with both mild and severe COVID-19. Clinical safety and efficacy of ivermectin in COVID-19 have yet to be confirmed.

Chloroquine and hydroxychloroquine are aminoquinoline antimalarials also used in the treatment of several autoimmune diseases. Both drugs have demonstrated a broad spectrum of antiviral activity *in vitro*—including against coronaviruses—through various mechanisms, such as blocking ACE2 terminal glycosylation and hindering endosome–lysosome fusion (De Wilde et al., 2014; Mauthe et al., 2018; Wang M. et al., 2020). In early clinical studies, chloroquine and hydroxychloroquine were suggested to reduce viral load and improve clinical outcomes in individuals with COVID-19 (Gao et al., 2020; Gautret et al., 2020) with an even better effect when combined with azithromycin (Gautret et al., 2020). However, such findings were not confirmed in later, well-designed clinical trials; both chloroquine and its hydroxy analog appear ineffective, whether used therapeutically or for postexposure prophylaxis, with conflicting evidence regarding their safety and toxicity (Borba et al., 2020; Boulware et al., 2020; Cavalcanti et al., 2020; Geleris et al., 2020; Lane et al., 2020; Molina et al., 2020).

Convalescent Plasma Therapy

Convalescent plasma (CP) is plasma rich in neutralizing antibodies which has been extracted from individuals who have recovered from an infection. This plasma is processed and then administered to infected individuals. CP has been demonstrated to reduce viral load and improve clinical outcomes in other coronavirus infections, specifically SARS and MERS (Cheng et al., 2005b; Ko et al., 2018). In early-stage clinical studies, CP therapy was well tolerated and improved clinical outcomes by neutralizing viremia in severe COVID-19 (Duan et al., 2020; Shen et al., 2020; Ye et al., 2020). Improvements in clinical scales as well as discharge and survival rate in individuals with severe COVID-19 were observed 14 days after CP therapy (Salazar et al., 2020; Zhang et al., 2020a). Several clinical trials of CP are in progress. Based on previous experience with influenza A (H1N1), some practical limitations may apply, and should be borne in mind when seeking to implement an effective CP therapy regimen for COVID-19. These limitations have been discussed elsewhere (Wong et al., 2010). Antibodies produced in humanized mouse or equine serum have been investigated as alternative strategies to neutralized SARS-CoV-2 with some promising results – up to 100 times more potent than convalescent plasma from COVID-19-recovered individuals (Cunha et al., 2020; Hansen et al., 2020).

Therapeutic Approaches Aimed at Immunomodulation and Tissue Repair

IL-6 Inhibitors

IL-6 is acutely induced by inflammatory stimuli and mediates a number of immune responses. Individuals with COVID-19 demonstrate an increase in serum levels of IL-6 within 3 days after disease onset, with even higher levels in severe cases compared to mild ones (Liu J. et al., 2020). A further increase in IL-6 levels has also been associated with increased risk of respiratory failure and death (Herold et al., 2020; Quartuccio et al., 2020; Ruan et al., 2020).

Tocilizumab (Actemra™) is a recombinant humanized monoclonal antibody that binds and inhibits IL-6 receptor activity. It is indicated for the treatment of autoimmune disorders and cytokine release syndrome and has been suggested as a potential therapeutic option for COVID-19-induced hyperinflammation (Zhang et al., 2020a). In early clinical studies, tocilizumab significantly improved several outcomes in severe and critical COVID-19 cases, including supplemental oxygen utilization and C-reactive protein and D-dimer levels (Sciascia et al., 2020; Xu X. et al., 2020). In a subsequent study, tocilizumab therapy was associated with clinical improvements, as well as rapid and sustained benefits in ICU and non-ICU patients with COVID-19 (Toniati et al., 2020). Alternatively, siltuximab (Sylvant™) is a chimeric monoclonal antibody that directly bind to IL-6. Siltuximab provides an advantage compared to tocilizumab as it neutralizes circulating IL-6, which could contribute to neurotoxic effects. In an early clinical study, siltuximab therapy at the onset of mechanical ventilation was associated with reduced occurrence of respiratory failure and death in severe COVID-19 cases (Gritti et al., 2020). Sarilumab is another IL-6R inhibitor that appears to be a potential therapy against severe COVID-19 (Gremese et al., 2020).

IL-1 Inhibitors

The ORF3a protein of coronaviruses can activate NF-κB signaling and the NLRP3 inflammasome. The inflammasome activates cleavage of pro-IL-1β by caspase-1 into active IL-1β, which mediates lung inflammation and fibrosis (Siu et al., 2019). Anakinra (Kineret™), a recombinant IL-1 receptor antagonist, was evaluated in individuals with severe COVID-19 and demonstrated effective reductions in need for mechanical ventilation and fatality rate in an early clinical study (Huet et al., 2020). A retrospective study also indicated that high-dose anakinra was safe and associated with clinical improvements in over 70% of individuals with severe COVID-19 (Cavalli et al., 2020). At least 10 additional clinical trials are ongoing with the aim of evaluating the effects of anakinra in targeting hyperinflammation in COVID-19 (King et al., 2020). Colchicine is another drug known to reduce neutrophil recruitment and IL-1β levels, and widely used for the treatment of gout. In hospitalized individuals with COVID-19, colchicine did not affect C-reactive protein or cardiac troponin levels, although an improved time to clinical deterioration was reported (Deftereos et al., 2020).

Janus Kinase/Signal Transducer and Activators of Transcription (JAK/STAT) Inhibitors

Targeting the JAK/STAT pathway appears to be a promising approach, given its role in cytokine receptors on immune cells. JAK/STAT inhibitors have also been used for the treatment of cytokine release syndrome. Several clinical trials evaluating the effects of JAK/STAT inhibitors for COVID-19 are ongoing. Fedratinib, a JAK2 inhibitor, has been hypothesized to inhibit SARS-CoV-2 and Th17-induced inflammation without modulating IFN signaling, but efficacy remains to be investigated (Wu and Yang, 2020). In a recent clinical study of individuals with COVID-19, ruxolitinib, a JAK1/2 inhibitor, was not significantly superior to placebo in terms of clinical parameters, although faster recovery from lymphopenia was observed (Cao et al., 2020b). On the other hand, baricitinib, a high-affinity JAK1/2 inhibitor, has been shown to improve some clinical and laboratory parameters (including C-reactive protein levels) in a pilot study of COVID-19 cases (Cantini et al., 2020).

Corticosteroids

There is some controversy regarding corticosteroid therapy in COVID-19 (Mattos-Silva et al., 2020). Although corticosteroids are widely used to suppress lung inflammation, they were associated with delayed viral clearance and no improvement in fatality rate during the SARS and MERS epidemics (Li et al., 2020a). Nevertheless, methylprednisolone therapy has been shown to improve chest imaging, reduce fatality rate, and shorten hospital stay in individuals with severe COVID-19 (Ramiro et al., 2020; Wang et al., 2020a).

Recent preliminary results of low-dose dexamethasone therapy also demonstrated a significant reduction in fatality rate (up to one-third) in critically ill individuals with COVID-19, although such benefits were not observed for the cohort of individuals who did not require oxygen therapy at admission (Horby et al., 2020). It is possible that corticosteroid therapy may be beneficial in certain phases of COVID-19, such as the hyperinflammatory stage, but certainly should be combined with an effective antiviral or antibiotic agent to reduce the risk of superinfection. Further clinical studies are necessary to better understand the effects of corticosteroid therapy in COVID-19.

Adjuvant Therapy

Unfractionated or low-molecular-weight heparin has been used prophylactically in hospitalized individuals with COVID-19 to prevent the occurrence of thromboembolic events. Heparin can also induce immunomodulatory effects and protect endothelial cells from oxidative stress, thus preventing increased vascular permeability, microthrombus formation, and leukocyte extravasation. In an observational study, anticoagulant therapy with low-molecular-weight heparin was associated with better prognosis in severe COVID-19 cases with high levels of D-dimer (Tang et al., 2020). Despite current recommendations for the prophylactic use of low-molecular-weight heparin in all hospitalized COVID-19 patients (except those with contraindications) (Thachil et al., 2020), individuals admitted to the ICU remain at high risk of pulmonary embolism (Klok et al., 2020).

The use of recombinant human DNase (dornase) to disrupt NETs has been proposed, as SARS-CoV-2 was found to induce excessive production of NETs, which may contribute to thromboembolism events, cytokine storm, and tissue injury (Barnes et al., 2020; Veras et al., 2020). Dornase alfa (Pulmozyme™) has been long used as an inhalation solution for mucus clearance in individuals with cystic fibrosis; however, its effects on COVID-19 remain to be investigated. Bevacizumab (Avastin™/Zaribev™) is an anti-VEGF humanized monoclonal antibody that has been investigated to reduce lung edema in COVID-19 (Samudrala et al., 2020). Finally, mepolizumab (Nucala™), an anti-CD147 humanized antibody used for

the treatment of severe asthma, demonstrated to improve the recovery of individuals with COVID-19-induced severe pneumonia in a small-scale clinical study (Bian et al., 2020).

Mesenchymal Stromal Cell (MSC)-Based Therapies

Mesenchymal stromal cells and their biologically active products, such as extracellular vesicles, are known to induce immunomodulatory and reparative effects, reducing lung and distal organ injury and improving survival in several preclinical models of ARDS and sepsis (Matthay et al., 2017; Silva et al., 2018, 2019; Lopes-Pacheco et al., 2020). In early clinical studies, MSC administration was well tolerated and caused no obvious safety concerns in critically ill individuals (McIntyre et al., 2018; Matthay et al., 2019; Khoury et al., 2020). Notably, SARS-CoV-2 is unable to infect MSCs, as these cells do not express ACE2 (Leng et al., 2020). In one case report of compassionate use in severe COVID-19, administration of Wharton's jelly-derived MSCs reduced plasma levels of IL-6, TNF-α, and C-reactive protein and improved lung function (Zhang Y. et al., 2020). In another early clinical study, bone marrow-derived MSCs were administered intravenously to seven individuals with severe COVID-19. MSC administration was safe and significantly reduced inflammation, resulting in improvements in symptoms and lung function (Leng et al., 2020). Alternatively, administration of bone marrow MSC-derived exosomes was evaluated in an early clinical study of individuals with severe COVID-19 and demonstrated no adverse events (Sengupta et al., 2020). Several other clinical trials are evaluating MSC-based therapies for COVID-19. Despite the great promise of MSCs for the treatment of COVID-19 complications, such as ARDS and sepsis, several open questions remain, including the best source, dose, route of administration, frequency, and timing (De Castro et al., 2019; Khoury et al., 2020; Lopes-Pacheco et al., 2020). Further understanding of the effects of MSCs on COVID-19 pathogenesis and their underlying mechanisms are also needed in order to translate MSC-based therapy into clinical practice with safety and effectiveness.

OUTLOOK AND CONSIDERATIONS

COVID-19 can cause not only severe lung injury but also multiple organ dysfunction with potential long-term effects on survivors. The downregulation of membrane-bound active ACE2 induced by SARS-CoV-2 infection can be detrimental to everyone, but is particular so for individuals whose baseline ACE2 expression is already deficient. Although infected individuals with certain pre-existing medical conditions are more prone to developing severe COVID-19, the disease is not exclusively restricted to this population, and a combination of multiple direct and indirect pathogenic factors contribute to disease severity and a broad spectrum of phenotypes. Even though vaccination has initiated in multiple countries, the production of vaccines on a global scale will take some time, despite having different vaccines approved against SARS-CoV-2. Accordingly, implementation of preventive measures remains crucial to limit rapid dissemination of the virus and potential COVID-19-induced organ injuries, as well as prevent saturation of national healthcare systems.

It is imperative to continuously monitor genetic modifications of coronaviruses, as any gain-of-function mutation affecting the life-cycle pathways of SARS-CoV-2 (and other viruses in this family) can make them more infectious and lethal to humans. In this context, a SARS-CoV-2 subtype with the D614G mutation in the spike protein was recently found to confer increased infectivity (Bhattacharyya et al., 2020; Hou et al., 2020); further investigations are ongoing to elucidate the lethality rate of this and other subtypes. More strict regulations against the trade of wild animals for domestication or food should also be implemented to prevent potential future pandemics.

Over the last two decades, we have witnessed three coronavirus outbreaks. The unprecedented consequences of COVID-19 pandemic have prompted a massive global research effort to better understand the pathological mechanisms underlying SARS-CoV-2 infection and—at an accelerated pace—develop safe and efficient therapies against this devastating condition. Although a number of these therapies have demonstrated promising results in early clinical studies, safety and efficacy remain to be demonstrated in large-scale clinical trials. A greater understanding of the clinical features has provided better guidance in managing the disease to prevent further complications. Furthermore, the phenotypical differences in COVID-19 manifestations suggest that personalized therapeutic regimens should be considered on a case-by-case basis, ranging from prevention of viral cell entry and replication to supportive, immunomodulatory and tissue reparative approaches. Therefore, multi-target therapeutic protocols may be the best option to achieve a higher number of individuals with severe COVID-19 and significantly reduce or even prevent multiple organ dysfunction.

AUTHOR CONTRIBUTIONS

ML-P contributed to the design and conceptualization, original draft, editing, and review for intellectual content. PS, FC, DB, CR, PP, MM, and CCN reviewed the intellectual content. PR did the conceptualization and edited and reviewed the intellectual content. All authors read and approved the final version of the manuscript.

FUNDING

This study was supported by the Brazilian Council for Scientific and Technological Development (CNPq) and Carlos Chagas Filho Rio de Janeiro State Research Foundation (FAPERJ). ML-P is a recipient of the 2018 Gilead Sciences Research Scholars for Cystic Fibrosis.

ACKNOWLEDGMENTS

We express our gratitude to Mrs. Moira Elizabeth Schottler and Mr. Filippe Vasconcellos for their assistance in editing the manuscript.

REFERENCES

Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al. (2020). Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N. Eng. J. Med.* 383, 120–128. doi: 10.1056/nejmoa2015432

Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C., and Garry, R. F. (2020). The Proximal Origin of SARS-CoV-2. *Nat. Med.* 26, 450–452. doi: 10.1038/s41591-020-0820-9

Arabi, Y. M., Shalhoub, S., Mandourah, Y., Al-Hammed, F., Al-Omari, A., Al Qasim, E., et al. (2020). Ribavirin and Interferon Therapy for Critically Ill Patients with Middle East Respiratory Syndrome: A Multicenter Observation Study. *Clin. Infect. Dis.* 70, 1837–1844. doi: 10.1093/cid/ciz544

Badgujar, K. C., Badgujar, V. C., and Badgujar, S. B. (2020). Vaccine development agains coronavirus (2003 to present): An orverview, recent advances, current scenario, opportunities and challenges. *Diabetes Metab. Syndr.* 14, 1361–1376. doi: 10.1016/j.dsx.2020.07.022

Barnes, N. J., Adrover, J. M., Baxter-Stoltzfus, A., Borczuk, A., Cools-Lartigue, J., Crawford, J. M., et al. (2020). Targeting Potential Driver of COVID-19: Neutrophil Extracellular Traps. *J. Exp. Med.* 217:e20200652.

Battaglini, D., Robba, C., Ball, L., Cruz, F. F., Silva, P. L., Pelosi, P., et al. (2020). Emerging therapies for COVID-19 pneumonia. *Expert Opin. Investig. Drugs* 29, 633–637.

Beigel, J. H., Tomashek, K. D., Dodd, L. E., Mehta, A. K., Zingman, B. S., Kalil, A. C., et al. (2020). Remdesivir for the Treatment of Covid-19 – Final Report. *N. Engl. J. Med.* 383, 1813–1826.

Bhattacharyya, C., Das, C., Ghosg, A., Singh, A. K., Mukherjee, S., Majumder, P. P., et al. (2020). Global Spread of SARS-CoV-2 Subtype with Spyke Protein Mutation D614G is Shaped by Human Genomic Variations that Regulate Expression of TMPRSS2 and MXI Genes. *bioRxiv* 2020:75911. doi: 10.1101/2020.05.04.075911

Bian, H., Zheng, Z.-H., Wei, D., Zhang, Z., Kang, W.-Z., Hao, C.-Q., et al. (2020). Meplazumab treats COVID-19 pneumonia: an open-labelled, concurrent controlled add-on clinical trial. *medRxiv* Preprint. doi: 10.1101/2020.03.21.20040691

Borba, M. G. S., Val, F. F. A., Sampaio, V. S., Alexandre, M. A. A., Melo, G. C., Brito, M., et al. (2020). Effect of High vs Low Doses of Chloroquine Diphosphate as Adjunctive Therapy for Patients with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection: A Randomized Clinical Trial. *JAMA Netw. Open* 3:e208857.

Boulware, D. R., Pullen, M. F., Bangdiwala, A. S., Pastick, K. A., Lofgern, S. M., Ofagor, E. C., et al. (2020). A Randomizes Trial of Hydroxychloroquine as Postexposure Prophylaxis for Covid-19. *N. Eng. J. Med.* 383, 517–525.

Bukowska, A., Spiller, L., Wolke, C., Lendeckel, U., Weinert, S., Hoffmann, J., et al. (2017). Protective Regulation of the ACE2/ACE Gene Expression by Estrogen in Human Atrial Tissue from Elderly Men. *Exp. Biol. Med.* 242, 1412–1423. doi: 10.1177/1535370217718808

Bunyavanich, S., Do, A., and Vicencio, A. (2020). Nasal Gene Expression of Angiotensin-Converting Enzyme 2 in Children and Adults. *JAMA* 323, 2427–2429. doi: 10.1001/jama.2020.8707

Butowt, R., and Bilinska, K. (2020). SARS-CoV-2: Olfaction, Brain Infection, and the Urgent Need for Clinical Samples Allowing Earlier Virus Detection. *ACS Chem. Neurosci.* 11, 1200–1203. doi: 10.1021/acschemneuro.0c00172

Cai, G. (2020). Bulk and single-cell transcriptomics identify tobacco-use disparity in lung gene expression of ACE2, the receptor of 2019-nCov. *medRxiv* Preprint. doi: 10.1101/2020.02.05.20020107

Cai, Q., Yang, M., Liu, D., Chen, J., Shu, D., Xia, J., et al. (2020). Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. *Engineering* 6, 1192–1198. doi: 10.1016/j.eng.2020.03.007

Calcagnile, M., Forgez, P., Iannelli, A., Bucci, C., Alifano, M., and Alifano, P. (2020). ACE2 Polymorphisms and Individual Susceptibility to SARS-CoV-2 Infection: Inseghts from an *In Silico* Study. *bioRxiv* Preprint. doi: 10.1101/2020.04.23.057042

Caly, L., Druce, J. D., Catton, M. G., Jans, D. A., and Wagstaff, K. M. (2020). The FDA-approved Drug Ivermectin Inhibits the Replication of SARS-CoV.2 in Vitro. *Antiviral Res.* 178:104787. doi: 10.1016/j.antiviral.2020.104787

Cantini, F., Niccoli, L., Matarrese, D., Nicastri, E., Stobbione, and Goletti, D. (2020). Baracitinib Therapy in COVID-19: A Pilot Study on Safety and Clinical Impact. *J. Infect.* 81, 318–356. doi: 10.1016/j.jinf.2020.04.017

Cao, B., Wang, Y., Wen, D., Liu, W., Wang, J., Fan, G., et al. (2020). A Trial of Lapinavir-Ritonavir in Adults Hospitalized with Several Covid-19. *N. Eng. J. Med.* 382, 1787–1799.

Cao, Y., Li, L., Feng, Z., Wan, S., Huang, P., Sun, X., et al. (2020a). Comparative Genetic Analysis of the Novel Coronavirus (2019-nCov/SARS-CoV-2) Receptors ACE2 in Different Populations. *Cell Discov.* 6:11.

Cao, Y., Wei, J., Zou, L., Jiang, T., Wang, G., Chen, L., et al. (2020b). Ruxolitinib in Treatment of Severe Coronavirus Dosease 2019 (COVID-19): A Multicenter, Single-Blind, Randomized Controlled Trial. *J. Allergy Clin. Immunol.* 146, 137.e–146.e.

Cavalcanti, A. B., Zampieri, F. G., Rosa, R. G., Azevedo, L. C. P., Veiga, V. C., Avezum, A., et al. (2020). Hydroxychloroquine with or without Azithromycin in Mild-to-Moderate Covid-19. *N. Eng. J. Med.* 383, 2041–2052.

Cavalli, G., de Luca, G., Campochiaro, C., Della-Torre, E., Ripa, M., Canetti, D., et al. (2020). Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. *Lancet Rheumatol.* 2, 325–331e.

Chai, X., Hu, L., Zhang, Y., Han, W., Lu, Z., Ke, A., et al. (2020). Specific ACE2 Expression in Cholangiocytes May Cause Liver Damage After 2019-nCov Infection. *bioRxiv* Preprint. doi: 10.1101/2020.02.03.931766

Chan, J. F.-W., Kok, K.-H., Zheng, Z., Chu, H., To, K. K.-W., Yuan, S., et al. (2020). Genomic Characterization of the 2019 Novel Human-Pathogenic Coronavirus Isolated From a Patients with Atypical Pneumonia After Visiting Wuhan. *Emerg. Microbes Infect.* 9, 221–236. doi: 10.1080/22221751.2020.1719902

Chan, J. F.-W., Yao, Y., Yeung, M.-L., Deng, W., Bao, L., Jia, L., et al. (2015). Treatment with Lopinavir/Ritonavir or Interferon-β1b Outcome of MERS-CoV Infection in a Nonhuman Primate Model of Common Marmoset. *J. Infect. Dis.* 212, 1904–1913. doi: 10.1093/infdis/jiv392

Channappanavar, R., Zhao, J., and Perlman, S. (2014). T Cell-Mediated Immune Response to Respiratory Coronaviruses. *Immunol. Res.* 59, 118–128. doi: 10.1007/s12026-014-8534-z

Chen, J., Jiang, Q., Xia, X., Liu, K., Yu, Z., Tao, W., et al. (2020). Individual Variation of the SARS-CoV-2 Receptor ACE 2 Expression and Regulation. *Aging Cell* 19:e13168.

Chen, L., Li, X., Chen, M., Feng, Y., and Xiong, C. (2020). The ACE2 Expression in Human Heart Indicates New Potential Mechanism of Heart Injury Among Patients Infected with SARS-CoV-2. *Cardiovasc. Res.* 116, 1097–1100. doi: 10.1093/cvr/cvaa078

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., et al. (2020). Epidemiological and Clinical Characteristics of 99 Cases of 2019 Novel Coronavirus Pneumonia in Wuhan, China: A Descriptive Study. *Lancet* 395, 507–513. doi: 10.1016/s0140-6736(20)30211-7

Chen, R., Wang, K., Yu, J., Chen, Z., Wen, C., Xu, Z., et al. (2020). The spatial and cell-type distribution of SARS-CoV-2 receptor ACE2 in human and mouse brain. *bioRxiv* Preprint. doi: 10.1101/2020.04.07.030650

Chen, X., Li, R., Pan, Z., Qian, C., Yang, Y., You, R., et al. (2020). Human monoclonal antibodies block the binding of SARS-CoV-2 spike protein to angiotensin converting enzyme 2 receptor. *Cell. Mol. Immunol.* 17, 647–649. doi: 10.1038/s41423-020-0426-7

Chen, Y., Guo, Y., Pan, Y., and Zhao, Z. J. (2020). Structure Analysis of the Receptor Binding of 2019-nCov. *Biochem. Biophys. Res. Commun.* 525, 135–140. doi: 10.1016/j.bbrc.2020.02.071

Cheng, Y., Cheng, G., Chui, C. H., Lau, F. Y., Chan, P. K. S., Ng, M. H. L., et al. (2005a). ABO blood group and susceptibility to severe acute respiratory syndrome. *JAMA* 293, 1450–1451.

Cheng, Y., Luo, R., Wang, K., Zhang, M., Wang, Z., Dong, L., et al. (2020). Kidney impairment is associated with in-hospital death of COVID-19 patients. *medRxiv* Preprint. doi: 10.1101/2020.02.18.20023242

Cheng, Y., Wong, R., Soo, Y. O. Y., Wong, W. S., Lee, C. K., Ng, M. H. L., et al. (2005b). Use of Convalescent Plasma Therapy in SARS Patients in Hong Kong. *Eur. J. Clin. Microbiol. Infect. Dis.* 24, 44–46. doi: 10.1007/s10096-004-1271-9

Chu, C. M., Cheng, V. C. C., Hung, I. F. N., Wong, M. M. L., Chan, K. H., Kao, R. Y. T., et al. (2004). Role of lopinavir/ritonavir in the Treatment of SARS: Initial Virological and Clinical Findings. *Thorax* 59, 252–256. doi: 10.1136/thorax.2003.012658

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses (2020). The Species Severe Acute Respiratory Syndrome-Related Coronavirus: Classifying 2019-nCoV and Naming It SARS-CoV-2. *Nat. Microbiol.* 5, 536–544. doi: 10.1038/s41564-020-0695-z

Coutard, B., Valle, C., de Lamballerie, X., Canard, B., Seidah, N. G., and Decroly, E. (2020). The Spike Glycoprotein of the New Coronavirus 2019-nCoV Contains a Furin-Like Clevage Site Absent in Cov of the same clade. *Antiviral Res.* 176:104742. doi: 10.1016/j.antiviral.2020.104742

Cui, J., Li, F., and Shi, Z.-L. (2019). Origin and Evolution of Pathogenic Coronaviruses. *Nat. Rev. Microbiol.* 17, 181–192. doi: 10.1038/s41579-018-0118-9

Cunha, L. E. R., Stolet, A. A., Strauch, M. A., Pereira, V. A. R., Dumard, C. H., Gomes, A. M. O., et al. (2020). Potent neutralizing equine antibodies raised against recombinant SARS-CoV-2 spike protein for COVID-19 passive immunization therapy. *bioRxiv* Preprint. doi: 10.1101/2020.08.17254375

Dai, W., Zhang, B., Jiang, X.-M., Su, H., Li, J., Zhao, Y., et al. (2020). Structure-based Design of Antiviral Candidates Targeting the SARS-CoV-2 Main Protease. *Science* 368, 1331–1335.

Danser, A. H. J., Epstein, M., and Battle, D. (2020). Renin-Angiotensin System Blockers and the COVID-19 Pandemic: At Present There Is No Evidence to Abandon Renin-Angiotensin System Blockers. *Hypertension* 75, 1382–1385. doi: 10.1161/hypertensionaha.120.15082

Davies, M., Osborne, V., Lane, S., Roy, D., Dhanda, S., Evans, A., et al. (2020). Remdesivir in Treatment of COVID-19: A Systematic Benefit-Risk Assessment. *Drug Saf.* 43, 645–656. doi: 10.1007/s40264-020-00952-1

De Castro, L. L., Lopes-Pacheco, M., Weiss, D. J., Cruz, F. F., and Rocco, P. R. M. (2019). Current Understanding of the Immunosuppressive Properties of Mesenchymal Stromal Cells. *J. Mol. Med.* 97, 605–618. doi: 10.1007/s00109-019-01776-y

De Meyer, S., Bojkova, D., Cinatl, J., Van Damme, E., Buyck, C., Van Loock, M., et al. (2020). Lack of Antiviral Activity of Darunavir Against SARS-CoV-2. *Int. J. Infect. Dis.* 97, 7–10. doi: 10.1016/j.ijid.2020.05.085

De Wilde, A. H., Jochmans, D., Posthuma, C. C., Zevenhoven-Dobbe, J. C., van Nieuwkoop, S., Bestebroer, T. M., et al. (2014). Screening of an FDA-approved Compound Library Identifies Four Small-Molecules Inhibitors of Middle East Respiratory Syndrome Coronavirus Replication in Cell Cultures. *Antimicrob. Agents Chemother.* 58, 4875–4884. doi: 10.1128/aac.03011-14

Deftereos, S. P., Giannopoulos, G., Vrachatis, D. A., Siasos, G. D., Giotaki, S. G., Gargalianos, P., et al. (2020). Effect of Colchicine vs Standard Care on Cardiac and Inflammarion Biomarkers and Clinical Outcomes in Patients Hospitalized with Coronavirus Disease 2019: The GRECCO-19 Randomized Clinical Trial. *JAMA Netw. Open* 3:e2013136. doi: 10.1001/jamanetworkopen.2020.13136

Deng, L., Li, C., Zeng, W., Liu, X., Li, X., Zhang, H., et al. (2020). Arbidol Combined with LPV/r versus LPV/r Alone Agains Corona Virus Disease 2019: A Retrospective Cohort Study. *J. Infect.* 81, 1–5e.

Devaux, C. A., Rolain, J.-M., and Raoult, D. (2020). ACE2 receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVD-19 disease outcome. *J. Microbiol. Immunol. Infect.* 53, 425–435. doi: 10.1016/j.jmii.2020.04.015

Diao, B., Wang, C., Wang, R., Feng, Z., Tan, Y., Wang, H., et al. (2020). Human Kidney is a Target for Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection. *medRxiv* Preprint. doi: 10.1101/2020.03.04.20031120

Duan, K., Liu, B., Li, C., Zhang, H., Yu, T., Qu, J., et al. (2020). Effectiveness of Convalescent Plasma Therapy in Severe COVID-19 Patients. *Proc. Natl. Acad. Sci. U S A.* 177, 9490–9496.

Dyer, O. (2020). Covid-19: Remdesivir has little or no impact on survival, WHO trial show. *BMJ* 371:m4057. doi: 10.1136/bmj.m4057

Elhabyan, A., Elyaacoub, S., Sanad, E., Abukhadra, A., Elhabyan, A., and Dinu, V. (2020). The role of host genetics in susceptibility to severe viral infections in human and insights into host genetic of severe COVID-19: A systematic review. *Virus Res.* 289:198163. doi: 10.1016/j.virusres.2020.198163

Ellinghaus, D., Degenhardt, F., Bujanda, L., Buti, M., Albillos, A., Invernizzi, P., et al. (2020). Gonemowide Association Study of Severe Covid-19 with Respiratory Failure. *N. Engl. J. Med.* 383, 1522–1534. doi: 10.1056/nejmoa2020283

Fan, C., Li, K., Ding, Y., Lu, W. L., and Wang, J. (2020). ACE2 Expression in Kidney and Testis May Cause Kidney and Testis Damage after 2019-nCov Infection. *medRxiv* Preprint. doi: 10.1101/2020.02.12.20022418

Fan, Z., Chen, L., Li, J., Cheng, X., Yang, H., Tian, C., et al. (2020). Clinical Features of COVID-19-Related Liver Functional Abnormality. *Clin. Gastroenterol. Hepatol.* 18, 1561–1566. doi: 10.1016/j.cgh.2020.04.002

Fehr, A. R., and Perlman, S. (2015). Coronaviruses: Na Overview of Their Replication and Pathogenesis. *Methods Mol. Biol.* 1282, 1–23. doi: 10.1007/978-1-0716-0900-2_1

Forrester, S., Booz, G. W., Sigmund, C. D., Coffman, T. M., Kawai, T., Rizzo, V., et al. (2018). Angiotensin II Signal Transduction: An Update on Mechanisms of Physiology and Pathophysiology. *Physiol. Rev.* 98, 1627–1738. doi: 10.1152/physrev.00038.2017

Fox, S. E., Akmatbekov, A., Harbert, J. L., Li, G., Brown, Q., and Heide, R. S. V. (2020). Pulmonary and Cardiac Pathology in Covid-19: The First Autopsy Series from New Orleans. *medRxiv* Preprint. doi: 10.1101/2020.04.06.20050575

Furuta, Y., Komeno, T., and Nakamura, T. (2017). Favipiravir (T-705), a Broad-Spectrum Inhibitor of Viral RNA Polymerase. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 93, 449–463. doi: 10.2183/pjab.93.027

Gao, J., Tian, Z., and Yang, X. (2020). Breakthrough: Chloroquine Phosphate Has Shown Apparent Efficacy in Treatment of COVID-19 Associated Pneumonia in Clinical Studies. *Biosci. Trends* 14, 72–73. doi: 10.5582/bst.2020.01047

Gautret, P., Lagier, J.-C., Parola, P., Hoang, V. T., Meddeb, L., Maihle, M., et al. (2020). Hydroxychloroquine and Azithromycin as a Treatment of COVID-19: Results of an Open-Label Non-Randomized Clinical Trial. *Int. J. Antimicrob. Agents* 56:105949.

Geleris, J., Sun, Y., Platt, J., Zucker, J., Baldwin, M., Hripcsak, G., et al. (2020). Observational Study of Hydroxychloroquine in Hospitalized Patients with COVID-19. *N. Eng. J. Med.* 382, 2411–2418.

Gheblawi, M., Wang, K., Viveiros, A., Nguyen, Q., Zhong, J.-C., Turner, A. J., et al. (2020). Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System: Celebrating the 20th Anniversary of the Discovery of ACE2. *Circ. Res.* 126, 1245–1274.

Glowacha, I., Bertran, S., Herzog, P., Pfefferle, S., Steffen, I., Muench, M. O., et al. (2010). Differential Downregulation of ACE2 by the Spike Protein of Severe Acute Respiratory Syndrom Coronavirus and Human Coronavirus NL63. *J. Virol.* 84, 1198–1205. doi: 10.1128/jvi.01248-09

Gordon, C. J., Tchesnokiv, E. P., Woolner, E., Perry, J. K., Feng, J. Y., Porter, D. P., et al. (2020). Remdesivir Is a Direct-Acting Antiviral That Inhibits RNA-dependent RNA Polymerase From Severe Acute Respiratory Syndrome Coronavirus 2 with High Potency. *J. Biol. Chem.* 295, 6785–6797. doi: 10.1074/jbc.ra120.013679

Gordon, D. E., Jan, G. M., Bouhaddou, M., Xu, J., Obernier, K., White, K. M., et al. (2020). A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug Repurposing. *Nature* 583, 459–468.

Grein, J., Ohmagar, N., Shin, D., Diaz, G., Asperges, E., Castagna, A., et al. (2020). Compassionate Use of Remdesivir for Patients with Severe Covid-19. *N. Engl. J. Med.* 382, 2327–2336.

Gremese, E., Cingolani, A., Bosello, S. L., Alivernini, S., Tolusso, B., Perniola, S., et al. (2020). Sarilumab use in severe SARS-CoV-2 pneumonia. *EClinicalMedicine* 27:100553. doi: 10.1016/j.eclinm.2020.100553

Gritti, G., Raimondi, F., Ripamonti, D., Riva, I., Landi, F., Alborghetti, L., et al. (2020). IL-6 signalling pathway inactivation with siltuximab in patients with COVID-19 respiratory failure: an observational cohort study. *medRxiv* Preprint. doi: 10.1101/2020.04.01.20048561

Guan, W.-J., Ni, Z.-Y., Hu, Y., Liang, W.-H., Ou, C.-Q., He, J.-X., et al. (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* 382, 1708–1720.

Gupta, A., Madhavan, M. V., Sehgal, K., Nair, N., Mahajan, S., Sehrawat, T. S., et al. (2020). Extrapulmonary manifestations of COVID-19. *Nat. Med.* 26, 1017–1032.

Haga, S., Nagata, N., Okamura, T., Yamamoto, N., Sata, T., Yamamoto, N., et al. (2010). TACE Antagonists Blocking ACE2 Shedding Caused by the Spike Protein of SARS-CoV Are Candidate Antiviral Compounds. *Antiviral Res.* 85, 551–555. doi: 10.1016/j.antiviral.2009.12.001

Hamming, I., Timens, W., Bulthuis, M. L. C., Lely, A. T., Navis, G. J., and van Good, H. (2004). Tissue Distribution of ACE2 Protein, the Functional Receptor for SARS Coronavirus. A First Step in Understandin SARS Pathogenesis. *J. Pathol.* 203, 631–637. doi: 10.1002/path.1570

Hansen, J., Baum, A., Pascal, K. E., Russo, V., Giordano, S., Wloga, E., et al. (2020). Studies in humanized mice and convalescente humans yield a SARS-CoV-2 antibody cocktail. *Science* 369, 1010–1014.

Hashimoto, T., Perlot, T., Rehman, A., Trichereau, J., Ishiguro, H., Paolino, M., et al. (2012). ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* 487, 477–481. doi: 10.1038/nature11228

He, X., Lau, E. H. Y., Wu, P., Deng, X., Wang, J., Hao, X., et al. (2020). Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat. Med.* 26, 672–675. doi: 10.1038/s41591-020-0869-5

Helms, J., Tacquard, C., Severac, F., Leonard-Lorant, I., Ohana, M., et al. (2020). High Risk of Thrombosis in Patients with Severe SARS-CoV-2 Infection: A Multicenter Prospective Cohort Study. *Intensive Care Med.* 46, 1089–1098. doi: 10.1007/s00134-020-06062-x

Herold, T., Jurinovic, V., Arnreich, C., Lipworth, B. J., Hellmuth, J. C., von Bergwelt-Baildon, M., et al. (2020). Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *J. Allergy Clin. Immunol.* 146, 128–136–e4.

Hirsch, J. S., Ng, J. H., Ross, D. W., Sharma, P., Shah, H. H., Barnett, R. L., et al. (2020). Acute kidney injury in patients hospitalized with COVID-19. *Kidney Int.* 98, 209–218.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020a). SARS-CoV-2 Cell Entry Dependes on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 181, 271–280. doi: 10.1016/j.cell.2020.02.052

Hoffmann, M., Schroeder, S., Kleine-Weber, H., Müller, M. A., Drosten, C., and Pöhlmann, S. (2020b). Nafamostat Mesylate Blocks Activation of SARS-CoV-2: New Treatment Option for COVID-19 *Antimicrob. Agents Chemother.* 64, 754–720e.

Holshue, M. L., DeBolt, C., Lindquist, S., Lofy, K. H., Wiesman, J., Bruce, H., et al. (2020). Fisrt Case of 2019 Novel Coronavirus in the United States. *N. Eng. J. Med.* 382, 929–936.

Horby, P., Lim, W. S., Emberson, J., Mafham, M., Bell, J., Linsell, L., et al. (2020). Dexamethasone in Hospitalized Patients with Covid-19 – Preliminary Report. *N. Eng. J. Med.* 2020:NEJMoa2021436. doi: 10.1056/NEJMoa2021436

Hou, Y. J., Chiba, S., Halfmann, P., Ehre, C., Kuroda, M., Dinnon, 3rd, et al. (2020). SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo. *Science* 370, 1464–1468.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical Features of Patients Infected with 2019 Novel Coronavirus in Wuahn. *China. Lancet* 395, 497–506.

Huang, Y. H., Jiang, D., and Huang, J. T. (2020). SARS-CoV-2 Detected in Cerebrospinal Fluid by PCR in a Case of COVID-19 Encephalitis. *Crain Behav. Immun.* 87:149. doi: 10.1016/j.bbi.2020.05.012

Huet, T., Beaussier, H., Voisin, O., Jouveshomme, S., Dauriat, G., Lazareth, I., et al. (2020). Anakinra for severe forms of COVID-19: a cohort study. *Lancet Rheum.* 2, 393–400e. doi: 10.1016/S2665.9913(20)30164-8

Hussain, M., Jabeen, N., Raza, F., Shabbir, S., Baig, A. A., Amanullah, A., et al. (2020). Structural Variations in Human ACE2 May Influence Its Binding With SARS-CoV-2 Spike Protein. *J. Med. Virol.* 92, 1580–1586. doi: 10.1002/jmv.25832

Iaccarino, G., Grassi, G., Borghi, C., Ferri, C., Salvetti, M., Volpe, M., et al. (2020). Age and Multimorbidity Predict Death Among COVID-19 Patients: Results of the SARS-RAS Study of the Italian Society of Hypertension. *Hypertension* 76, 366–372.

Jackson, L. A., Anderson, E. J., Rouphael, N. G., Roberts, P. C., Makhene, M., Coler, R. N., et al. (2020). An mRNA Vaccine against SARS-CoV-2 – Preliminary Report. *N. Engl. J. Med.* 383, 1920–1931. doi: 10.1056/NEJMoa2022483

Jia, H. P., Look, D. C., Tan, P., Shi, L., Hickey, M., Gakhar, L., et al. (2009). Ectodomain Shedding of Angiotensin Converting Enzyme 2 in Human Airway Epithelia. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 297, L84–L96.

Jiang, S., Hillyer, C., and Du, L. (2020). Neutralizing Antibodies Against SARS-CoV-2 and Other Human Coronaviruses. *Trends Immunol.* 41, 355–359. doi: 10.1016/j.it.2020.03.007

Jin, J.-M., Bai, P., He, W., Wu, F., Liu, X.-F., Han, D.-M., et al. (2020). Gender Differences in Patients with COVID-19: Focus on Severity and Mortality. *Front. Public Health* 8:152. doi: 10.3389/fpubh.2020.00152

Kahn, A., Benthin, C., Zeno, B., Albertson, T. E., Boyd, J., Christie, J. D., et al. (2017). A Pilot Clinical Trial of Recombinant Human Angiotensin-Converting Enzyme 2 in Acute Respiratory Distress Syndrome. *Crit. Care* 21:234.

Kawase, M., Shirato, K., van der Hoek, L., Taguchi, F., and Matsuyama, S. (2012). Simultaneous Treatment of Human Bronchial Epithelial Cells with Serine and Cysteine Protease Inhibitors Prevents Severe Acute Respiratory Syndrome Coronavirus Entry. *J. Virol.* 86, 6537–6545. doi: 10.1128/jvi.00094-12

Khera, R., Clark, C., Lu, Y., Guo, Y., Ren, S., Truax, B., et al. (2020). Association of Angiotensin-Converting Enzyme Inhibitors and Angiotensin Receptor Blockers with the Risk of Hospitalization and Death in Hypertensive Patients with Coronavirus Doseiase-19. *medRxiv* Preprint. doi: 10.1101/2020.05.17.20104943

Khoury, M., Cuenca, J., Cruz, F. F., Figueroa, F. E., Rocco, P. R. M., and Weiss, D. J. (2020). Current status of cell-based therapies for respiratory virus infections: applicability to COVID-19. *Eur. Respir. J.* 55:2000858. doi: 10.1183/13993003.00858-2020

Kim, D., Lee, J.-Y., Yang, J.-S., Kim, J. W., Kim, V. N., and Chang, H. (2020). The Architecture of SARS-CoV-2 Transcriptome. *Cell* 181, 914–921.

Kim, U. J., Won, E.-J., Kee, S.-J., Jung, S.-I., and Jang, H.-C. (2016). Combination Therapy with Lopinavir/Ritonavir, Ribavirin and Interferon-α for Middle East Respiratory Syndrome. *Antivir. Ther.* 21, 455–459. doi: 10.3851/imp3002

King, A., Vail, A., O'Leary, C., Hannan, C., Brough, D., Patel, H., et al. (2020). Anakinra in COVID-19: important considerations for clinical trials. *Lancet Rheum.* 2, 30160–30160.

Klok, F. A., Kruip, M. J. H. A., van der Meer, N. J. M., Arbous, M. S., Gommers, D. A. M. P. J., Kant, K. M., et al. (2020). Incidence of Thrombotic Complications in Critically Ill ICU Patients with COVID-19. *Thromb. Res.* 191, 145–147. doi: 10.1016/j.thromres.2020.04.013

Ko, J.-H., Seok, H., Cho, S. Y., Ha, Y. E., Baek, J. Y., Kim, S. H., et al. (2018). Challenges of Convalescent Plasma Infusion Therapy in Middle East Respiratory Coronavirus Infection: A Single Centre Experience. *Antivir. Ther.* 23, 617–622. doi: 10.3851/imp3243

Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., et al. (2005). A Crucial Role of Angiotensins Converting Enzyme 2 (ACE2) in SARS Coronavirus-Induced Lung Injury. *Nat. Med.* 11, 875–879. doi: 10.1038/nm1267

Lam, T. T.-Y., Jia, N., Zhang, Y.-W., Shum, M. H.-H., Jiang, J.-F., Zhu, H.-C., et al. (2020). Identifying SARS-CoV-2-related Coronaviruses in Malayan Pangolins. *Nature* 583, 282–285.

Lamers, M. M., Beumer, J., van der Vaart, J., Knoops, K., Puschhof, J., Breugen, T. I., et al. (2020). SARS-CoV-2 productively infects human gut enterocytes. *Science* 369, 50–54. doi: 10.1126/science.abc1669

Lane, J. C. E., Weaver, J., Kostka, K., Duarte-Salle, T., Abrahao, M. T. F., Alghoul, H., et al. (2020). Safety of hydroxychloroquine, alone and in combination with azithromycin, in light of rapid wide-spread use for COVID-19: a multinational, network cohort and self-controlled case series study. Preprint. doi: 10.1101/2020.04.08.20054551

Lauer, S. A., Grantz, K. H., Bi, Q., Jones, F. K., Zheng, Q., Meredith, H. R., et al. (2020). The Incubation Periodo of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. *Ann. Intern. Med.* 172, 577–582. doi: 10.7326/m20-0504

Lax, S. F., Skok, K., Zechner, P., Kessler, H. H., Kaufmann, N., Koelblinger, C., et al. (2020). Pulmonary Arterial Thrombosis in COVID-19 with Fatal Outcome: Results from a Prospective, Single-Center, Clinicopathologic Case Series. *Ann. Intern. Med.* 173, M20–M2566.

Leng, Z., Zhu, R., Hou, W., Feng, Y., Yang, Y., Han, Q., et al. (2020). Transplantation of ACE2- Mesenchymal Stem Cells Improves the Outcomes of Patients with COVID-19 Pneumonia. *Aging Dis.* 11, 216–228.

Leung, J. M., Yang, C. X., Tam, A., Shaipanich, T., Hackett, T.-L., Singhera, G. K., et al. (2020). ACE-2 Expression in the Small Airway Epithelial of Smokers and COPD Patients: Implications for COVID-19. *Eur. Respir. J.* 55:2000688. doi: 10.1183/13993003.00688-2020

Levi, M., Thachil, J., Iba, T., and Levy, J. H. (2020). Coagulation Abnormalities and Thrombosis in Patients with COVID-19. *Lancet Haematol.* 7, 438–440e.

Li, G., Fan, Y., Lai, Y., Han, T., Li, Z., Zhou, P., et al. (2020). Coronavirus Infections and Immune Responses. *J. Med. Virol.* 92, 424–432.

Li, H., Chen, C., Hu, F., Wang, J., Zhao, Q., Gale, R. P., et al. (2020a). Impact of Corticosteriod Therapy on Outcomes of Persons with SARS-CoV-2, SARS-CoV, or MERS-CoV Infection: A Systematic Review and Meta-Analysis. *Leukemia* 34, 1503–1511. doi: 10.1038/s41375-020-0848-3

Li, H., Liu, L., Zhang, D., Xu, J., Dai, H., Tang, N., et al. (2020b). SARS-CoV-2 and Viral Sepsis: Observations and Hypotheses. *Lancet* 395, 1517–1520. doi: 10.1016/s0140-6736(20)30920-x

Li, Y.-C., Bai, W.-Z., and Hashikawa, T. (2020). The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. *J. Med. Virol.* 92, 552–555. doi: 10.1002/jmv.25728

Li, Z., Wi, M., Yao, J., Guo, J., Liao, X., Song, S., et al. (2020). Caution on Kidney Dysfunctions of COVID-19 Patients. *medRxiv* Preprint. doi: 10.1101/2020.02.08.20021212

Ling, T.-Y., Kuo, M.-D., Li, C.-L., Yu, A. L., Huang, Y.-H., Wu, T.-J., et al. (2006). Identification pf Pulmonary Oct-4^{+} stem-progenitor Cells and Demonstration of Their Susceptibility to SARS Coronavirus (SARS-CoV) Infection in Vitro. *Proc. Natl. Acad. Sci. U S A.* 103, 9530–9535. doi: 10.1073/pnas.0510232103

Liu, J., Li, S., Liu, J., Liang, B., Wang, X., Wang, H., et al. (2020). Longitudinal characteristics of lymphocyte responses and cytokine profile in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine* 55:102763.

Liu, Y., Du, X., Chen, J., Jin, Y., Peng, L., Wang, H. H. X., et al. (2020a). Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. *J. Infect.* 81, 6–12e.

Liu, Y., Yan, L.-M., Wan, L., Xiang, T.-X., Le, A., Liu, J.-M., et al. (2020b). Viral Dynamics in Mild and Severe Cases of COVID-19. *Lancet Infect. Dis.* 20, 656–657. doi: 10.1016/s1473-3099(20)30232-2

Lodigiani, C., Iapichino, G., Carenzo, L., Cecconi, M., Ferrazzi, P., Sebastian, T., et al. (2020). Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan. Italy. *Thromb. Res.* 191, 9–14. doi: 10.1016/j.thromres.2020.04.024

Logunov, D. Y., Dolzhikova, I. V., Zubkova, O. V., Tukhvatullin, A. I., Skcheblyakov, D. V., Dzharullaeva, A. S., et al. (2020). Safety and immunogenicity of ah rAD26 and rAd5 vactor-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. *Lancet* 396, 887–897. doi: 10.1016/S0140-6736(20)31866-3

Lopes-Pacheco, M. (2020). CFTR Modulators: The Changing Face of Cystic Fibrosis in the Era of Precision Medicine. *Front. Pharmacol.* 10:1662. doi: 10.3389/fphar.2019.01662

Lopes-Pacheco, M., Robba, C., Rocco, P. R. M., and Pelosi, P. (2020). Current Understanding of the Therapeutic Benefits of Mesenchymal Stem Cells in Acute Respiratory Distress Syndrome. *Cell. Biol. Toxicol.* 36, 83–102. doi: 10.1007/s10565-019-09493-5

Mancia, G., Rea, F., Ludergnani, M., Apolone, G., and Corrao, G. (2020). Renin-Angiotensin-Aldosterone System Blockers and the Risk of Covid-19. *N. Engl. J. Med.* 382, 2431–2440. doi: 10.1056/nejmoa2006923

Mao, L., Wang, M., Chen, S., He, Q., Chang, J., Hong, C., et al. (2020). Neurological Manifestations of Hospitalized Patients with COVID-19 in Wuhan, China: a retrospective case series study. *medRxiv* Preprint. doi: 10.1101/2020.02.22.20026500

Mao, R., Qiu, Y., Tan, J.-Y., Li, X.-H., Liang, J., Shen, J., et al. (2020). Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol. Hepatol.* 5, 667–678. doi: 10.1016/s2468-1253(20)30126-6

Matsuyama, S., Nao, N., Shirato, K., Kawasa, M., Saito, S., Takayama, I., et al. (2020). Enhanced Isolation of SARS-CoV-2 by TMPRSS2-expressing Cells. *Proc. Natl. Acad. Sci. U S A.* 117, 7001–7003. doi: 10.1073/pnas.2002589117

Matthay, M. A., Calfee, C. S., Zhuo, H., Thompsom, B. T., Wilson, J. G., Levitt, J. E., et al. (2019). Treatment with Allogeneic Mesenchymal Stromal Cells for Moderate to Severe Acute Respiratory Distress Syndrom (START Study): A Randomised Phase 2a Safety Trial. *Lancet Respir. Med.* 7, 154–162. doi: 10.1016/s2213-2600(18)30418-1

Matthay, M. A., Pati, S., and Lee, J.-W. (2017). Concise Review: Mesenchymal Stem (Stromal) Cells: Biology and Preclinical Evidence for Therapeutic Potential for Organ Dysfunction Following Trauma os Sepsis. *Stem Cells* 35, 316–324. doi: 10.1002/stem.2551

Mattos-Silva, P., Felix, N. S., Silva, P. L., Rocca, C., Battaglini, D., Pelosi, P., et al. (2020). Pros and cons of corticosteriod therapy for COVID-19 patients. *Respir. Physiol. Neurobiol.* 280:103492.

Mauthe, M., Orhon, I., Rocchi, C., Zhou, X., Luhr, X., Luhr, M., et al. (2018). Chloroquine Inhibits Autophagic Flux by Decreasing Autophagosome-Lysosome Fusion. *Autophagy* 14, 1435–1455. doi: 10.1080/15548627.2018.1474314

McIntyre, L. A., Stewart, D. J., Mei, S. H. J., Courtman, D., Watpool, I., Granton, J., et al. (2018). Cellular Immunotherapy for Septic Shock. A Phase I Clinical Trial. *Am. J. Respir. Crit. Care Med.* 197, 337–347. doi: 10.1164/rccm.201705-1006oc

Molina, J. M., Celaugerre, C., Goff, J. L., Mela-Lima, B., Ponscarme, D., Goldwirt, L., et al. (2020). No Evidence of Rapid Antiviral Clearance or Clinical Benefit with the Combination of Hydroxychloroquine and Azithromycin in Patients with Severe COVID-19. *Med. Mal. Infect.* 50:384. doi: 10.1016/j.medmal.2020.03.006

Monteil, V., Kwon, H., Prado, P., Hagelkrüys, A., Wimmer, R. A., Stahl, M., et al. (2020). Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. *Cell* 181, 905–913. doi: 10.1016/j.cell.2020.04.004

Moriguchi, T., Harii, N., Goto, J., Harada, D., Sugawara, H., Takamino, J., et al. (2020). A first case of meningitis/enchephalitis associated with SARS-Coronavirus-2. *Int. J. Infect. Dis.* 94, 55–58.

Ni, W., Yang, X., Liu, J., Bao, J., Li, R., Xu, Y., et al. (2020). Acute Myocardical Injury at Hospital Admission Is Associated with All-Cause Mortality in COVID-19. *J. Am. Coll. Cardiol.* 76, 124–125. doi: 10.1016/j.jacc.2020.05.007

Osborne, V., Davies, M., Lane, S., Evans, A., Denyer, J., Dhanda, S., et al. (2020). Lopanavir-Ritonavir in the Treatment of COVID-19: A Dynamic Systematic Benefit-Risk Assessment. *Drug Saf.* 43, 809–821. doi: 10.1007/s40264-020-00966-9

Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., et al. (2020). Characterization of Spike Glycoprotein of SARS-CoV-2 on Virus Entry and Its Immune Cross- Reactivity with SARS-CoV. *Nat. Commun.* 11:1620.

Pan, L., Mu, M., Yang, P., Sun, Y., Wang, R., Yan, J., et al. (2020). Clinical Characteristics of COVID-19 Patients with Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *Am. J. Gastroenterol.* 115, 766–773. doi: 10.14309/ajg.0000000000000620

Pan, X.-W., Xu, D., Zhang, H., Zhou, W., Wang, L.-H., and Cui, X.-G. (2020). Identification of a Potential Mechanims of Acute Kidney Injury During the COVID-19 Outbreak: A Study Based on Single-Cell Transcriptome Analysis. *Intesive Care Med.* 46, 1114–1116. doi: 10.1007/s00134-020-06026-1

Pécheur, E.-I., Borisevich, V., Halfmann, P., Morrey, J. D., Smee, D. F., Prichard, M., et al. (2016). The Synthetic Antiviral Drug Arbidol Inhibits Globally Prevalent Pathogenic Viruses. *J. Virol.* 90, 3086–3092. doi: 10.1128/jvi.02077-15

Polack, F. P., Thomas, S. J., Kitchin, N., Absalon, J., Gurtman, A., Locklart, S., et al. (2020). Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N. Engl. J. Med.* 383:2034577. doi: 10.1056/NEJMoa2034577

Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., et al. (2019). Drug Repurposing: Progress, Challenges and Recommendations. *Nat. Rev. Drug Discov.* 18, 41–58. doi: 10.1038/nrd.2018.168

Quartuccio, L., Sonaglia, A., Pecori, D., Peghin, M., Fabris, M., Tascini, C., et al. (2020). Higher levels of IL-6 early after toxilizumab distinguish survivors from nonsurvivors in COVID-19 pneumonia: A possible indication for deeper targeting of IL-6. *J. Med. Virol.* 92, 2852–2856. doi: 10.1002/jmv.26149

Ramiro, S., Mostard, R. L. M., Magro-Checa, C., van Dongen, C. M. P., Dormans, T., Buijs, J., et al. (2020). Historically controlled comparison of glucocorticoids with and without tocilizumab versus supportive care only in patients with COIVD-19-associated cytokine storm syndrome: results of the CHIC study. *Ann. Rheum. Dis.* 79, 1143–1151. doi: 10.1136/annrheumdis-2020-218479

Rawat, K., Kumari, P., and Saha, L. (2020). COVID-19 vaccine: A recent update in pipeline vaccines, their design and development strategies. *Eur. J. Pharmacol.* 892:173751. doi: 10.1016/j.ejphar.2020.173751

Reynolds, H. R., Adhihari, S., Pulgarin, C., Troxel, A. B., Iturrate, E., Johnson, S. B., et al. (2020). Renin-Angiotensin-Aldosterone System Inhibitos and Risk of Covid-19. *N. Engl. J. Med.* 382, 2441–2448.

Robba, C., Battaglini, D., Ball, L., Patroniti, N., Loconte, M., Brunetti, I., et al. (2020a). Distinct Phenotypes Require Distinct Respiratory Management Strategies in Severe COVID-19. *Respir. Physiol. Neurobiol.* 279:103455. doi: 10.1016/j.resp.2020.103455

Robba, C., Battaglini, D., Pelosi, P., and Rocco, P. R. M. (2020b). Multiple organ dysfunction in SARS-CoV-2: MODS-CoV-2. *Expert Rev. Respir. Med.* 14, 865–868. doi: 10.1080/17476348.2020.1778470

Rocco, P. R., Silva, P. L., Cruz, F. F., Junior, M. A. C. M., Tierno, P. F. G. M. M., Moura, M. A., et al. (2020). Early use of nitazoxanide in mild Covid-19 disease: randomised, placebo-controlled trial. *Eur. Respir. J.* 2020:2003725. doi: 10.1183/13993003.03725-2020

Ruan, Q., Yang, K., Wang, W., Jiang, L., and Song, J. (2020). Clinical Predictors of Mortality Due to COVID-19 Based on an Analysis of Data of 150 Patients From Wuhan, China. *Intensive Care Med.* 46, 846–848. doi: 10.1007/s00134-020-05991-x

Russo, R., Andolfo, I., Lasorca, V. A., Iolascon, A., and Capasso, M. (2020). Genetic Analysis of the Coronavirus SARS-CoV-2 Host Protease TMPRSS2 in Different Populations. *Front. Genet.* 11:872. doi: 10.3389/fgene.2020.00872

Sakurai, A., Sasaki, T., Kato, S., Hayashi, M., Tsuzuki, S.-I., Ishihara, T., et al. (2020). Natural History of Asymptomatic SARS-CoV.2 Infection. *N. Engl. J. Med.* 383, 885–886.

Salazar, E., Perez, K. K., Ashraf, M., Chen, J., Castillo, B., Christensen, P. A., et al. (2020). Treatment of Coronavirus Disease 2019 (COVID-19) Patients with Convalescent Plasma. *Am. J. Pathol.* 190, 1680–1690.

Sama, I. E., Ravera, A., Santema, B. T., van Goor, H., Ter Maaten, J. M., Cleland, J. G. F., et al. (2020). Circulating Plasma Concentrations of Angiotensin-Converting Enzyme 2 in Men and Women with Heart Failure and Effects of Renin-Angiotensin-Aldosterone Inhibitors. *Eur. Heart J.* 41, 1810–1817.

Samudrala, P. K., Kumar, P., Choudhary, K., Thakur, N., Wadekar, G. S., Dayaramani, R., et al. (2020). Virology, pathogenesis, diagnosis and in-line treatment of COVID-19. *Eur. J. Pharmacol.* 883:173375. doi: 10.1016/j.ejphar.2020.173375

Santos, R. A. S., Sampaio, W. O., Alzamora, A. C., Motta-Santos, D., Alenina, N., Bader, M., et al. (2018). The ACE2/Angiotensin-(1-7)/MAS Axis of the Renin-Angiotensin-System: Focus on Angiotensin-(1-7). *Physiol. Rev.* 98, 505–553. doi: 10.1152/physrev.00023.2016

Sciascia, S., Aprà, F., Baffa, A., Baldovino, S., Boaro, D., Boero, R., et al. (2020). Pilot Prospective Open, Single-Arm Multicentre Study on Off-Label Use of Tocilizumab in Patients with Severe COVID-19. *Clin. Exp. Rheumatol.* 38, 529–532.

Sengupta, V., Sengupta, S., Lazo, A., Woods, P., Nolan, A., and Bremer, N. (2020). Exosomes Derived From Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19. *Stem Cells Dev.* 29, 747–754. doi: 10.1089/scd.2020.0080

Shah, P. P., Wang, T., Kaletsky, R. L., Myers, M. C., Purvir, J. E., Jing, H., et al. (2010). A Small-Molecule Oxocarbazate Inhibitor of Human Cathepsin L Blocks Severe Acute Respiratory Syndrome and Ebola Pseudotype Virus Infection Into Human Embryonic Kidney 293T Cells. *Mol. Pharmacol.* 78, 319–324. doi: 10.1124/mol.110.064261

Sharif-Askari, N. D., Sharif-Askari, F. S., Alabed, M., Temsah, M.-H., Heialy, S. A., Hamid, Q., et al. (2020). Airways Expression of SARS-CoV-2 Receptor, ACE2, and TMPRSS2 is Lower in Children than Adults and Increases with Smoking and COPD. *Mol. Ther. Methods Clin. Dev.* 18, 1–6. doi: 10.1016/j.omtm.2020.05.013

Shastri, A., Wheat, J., Agrawal, S., Chaterjee, N., Pradhan, K., Goldfinger, M., et al. (2020). Delayed clearance of SARS-CoV2 in male compared to female patients: High ACE2 expression in testes suggests possible existence of gender-specific viral reservoirs. *medRxiv* Preprint. doi: 10.1101/2020.04.16.20060566

Sheahan, T. P., Sims, A. C., Leist, S. R., Schäfer, A., Won, J., Brown, A. J., et al. (2020). Comparative Therapeutic Efficact of Remdesivir and Combination Lopinavir, Ritonavir, and Interferon Beta Against MERS-CoV. *Nat. Commun.* 11:222.

Shen, C., Wang, Z., Zhao, F., Yang, Y., Li, Z., Yuan, J., et al. (2020). Treatment of 5 Critically Ill Patients with COVID-19 with Convalescent Plasma. *JAMA* 323, 1582–1589.

Shi, S., Qin, M., Shen, B., Cai, Y., Liu, T., Yang, F., et al. (2020). Association of Cardiac Injury with Mortality in Hospitalized Patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* 5, 802–810. doi: 10.1001/jamacardio.2020.0950

Silva, J. D., de Castro, L. L., Braga, C. L., Oliveira, G. P., Trivelin, A. S., Barbosa-Junior, C. M., et al. (2019). Mesenchymal Stromal Cells Are More Effective Than Theirs Extracellular Vesicles at Reducing Lung Injury of Acute Respiratory Distress Syndrome Etiology. *Stem Cells Int.* 2019:8262849.

Silva, J. D., Lopes-Pacheco, M., Paz, A. H. R., Cruz, F. F., Melo, E. B., de Oliveira, M. V., et al. (2018). Mesenchymal Stem Cells From Bone Marrow, Adipose Tissue, and Lung Tissue Differentially Mitigate Lung and Distal Organ Damage in Experimental Acute Respiratory Distress Syndrome. *Crit. Care Med.* 46, 132–140e.

Siu, K.-L., Yuen, K.-S., Castaño-Rodriguez, C., Ye Zi-Wei, Yeung, M.-L., et al. (2019). Severe Acute Respiratory Syndrome Coronavirus ORF3a Protein Activates the NLRP3 Inflammasome by Promoting TRAF3-dependent Ubiquitination of ASC. *FASEB J.* 33, 8865–8877. doi: 10.1096/fj.201802418r

Solomon, I. H., Normandin, E., Bhattacharyya, S., Mukerji, S., Keller, K., Ali, A. S., et al. (2020). Neuropathological Features of Covid-19. *N. Eng. J. Med.* 383, 989–992.

Su, H., Yang, M., Wan, G., Yi, L.-X., Tang, F., Zhu, H.-Y., et al. (2020). Renal Histopathological Analysis of 26 Postmortem Findings of Patients with COVID-19 in China. *Kidney Int.* 98, 219–227. doi: 10.1016/j.kint.2020.04.003

Sungnak, W., Huang, N., Bécavin, C., Berg, M., Queen, R., Litvinukova, M., et al. (2020). SARS-CoV-2 Entry Factors Are Highly Expressed in Nasal Epithelial Cells Together with Innate Immune Genes. *Nat. Med.* 26, 681–687. doi: 10.1038/s41591-020-0868-6

Takahashi, T., Wong, P., Ellingson, M., Lucas, C., Klein, J., Israelow, B., et al. (2020). Sex differences in immune responses to SARS-CoV-2 that underlie disease outcomes. *medRxiv* Preprint. doi: 10.1101/2020.06.06.20123414

Taneja, V. (2018). Sex Hormones Determine Immune Response. *Front. Immunol.* 9:1931. doi: 10.3389/fimmu.2018.01931

Tang, N., Bai, H., Chen, X., Gong, J., Li, D., and Sun, Z. (2020). Anticoagulant Treatment Is Associated with Decreased Mortality in Severe Coronavirus Disease 2019 Patients with Coagulopathy. *J. Thromb. Harmost.* 18, 1095–1099.

Thachil, J., Tang, N., Gando, S., Falanga, A., Cattaneo, M., Levi, M., et al. (2020). ISTH Interim Guidance on Recognition and Management of Coagulopathy in COVID.19. *J. Thromb. Haemost.* 18, 1023–1026. doi: 10.1111/jth.14810

Tian, S., Xiong, Y., Liu, H., Niu, L., Guo, J., Miao, M., et al. (2020). Pathological Study of the 2019 Novel Coronavirus Disease (COVID-19) Through Postmortem Core Biopsies. *Mol. Pathol.* 33, 1007–1014. doi: 10.1038/s41379-020-0536-x

Tian, X., Li, C., Huang, A., Xia, S., Lu, S., Shi, Z., et al. (2020). Potent Binding of 2019 Novel Coronavirus Spike Protein by a SARS Coronavirus-Specific Human Monoclonal Antibody. *Emerg. Microbes Infect.* 9, 382–385. doi: 10.1080/22221751.2020.1729069

Toniati, P., Piva, S., Cattalini, M., Garrafa, E., Regola, F., Castelli, F., et al. (2020). Toxilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single center study of 100 patients in Brescia, Italy. *Autoimmun. Rev.* 19:102568.

Uhal, B. D., Li, X., Xue, A., Gao, X., and Abdul-Hafez, A. (2011). Regulation of Alveolar Epithelial Cell Survival by the ACE-2/angiotensin1-7/Mas Axis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 301, L269–L274.

Úri, K., Fagyas, M., Kertész, Borbély, A., Jenei, C., Bene, O., et al. (2016). Circulating ACE2 Activity Correlates with Cardiovascular Disease Development. *J. Renin Angiotensin Aldosterone Syst.* 17:1470320316668435.

Van Doremalen, N., Lambe, T., Spencer, A., Belij-Rammerstorfer, S., Purushotham, J. N., Port, J. R., et al. (2020). ChAdOx1 nCov-19 vaccination prevents SARS-CoV-2 pneumonia in rhesus macaques. *bioRxiv* Preprint. doi: 10.1101/2020.05.13.093195

Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., et al. (2020). Endothelial Cell Infection and Endotheliitis in COVID-19. *Lancet* 395, 1417–1418.

Veras, F. P., Pontelli, M., Silva, C., Toller-Kawahisa, J., de Lima, M., Nascimento, D., et al. (2020). SARS-CoV-2 triggered neutrophil extracelular traps (NETs) mediated COVID-19 pathology. *medRxiv* Preprint. doi: 10.1101/2020.06.08.20125823

Von Weyhern, C. H., Kaufmann, I., Neff, F., and Kremer, M. (2020). Early evidence of pronounced brain involvement in fatal COVID-19 outcomes. *Lancet* 395:e109. doi: 10.1016/s0140-6736(20)31282-4

Voysey, M., Clemens, S. A. C., Madhi, S. A., Weckx, L. Y., Folegatti, P. M., Aley, P. K., et al. (2020). Safety and efficacy of ChAdOx1 nCov-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomized controlled trials in Brazil, South Africa, and the UK. *Lancet* 397, 99–111. doi: 10.1016/S0140-3736(20)32661-1

Walls, A. C., Park, Y.-J., Tortorici, M. A., Wall, A., McGuire, A. T., and Veesler, D. (2020). Structure, Function and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* 181, 281–292. doi: 10.1016/j.cell.2020.02.058

Wan, Y., Shang, J., Graham, R., Baric, R. S., and Li, F. (2020). Receptor Recognition by the Novel Coronavirus From Wuhan: An Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J. Virol.* 94, 127–120e.

Wang, C., Horby, P. W., Hayden, F. G., and Gao, G. F. (2020). A Novel Coronavirus Outbreak of Global Health Concern. *Lancet* 395, 470–473. doi: 10.1016/s0140-6736(20)30185-9

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. (2020). Clinical Characteristics of 138 Hospitalized Patients with 2019 Novel Coronavirus-Infected Pneumonia in Wuhan. China. *JAMA* 323, 1061–1069. doi: 10.1001/jama.2020.1585

Wang, K., Gheblawi, M., and Oudit, G. Y. (2020). Angiotensin Converting Enzyme 2: A Double-Edged Sword. *Circulation* 142, 426–428. doi: 10.1161/CIRCULATIONAHA.120.047049

Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., et al. (2020). Remdesivir and Chloroquine Effectively Inhibit the Recently Emerged Novel Coronavirus (2019-nCoV) in Vitro. *Cell Res.* 30, 269–271. doi: 10.1038/s41422-020-0282-0

Wang, S., Guo, F., Liu, K., Wang, H., Rao, S., Yang, P., et al. (2008). Endocytosis of the Receptor-Binding Domain of SARS-CoV Spike Protein Together with Virus Receptor ACE2. *Virus Res.* 136, 8–15. doi: 10.1016/j.virusres.2008.03.004

Wang, W., Patel, V. B., Parajuli, N., Fan, D., Basu, R., Wang, Z., et al. (2014). Heterozygote loss of ACE2 is sufficient to increae the susceptibility to heart disease. *J. Mol. Med.* 92, 847–858. doi: 10.1007/s00109-014-1149-y

Wang, Y., Jiang, W., He, Q., Wang, C., Wang, B., Zhou, P., et al. (2020a). A retrospective cohort study of methylprednisolone therapt in severe patients with COVID-19 pneumonia. *Signal Transduct. Target Ther.* 5:57.

Wang, Y., Zhang, D., Du, G., Du, R., Zhao, J., Jin, Y., et al. (2020b). Remdesivir in Adults with Severe COVID-19: A Randomised, Double-Blind, Placebo-Controlled, Multicentre Trial. *Lancet* 395, 1569–1579.

Wang, Z., and Xu, X. (2020). scRNA-seq Profiling of Human Testes Reveals the Presence of ACE2 Receptor, A Target for SARS-CoV-2 Infection in Spermatogonia, Leydig and Sertoli Cells. *Cells* 9:920. doi: 10.3390/cells9040920

Wang, Z., Yang, B., Li, Q., Wen, L., and Zhang, R. (2020). Clinical Features of 69 Cases with Coronavirus Disease 2019 in Wuhan, China. *Clin. Infect. Dis.* 71, 769–777. doi: 10.1096/cid/ciaa272

Williamson, E., Walker, A. J., Bhaskaran, K., Bacon, S., Bates, C., Morton, C. E., et al. (2020). OpenSAFELY: factors associated with COVID-19 death in 17 million patients. *Nature* 584, 430–436. doi: 10.1038/s41586-020-2521-4

Wölfel, R., Corman, V. M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M. A., et al. (2020). Virological assessment of hospitalized patients with COVID-19. *Nature* 581, 465–469.

Wong, H. K., Lee, C. K., Hung, I. F. N., Leung, J. N. S., Hong, J., Yuen, K. Y., et al. (2010). Practical limitations of Convalescent Plasma Collection: A Case Scenation in Pandemic Preparation for Influenza A (H1N1) Infection. *Transfusion* 50, 1967–1971. doi: 10.1111/j.1537-2995.2010.02651.x

Wrapp, D., Wang, N., Corbett, K., Goldsmith, J. A., Hsieh, C.-L., Abiona, O., et al. (2020). Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation. *Science* 367, 1260–1263.

Wu, A., Peng, Y., Huang, B., Ding, X., Wang, X., Niu, P., et al. (2020). Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. *Cell Host Microbe* 27, 325–328. doi: 10.1016/j.chom.2020.02.001

Wu, D., and Yang, X. O. (2020). Th17 Responses in Cytokine Storm of COVID-19: An Emerging Target of JAK2 Inhibitor Fedratinib. *J. Microbiol. Immunol. Infect.* 53, 368–370. doi: 10.1016/j.jmii.2020.03.005

Wu, P., Duan, F., Luo, C., Liu, Q., Qu, X., Liang, L., et al. (2020). Characteristics of Ocular Findings of Patients with Coronavirus Disease 2019 (COVID-19) in Hubei Province, China. *JAMA Ophthalmol.* 138, 575–578. doi: 10.1001/jamaophthalmol.2020.1291

Wu, Z., and McGoogan, J. M. (2020). Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From Chinese Center for Disease Control and Prevention. *JAMA* 323, 1239–1242. doi: 10.1001/jama.2020.2648

Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X., and Shan, H. (2020). Evidence for Gastrointestinal Infection of SARS-CoV-2. *Gastroenterology* 158, 1831–1833. doi: 10.1053/j.gastro.2020.02.055

Xu, J., Sriramula, S., Xia, H., Moreno-Walton, L., Culicchia, F., Domenig, O., et al. (2017). Clinical Relevance and Role of Neuronal AT_1 Receptors in ADAM17-Mediated ACE2 Shedding in Neurogenic Hypertension. *Circ. Res.* 121, 43–55.

Xu, X., Han, M., Li, Y., Sun, W., Wang, D., Fu, B., et al. (2020). Effective Treatment of Severe COVID-19 Patients with Tocilizumab. *Proc. Natl. Acad. Sci. U S A.* 117, 10970–10975.

Xu, Y., Sriramula, S., Xia, H., Moreno-Walton, L., Culicchia, F., Domening, O., et al. (2017). Clinical Relevance and Role of Neuronal AT_1 Receptor in ADAM17-Mediated ACE2 Shedding in Neurogenic Hypertension. *Circ. Res.* 121, 43–55. doi: 10.1161/circresaha.116.310509

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., et al. (2020). Pathological Findings of COVID-19 Associated with Acute Respiratory Distress Syndrome. *Lancet Respir. Med.* 8, 420–422. doi: 10.1016/s2213-2600(20)30076-x

Yamamoto, M., Kiso, M., Sakai-Tagawa, Y., Iwatsuki-Horimoto, K., Imai, M., Takeda, M., et al. (2020). The anticoagulante nafamostat potently inhibits SARS-CoV-2 infection in vitro: an existing drug with multiple possible therapeutic effects. *bioRxiv* Preprint. doi: 10.1101/2020.04.22.054981

Yamamoto, M., Matsuyama, S., Li, X., Takeda, M., Kawaguchi, Y., Inoue, J.-I., et al. (2016). Identification of Nafamostat as a Potent Inhibitor of Middle East Respiratory Syndrome Coronavirus S Protein-Mediated Membrane Fusion Using the Split-Protein-Based Cell-Cell Fusion Assay. *Antimicrob. Agents Chemother.* 60, 6532–6539. doi: 10.1128/aac.01043-16

Ye, M., Fu, D., Ren, Y., Wang, F., Wang, D., Zhang, F., et al. (2020). Treatment with convalescent plasma for COVID-19 patients in Wuhan, China. *J. Med. Virol.* 92, 1890–1901. doi: 10.1002/jmv.25882

Zhang, B., Liu, S., Tan, T., Huang, W., Dong, Y., Chen, L., et al. (2020a). Treatment with Convalescent Plasma for Critically Ill Patients with Severe Acute Respiratory Syndrome Coronavirus 2 Infection. *Chest* 158, 9–13e.

Zhang, B., Zhou, X., Qiu, Y., Feng, F., Feng, J., Jia, Y., et al. (2020b). Clinical characteristics of 82 death cases with COVID-19. *medRxiv* Preprint. doi: 10.1101/2020.02.26.20028191

Zhang, C., Shi, L., and Wang, F.-S. (2020a). Liver Injury in COVID-19: Management and Challenges. *Lancet Gastroenterol. Hepatol.* 5:430.

Zhang, C., Wu, Z., Li, J.-W., Zhao, H., and Wang, G.-Q. (2020b). Cytokine Release Syndrome in Severe COVID-19: Interleukin-6 Receptor Antagonist Tocilizumab May Be the Key to Reduce Mortality. *Int. J. Antimicrob. Agents* 55:105954. doi: 10.1016/j.ijantimicag.2020.105954

Zhang, H., Zhou, P., Wei, Y., Yue, H., Wang, Y., Hu, M., et al. (2020). Histopathologic Changes and SARS-CoV-2 Immunostaining in the Lung of a Patients with COVID-19. *Ann. Intern. Med.* 172, 629–632. doi: 10.7326/m20-0533

Zhang, P., Zhu, L., Cai, J., Lei, F., Qin, J.-J., Xie, J., et al. (2020). Association of Inpatient Use of Angiotensin-Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers with Mortality Among Patients with Hypertension Hospitalized with COVID-19. *Circ. Res.* 126, 1671–1681.

Zhang, T., Wu, Q., and Zhang, Z. (2020). Probable Pangolin Origin of SARS-CoV-2 Associated with the COVID-19 Outbreak. *Corr. Biol.* 30, 1346–1351. doi: 10.1016/j.cub.2020.03.022

Zhang, W., Du, R.-H., Li, B., Zheng, X.-S., Yang, X.-L., Hu, B., et al. (2020). Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg. Microbes Infect.* 9, 386–389.

Zhang, Y., Zheng, G., Pan, H., Li, C., Hu, Y., Chu, K., et al. (2020). Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomized, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect. Dis.* 2020:4. doi: 10.1016/S1473-3099(20)30843-4

Zhao, J., Yang, Y., Huang, H., Li, D., Gu, D., Li, X., et al. (2020). Relationship between the ABO blood groups and the COVID-19 susceptibility. *Preprint* doi: 10.1101/2020.03.11.20031096

Zhao, X., Nichills, J. M., and Chen, Y.-G. (2008). Severe acute respiratory syndrom-associated coronavirus nucleocapsid protein interacts with Smad3 and modulates transforming growth factor-beta signaling. *J. Biol. Chem.* 283, 3272–3280.

Zhao, Y., Zhao, Z., Wang, Y., Zhou, Y., Ma, Y., and Zuo, W. (2020). Single-cell RNA expression profiling of ACE2, the receptor of SARS-CoV-2. *Preprint* doi: 10.1101/2020.01.26.919985

Zhao, Z., Zhang, F., Xu, M., Huang, K., Zhong, W., Cai, W., et al. (2003). Description and clinical treatment of an early outbreak of severe acute respiratory syndrome (SARS) in Guangzhou. *PR China. J. Med. Microbiol.* 52, 715–720.

Zhou, F., Yu, T., Fan, G., Liu, Y., Liu, Z., Xiang, J., et al. (2020). Clinical course and risk factor for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395, 1054–1062.

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273.

Zhou, Y., Fu, B., Zheng, X., Wang, D., Zhao, C., Qi, Y., et al. (2020). Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients. *Natl. Sci. Rev.* nwaa041. doi: 10.1093/nsr/nwaa041

Zhou, Y., Vedantham, P., Kai, L., Agudelo, J., Carrion, R. Jr., Nunneley, J. W., et al. (2015). Protease inhibitors targeting coronavirus and filovirus entry. *Antiviral Res.* 116, 76–84.

Zietz, M., and Tatonetti, N. P. (2020). Testing the association between blood type and COVID-19 infection, intubation and death. *Preprint* doi: 10.1101/2020.04.08.20058073

Zuo, Y., Yalavartly, S., Shi, H., Gockman, K., Zuo, M., Madison, J. A., et al. (2020). Neutrophil extracellular traps in COVID-19. *JCI Insight* 5:138999.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 02 February 2021
doi: 10.3389/fphys.2021.624154

Pain in Covid Era

Heloísa Alonso-Matielo, Victória Regina da Silva Oliveira, Victhor Teixeira de Oliveira and Camila Squarzoni Dale**

Department of Anatomy, Laboratory of Neuromodulation and Experimental Pain, University of São Paulo, São Paulo, Brazil

The COVID19 pandemic has impacted the lives and health of persons worldwide and although majority of COVID19 patients present with respiratory symptoms, pain emerges as an important feature of COVID19 infection. About 15–20% of patients progress to a severe condition that requires hospitalization. Although the disease was initially reported as a respiratory syndrome, other systems such as cardiovascular, renal, and nervous systems may be affected in the acute stages, increasing the need for continuous support to treat multiple sequelae caused by the disease. Due to the severity of the disease, damages found after discharge should also be considered. Providing multidisciplinary interventions promoting physical and psychological recovery in the first stages of hospitalization can minimize these damages. Cognitive, physical and psychological dysfunction reported by COVID19 patients after discharge can have profound effects on quality of life. Pain is usually part of this dysfunction, but it is still poorly understood how it affects survivors of COVID19 infections. There is limited information about the clinical characteristics, treatment and outcome of maintenance of pain in COVID19 patients. The purpose of this narrative review is to provide an overview of the implications of COVID19 on acute and chronic pain states.

Keywords: chronic pain, acute pain, comorbidity, SARS-CoV-2, pandemic (COVID19)

OPEN ACCESS

Edited by:
Georges Leftheriotis, Université Côte d'Azur, France

Reviewed by:
Rosario Vasta, University of Turin, Italy
Alberito Rodrigo Carvalho, Universidade Estadual do Oeste do Paraná, Brazil

****Correspondence:***
Camila Squarzoni Dale
camila.dale@usp.br
Victória Regina da Silva Oliveira
victoria.oliveira.92@hotmail.com

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *30 October 2020*
Accepted: *11 January 2021*
Published: *02 February 2021*

Citation:
Alonso-Matielo H, da Silva Oliveira VR, de Oliveira VT and Dale CS (2021) Pain in Covid Era. Front. Physiol. 12:624154. doi: 10.3389/fphys.2021.624154

INTRODUCTION

The COVID19 pandemic has significantly impacted the lives and health of people worldwide, with potential for further effects in the future. The unprecedented changes which developed quickly due to the pandemic, have disrupted and affected everyone's daily life, including those living with chronic pain (Puntillo et al., 2020). About 15–20% of patients infected with SARS-CoV-2 progress to a severe condition that requires hospitalization (Nanjayya, 2020). It is known that comorbidities such as diabetes, obesity, hypertension, cardiovascular diseases, immunodeficiency, among others, play an important role in the severity of COVID19, however, patients without comorbidities can also progress to severe cases requiring hospitalization. Although the disease was initially reported as a respiratory syndrome, other systems such as cardiovascular, renal and nervous systems may be affected in the acute stages, increasing the need for continuous support to treat multiple sequelae caused by the disease.

Chronic pain, as defined by the International Association for the Study of Pain (IASP), is a persistent or recurrent pain lasting more than 3 months or beyond the normal tissue healing (Puntillo et al., 2020). The overall prevalence of chronic pain in the general population is around 30% and its burden is huge in terms of personal and socioeconomic costs (Van Hecke et al., 2014). The SARS-CoV-2 pandemic has increased the risk of developing chronic pain due to viral infection, pain management or as a consequence of social isolation.

A consistent risk factor for the development of chronic pain is the occurrence of acute pain, it is worth considering how this is managed in hospitalized patients. Those who remember higher pain and distress during an ICU stay appear to be at greater risk of developing chronic pain after discharge (Nanjayya, 2020). It is likely that those who survive critical illnesses with COVID19 are at particular risk of developing chronic diseases such as chronic pain.

Cognitive, physical, and psychological dysfunction reported by COVID19 patients can have profound effects on quality of life (Nanjayya, 2020). Chronic pain is usually part of this dysfunction, but it is still poorly understood how it affects survivors of intensive care units (ICU). A main concern due to the severity of the disease, are damages found during and after hospital discharge. Providing multidisciplinary interventions promoting physical and psychological recovery in the first stages of hospitalization can minimize these damages (Eccleston et al., 2020).

Additionally, patients with chronic pain also have a higher risk of depression (Williams, 2003). Another concerning factor is that social isolation itself is a risk factor for the development of depressive symptoms. The present population suffering from chronic pain was seriously affected by social isolation, usually in-home confinement, as an important measure to mitigate the risk of COVID19 infection. Also, pain management services have been postponed or canceled, considerably diminishing the condition of the general population suffering from chronic pain (Eccleston et al., 2020; Shanthanna et al., 2020). In addition, physical well-being and mental health were deeply harmed, enhancing symptoms such as depression, anxiety, disruption of sleep, worsening pain status and resulting in a poorly quality of life. It is obvious that the relationship between chronic pain, COVID19-related mental disorders and those affected by social isolation can be drastic for chronic pain patients, with an additional impairment of their conditions and quality of life in general.

In this narrative review, we will examine the potential health consequences of COVID19 on chronic pain, by providing a summary and an argumentation of relevant published topics, in three different scenarios, including: (1) chronic pain as part of a post-viral syndrome or the result of viral-associated organ damage; (2) worsening of chronic pain due to exacerbation of preexisting pain physical or mental complaints; and (3) chronic pain by exacerbation of risk factors. **Figure 1** summarizes the evaluate scenario.

ACUTE PAIN ASSOCIATED WITH SARS-CoV-2 INFECTION PATHOLOGY

COVID19 is characterized as the novel coronavirus disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that drives common or atypical symptoms such as fever, dry cough, fatigue, dyspnea, anosmia, diarrhea, and possibly resulting in patient's death (Drożdżal et al., 2020; Shanthanna et al., 2020). Like most viral infections in which pain is a very common symptom, COVID19-infected patients commonly manifest headaches, sore throat, myalgia, arthralgia or peripheral neuralgias, not so different from what has been observed in many COVID19 patients, in which pain is also considered a major symptom (Drożdżal et al., 2020). However, different pain symptoms were linked to the current COVID19 infection as cofactors associated with the disease.

Muscle Pain-Myalgia

Myalgia is one of the most common manifestations observed in COVID19 patients, appearing in nearly 36% of them (Li et al., 2020). In a first estimation it was pointed out that 14.8% of infections presented myalgia or arthralgia (Drożdżal et al., 2020; Nanjayya, 2020). In Hubei, China, one of the first affected areas, myalgia and fatigue were presented by 32.1% of patients (Liu et al., 2020). Increased release of cytokines such as IL-6, IL-10 and TNF-a (Drożdżal et al., 2020), as well as clinical laboratory markers of inflammation such as C-reactive protein (CRP), lactate dehydrogenase (LDH), and erythrocyte sedimentation rate (ESR) are elevated in patients with COVID19 with moderate to severe rates, thus suggesting the presence of generalized inflammatory response (Chen et al., 2020; Rodriguez-Morales et al., 2020), which could explain the presence of myalgia.

Weakness and hyporeflexia of the lower limbs have also been reported in association with the new coronavirus infection. Thus, not classical pain, but weakness of the lower limbs can be reported as suggestive of a motor peripheral neuropathy and can be present even before the appearance of first symptoms and clinical confirmation of COVID19 (Abdelnour et al., 2020).

Abdominal Pain

Gastrointestinal symptoms are less common and more difficult to recognize as part of a COVID19 syndrome. Abdominal pain was reported following gastrointestinal manifestations, in 2–6% of infected general adults, teens or children (Oba et al., 2020). In most cases, pain was associated with the presence of diarrhea or anorexia (Dong et al., 2020). In this sense, Gahide et al. (2020) reported three cases of patients, clinically diagnosed with COVID19, who presented acute abdominal pain and lung injuries, without other major respiratory symptoms or fever at the time of hospitalization, indicating that abdominal pain is a relevant occurrence for COVID19 diagnosis and treatment. Also, Poggiali et al. (2020) reported 10 cases of patients presenting fever and flu like symptoms in the previous 5–10 days, with general malaise, decreased appetite, abdominal pain and diarrhea. Angiotensin-converting enzyme 2 (ACE2) highly expressed in the human small intestine, is the main described cell receptor for the novel coronavirus (Su et al., 2020). Diarrhea occurs secondary to the interaction between ACE2 receptor and SARS-CoV-2. Recent studies show that SARS-CoV-2 RNA was detected in stool samples, confirming fecal-oral transmission (Poggiali et al., 2020). These data reinforce the presence of abdominal pain as a differential symptom in COVID19 infection.

Generalized inflammation in gastrointestinal system (gastroenteritis) was suggested as possible mechanism associated

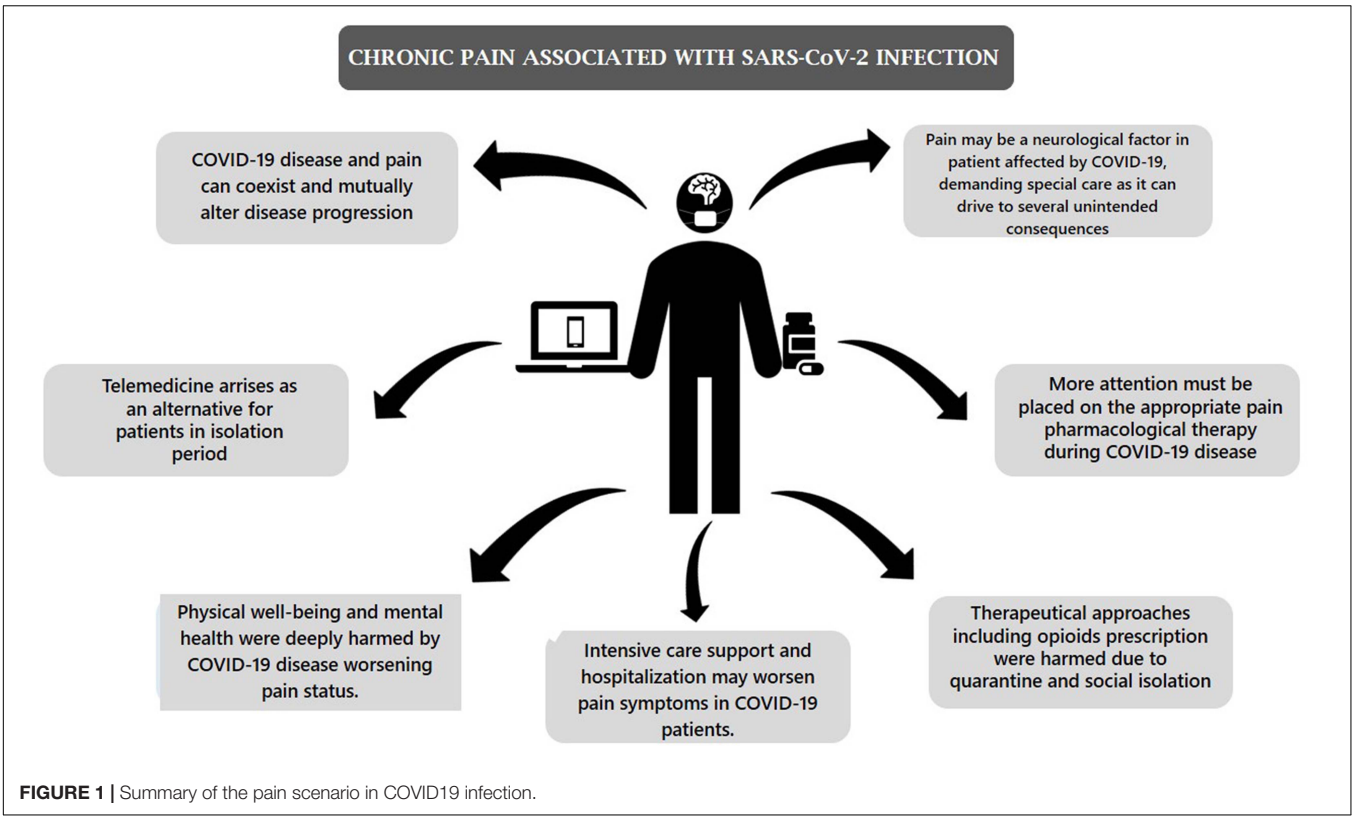


FIGURE 1 | Summary of the pain scenario in COVID19 infection.

to abdominal pain in COVID19 patients, however, it should be noted that patients diagnosed with inflammatory bowel disease or chronic liver disease do not present elevated risk for COVID19 compared to the general population (Oba et al., 2020).

Neurological Manifestations

Central pain has also been suggested as a possible COVID19 neurological manifestation (Drożdżal et al., 2020), thus increasing the concern for the development of pain accompanied by other coronavirus sequels. Headache is highly prevalent in infected individuals with COVID19, and in some surveys up to 90.5% of infected patients reported headaches as a first symptom (26%) or a symptom that appeared up to 48 h (62.5%) after admission at the emergency service (Lechien et al., 2020; Trigo et al., 2020). Headache was accompanied by anosmia, arthralgia, cough, light headedness, and myalgia (Trigo et al., 2020). Generalized inflammation, release and increase of cytokines, injury to endothelial vessels, macrophage activation, and increased glial activation are some of the mechanisms suggested for pain (Drożdżal et al., 2020; Kanberg et al., 2020; Trigo et al., 2020). Increased expression of ACE2 receptors in spinal neurons would play a role in pain sensibility, and therefore, central pain. However, further investigation are still necessary to demonstrate the exact mechanisms leading to central pain as well as causes and correlation of headache with COVID19 severity (Su et al., 2020; Trigo et al., 2020).

CHRONIC PAIN AND COVID-19

Worsening of Chronic Pain Due to Exacerbation of Preexisting Pain, Physical, or Mental Complaints

Of the people affected with chronic pain in the general population, 85% suffer from severe depression (Williams, 2003). Chronic pain patients that do not receive adequate treatment for their pain condition, present drastic levels of depression (Choinière et al., 2010). A specific serotonergic pathway from the dorsal raphe nucleus to the lateral habenula, via the central amygdala was identified as a key neural circuit governing depressive symptoms in chronic pain (Tappe-Theodor and Kuner, 2019; Zhou et al., 2019). Chronic pain may induce depression and vice versa, depression may cause abnormal pain perception and modulation, with increased risk of developing chronic pain (Currie and Wang, 2005; Tappe-Theodor and Kuner, 2019). High levels of anxiety or presence of anxiety disorder have been observed in more than 50% of individuals with chronic pain. Neuroimaging studies suggest that overlapping brain areas, such as thalamus, prefrontal cortex, and anterior cingulate cortex are activated by both chronic pain and anxiety (Hsieh et al., 1996; Davidson et al., 1999).

Almost 60% of the people affected by COVID19 have been affected in one or more social and daily activities such as; sleep, diet, and exercise. The most frequently reported problem was pain/discomfort (19.0%) and anxiety/depression (17.6%). Logistic regression models demonstrate that the risk

of pain/discomfort and anxiety/depression triggering factors related to mental disorders have significantly raised in particular population groups (Lei et al., 2020; Majumdar et al., 2020; Ping et al., 2020). Among them elderly and people affected by chronic diseases, lower income, and those concerned with acquiring the COVID19 which also develop stress, anxiety and depression acquire higher risk of pain (Lei et al., 2020; Majumdar et al., 2020; Ping et al., 2020).

Individuals with chronic diseases report more psychological symptoms than the rest of the population (Ozamiz-Etxebarria et al., 2020) and social isolation due to COVID19 pandemic intensified those symptoms. Thus, social isolation itself added to all reported consequences of COVID19 outbreak, are considered risk factors in respect to the development or even decreasing mental health and exacerbate pre-existing conditions which, in turn, could adversely impact pain-related treatment outcomes (Cohen et al., 2020). Moreover, the number of people suffering from mental illness after a major event is often greater and its effects may last longer (Ping et al., 2020), especially in people with chronic disease, elderly and lower income population.

Chronic Pain by Exacerbation of Risk Factors

Chronic pain is a highly prevalent condition which has high cost while impairing quality of life and implying personal disabilities requiring health, economic and social efforts (Mills et al., 2019). Of notice, chronic pain occurs significantly more in the elderly population, already reported as higher risk for developing severe COVID19 (Chen et al., 2020; Shanthanna et al., 2020).

Patients are more likely to develop pain or discomfort and anxiety/depression, while worrying about being infected with SARS-CoV-2 and developing severe symptoms of COVID19 (Ping et al., 2020). Data peaks in the elderly, people with chronic diseases and individuals with low incomes or worried about get COVID19 during the COVID19 pandemic (Ping et al., 2020). This data reinforces the urgency to observe, diagnose and address painful symptoms during COVID19 outbreak.

During the novel COVID19 pandemic, chronic neuropathic pain, neck and back pain, orofacial pain, or headaches, besides being consequences of major SARS-CoV-2 infection, may also be increased by intensive care support during hospitalization, or directly influenced by the loss of health care facilities which stopped their activities following governmental orientation. Unpleasant sensations, discomfort and continuous ongoing pain are marked outcomes in patients hospitalized in ICUs that require interventive life supporting procedures such as sedation and mechanical ventilation (Drożdżal et al., 2020). Also, under ICU treatment conditions, COVID19 patients frequently are unable to personally report scales of pain, increasing the need for caring for patients pain as a potential underestimated sequel and suggestive for the use of others assessment tools (Drożdżal et al., 2020), as shown previously for assessment of pain scores in intubated patients (Critical Care Pain Observation Tool–CPOT) (Kotfis et al., 2018) or patients under sedation (Behavioral Pain Scale–BPS) (Payen et al., 2001) allowing to start pain monitoring still during hospitalization as a way to prevent further aggravation on pain reports after COVID19 recovery.

Treating Chronic Pain on COVID19 Infections

Patients suffering any kind of pain during COVID19 pandemic demand special attention, as it can be driven by several neurological factors (Drożdżal et al., 2020) and may potentially be aggravated by pain or lead to chronic pain, a condition in coronavirus survivors that will require professional assistance for adequate analgesia and pain relief (Su et al., 2020). Thus, healthcare professionals urge to ensure continued care of acute and chronic pain in patients.

Facing this isolation period, individuals with higher pain burden (including chronic pain) are more likely to experience higher incidence of COVID19 infections. Thus, with the disruption of their usual healthcare access, the consequences of abruptly interrupted/altered healthcare treatment will diminish patients conditions (Eccleston et al., 2020). The risks of harm from under treatment can be aggravated further by inadequate treatment (Eccleston et al., 2020). Despite the fact that pharmacological therapies for pain management and related syndromes tend to be ineffective, negatively affecting the quality of life of individuals (Shamji et al., 2017; Campos et al., 2020), opioids and non-steroidal anti-inflammatory drugs are commonly used in the treatment of acute and chronic pain, even considering their adverse effects, tolerance and potential for addiction (Busse et al., 2018; Szok et al., 2019; Campos et al., 2020). Thus, the impact of the cessation of pain treatment caused by the COVID19 pandemic can lead to several unintended consequences, such as increased pain, reduced function, increased reliance on opioid medications and potential increased morbidity (Deer et al., 2020; El-Tallawy et al., 2020). The effect of opioids on the immune system seems to be complex and have been linked to infection in individuals on chronic opioid therapy. Therefore, its use by immunocompromised patients should be cautious and limited (Plein and Rittner, 2018; Cohen et al., 2020; El-Tallawy et al., 2020). With quarantine and social distancing in COVID19 pandemic there was a worsening of opioid use disorders, hence more attention must be placed on the appropriate prescription of these medications. Non-opioid strategies were suggested (e.g., using clonidine) to prevent opioid withdrawal and in last case, for patients at risk of opioid withdrawal, an in-person visit should be scheduled (El-Tallawy et al., 2020).

Notwithstanding, chronic pain management during COVID19 pandemic must be considered as important as the need of continuous supportive care, to recognize that health professionals worldwide could lack guidelines to deal remotely with patient's pain therapeutics, from diagnosis to analgesics prescription and readily a new way of work, had to be carried out, once health facilities have paused activities and telemedicine was adopted (Shanthanna et al., 2020), a fact that may also influence the incidence of chronic pain.

DISCUSSION AND CONCLUSION

In the present COVID19 pandemic many unknown factors still have to be identified, in order to understand the relationship of pain in COVID19-patients. Mechanisms for shutting down pain triggered by the virus have also been recently suggested and should be further explored (Moutal et al., 2020) since it may help in explaining the variability of pain symptoms. Despite the large number of COVID19 patients and manuscripts, there is still a lack of epidemiological studies focusing on pain symptoms. These studies should have more comparable criteria for selecting subjects in order to obtain a more representative picture of pain symptoms in COVID19. To better comprehend the mechanisms involved in the disease and the role of pain in the development of the infectious condition, pain should be analyzed as a consequence of the disease. Admitting that social isolation play a very important role in worsening pain cases is an extreme need for treatment and improvement in the quality of life of patients with chronic pain and other psychiatric comorbidities. Epidemiological data should be used to assist future health policies that seek to reduce the magnitude of future epidemics and their many consequences for chronic pain. The recognition that COVID19 induces chronic pain and exacerbates pre-existing chronic pain will be of utmost importance for a better understanding of the disease. In addition, immediate and targeted treatment as well as strategies to reduce the potential impact of chronic pain should be strongly encouraged.

AUTHOR CONTRIBUTIONS

All authors participated in the writing of this manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; 2018/14660-1) and by Brazilian National Research Council (CNPq; 472876/2012-0). HA-M was supported by FAPESP (2017/25399-4), VRSO was supported by FAPESP (2018/18483-1), VTO was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; 88882.377357/2019-01).

ACKNOWLEDGMENTS

We would like to thank Dr. Deborah Schechtman for critical review of the manuscript.

REFERENCES

Abdelnour, L., Eltahir Abdalla, M., and Babiker, S. (2020). COVID 19 infection presenting as motor peripheral neuropathy. *J. Formos. Med. Assoc.* 119, 1119–1120. doi: 10.1016/j.jfma.2020.04.024

Busse, J. W., Wang, L., Kamaleldin, M., Craigie, S., Riva, J. J., Montoya, L., et al. (2018). Opioids for chronic noncancer pain: a systematic review and meta-analysis. *JAMA J. Am. Med. Assoc.* 320, 2448–2460. doi: 10.1001/jama.2018.18472

Campos, A. C. P., Antunes, G. F., Matsumoto, M., Pagano, R. L., and Martinez, R. C. R. (2020). Neuroinflammation, pain and depression: an overview of the main findings. *Front. Psychol.* 11:1825. doi: 10.3389/fpsyg.2020.01825

Chen, G., Wu, D., Guo, W., Cao, Y., Huang, D., Wang, H., et al. (2020). Clinical and immunological features of severe and moderate coronavirus disease 2019. *J. Clin. Invest.* 130, 2620–2629. doi: 10.1172/JCI137244

Choinière, M., Dion, D., Peng, P., Banner, R., Barton, P. M., Boulanger, A., et al. (2010). The canadian STOP-PAIN project – Part 1: who are the patients on the waitlists of multidisciplinary pain treatment facilities? *Can. J. Anesth.* 57, 539–548. doi: 10.1007/s12630-010-9305-5

Cohen, S. P., Baber, Z. B., Buvanendran, A., McLean, B. C., Chen, Y., Hooten, W. M., et al. (2020). Pain management best practices from multispecialty organizations during the COVID-19 pandemic and public health crises. *Pain Med.* 21, 1331–1346. doi: 10.1093/pm/pnaa127

Currie, S. R., and Wang, J. (2005). More data on major depression as an antecedent risk factor for first onset of chronic back pain. *Psychol. Med.* 35, 1275–1282. doi: 10.1017/S0033291705004952

Davidson, R. J., Abercrombie, H., Nitschke, J. B., and Putnam, K. (1999). Regional brain function, emotion and disorders of emotion. *Curr. Opin. Neurobiol.* 9, 228–234. doi: 10.1016/S0959-4388(99)80032-4

Deer, T. R., Sayed, D., Pope, J. E., Chakravarthy, K. V., Petersen, E., Moeschler, S. M., et al. (2020). Emergence from the COVID-19 pandemic and the care of chronic pain: guidance for the interventionalist. *Anesth. Analg.* 131, 387–394. doi: 10.1213/ANE.0000000000005000

Dong, Z. Y., Xiang, B. J., Jiang, M., Sun, M. J., and Dai, C. (2020). The prevalence of gastrointestinal symptoms, abnormal liver function, digestive system disease and L. *J. Clin. Gastroenterol.* 55, 67–76. doi: 10.1097/MCG.0000000000001424

Drożdżal, S., Rosik, J., Lechowicz, K., Machaj, F., Szostak, B., Majewski, P., et al. (2020). COVID-19: pain management in patients with SARS-CoV-2 infection-molecular mechanisms, challenges, and perspectives. *Brain Sci.* 10:465. doi: 10.3390/brainsci10070465

Eccleston, C., Blyth, F. M., Dear, B. F., Fisher, E. A., Keefe, F. J., Lynch, M. E., et al. (2020). Managing patients with chronic pain during the COVID-19 outbreak. *Pain* 161, 889–893. doi: 10.1097/j.pain.0000000000001885

El-Tallawy, S. N., Nalamasu, R., Pergolizzi, J. V., and Gharibo, C. (2020). Pain management during the COVID-19 pandemic. *Pain Ther.* 9, 453–466. doi: 10.1007/s40122-020-00190-4

Gahide, G., Frandon, J., and Vendrell, J. F. (2020). COVID-19 patients presenting with afebrile acute abdominal pain. *Clin. Med. J. R. Coll. Phys. Lond.* 20, E4–E6. doi: 10.7861/clinmed.2020-0150

Hsieh, J. C., Hannerz, J., and Ingvar, M. (1996). Right-lateralised central processing for pain of nitroglycerin-induced cluster headache. *Pain* 67, 59–68. doi: 10.1016/0304-3959(96)03066-7

Kanberg, N., Ashton, N. J., Anderson, L. M., Yilmaz, A., Lindh, M., Nilsson, S., et al. (2020). Neurochemical evidence of astrocytic and neuronal injury commonly found in COVID-19. *Neurology* 95, e1754–e1759. doi: 10.1212/WNL.0000000000010111 t

Kotfis, K., Zegan-Barańska, M., Strzelbicka, M., Safranow, K., Zukowski, M., and Wesley Ely, E. (2018). Validation of the polish version of the critical care pain observation tool (CPOT) to assess pain intensity in adult, intubated intensive care unit patients: the POL-CPOT study. *Arch. Med. Sci.* 14, 880–889. doi: 10.5114/aoms.2017.69752

Lechien, J. R., Chiesa-Estomba, C. M., Place, S., Van Laethem, Y., Cabaraux, P., Mat, Q., et al. (2020). Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019. *J. Intern. Med.* 288, 335–344. doi: 10.1111/joim.13089

Lei, L., Huang, X., Zhang, S., Yang, J., Yang, L., and Xu, M. (2020). Comparison of prevalence and associated factors of anxiety and depression among people

affected by versus people unaffected by quarantine during the COVID-19 Epidemic in Southwestern China. *Med. Sci. Monit.* 26, 1–12. doi: 10.12659/MSM.924609

Li, L. Q., Huang, T., Wang, Y. Q., Wang, Z. P., Liang, Y., Huang, T. B., et al. (2020). COVID-19 patients' clinical characteristics, discharge rate, and fatality rate of meta-analysis. *J. Med. Virol.* 92, 577–583. doi: 10.1002/jmv.25757

Liu, K., Fang, Y. Y., Deng, Y., Liu, W., Wang, M. F., Ma, J. P., et al. (2020). Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. *Chin. Med. J. Engl.* 133, 1025–1031. doi: 10.1097/CM9.0000000000000744

Majumdar, P., Biswas, A., and Sahu, S. (2020). COVID-19 pandemic and lockdown: cause of sleep disruption, depression, somatic pain, and increased screen exposure of office workers and students of India. *Chronobiol. Int.* 37, 1191–1200. doi: 10.1080/07420528.2020.1786107

Mills, S. E. E., Nicolson, K. P., and Smith, B. H. (2019). Chronic pain: a review of its epidemiology and associated factors in population-based studies. *Br. J. Anaesth.* 123, e273–e283. doi: 10.1016/j.bja.2019.03.023

Moutal, A., Martin, L. F., Boinon, L., Gomez, K., Ran, D., and Zhou, Y. (2020). SARS-CoV-2 Spike protein co-opts VEGF-A / neuropilin-1 receptor signaling. *Pain.* 162, 243–252. doi: 10.1097/j.pain.0000000000002097

Nanjayya, V. (2020). *Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19).* WHO-China Jt. Mission Coronavirus Dis. 2019 2019, 16–24. Available online at: https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf. (accessed April 7, 2020).

Oba, J., Carvalho, W. B., de Silva, C. A., and Delgado, A. F. (2020). Gastrointestinal manifestations and nutritional therapy during COVID-19 pandemic: a practical guide for pediatricians. *Einstein (Sao Paulo)* 18:eRW5774. doi: 10.31744/einstein_journal/2020rw5774

Ozamiz-Etxebarria, N., Dosil-Santamaria, M., Picaza-Gorrochategui, M., and Idoiaga-Mondragon, N. (2020). Stress, anxiety, and depression levels in the initial stage of the COVID-19 outbreak in a population sample in the northern Spain. *Cad. Saude Publica.* 36, 1–9. doi: 10.1590/0102-311X00054020

Payen, J. F., Bru, O., Bosson, J. L., Lagrasta, A., Novel, E., Deschaux, I., et al. (2001). Assessing pain in critically ill sedated patients by using a behavioral pain scale. *Crit. Care Med.* 29, 2258–2263. doi: 10.1097/00003246-200112000-00004

Ping, W., Zheng, J., Niu, X., Guo, C., Zhang, J., Yang, H., et al. (2020). Evaluation of health-related quality of life using EQ-5D in China during the COVID-19 pandemic. *PLoS One* 15:1–12. doi: 10.1371/journal.pone.0234850

Plein, L. M., and Rittner, H. L. (2018). Opioids and the immune system – friend or foe. *Br. J. Pharmacol.* 175, 2717–2725. doi: 10.1111/bph.13750

Poggiali, E., Ramos, P. M., Bastoni, D., Vercelli, A., and Magnacavallo, A. (2020). Abdominal pain: a real challenge in novel COVID-19 infection. *Eur. J. Case Rep. Intern. Med.* 7, 001632. doi: 10.12890/2020_001632

Puntillo, F., Giglio, M., Brienza, N., Viswanath, O., Urits, I., Kaye, A. D., et al. (2020). Impact of COVID-19 pandemic on chronic pain management: looking for the best way to deliver care. *Best Pract. Res. Clin. Anaesthesiol.* 34, 529–537. doi: 10.1016/j.bpa.2020.07.001

Rodriguez-Morales, A. J., Cardona-Ospina, J. A., Gutiérrez-Ocampo, E., Villamizar-Peña, R., Holguin-Rivera, Y., Escalera-Antezana, J. P., et al. (2020). Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. *Travel Med. Infect. Dis.* 34, 101623. doi: 10.1016/j.tmaid.2020.101623

Shamji, M. F., De Vos, C., and Sharan, A. (2017). The advancing role of neuromodulation for the management of chronic treatment-refractory pain. *Clin. Neurosurg.* 80, S108–S113. doi: 10.1093/neuros/nyw047

Shanthanna, H., Strand, N. H., Provenzano, D. A., Lobo, C. A., Eldabe, S., Bhatia, A., et al. (2020). Caring for patients with pain during the COVID-19 pandemic: consensus recommendations from an international expert panel. *Anaesthesia* 75, 935–944. doi: 10.1111/anae.15076

Su, S., Cui, H., Wang, T., Shen, X., and Ma, C. (2020). Pain: a potential new label of COVID-19. *Brain. Behav. Immun.* 87, 159–160. doi: 10.1016/j.bbi.2020.05.025

Szok, D., Tajti, J., Nyári, A., and Vécsei, L. (2019). Therapeutic approaches for peripheral and central neuropathic pain. *Behav. Neurol.* 2019:8685954. doi: 10.1155/2019/8685954

Tappe-Theodor, A., and Kuner, R. (2019). A common ground for pain and depression. *Nat. Neurosci.* 22, 1612–1614. doi: 10.1038/s41593-019-0499-8

Trigo, J., García-Azorín, D., Planchuelo-Gómez, Á, Martínez-Pías, E., Talavera, B., Hernández-Pérez, I., et al. (2020). Factors associated with the presence of headache in hospitalized COVID-19 patients and impact on prognosis: a retrospective cohort study. *J. Headache Pain* 21, 1–10. doi: 10.1186/s10194-020-01165-8

Van Hecke, O., Austin, S. K., Khan, R. A., Smith, B. H., and Torrance, N. (2014). Neuropathic pain in the general population: a systematic review of epidemiological studies. *Pain* 155, 654–662. doi: 10.1016/j.pain.2013.11.013

Williams, L. S. (2003). Prevalence and impact of depression and pain in neurology outpatients. *J. Neurol. Neurosurg. Psychiatry* 74, 1587–1589. doi: 10.1136/jnnp.74.11.1587

Zhou, W., Jin, Y., Meng, Q., Zhu, X., Bai, T., Tian, Y., et al. (2019). A neural circuit for comorbid depressive symptoms in chronic pain. *Nat. Neurosci.* 22, 1649–1658. doi: 10.1038/s41593-019-0468-2

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

BRIEF RESEARCH REPORT
published: 05 February 2021
doi: 10.3389/fphys.2021.624169

Determinants of Health and Physical Activity Levels Among Breast Cancer Survivors During the COVID-19 Pandemic: A Cross-Sectional Study

*Aline Rachel Bezerra Gurgel[1], Pedro Mingroni-Netto[1], Jose Carlos Farah[2], Christina May Moran de Brito[3], Remama Group[†], Anna S. Levin[4] and Patricia Chakur Brum[1]**

[1] School of Physical Education and Sport, University of São Paulo, São Paulo, Brazil, [2] Centro de Práticas Esportivas da Universidade de São Paulo (CEPEUSP), São Paulo, Brazil, [3] Instituto do Câncer do Estado de São Paulo, ICESP, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [4] Department of Infectious Diseases and LIM49, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil

OPEN ACCESS

Edited by:
Georges Leftheriotis, Université Côte d'Azur, France

Reviewed by:
Dileep Rohra, Alfaisal University, Saudi Arabia
Hamid Reza Marateb, Universitat Politecnica de Catalunya, Spain

***Correspondence:**
Patricia Chakur Brum
pcbrum@usp.br

[†]*On behalf of Remama Group: Luiz Augusto Riani Costa, Raphael Barreto, Sarah Milani de Moraes Leandrini, and Christian Klausener*

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *30 October 2020*
Accepted: *11 January 2021*
Published: *05 February 2021*

Citation:
Gurgel ARB, Mingroni-Netto P, Farah JC, de Brito CMM, Remama Group, Levin AS and Brum PC (2021) Determinants of Health and Physical Activity Levels Among Breast Cancer Survivors During the COVID-19 Pandemic: A Cross-Sectional Study. Front. Physiol. 12:624169. doi: 10.3389/fphys.2021.624169

Background: Increased exercise and physical activity levels are recommended throughout cancer therapy and survivorship. Nonetheless, the COVID-19 pandemic and consequent social distancing are likely to cause a decline in physical activity.

Objective: to evaluate the level of unsupervised physical activity of breast cancer survivors during the COVID-19 pandemic, and the factors associated with difficulties in engaging and maintaining recommended physical activity levels.

Methods: This is a cross-sectional epidemiological study with a sample of 37 breast cancer survivors. They participated in a canoeing training program (project Remama) at the University of São Paulo before the COVID-19 pandemic. Socioeconomic aspects, engagement in physical activity, motivation, and potential exposure to COVID-19 were investigated through an online survey, administered in September of 2020.

Results: During the pandemic, participants increased their body weight (5 ± 3.4 kg); 90% reported decreasing physical activity levels associated with increased sedentary time. Twenty-one (58%) participants exhibited some COVID-19-related symptoms, most used public transportation (59%), or returned to work during the period of a high incidence of COVID-19. The only factor associated with perceived difficulty in engaging in physical activities was having had more than three cancer treatments (RR: 2.14; 95% CI: 1.07–4.27).

Conclusion: The COVID-19 pandemic led to a group of previously active breast cancer survivors to decrease their physical activity, gain weight, and have sedentary behavior. Specific tailored-care interventions are needed to prevent these occurrences, as overweight and physical inactivity may impose an additional risk for breast cancer recurrence and a severe course of COVID-19 in cancer patients.

Keywords: physical activity, COVID-19, breast neoplasms, survivorship, pandemic (COVID-19)

INTRODUCTION

A series of pneumonia cases of unknown etiology was reported in the city of Wuhan, China, in late 2019 (Xu et al., 2020). By sequencing a patient's lower respiratory tract, a new virus was identified called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its disease, coronavirus disease 2019 (COVID-19). It rapidly spread across countries and a new pandemic was declared by the World Health Organization (WHO) in March 2020. COVID-19 reached a mark of 20,162,474 cases and 737417 deaths globally in August of 2020 (World Health Organization, 2020). In Brazil, the Ministry of Health notified the first confirmed case on February 26, 2020. From that date until August 8, 2020, 3,012,412 cases were confirmed, and 100,477 deaths occurred as a result of COVID-19 in the country (Ministério da Saúde, 2020).

Social distancing recommendations significantly reduced levels of physical activity of the overall population and more profoundly in the population at increased risk, such as the elderly and those living with chronic non-communicable diseases (Damiot et al., 2020). Periods of confinement can be identified as a barrier to regular physical activity. A recent study showed that middle-aged individuals who adopt sedentary behavior have an increased risk of cancer mortality (Gilchrist et al., 2020). Physical inactivity may exacerbate comorbidities amongst older adults, including cardiovascular disease, cancer, and dysfunctional inflammatory responses (Flynn et al., 2019). In this scenario, older adults and individuals living with underlying conditions are at a greater risk for complications during COVID-19 disease (Damiot et al., 2020).

Worldwide, the incidence of cancer and mortality remain high. Over 18 million new cases were registered in 2018, alongside 9.6 million deaths (Bray et al., 2018). Among women, the most diagnosed neoplasm is breast cancer (excluding non-melanoma skin cancer), which also represents the major oncological cause of death in this population, with 2.1 million diagnosed cases in 2018, accounting for almost one in four cancer cases (Bray et al., 2018). Despite the escalating cancer incidence, advances in early diagnosis and breast cancer therapy improved five-year overall survival rates, now exceeding 90% when diagnosed in the early stages (Simon et al., 2019; Siegel et al., 2020).

In this context, particular attention should be dedicated to metabolic aspects including weight control and management of physical inactivity, through lifestyle interventions. In fact, increased physical activity levels, exercise, and body weight control have been considered strong allies to cancer patients and survivors, as they positively impact physical capacities, fatigue, depressive symptoms, anxiety, and quality of life (Campbell et al., 2019; Mctiernan et al., 2019; Nardin et al., 2020). Since the literature of the past two decades has provided evidence that supports the practice of physical activity during and after cancer treatment (Schmitz et al., 2019); and that physical activity helps to prevent neoplastic recurrence (Patel et al., 2019), it is of utmost importance to develop public health strategies to encourage this practice.

The present study aimed to evaluate the level of unsupervised physical activity of breast cancer survivors during the COVID-19 pandemic, and the factors associated with difficulties in engaging and maintaining recommended physical activity levels.

MATERIALS AND METHODS

This is a cross-sectional study designed to address physical activity levels in a convenience sample of breast cancer survivors. All subjects of this study participate in a group canoeing training project called Remama at the University of São Paulo, in which they are longitudinally followed-up.

Remama is a collaborative project between the Cancer Institute of the State of Sao Paulo, the School of Physical Education of the University of São Paulo (USP), and the Sports Center of USP. The inclusion criteria were participants who have finished their prescribed breast cancer treatment with curative intent, including surgery, systemic cytotoxic chemotherapy, and radiotherapy; who are aged between 35 and 75 years old; and have concluded their treatment within a time span of at least 6 months up to 3 years. The exclusion criteria were patients who had metastatic disease, severe lymphedema, organic dysfunction, or uncontrolled risk factors (hypercholesterolemia, diabetes, hypertension).

Previously to the pandemic, Remama participants received a physical activity recommendation booklet (based on WHO guidelines). The booklet suggests different exercise modalities such as aerobic, strength and flexibility, and an increase in overall physical activity level as part of a behavioral change. It also recommends precautions participants should adopt while exercising. Due to the COVID-19 pandemic, access to public and private spaces is restricted. The participants were instructed to increase physical activity levels at home because of the discontinuation of face-to-face training sessions. We sent online questionnaires to 41 Remama participants in September 2020. The participants were instructed through videoconference.

Instruments

The level of physical activity, sport, and leisure was assessed using an instrument built especially for this study and adapted from the Minnesota Leisure Time Physical Activity Questionnaire (Taylor et al., 1978), that is a widely used tool to address physical activity levels in different populations (Elosua et al., 2000; Lozano-Lozano et al., 2016) and that has been validated for Brazilian population (Lustosa et al., 2011). It quantified: (a) the time that participants spent on accumulated physical activities in daily life (lifestyle), called "not programmed movement"; (b) the time spent on physical activities to meet the goal of 150 min per week of physical exercise; and (c) the total time they spent sitting, whether in leisure activities (such as watching television, cell phones, etc.) or in professional work-related activities.

To assess current patterns of physical activity upon the COVID-19 pandemic, a survey adapted from a questionnaire used by Lesser and Nienhuis (2020) was applied. This was based on the Nature Relatedness scale (Nguyen and Brymer, 2018) and on the Godin Leisure Questionnaire, which has been

TABLE 1 | Demographic and clinical characteristics, and responses of 37 breast cancer survivors, enrolled in a program for physical activity, who answered a survey on their level of activity and perceptions on the subject, on possible COVID-19 symptoms, and on potential exposure to the disease during the pandemic.

Age, years, mean ± SD	57 ± 7.4
Ethnicity *n* (%)	
White	19 (51%)
Black/brown	13 (35%)
Others	5 (14%)
Education *n* (%)	
Up to high school	15 (41%)
University	12 (32%)
Post-graduation	10 (27%)
Menopausal *n* (%)	22 (59%)
Obesity status *n* (%)	
Overweight	22 (59%)
Weight unchanged	7 (19%)
Weight loss	6 (16%)
Unknown	2 (5%)
Weight gain (Kg), median (range)	3.75 (1–15)
Type of treatment *n* (%)	
Surgery	32 (86%)
Chemotherapy	32 (86%)
Radiotherapy	31 (84%)
Endocrine	12 (32%)
Immunotherapy	2 (5%)
Type of malignancy *n* (%)	
Invasive ductal carcinoma	15 (41%)
In situ ductal carcinoma	5 (14%)
Invasive lobular carcinoma	3 (8%)
Unknown	10 (27%)
Other	4 (11%)
Time since treatment completion, months, median (range)	46 (1–95)
Use of tamoxifen *n* (%)	15 (41%)
Under cancer treatment *n* (%)	4 (11%)
Current treatments *n* (%) Systemic arterial hypertension	11 (30%)
Diabetes mellitus	5 (14%)
Symptoms suggestive of COVID-19 *n* (%)	
No symptoms	16 (43%)
Headache	13 (35%)
Myalgia	7 (19%)
Cough	5 (14%)
Coryza	5 (14%)
Sore throat	4 (11%)
Other	5 (14%)
Duration of symptoms (days), median (range)	4 (4–20)
Admitted to hospital *n* (%)	3 (8%)
Contact with suspected or confirmed case of COVID-19 at home *n* (%)	5 (14%)
Duration of exposure (days), median (range)	3 (3–10)
Contact with suspected or confirmed case of COVID-19 outside the home *n* (%)	8 (22%)
Worked outside the home *n* (%)	13 (35%)
Number of times per week worked outside the home, median (range)	3.5 (1–6)
Used public transportation *n* (%)	22 (59%)
Number of times per week used public transportation, median (range)	2 (0–6)
Levels and characteristics of physical activity during the COVID-19 pandemic	
Prefers outdoor training *n* (%)	22 (59%)
Considers outdoor activities very important *n* (%)	25 (68%)
Reduced physical activity levels during the pandemic *n* (%)	33 (89%)

SD, standard deviation.

validated for the Brazilian population (São-João et al., 2013). This included questions on the (a) the importance of carrying out outdoor activities (Nisbet et al., 2009); (b) sedentary behavior, questions based on a study by Ekelund et al. (2016); and, (c) the Behavioral Regulations in Exercise questionnaire (BREQ-3), also validated to adult Brazilian population (Guedes and Sofiati, 2015) to assess the participants' motivation for physical activity at home (Rutten et al., 2014). Participants were asked to answer questions related to motivation and training opportunities, indicating answers on eight statements using a 5-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). Finally, we also investigated the potential exposure to SARS-CoV-2. A validated questionnaire adapted from the Mount Sinai Hospital (2020) survey was administered. The participants were asked to answer the questions considering the period starting on March 1, 2020.

Dependent Variable

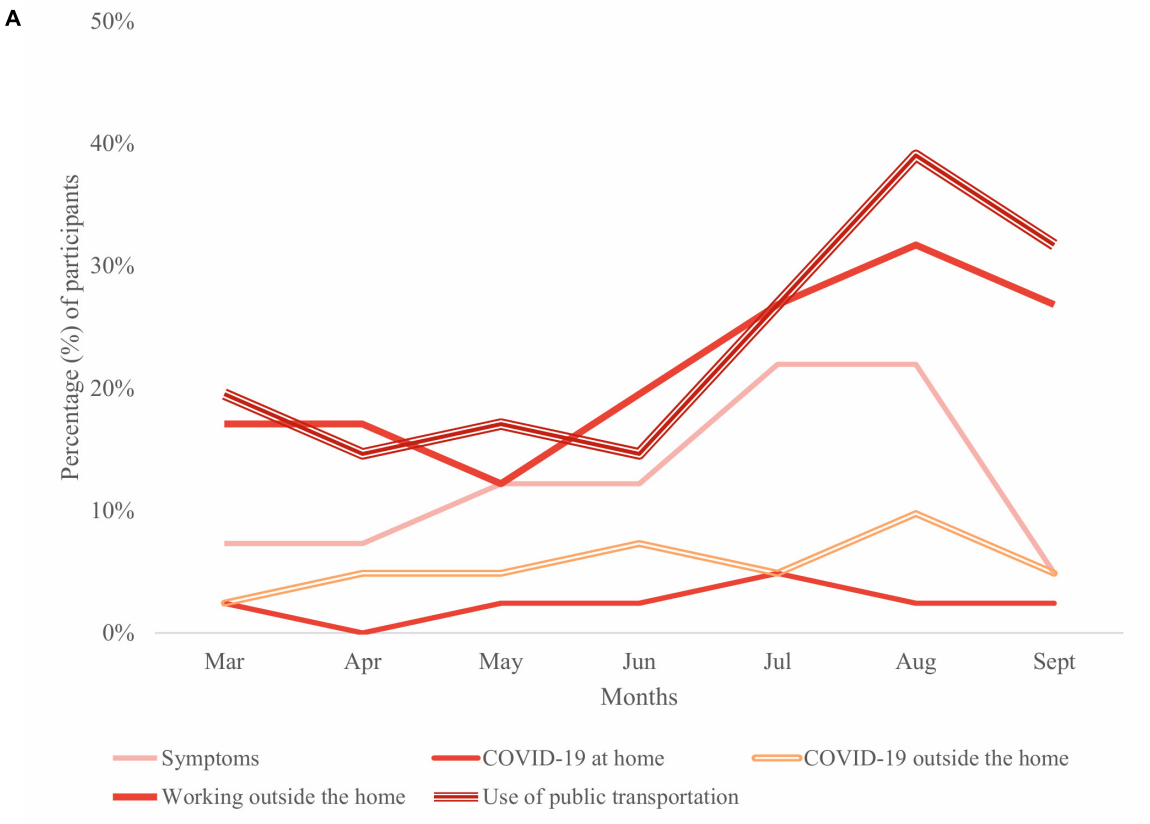
Participants were asked about adherence to physical activity in the context of measuring an individuals' motivation, support, and opportunity to engage in physical activity. Each answer was scored on the Likert scale from one (strongly disagree), two (partially disagree), three (neutral), four (partially agree) to five (strongly agree). Next, we generated dichotomous variables from the Likert scale and used them for measuring the participants' difficulty in engage with physical activity. The scores 1 and 2 were accounted as "no," while the scores from three to five were considered "yes."

Independent Variables

Demographic data were evaluated, including age, education, occupation, return to work, and professional working routine. Clinical data were retrieved on body mass index, menopausal status, presence of underlying conditions, and cancer treatment adopted (chemotherapy, radiotherapy, surgery, immunotherapy, and hormone-based therapy). Behavioral factors included opportunity and motivation for physical activity. Lastly, patients were asked whether they had experienced any symptoms of COVID-19 during social distancing.

Statistical Analyses

Two groups were compared: participants who did experience difficulty in engaging in physical activity against those who did not. Results are presented as relative risk (RR) and 95% confidence intervals (CI). A 95% CI that did not include 1.00 was considered statistically significant. Statistical analysis was performed using EpiInfo™ version 7.2.4.0.



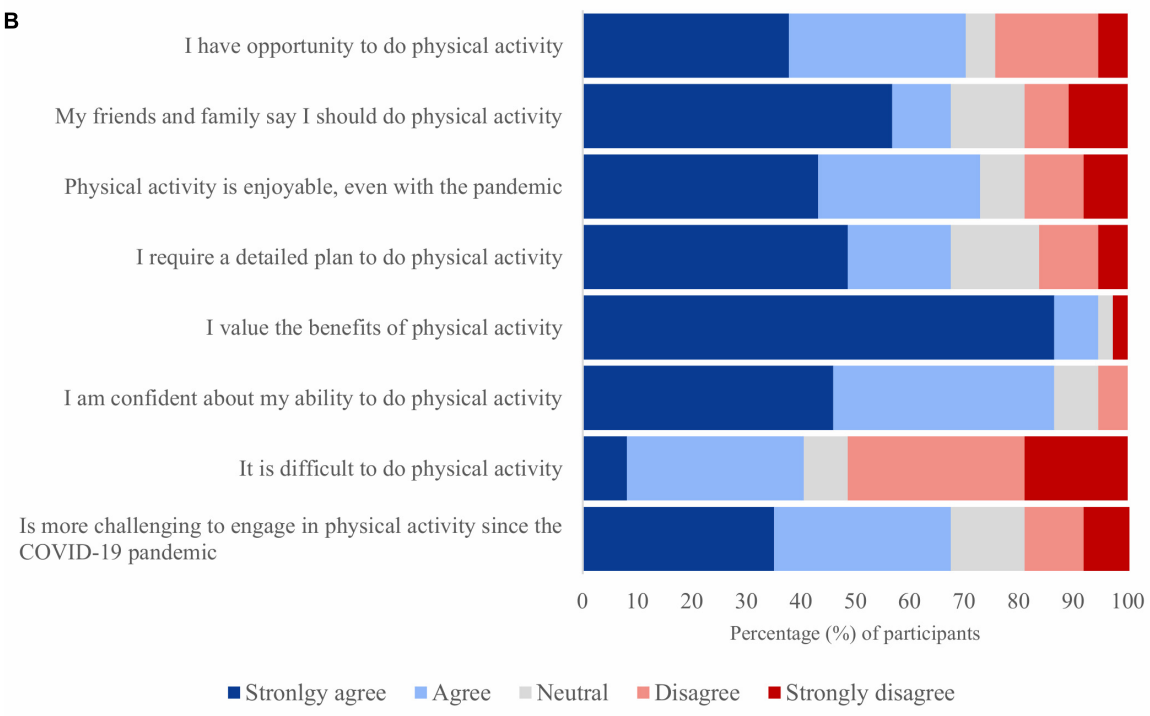


FIGURE 1 | Participants' behavior outside and inside the home during the COVID-19 pandemic. **(A)** Proportion of participants who reported exposure, from March to September 2020, to suspected or confirmed cases of COVID-19 at home, outside the home, and who used public transportation (n = 37). **(B)** Barriers and facilitators for physical activity perceived in September 2020 (n = 37). Results are presented in percentage.

Ethical Considerations

This study was approved by the School of Physical Education Research Ethical Board (CAAE: 12242919.9.0000.5391) in accordance with the 1964 Declaration of Helsinki amendment in 2013. All participants signed a consent form. The identity and individual information of the subjects are confidential, and the data were analyzed in an aggregate form. The raw data supporting the conclusions of this article will be made available by the authors upon request.

RESULTS

The survey was sent to 41 members of the project Remama of which 37 responded (90%). The demographic and clinical data and, the characteristics of their breast cancer can be seen in **Table 1**.

The mean age of the volunteers was 57 years old, with the majority aged 55 years or older. Twenty-two (59%) participants reported increase in bodyweight (5 kg, ranging from 1 to 15 kgs). The five most frequently reported symptoms were headache, myalgia, cough, coryza, and sore throat. These symptoms occurred mainly in July and lasted a few days. Although three participants were hospitalized, no subject from the population developed severe COVID-19 or post-COVID19 complications.

Participants worked outside the home mainly in July, August, and September, roughly between three and four times a week. This coincides with the use of public transportation in July, August, and September. They used public transportation between two and three times a week. **Figure 1A** summarizes participants' potential exposure to SARS-CoV-2 reported from March to September 2020.

Barriers and facilitators of physical activity perceived by the participants can be seen in **Figure 1B**. The vast majority reported that it is challenging to engage in physical activity since the pandemic, although most had the opportunity and valued the effect of exercise and being physically active.

The levels of programmed and not programmed movement are described in **Table 2**. Most volunteers reported having adopted alternative movement and used stairs. Besides, the majority stated they performed housework activities. Roughly half of them achieved 150 min/week of physical activity in September 2020. As to sedentary behavior, 20 (54%) participants reported remaining seated for over two consecutive hours during the week, and 19 (51%), during the weekend.

The potential association between the patients having reported having difficulty engaging in physical activities can be seen in **Table 3**. Women who underwent at least three anticancer treatments found difficulty in doing physical activity.

TABLE 2 | Evaluation of programmed and non-programmed physical activities of breast cancer survivors enrolled in a program for physical activity, who answered a survey on their level of activity, (*n* = 37).

Non-programmed movement/daily activities	
Adopted alternative movement *n* (%)	28 (76%)
Used stairs *n* (%)	26 (70%)
Walked *n* (%)	24 (65%)
Worked in the last 2 weeks *n* (%)	25 (68%)
Movement in professional activities *n* (%)	16 (43%)
Movement in leisure activities *n* (%)	15 (41%)
Performed housework *n* (%)	31 (84%)
Felt discomfort during housework activities *n* (%)	11 (30%)
Programmed activities	
Achieved 150 min/week physical activity *n* (%)	18 (49%)
Sedentary behavior during week/weekend	
Remained seated for more than two consecutive hours during week *n* (%)	20 (54%)
Remained seated for more than two consecutive hours during weekend *n* (%)	19 (51%)

TABLE 3 | Evaluation of factors potentially associated with the perception of the patients that it is difficult to engage in physical activities (Survey taken during the COVID-19 pandemic with breast cancer survivors previously enrolled in a canoeing program, *n* = 37).

	Find it difficult to engage in physical activity		
	Yes (%) *n* = 15	**No (%) *N* = 22**	**RR (CI 95%)**
Age > 55 years old	9 (60%)	12 (55%)	1.14 (0.51–2.55)
Obesity	11 (73%)	14 (64%)	1.03 (0.53–3.29)
Studied up to high school	9 (40%)	13 (59%)	1.02 (0.46–2.27)
Menopause	6 (40%)	16 (73%)	0.75 (0.32–1.78)
Has hypertension	5 (33%)	6 (27%)	1.18 (0.52–2.65)
Had > 3 cancer treatments	5 (33%)	2 (9%)	2.14 (1.07–4.27) *
Prefers outdoor activities	8 (53%)	14 (64%)	0.72 (0.33–1.55)
Has opportunity to do physical activity	9 (60%)	18 (82%)	0.55 (0.26–1.15)
Works outside the home	7 (47%)	6 (27%)	1.60 (0.75–3.44)
Presented COVID-19 symptoms	9 (60%)	12 (55%)	1.14 (0.51–2.55)

**Statistically significant.*

DISCUSSION

Our survey with 37 breast cancer survivors revealed that most of the participants reduced their physical activity level and gained weight upon temporary suspension of the canoeing training project Remama at COVID-19 pandemic onset. Although no factors were associated with reducing activity, our study discloses that having been submitted to more than three cancer treatments was associated with their perception of difficulty to do physical activity. Taken together, these aspects may impose an additional risk of a severe course of COVID-19 with a worse prognosis. No other modifiable factor could be associated with this outcome. This finding was crucial to support our research group in developing a new strategy to engage participants in a physical activity program that is active *via* online classes currently.

Exercise, preferably following an exercise program (Newton et al., 2020), can improve outcomes in people who have or have had cancer, such as well-being, body weight control, and reduce the cancer recurrence risk. Bodyweight gain and physical inactivity are known to increase the cancer recurrence risk (Meyerhardt et al., 2006; Renehan et al., 2008), cardiovascular disease, and metabolic disorders (Ford and Caspersen, 2012). Physical activity enhances quality of life and

improves the effectiveness of therapies, mitigating potential adverse effects inherent to antineoplastic therapy and drug toxicity. Furthermore, it may minimize or reverse the progression of other chronic diseases. Besides that, cancer patients may be at an increased risk of developing severe COVID-19 due to the presence of underlying conditions, anticancer therapy, and old age, among others (Galluzzi et al., 2015; Dai et al., 2020; Damiot et al., 2020).

In our study, most women reported they preferred outdoor activities, and the majority considered outdoor spaces especially important for physical activity. However, due to the pandemic, the health authorities restricted these outdoor activities. This may have been one of the contributing factors to our results. A larger number of cancer treatments was significantly associated with perceived difficulty in being physically active. This might be due to the long-term side effects associated with antineoplastic therapies. This includes fatigue, insomnia, persistent pain, lymphedema, among other unwanted effects that might impose barriers to physical activity engagement (Campbell et al., 2019). In fact, the probability of developing long-term side effects increases as the number of antineoplastic treatment increases (Cheville, 2001; Stout and Sabo Wagner, 2019).

A large proportion of the participants reported having had symptoms, mostly during July and August, the months with the highest incidence of COVID-19 in São Paulo. This was also the period in which the participants reported more frequent use of public transportation and return to work. Many of our participants were self-employed. They may have found themselves pushed back to work to provide for their families. This behavior possibly increased the chance of exposure to symptomatic or asymptomatic people outside the home. Brazilian women who are workers in the informal economy were disproportionally affected by the pandemic (International Labour Organization, 2020a), as they do not have access to social protection mechanisms. Thus, we believe that our study reflects a particular socioeconomic scenario that exacerbates social inequality. This scenario differs from that of other countries that provide social protection, allowing the population to stay at home and follow social distancing orders, minimizing the spread of SARS-CoV-2. We believe that adequate responses to the pandemic should observe the particularities of each country, and policies ensuring measures for social health protection and extending financial protection should prioritize vulnerable workers (International Labour Organization, 2020b).

Our study has limitations. Self-reported physical activity levels may impose imprecision on the data. Furthermore, this survey does not fully report all aspects of BREQ-3 or Minnesota questionnaire, including psychological aspects. The interpretation of our findings might be limited by the sample size. Nevertheless, understanding the practice of physical activity in the context of the life cycle and macro determinants of behavior is of vital importance (Hallal, 2014). Although using a small sample, this study provides fresh insights into a complex problem and may provide a direction for interventions. In this sense, alternative ways of delivering supervised and structured physical activity (exercise) based on telehealth should be studied as an alternative for increasing the adherence to exercise aiding to maintain an active lifestyle in the pandemic and even afterward (Newton et al., 2020).

In conclusion, previously active breast cancer survivors found themselves inactive or with reduced physical activity levels and gained weight during the pandemic. Those who underwent multiple antineoplastic treatments found it difficult to engage in physical activity. Therefore, our study calls for tailored-care interventions and alternative ways of delivering supervised exercise to cancer survivors during the COVID-19 pandemic and beyond.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of School of Physical Education and Sports of University of São Paulo. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PB, AL, and AG planned the design of the study and data collection. PM-N carried out data collection through an online survey. AG and PM-N worked on data organization and treatment. AL provided guidance on statistical analysis. PB and AL contributed to the interpretation of the results. AG drafted the manuscript and designed the figures. JF is the coordinator of the Cancer institute rehabilitation program supporting Remama group health care. AG and PB drafted the manuscript with critical revision from AL and CB. All authors provided critical feedback on the present manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.624169/full#supplementary-material

REFERENCES

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L, Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* 68, 394–424. doi: 10.3322/caac.21492

Campbell, K. L., Winters-Stone, K. M., Wiskemann, J., May, A. M., Schwartz, A. L., Courneya, K. S., et al. (2019). Exercise guidelines for cancer survivors:

consensus statement from international multidisciplinary roundtable. *Med. Sci. Sports Exerc.* 51, 2375–2390. doi: 10.1249/MSS.0000000000002116

Cheville, A. (2001). Rehabilitation of patients with advanced cancer. *Cancer* 92, 1039–1048.

Dai, M., Liu, D., Liu, M., Zhou, F., Li, G., Chen, Z., et al. (2020). Patients with cancer appear more vulnerable to SARS-CoV-2: a multicenter study during the COVID-19 outbreak. *Cancer Discov.* 10, 783–791. doi: 10.1158/2159-8290.CD-20-0422

Damiot, A., Pinto, A. J., Turner, J. E., and Gualano, B. (2020). Immunological implications of physical inactivity among older adults during the COVID-19 pandemic. *Gerontology* 66, 431–438. doi: 10.1159/000509216

Ekelund, U., Steene-Johannessen, J., Brown, W. J., Fagerland, M. W., Owen, N., Powell, K. E., et al. (2016). Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women. *Lancet* 388:10051. doi: 10.1016/S0140-6736(16)30370-1

Elosua, R., Garcia, M., Aguilar, A., Molina, L., Covas, M.-I., Marrugat, J., et al. (2000). Validation of the minnesota leisure time Spanish women. *Med. Sci. Sports Exerc.* 32, 1431–1437. doi: 10.1097/00005768-200008000-00011

Flynn, M. G., Markofski, M. M., and Carrillo, A. E. (2019). Elevated inflammatory status and increased risk of chronic disease in chronological aging: inflamm-aging or *inflamm-inactivity*? *Aging Dis.* 10, 147–156. doi: 10.14336/AD.2018.0326

Ford, E. S., and Caspersen, C. J. (2012). Sedentary behaviour and cardiovascular disease: a review of prospective studies. *Int. J. Epidemiol.* 41, 1338–1353. doi: 10.1093/ije/dys078

Galluzzi, L., Buqué, A., Kepp, O., Zitvogel, L., and Kroemer, G. (2015). Immunological effects of conventional chemotherapy and targeted anticancer agents. *Cancer Cell* 28, 690–714. doi: 10.1016/j.ccell.2015.10.012

Gilchrist, S. C., Howard, V. J., Akinyemiju, T., Judd, S. E., Cushman, M., Hooker, S. P., et al. (2020). Association of sedentary behavior with cancer mortality in middle- aged and older US adults. *JAMA Oncol.* 6, 1210–1217. doi: 10.1001/jamaoncol.2020.2045

Guedes, D. P., and Sofiati, S. L. (2015). Translation and psicometric validation of the behavioral regulation in exercise questionnaire for use in Brazilian adults. *Rev. Bras. Ativ. Fís. Saúde* 20, 397–412. doi: 10.12820/rbafs.v.20n4p397

Hallal, P. C. (2014). Atividade física e saúde no Brasil: Pesquisa, vigilância e políticas. *Cad. Saude Publica* 30, 2487–2489. doi: 10.1590/0102-311XPE011214

International Labour Organization (2020a). *COVID-19 and the World of Work: Impact and Policy Responses*. Available online at: https://www.ilo.org/wcmsp5/groups/public/---dgreports/---dcomm/documents/briefingnote/wcms_738753.pdf (Acessed September, 2020).

International Labour Organization (2020b). *Extending Social Protection to Informal Workers in the COVID-19 Crisis: Country Responses and Policy Considerations*. Available online at: https://www.ilo.org/wcmsp5/groups/public/---ed_protect/---soc_sec/documents/publication/wcms_754731.pdf (acessed September, 2020).

Lesser, I. A., and Nienhuis, C. P. (2020). The impact of COVID-19 on physical activity behavior and well-being of canadians. *Int. J. Environ. Res. Public Health* 17:3899. doi: 10.3390/ijerph17113899

Lozano-Lozano, M., Martín-Martín, L., Galiano-Castillo, N., Álvarez-Salvago, F., Cantarero-Villanueva, I., Fernández-Lao, C., et al. (2016). Integral strategy to supportive care in breast cancer survivors through occupational therapy and a m-health system: design of a randomized clinical trial. *BMC Med. Inform. Decis. Mak.* 16:150. doi: 10.1186/s12911-016-0394-0

Lustosa, L. P., Pereira, D. S., Dias, R. C., Britto, R. R., Parentoni, A. N., Pereira, L. S. M., et al. (2011). Tradução e adaptação transcultural do minnesota leisure time activities questionnaire em idosos. *Geriatr. Gerontol. Aging* 5, 57–65.

Mctiernan, A., Friedenreich, C. M., Katzmarzyk, P. T., Powell, K. E., Macko, R., Buchner, D., et al. (2019). Physical activity in cancer prevention and survival: a systematic review. *Med. Sci. Sports Exerc.* 51, 1252–1261. doi: 10.1249/MSS.0000000000001937

Meyerhardt, J. A., Heseltine, D., Niedzwiecki, D., Hollis, D., Saltz, L. B., Mayer, R. J., et al. (2006). Impact of physical activity on cancer recurrence and survival in patients with stage III colon cancer: findings from CALGB 89803. *J. Clin. Oncol.* 24, 3535–3541. doi: 10.1200/JCO.2006.06.0863

Ministério da Saúde (2020). *Boletim Epidemiológico Especial.* Doença pelo Coronavírus COVID-19. Available online at: http://antigo.saude.gov.br/images/pdf/2020/August/12/Boletim-epidemiologico-COVID-26.pdf (accessed August 23, 2020).

Mount Sinai Hospital (2020). *COVID-19 Symptoms*. Available online at: https://www.mountsinai.org/health-library/symptoms/covid-19-symptoms (Accessed August, 2015).

Newton, R. U., Hart, N. H., and Clay, T. (2020). Keeping patients with cancer exercising in the age of COVID-19. *JCO Oncol. Pract.* 16, 656–664. doi: 10.1200/OP.20.00210

Nguyen, J., and Brymer, E. (2018). Nature-based guided imagery as an intervention for state anxiety. *Front. Psychol.* 9:1858. doi: 10.3389/fpsyg.2018.01858

Nardin, S., Mora, E., Varughese, F. M., D'Avanzo, F., Vachanaram, A. R., Rossi, V., et al. (2020). Breast cancer survivorship, quality of life, and late toxicities. *Front. Oncol.* 10:864. doi: 10.3389/fonc.2020.00864

Nisbet, E. K., Zelenski, J. M., and Murphy, S. A. (2009). The nature relatedness scale: linking individuals' connection with nature to environmental concern and behavior. *Environ. Behav.* 41, 715–740. doi: 10.1177/0013916508318748

Patel, A. V., Friedenreich, C. M., Moore, S. C., Hayes, S. C., Silver, J. K., Campbell, K. L., et al. (2019). American College of Sports Medicine roundtable report on physical activity, sedentary behavior, and cancer prevention and control. *Med. Sci. Sports Exerc.* 51, 2391–2402. doi: 10.1249/MSS.0000000000002117

Renehan, A. G., Roberts, D. L., and Dive, C. (2008). Obesity and cancer: pathophysiological and biological mechanisms. *Arch. Physiol. Biochem.* 114, 71–83. doi: 10.1080/13813450801954303

Rutten, G. M., Meis, J. J. M., Hendriks, M. R. C., Hamers, F. J. M., Veenhof, C., and Kremers, S. P. J. (2014). The contribution of lifestyle coaching of overweight patients in primary care to more autonomous motivation for physical activity and healthy dietary behaviour: results of a longitudinal study. *Int. J. Behav. Nutr. Phys. Act.* 11:86. doi: 10.1186/s12966-014-0086-z

São-João, T. M., Rodrigues, R. C. M., Gallani, M. C. B. J., Miura, C. T. D. P., Domingues, G. D. B. L., and Godin, G. (2013). Cultural adaptation of the Brazilian version of the godin-shephard leisure-time physical activity questionnaire. *Rev. Saude Publica* 47, 3. doi: 10.1590/S0034-8910.2013047003947

Schmitz, K. H., Campbell, A. M., Stuiver, M. M., Pinto, B. M., Schwartz, A. L., Morris, G. S., et al. (2019). Exercise is medicine in oncology: engaging clinicians to help patients move through cancer. *CA Cancer J. Clin.* 69, 468–484. doi: 10.3322/caac.21579

Siegel, R. L., Miller, K. D., and Jemal, A. (2020). Cancer statistics, 2020. *CA Cancer J. Clin.* 70, 7–30. doi: 10.3322/caac.21590

Simon, S. D., Bines, J., Werutsky, G., Nunes, J. S., Pacheco, F. C., Segalla, J. G., et al. (2019). Characteristics and 238 prognosis of stage I-III breast cancer subtypes in Brazil: the AMAZONA retrospective cohort 239 study. *Breast* 44, 113–119. doi: 10.1016/j.breast.2019.01.008

Stout, N. L., and Sabo Wagner, S. (2019). Antineoplastic therapy side effects and polypharmacy in older adults with cancer. *Top Geriatr. Rehabil.* 35, 15–30. doi: 10.1097/TGR.0000000000000212

Taylor, H. L., Jacobs, D. R., Schucker, B., Knudsen, J., Leon, A. S., and Debacker, G. (1978). A questionnaire for the assessment of leisure time physical activities. *J Chronic Dis.* 31, 741–755. doi: 10.1016/0021-9681(78)90058-9

World Health Organization (2020). *Coronavirus Disease 2019.* Situation Report – 205. Available online at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200812-covid-19-sitrep-205.pdf?sfvrsn=627c9aa8_2 (accessed August 23, 2020).

Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., et al. (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci. China Life Sci.* 63, 457–460. doi: 10.1007/s11427-020-1637-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

CORRECTION
published: 26 July 2021
doi: 10.3389/fphys.2021.719688

Corrigendum: Determinants of Health and Physical Activity Levels Among Breast Cancer Survivors During the COVID-19 Pandemic: A Cross-Sectional Study

Aline Rachel Bezerra Gurgel[1], *Pedro Mingroni-Netto*[1], *Jose Carlos Farah*[2], *Christina May Moran de Brito*[3], *Remama Group*[†], *Anna S. Levin*[4] and *Patricia Chakur Brum*[1*]

[1] School of Physical Education and Sport, University of São Paulo, São Paulo, Brazil, [2] Centro de Práticas Esportivas da Universidade de São Paulo (CEPEUSP), São Paulo, Brazil, [3] Instituto Do Câncer Do Estado de São Paulo, ICESP, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [4] Department of Infectious Diseases and LIM49, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil

Keywords: physical activity, COVID-19, breast neoplasms, survivorship, pandemic (COVID-19)

OPEN ACCESS

Approved by:
Frontiers Editorial Office, Frontiers Media SA, Switzerland

***Correspondence:**
Patricia Chakur Brum
pcbrum@usp.br

[†] *On behalf of Remama Group: Luiz Augusto Riani Costa, Raphael Barreto, Sarah Milani de Moraes Leandrini, and Christian Klausener*

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *02 June 2021*
Accepted: *02 July 2021*
Published: *26 July 2021*

Citation:
Gurgel ARB, Mingroni-Netto P, Farah JC, de Brito CMM, Remama Group, Levin AS and Brum PC (2021) Corrigendum: Determinants of Health and Physical Activity Levels Among Breast Cancer Survivors During the COVID-19 Pandemic: A Cross-Sectional Study. Front. Physiol. 12:719688. doi: 10.3389/fphys.2021.719688

A Corrigendum on

Determinants of Health and Physical Activity Levels Among Breast Cancer Survivors During the COVID-19 Pandemic: A Cross-Sectional Study
by Gurgel, A. R. B., Mingroni-Netto, P., Farah, J. C., de Brito, C. M. M., Remama Group, Levin, A. S., et al. (2021). Front. Physiol. 12:624169. doi: 10.3389/fphys.2021.624169

In the original article, the reference for "**A series of pneumonia cases of unknown etiology was reported in the city of Wuhan, China, in late 2019**" was incorrectly written as **Taylor, H. L., Jacobs, D. R., Schucker, B., Knudsen, J., Leon, A. S., and Debacker, G. (1978). A questionnaire for the assessment of leisure time physical activities. *J Chronic Dis.* 31, 741–755. doi: 10.1016/0021-9681(78)90058-9**. It should be **Xu, X., Chen, P., Wang, J., Feng, J., Zhou, H., Li, X., et al. (2020). Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci. China Life Sci.* 63, 457–460. doi: 10.1007/s11427-020-1637-5.**

In the original article, the reference for "**COVID- 19 reached a mark of 20,162,474 cases and 737417 deaths globally in August of 2020**" was incorrectly written as **Elosua, R., Garcia, M., Aguilar, A., Molina, L., Covas, M.-I., Marrugat, J., et al. (2000). Validation of the minnesota leisure time Spanish women. *Med. Sci. Sports Exerc.* 32, 1431–1437. doi: 10.1097/00005768-200008000-00011.** It should be **World Health Organization (2020). *Coronavirus Disease 2019*. Situation Report – 205. Available online at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200812-covid-19-sitrep-205.pdf?sfvrsn=627c9aa8_2 (accessed August 23, 2020)**.

In the original article, the reference for "**From that date until August 8, 2020, 3,012,412 cases were confirmed, and 100,477 deaths occurred as a result of COVID-19 in the country**" was incorrectly written as **Lozano-Lozano, M., Martín-Martín, L., Galiano-Castillo, N., Álvarez-Salvago, F., Cantarero-Villanueva, I., Fernández-Lao, C., et al. (2016). Integral strategy to supportive care in breast cancer survivors through occupational therapy and a m-health system: design of a randomized clinical trial. *BMC Med. Inform. Decis.***

***Mak.* 16:150. doi: 10.1186/s12911-016-0394-0.** It should be **Ministério da Saúde (2020). *Boletim Epidemiológico Especial.* Doença pelo Coronavírus COVID-19. Available online at: http://antigo.saude.gov.br/images/pdf/2020/August/12/Boletim-epidemiologico-COVID-26.pdf (accessed August 23, 2020)**.

In the original article, the reference for "**Social distancing recommendations significantly reduced levels of physical activity of the overall population and more profoundly in the population at increased risk, such as the elderly and those living with chronic non-communicable diseases**" was incorrectly written as **Lustosa, L. P., Pereira, D. S., Dias, R. C., Britto, R. R., Parentoni, A. N., Pereira, L. S. M., et al. (2011). Tradução e adaptação transcultural do minnesota leisure time activities questionnaire em idosos. *Geriatr. Gerontol. Aging* 5, 57–65**. It should be **Damiot, A., Pinto, A. J., Turner, J. E., and Gualano, B. (2020). Immunological implications of physical inactivity among older adults during the COVID-19 pandemic. *Gerontology* (2020) 66, 431–438. doi: 10.1159/000509216.**

In the original article, the reference for "**A recent study showed that middle-aged individuals who adopt sedentary behavior have an increased risk of cancer mortality**" was incorrectly written as **Lesser, I. A., and Nienhuis, C. P. (2020). The impact of COVID-19 on physical activity behavior and well-being of canadians. *Int. J. Environ. Res. Public Health* 17:3899. doi: 10.3390/ijerph17113899.** It should be **Gilchrist, S. C., Howard, V. J., Akinyemiju, T., Judd, S. E., Cushman, M., Hooker, S. P., et al. (2020). Association of sedentary behavior with cancer mortality in middle-aged and older US adults. *JAMA Oncol.* 6, 1210–1217. doi: 10.1001/jamaoncol.2020.2045.**

In the original article, the reference for "**Physical inactivity may exacerbate comorbidities amongst older adults, including cardiovascular disease, cancer, and dysfunctional inflammatory responses**" was incorrectly written as **Nguyen, J., and Brymer, E. (2018). Nature-based guided imagery as an intervention for state anxiety. *Front. Psychol.* 9:1858. doi: 10.3389/fpsyg.2018.01858.** It should be **Flynn, M. G., Markofski, M. M., and Carrillo, A. E. (2019). Elevated inflammatory status and increased risk of chronic disease in chronological aging: inflamm-aging or inflamm-inactivity? *Aging Dis.* (2019) 10, 147–156. doi: 10.14336/AD.2018.0326.**

In the original article, the reference for "**In this scenario, older adults and individuals living with underlying conditions are at a greater risk for complications during COVID-19 disease**" was incorrectly written as **Lustosa, L. P., Pereira, D. S., Dias, R. C., Britto, R. R., Parentoni, A. N., Pereira, L. S. M., et al. (2011). Tradução e adaptação transcultural do minnesota leisure time activities questionnaire em idosos. *Geriatr. Gerontol. Aging* 5, 57–65.** It should be **Damiot, A., Pinto, A. J., Turner, J. E., and Gualano, B. (2020). Immunological implications of physical inactivity among older adults during the COVID-19 pandemic. *Gerontology* 66, 431–438. doi: 10.1159/000509216.**

In the original article, the reference for "**Worldwide, the incidence of cancer and mortality remain high. Over 18 million new cases were registered in 2018, alongside 9.6 million deaths**" was incorrectly written as **São-João, T. M., Rodrigues, R. C. M., Gallani, M. C. B. J., Miura, C. T. D. P., Domingues, G. D. B. L., and Godin, G. (2013). Cultural adaptation of the Brazilian version of the godin-shephard leisure-time physical activity questionnaire. *Rev. Saude Publica* 47, 3. doi: 10.1590/S0034-8910.2013047003947.** It should be **Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L, Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* (2018) 68, 394–424. doi: 10.3322/caac.21492**.

In the original article, the reference for "**Among women, the most diagnosed neoplasm is breast cancer (excluding non-melanoma skin cancer), which also represents the major oncological cause of death in this population, with 2.1 million diagnosed cases in 2018, accounting for almost one in four cancer cases**" was incorrectly written as **São-João, T. M., Rodrigues, R. C. M., Gallani, M. C. B. J., Miura, C. T. D. P., Domingues, G. D. B. L., and Godin, G. (2013). Cultural adaptation of the Brazilian version of the godin-shephard leisure-time physical activity questionnaire. *Rev. Saude Publica* 47, 3. doi: 10.1590/S0034-8910.2013047003947.** It should be **Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L, Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* (2018) 68, 394–424. doi: 10.3322/caac.21492.**

In the original article, the reference for "**Despite the escalating cancer incidence, advances in early diagnosis and breast cancer therapy improved five-year overall survival rates, now exceeding 90% when diagnosed in the early stages**" was incorrectly written as **Nisbet, E. K., Zelenski, J. M., and Murphy, S. A. (2009). The nature relatedness scale: linking individuals' connection with nature to environmental concern and behavior. *Environ. Behav.* 41, 715–740. doi: 10.1177/0013916508318748; Ekelund, U., Steene-Johannessen, J., Brown, W. J., Fagerland, M. W., Owen, N., Powell, K. E., et al. (2016). Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women. *Lancet* 388:10051. doi: 10.1016/S0140-6736(16)30370-1.** It should be **Simon, S. D., Bines, J., Werutsky, G., Nunes, J. S., Pacheco, F. C., Segalla, J. G., et al. (2019). Characteristics and 238 prognosis of stage I-III breast cancer subtypes in Brazil: the AMAZONA retrospective cohort 239 study. *Breast* 44, 113–119. doi: 10.1016/j.breast.2019.01.008; Siegel, R. L., Miller, K. D., and Jemal, A. (2020). Cancer statistics, 2020. *CA Cancer J. Clin.* 70, 7–30. doi: 10.3322/caac.21590.**

In the original article, the reference for "**In fact, increased physical activity levels, exercise, and body weight control have been considered strong allies to cancer patients and survivors, as they positively impact physical capacities, fatigue, depressive symptoms, anxiety, and quality of life**" was incorrectly written as **Guedes, D. P., and Sofiati, S. L. (2015). Translation and psicometric validation of the behavioral regulation in exercise questionnaire for use in**

Brazilian adults. *Rev. Bras. Ativ. Fís. Saúde* 20, 397–412. doi: 10.12820/rbafs.v.20n4p397; Rutten, G. M., Meis, J. J. M., Hendriks, M. R. C., Hamers, F. J. M., Veenhof, C., and Kremers, S. P. J. (2014). The contribution of lifestyle coaching of overweight patients in primary care to more autonomous motivation for physical activity and healthy dietary behaviour: results of a longitudinal study. *Int. J. Behav. Nutr. Phys. Act.* 11:86. doi: 10.1186/s12966-014-0086-z; and **Mount Sinai Hospital (2020). *COVID-19 Symptoms.* Available online at: https://www.mountsinai.org/health-library/symptoms/covid-19-symptoms (Accessed August, 2015)**. It should be **Campbell, K. L., Winters-Stone, K. M., Wiskemann, J., May, A. M., Schwartz, A. L., Courneya, K. S., et al. (2019). Exercise guidelines for cancer survivors: consensus statement from international multidisciplinary roundtable. *Med. Sci. Sports Exerc.* 51, 2375–2390. doi: 10.1249/MSS.0000000000002116; Nardin, S., Mora, E., Varughese, F. M., D'Avanzo, F., Vachanaram, A. R., Rossi, V., et al. (2020). Breast cancer survivorship, quality of life, and late toxicities. *Front. Oncol.* (2020) 10:864. doi: 10.3389/fonc.2020.00864;** and **Mctiernan, A., Friedenreich, C. M., Katzmarzyk, P. T., Powell, K. E., Macko, R., Buchner, D., et al. (2019). Physical activity in cancer prevention and survival: a systematic review. *Med. Sci. Sports Exerc.* (2019) 51, 1252–1261. doi: 10.1249/MSS.0000000000001937.**

In the original article, the reference for "**Since the literature of the past two decades has provided evidence that supports the practice of physical activity during and after cancer treatment**" was incorrectly written as **Newton, R. U., Hart, N. H., and Clay, T. (2020). Keeping patients with cancer exercising in the age of COVID-19. *JCO Oncol. Pract.* 16, 656–664. doi: 10.1200/OP.20.00210.** It should be **Schmitz, K. H., Campbell, A. M., Stuiver, M. M., Pinto, B. M., Schwartz, A. L., Morris, G. S., et al. (2019). Exercise is medicine in oncology: engaging clinicians to help patients move through cancer. *CA Cancer J. Clin.* (2019) 69, 468–484. doi: 10.3322/caac.21579**.

In the original article, the reference for "**and that physical activity helps to prevent neoplastic recurrence**" was incorrectly written as **Renehan, A. G., Roberts, D. L., and Dive, C. (2008). Obesity and cancer: pathophysiological and biological mechanisms. *Arch. Physiol. Biochem.* 114, 71–83. doi: 10.1080/13813450801954303.** It should be **Patel, A. V., Friedenreich, C. M., Moore, S. C., Hayes, S. C., Silver, J. K., Campbell, K. L., et al. American College of Sports Medicine roundtable report on physical activity, sedentary behavior, and cancer prevention and control. *Med. Sci. Sports Exerc.* (2019) 51, 2391–2402. doi: 10.1249/MSS.0000000000002117.**

In the original article, the reference for "**This behavior possibly increased the chance of exposure to symptomatic or asymptomatic people outside the home. Brazilian women who are workers in the informal economy were disproportionally affected by the pandemic**" was incorrectly written as **International Labour Organization (2018). *COVID-19 and the World of Work: Impact and Policy Responses.* Available online at: https://www.ilo.org/wcmsp5/groups/public/---dgreports/---dcomm/documents/briefingnote/wcms_738753.pdf (accessed September, 2020)**. It should be **International Labour Organization (2020). *COVID-19 and the World of Work: Impact and Policy Responses.* Available online at: https://www.ilo.org/wcmsp5/groups/public/---dgreports/---dcomm/documents/briefingnote/wcms_738753.pdf (accessed September, 2020)**.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

frontiers in Physiology

MINI REVIEW
published: 09 February 2021
doi: 10.3389/fphys.2021.627260

The Physiological Mechanisms of the Sex-Based Difference in Outcomes of COVID19 Infection

Susan Wray and Sarah Arrowsmith*

Department of Women's and Children's Health, University of Liverpool, Liverpool, United Kingdom

The scale of the SARS-CoV-2 pandemic has thrust a spotlight on the sex-based differences in response to viral diseases; morbidity and mortality are greater in men than women. We outline the mechanisms by which being female offers a degree of protection from COVID19, that persists even when confounders such as comorbidities are considered. The physiological and immunological mechanisms are fascinating and range from incomplete X chromosome inactivation of immune genes, a crucial role for angiotensin converting enzyme 2 (ACE2), and regulation of both immune activity and ACE2 by sex steroids. From this flows understanding of why lung and other organs are more susceptible to COVID19 damage in men, and how their distinct immunological landscapes need to be acknowledged to guide prognosis and treatment. Pregnancy, menopause, and hormone replacement therapy bring changed hormonal environments and the need for better stratification in COVID19 studies. We end by noting clinical trials based on increasing estrogens or progesterone or anti-testosterone drugs; excellent examples of translational physiology.

Keywords: steroid hormones, sexual dimorphism, SARS-CoV-2, pregnancy, ACE2, hormones

OPEN ACCESS

Edited by:
George Grant,
University of Aberdeen,
United Kingdom

Reviewed by:
Ajay Pradhan,
Örebro University, Sweden
Maurizio Acampa,
Siena University Hospital, Italy
Walter Malorni,
National Institute of Health (ISS), Italy

****Correspondence:***
Susan Wray
s.wray@liv.ac.uk

Specialty section:
This article was submitted to Clinical and Translational Physiology, a section of the journal Frontiers in Physiology

Received: *08 November 2020*
Accepted: *15 January 2021*
Published: *09 February 2021*

Citation:
Wray S and Arrowsmith S (2021) The Physiological Mechanisms of the Sex-Based Difference in Outcomes of COVID19 Infection. Front. Physiol. 12:627260. doi: 10.3389/fphys.2021.627260

INTRODUCTION

This short review focuses on how differences in the physiology of women and men affect the outcome and survival of patients with COVID19. We first review the evidence that outcomes for females are more favorable, before considering the mechanisms and relating them to viral infection. We use the binary terms "male" and "female" so we can correctly report data in published studies, which so far have not considered if COVID19 has particular effects on trans and non-binary people.

COVID19 Outcomes Are Worse in Males

At the time of writing (October 2020), it is almost a year since the first reports of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus-2019 (COVID19), appeared. Since then, there has been concerted international effort to understand the virus, the disease it produces and develop strategies to combat it. From the earliest findings, it emerged that more men than women suffer severe COVID19 disease and die from it (Jin et al., 2020; Pradhan and Olsson, 2020; Scully et al., 2020). This finding of men succumbing to more severe disease and dying, was also a feature in the two previous, smaller coronavirus diseases, Middle East Respiratory System (MERS-CoV) in 2012 and SARS-CoV in 2002 (Channappanavar et al., 2017; Lu et al., 2020). For SARS-CoV-2, with its global reach and high infectivity, the continued analysis of large global data sets of sex-disaggregated data has been possible, and the data are clear; women

fare better with COVID19 (Raparelli et al., 2020; Williamson et al., 2020). For updated statistical information, from ∼180 countries, the "COVID19 sex-disaggregated tracker update," from (https://globalhealth5050.org/the-sex-gender-and-covid-19-project/) is recommended. **Figure 1A** is taken from their 19th October 2020 report and shows some clinical stages of COVID19 by sex. Examples of regional case fatality rates by sex can be seen in **Figure 1B**.

Sex Differences Remain After Accounting for Infection Rates, Age, and Comorbidities

With the increases in available data it is possible to interrogate the statistics further and ask whether differences such as infection rates, age and co-morbidities can explain the sex differences in the outcomes of COVID19, (Abate et al., 2020). It appears that none can. Infection rates are approximately similar in women and men throughout the world (Chakravarty et al., 2020; Williamson et al., 2020)—the effects of gender and social norms, are discussed below. The effects of COVID19, and death from it, are known to increase with age. This is true for both sexes, and a variety of explanations suggested, from access to hospital and intensive care facilities, comorbidities, and immunosenescence. The latter may be due to decline in sex hormones in both sexes (Gomez et al., 2019). Menopause is specifically addressed later. When however, COVID19 deaths rates are disaggregated by age and sex, the disproportionate effect on males remains, see **Figure 1C**. Like age, comorbidities also worsen the progress of the disease and fatalities from it. More men die even when these factors are adjusted for. A recent study undertaken to determine who is most at risk of a severe outcome from SAR-CoV-2 infection, used a health analytical platform to obtain data from >17 million patients in the UK, within which were almost 11,000 COVID19 deaths (Williamson et al., 2020). These deaths were associated with being male, and various medical conditions, including asthma and diabetes. A multivariate analysis confirmed the sex difference in deaths, even when adjusted for all other factors, including age, obesity and diseases (diabetes, cancer, kidney, asthma, and ten others). Although beyond the scope of this review, a substantially higher death rate was found in South Asian and black people compared to white people, that was only partially attributable to comorbidities and deprivation.

While future studies will further refine our knowledge concerning disease outcomes, sex makes a significant contribution to outcomes; in COVID19, women have a degree of protection compared to men. We are not saying that all the aspects of COVID19 can be attributed to sex differences, but rather, that benefits will follow from understanding the disease better, and biological sex is a part of this. We will briefly mention how gender may impact on these data, before a more detailed discussion of the physiological mechanisms of the reported sex differences.

Gender

There are many ways that gender can impact on COVID19 statistics. Compared to men, women may be more concerned about COVID19 (Brooks and Saad, 2020). This may lead to greater compliance with public health policies such as mask wearing, hand washing, and social distancing. In addition, globally women spend less time out of the home. These factors may reduce their infection rates, but are countered by the fact that they contribute significantly higher to the healthcare work force—an analysis of 104 countries by the World Health Organization in 2019 found that women represent around 70% of the health workforce. Men may wait longer to seek a doctor after infection and therefore be sicker before treatment. In addition, more men are, or have been, smokers, drink alcohol and have cardiovascular disease. These factors will all increase susceptibility to COVID19, but as discussed earlier, cannot explain the findings of sex differences.

MECHANISM FOR SEX DIFFERENCES DURING COVID19

We first overview how SARS-CoV-2 infects humans as the basis for understanding how sex-based differences can arise. We then focus on the role of angiotensin converting enzyme 2 (ACE2) and infection, and then sex differences in immunological responses to infection.

Overview of SARS-CoV-2

Coronaviruses are large-enveloped, single stranded, positive-sense RNA viruses. They contain transmembrane spike glycoproteins; composed of heads, which have host receptor binding domains and stalks, responsible for membrane fusion and infection of the host cell (**Figure 2A**). Compared to the 2003 SARS-CoV outbreak, SARS-CoV-2 is better able to evade our immune defenses and is highly infectious, hence the current pandemic. Both viruses use the receptor for ACE2 as their attachment target, starting in the lungs (Li et al., 2003; Tai et al., 2020; **Figures 2B,C**.[1]That this is essential for viral entry was shown using ACE2 knock out mice (Kuba et al., 2005). Infection is associated with both shedding and down-regulation of the ACE2 receptor, which as discussed below, will have physiological consequences (Heurich et al., 2014; **Figure 2D**). The SARS-CoV-2 virus has a higher binding affinity than SARS-CoV. For infection, the stalks must be activated, and this is achieved by proteases, specifically the host cell's transmembrane serine protease 2 (TMPRSS2, see **Figure 2D**; Belouzard et al., 2009). It has however been found that with SARS-CoV-2, there is an element of self-activation performed by the viral proprotein convertase furin. This facilitates SARS-CoV-2 entry, a property that it exploits in those cells that have low TMPRSS2 expression (Shang et al., 2020).

ACE2

Our knowledge of ACE2 and its relation to the classic renin-angiotensin-aldosterone system (RAAS), is relatively recent; (Donoghue et al., 2000; Tipnis et al., 2000) see **Figure 3** for a simple scheme. It has been labeled the protective

[1]https://scx1.b-cdn.net/csz/news/800/2020/whatistheace.jpg

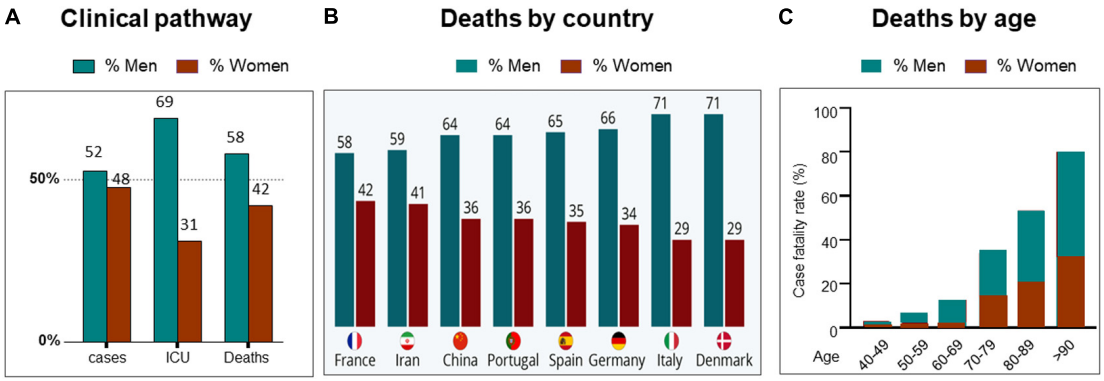


FIGURE 1 | Sex differences and COVID19. **(A)** Clinical pathway from confirmed cases, intensive care unit (ICU) admissions and deaths from COVID19. Data from countries providing sex-disaggregated data in October 2020. Redrawn from Global health 5050 at https://globalhealth5050.org/wp-content/uploads/October-2020-The-COVID-19-Sex-Disaggregated-Data-Tracker-Update.pdf, accessed October 16th 2020. **(B)** Covid19 deaths by country and sex, in March 2020. Chart from Statista at: https://www.statista.com/chart/21345/coronavirus-deaths-by-gender/. **(C)** deaths from COVID19 grouped by age and sex. Data obtained from 12 countries in May 2020. Redrawn from Global health 5050.

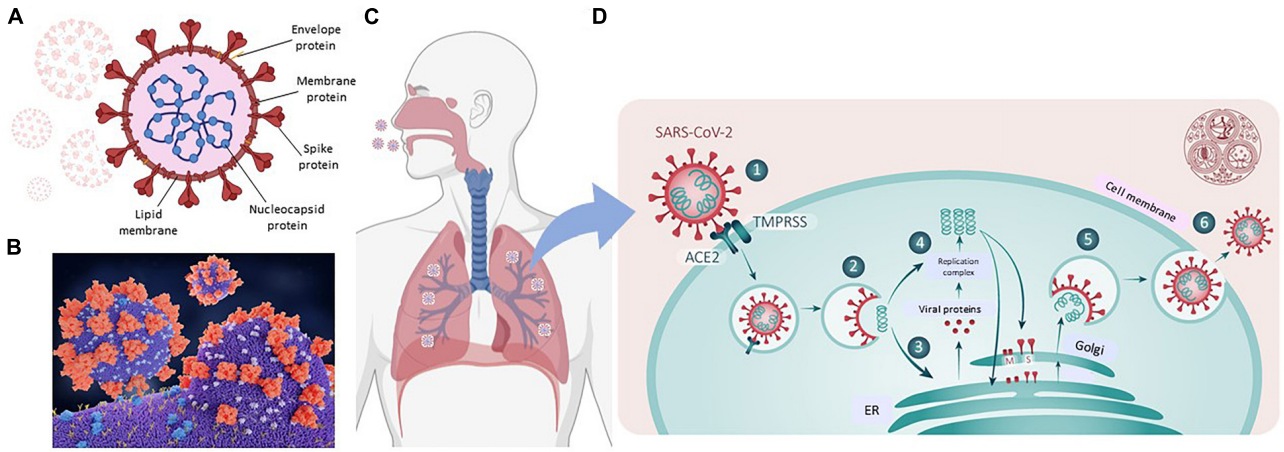


FIGURE 2 | SARS-CoV-2 and COVID19. **(A)** Representation of SARS-CoV-2 virus. **(B)** A molecular model showing the virus with spike proteins (red) and ACE2 (angiotensin converting enzyme 2) receptor (blue) on host cell surface. From Juan Gaertner/Science Photo Library, accessed 8/11/2020. **(C)** Covid19 entry via airways. **(D)** scheme showing TMPRSS activation of virus, followed by its internalization, processing and replication, Adapted from Ward P. et al. (2020). Available at: https://www.fpm.org.uk/blog/covid-19-sars-cov-2-pandemic. Accessed 8/11/2020.

counter arm of RAAS as it has positive metabolic effects, and is vasodilating, anti-proliferation, and anti-inflammatory, balancing angiotensin II's vasoconstrictive role (White et al., 2019; Samavati and Uhal, 2020).

ACE2 is a zinc containing, carboxy peptidase that removes an amino acid and converts angiotensin 1 to angiotensin 1–9 and angiotensin 11 into the vasodilator, angiotensin(1-7), and may have additional substrates (Hamming et al., 2007; **Figure 3**). Its catalytic site is extracellular. ACE2 is cleaved from cells by metalloproteases such as ADAM10 and ADAM17 and is shed with an active catalytic site into plasma (Turner, 2015), see **Figure 3**). Men appear to have higher plasma ACE2 levels than women (see Salah and Mehta, 2020). Physiologically, Ang-(1-7) has been shown to signal via a novel GPCR, Mas (Bader et al., 2018). As infection produces a down-regulation of ACE2, this may contribute to the hypertension and inflammation seen with COVID19, as the vasodilator Ang(1-7) is decreased (Povlsen et al., 2020; Samavati and Uhal, 2020), and has led to the suggestion that exogenous ACE2 could be therapeutic (Verdecchia et al., 2020). ACE2 expression can be modulated by peptides and hypoxia (Melo Junior et al., 2020), and it and TMPRSS2 are modulated by steroid hormones (Baratchian et al., 2020; Young et al., 2020), as described next.

ACE2, TMPRSS, and Sex

Until menopause, women are relatively protected from a variety of cardiovascular risks, including high blood pressure (Reckelhoff, 2018). Part of the underlying reason for this is the effect of sex steroid hormones on RAAS, (Dalpiaz et al., 2015; Turner, 2015; Melo Junior et al., 2020). Although it seems reasonable to anticipate sex-based differences and regulation of ACE2, research is limited, especially on human tissues

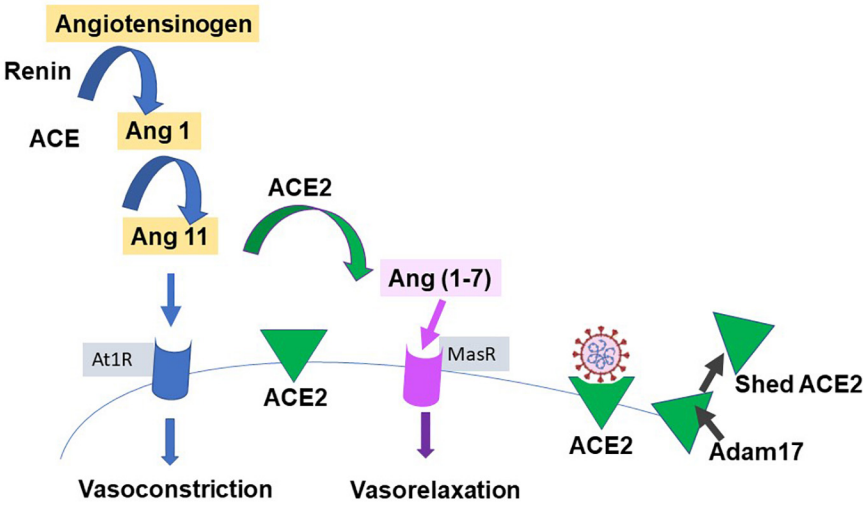


FIGURE 3 | Simplified renin-angiotensin system. Scheme shows the role of ACE2 as both the catalysis for Ang(1-7) creation, with a vasodilatory effect on vascular smooth muscle, and as the receptor for SARS-CoV-2. Proteases such as those of the Adam group act to cause shedding of ACE2 into plasma.

(Salah and Mehta, 2020; Samavati and Uhal, 2020; Song et al., 2020). With the COVID19 pandemic, attention has focused on ACE2 in the alveoli, but it has a wide tissue distribution. Specifically, it is only moderately expressed in lung, compared to kidney, heart, fat cells, and oral mucosa, and in comparable amounts to those reported in gut, bladder, brain and adrenals (Hamming et al., 2004; Zou et al., 2020). This tissue-wide distribution probably contributes to the multi organ pathologies brought on by infection. Of note with respect to COVID19, greater ACE2 expression was found in pneumocytes from men compared to women (Song et al., 2020). In differentiated human airway epithelial cells, treated either with vehicle or estradiol, the latter expressed lower levels of ACE2 mRNA (Stelzig et al., 2020) (TMPRSS2 mRNA levels were not affected). Estradiol may also positively regulates kidney, cardiac and adipose ACE2 expression (Gupte et al., 2012; Dalpiaz et al., 2015). In rats, both sexes have age-related declines in ACE2 expression, but to a greater extent in males (Xie et al., 2006). It is important to see if ACE2 transcript are translated to protein levels on the cell membrane, but it seems likely that there is sexual dimorphism in the availability of a key infectivity component, ACE2, necessary for COVID19.

TMPRSS2 is also widely distributed and highly expressed in epithelial cells in lungs, small intestine, heart, liver, and prostate. No significant difference in TMPRSS2 expression between males and females in human lung were found (Song et al., 2020). Its transcription and activity are controlled by androgens and discussed again in the section on males and COVID19.

Both epidemiological and experimental studies have reported sex differences in the therapeutic benefits of modulators of the RAAS pathway. It was noted that "Despite these differences, RAS inhibitors are the most commonly prescribed drugs for the treatment of chronic renal disease, irrespective of sex" (Sullivan, 2008). We consider that this point remains valid for therapeutic approaches using RAAS modulating drugs during COVID19, and could skew findings if not considered (Furuhashi et al., 2020; Reynolds et al., 2020; Young et al., 2020).

IMMUNOLOGICAL RESPONSES AND SEX DIFFERENCES

Background

During COVID19, immune cells in the lungs produce a "cytokine storm"; specifically, interleukin-6, interleukin-1β, tumor necrosis factor α, along with infiltration of chemokines, occurs. This hypercytokinemia and infiltration of monocytes and neutrophils, produces lung injury and respiratory difficulties. This pathological consequence of the immune response underlies the use of blockers of these cytokines as therapeutic approaches (Tang et al., 2020). These differences in male and female immunological activity can be related to their differing vulnerability to the disease.

Women and men differ in their physiological responses to viral diseases (Klein and Flanagan, 2016). Compared to males, females mount stronger immune responses to combat and clear viral loads (Klein, 2012). With vaccines this can lead to females producing over-exuberant responses, of both the innate and adaptive immune systems, estimated to be twice as strong as in males. This can cause increased adverse outcomes (Klein et al., 2010), as well as the increased incidence of auto-immune and inflammatory diseases found in females. Sex-dependent steroid hormones and genes, have been linked to the mechanism determining differences between the sexes in response to viral infection (Forsyth and Anguera, 2021). Many genes associated with immune responses are present on the X chromosome. Although in females one copy of these should be inactivated, there is evidence for gene imbalance, favoring females and their immunological responses to viral infections (Wang et al., 2016; Schurz et al., 2019). One example is Toll-like receptor 7

(TLR7). The gene for this receptor which senses RNA viruses such as SARS-CoV-2, is present on the X chromosome and may escape X cell inactivation (Souyris et al., 2018). All types of immune cells have estrogen and progesterone receptors which will act as transcriptional regulators. The effects of testosterone on immune responses are not as marked as those of female hormones and there will only be one copy of the X chromosome. In addition, it has been speculated that microRNAs., which act as post−transcriptional modulators of gene expression, and are also regulated by sex hormones., may also contribute to sex-based differences, especially as the X−chromosome has a particular abundance of microRNAs (see e.g., for further details Pontecorvi et al., 2020). Although too large a topic to be covered in detail here (Channappanavar et al., 2017; Jakovac, 2020), the protective effects of estrogen (and progesterone) have been attributed to (and see also **Figure 5**): (i) their promotion of production of anti-inflammatory cytokines (e.g., such as interleukins 4 and 10), (ii) increasing helper T cells, (iii) increasing B cells and thereby antibodies, and (iv) suppressing production of pro-inflammatory cytokines and migration of macrophages and monocytes into infected tissue (Mauvais-Jarvis et al., 2020). These protective advantages decline with age. A different but related point concerns Vitamin D, as it has been suggested that low levels of D3 may correlate with poorer infection outcomes. Estrogen may enhance vitamin D's actions, which include reducing the cytokine storm, and in this way contribute to sex-based differences (Pagano et al., 2020). A collection of papers covering endocrinology and COVID19 was published in 2020[2]. For a comprehensive account of the endocrinological effects on the immune system recent reviews are recommended (Gadi et al., 2020; Mauvais-Jarvis et al., 2020; Young et al., 2020). Thus, we expect that the immune landscape during a SARS-CoV-2 infection will differ between men and women and make the former more vulnerable to COVID19.

Different inflammatory patterns are also thought to lead to different occurrences of cardiac arrhythmias which are also burdening patients with COVID-19. Systemic infection and inflammatory cytokines, such as IL-6, have been shown to prolong the QT-interval and alter

[2]https://www.frontiersin.org/research-topics/13975/endocrinology-and-covid--a-cross-disciplinary-topic#articles

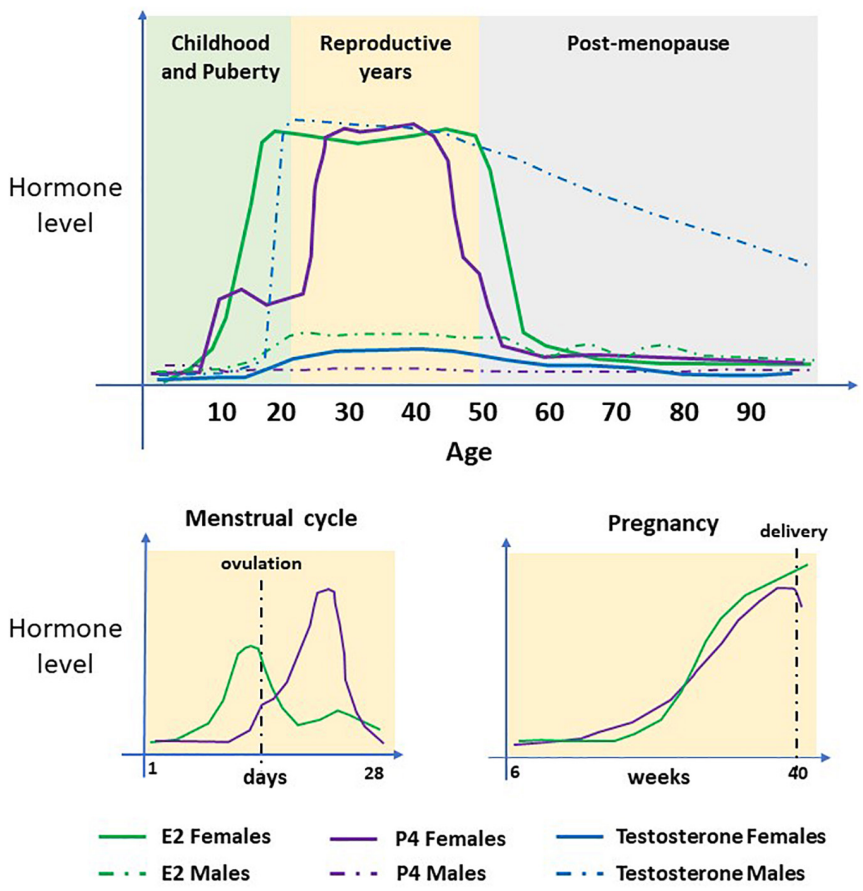


FIGURE 4 | Serum concentrations of sex steroid hormones. The hormones 17β-estradiol (E2), Progesterone (P4) and Testosterone (T/DHT) in women and men during their life course. In females, E2 and P4 are the predominant hormones. Concentrations increase at puberty, undergo cyclical changes during the menstrual cycle and steadily increase during pregnancy. At menopause, concentrations decline to pre-puberty levels. T/DHT is the predominant male hormone which increases at puberty and remains high until late in life when levels decline steadily.

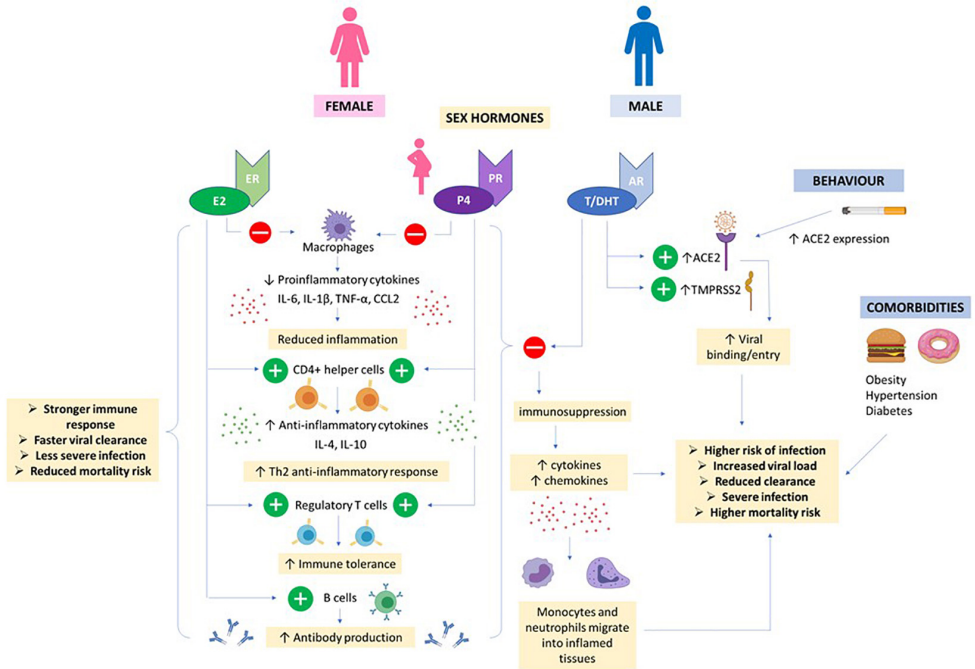


FIGURE 5 | Hormones and COVID19. High E2 and P4 concentrations in females (even higher in pregnancy) helps to suppress proinflammatory cytokine production by macrophages and prevent migration of monocytes and neutrophils into inflamed tissues. CD4 + helper cells are stimulated to produce anti-inflammatory cytokines and T regulatory cells promote immune tolerance. E2 also stimulates production of antibodies. Together, this results in a stronger immune response, faster viral clearance and less severe COVID infection in women. Androgens e.g., T/DHT and AR signaling increases expression of ACE2 and TMPRSS2 promoting viral entry. Together, T/DHT's immunosuppressive effects, male behavioral factors and co-morbidities can contribute to a more severe COVID infection and worse outcome in males.

repolarizations (Lazzerini et al., 2020a,b). Hence, sex-based differences in cytokine expression may also be attributing to differences in mortality rates via alterations in risk of life-threatening cardiac events.

Immune Differences With COVID19

Studies of lung injury have demonstrated increased damage in male mice and ovariectomized females, which could be reduced by estradiol administration (Speyer et al., 2005). Similar protective effects of estradiol and progesterone were observed in studies of influenza-infected animals (Robinson et al., 2011; Hall et al., 2016). When considering the role of the immune system in sex based COVID19 differences, a key question is whether they are due to differences in viral load, antibody response or plasma cytokines. With SARS female mice had lower viral loads, lower inflammatory responses, reduced lung damage and death, compared to males; this protection was lost with ovariectomy or treatment with the estrogen receptor antagonist, fulvestrant (Channappanavar et al., 2017). The detailed answers to how women and men differ in their immune responses to COVID19 has been directly addressed in a recent comprehensive study (Takahashi et al., 2020). Patients with a clinical diagnosis of moderate COVID19 who were not taking immunomodulatory medicines, were studied. No sex difference was found in viral RNA concentrations. Follow up of these patients found, however, that those females with higher salivary viral load deteriorated, whereas this correlation was not found in males. Males had higher plasma levels of immune cytokines of the innate immune system such as IL-8 and IL-18. Women had more robust T cell responses, which is consistent with findings during other infections (Amadori et al., 1995). This is an important difference, as a poor T cell response was associated with poor disease outcome in men but not in women. In women but not men, worse outcome was associated with high levels of innate immune cytokines. There were some innate immune factors, such as Il-15 that increased only in females who progressed to worse disease, an association not found in males. Women could benefit more from therapies that that dampened their innate immunity responses during initial infection period.

Thus, by disaggregating patient data by sex, key differences in the immune landscapes have been identified. This heterogeneity in immune capabilities and responses helps understanding of the distinct COVID19 progression in women and men, and may be used to guide disease prognosis and sex-specific treatments (Tang et al., 2020; Ursin et al., 2020). Of relevance here also is the use of COVID19 convalescent plasma donor therapy. As males tend to have more severe COVID19, their enhanced inflammatory responses and higher B cell recruitment, and antibodies, suggests that older males may be more useful plasma donors (Klein et al., 2020). As described below, the protective effects of estrogen and progesterone (or anti-testosterone treatments) have stimulated novel treatment trials.

PREGNANCY

Pregnancy presents a unique and complex immunological scenario; the maternal immune system needs to be able to tolerate a "foreign" developing fetus whilst also protecting the mother against infections and favoring the transfer of maternal antibodies to the fetus. Elements of host defense and innate and adaptive immunity are altered during pregnancy to provide this co-operation (Racicot et al., 2014). Whilst protecting the fetus, this immune modulation could predispose pregnant women to increased susceptibility to infection from pathogens such as viruses (Robinson and Klein, 2012). Indeed, pregnant women have been shown to be disproportionately affected by respiratory illnesses, e.g., influenza (Robinson and Klein, 2012). During the MERS and SARS outbreaks, increased morbidity and higher maternal mortality rates were found (Wong et al., 2004). Hence during the current COVID19 pandemic, higher rates of mortality and disease severity were expected in pregnant women, and shielding was recommended.

The potential increased seriousness of COVID19 in pregnancy, however, has not been observed. So far, despite ACE2 being highly expressed in the placenta (Levy et al., 2008), vertical transmission to the fetus has not been seen (Chen H. et al., 2020). There is no consensus of an effect on rates of miscarriage, stillbirth or preterm birth in COVID19-infected mothers (Dubey et al., 2020; Pettirosso et al., 2020). In terms of maternal morbidity and mortality, despite the heightened severity experienced in other viral diseases (Siston et al., 2010), studies so far have not put pregnant women at any greater risk of disease severity or complications from COVID19 compared to their non-pregnant counterparts (Chen L. et al., 2020; Collin et al., 2020). How these differences relate to the specific differences between the corona viruses has not been elucidated. Epidemics can lead to resources being removed from obstetrics, maternity, and sexual health, and diverted to emergency responses, and hence increasing maternal deaths, with or without infection.

Physiological Protective Mechanisms and COVID19 in Pregnancy

We know from sex-based studies that females mount a greater immune response to many viral infections and this is largely due to the protective and acute effects of estrogen (Robinson et al., 2011). In pregnancy, the concentrations of 17β-estradiol (E2), estriol (E3), and progesterone are significantly increased (see **Figure 4**). These hormonal changes underly the immunological changes required to provide a pregnancy-supportive immune environment, as well as stimulating antibody production by B cells. Both E2 and progesterone are known to alter the number and function of multiple immune cell types producing an immunologic switch from a pro- to an anti-inflammatory state, with T-helper 2 cell dominance elevating IL-4, IL-10, IL-13, and TGF-beta (Mauvais-Jarvis et al., 2020), see **Figure 5**).

These changes in the hormonal milieu which shift the cytokine signature toward an anti-inflammatory state in pregnancy, may support an early adaptive immune response which helps to blunt early COVID19 infection and inflammation. In turn, this would help prevent the "cytokine storm" and its associated pulmonary pathologies, in pregnant women infected with SARS-CoV-2.

Others have suggested that folic acid supplementation during pregnancy may provide protection (Acosta-Elias and Espinosa-Tanguma, 2020). Computer simulation studies indicated that folic acid can reduce viral replication by inhibiting its furin endoprotease (Coutard et al., 2020) which is part of SARS-CoV-2 host cell entry mechanism or inhibit the coronavirus 3C-like protease, $3CL_{pro}$, (Serseg et al., 2020) required for its replication (Hsu et al., 2005). Hence the severity of infection may be inversely proportional to the concentration of folic acid but more work is required (Acosta-Elias and Espinosa-Tanguma, 2020).

As the data on COVID19 in pregnancy come from small studies and sometimes lack controls including age-matching, conclusion remains tentative but cautiously optimistic. Of note also, pregnant women may visit care settings frequently and so signs of infection may be detected and treated earlier.

POST-MENOPAUSAL WOMEN

That adult men of all ages and older women pose the highest risk of developing serious complications from COVID19 infection (Scully et al., 2020), again raises the question of the role of sex steroid hormones on infectivity. In women the increase in risk begins in their late 50s, see **Figure 1C**, around the time of the menopause (Ding et al., 2020), which is characterized by female sex hormone deficiency (**Figure 4**).

Animal studies of SARS-CoV and MERS, showed that absence of E2 signaling following ovariectomy or estrogen receptor antagonist treatment is associated with more severe disease in female mice (Channappanavar et al., 2017). Moreover, hormones associated with having a higher ovarian reserve (anti-Mullerian hormone and E2) negatively correlate with COVID severity (Ding et al., 2020), further suggesting that pre-menopausal women are protected.

Large-scale self-reported data obtained from the UK COVID19 symptom tracker application (C-19) showed a positive association between COVID19 and menopausal status, and a negative association with combined oral contraceptive pill use (Costeira et al., 2020), supporting the hypothesis that E2 offers protection against disease severity. Hormone replacement therapy (HRT) use, however, was positively associated with COVID19 symptoms. The route of administration, dose and type of HRT however, was not recorded and further investigations are needed (Gargaglioni and Marques, 2020). HRT is also usually only estrogenic and at physiological concentrations, whilst the combined oral contraceptive pill has E2 and progesterone and at supra-physiological concentrations.

MEN

The differences between men and women has been emphasized throughout. A few additional points can be made. Testosterone exerts immunosuppressive effects (Foo et al., 2017; **Figure 5**)

which may contribute to a blunted antibody response in men and result in a worse prognosis compared to females (Chanana et al., 2020). Androgens, including testosterone, enhance expression of $TPMRSS_2$ facilitating viral fusion with host cell membranes (Asselta et al., 2020; Hoffmann et al., 2020). Male sex hormones are also thought to increase the activity of the ACE2 receptor (Dalpiaz et al., 2015) further enabling SARS-COV-2 viral infectivity. Men with androgenetic alopecia or male pattern hair loss, a condition associated with genetic variations in the androgen receptor gene and signaling (Hillmer et al., 2005), are also thought to be at a greater risk of COVID19 severity: small studies have indicated high incidence of male pattern baldness in patients hospitalized with COVID19 (Goren et al., 2020). Along with the gender differences and detailed immunological differences reported in men with COVID19 disease discussed above, it is suggested that men will benefit from treatments that increase their T cell immune responses, and anti-testosterones.

CLINICAL TRIALS

A SARS-CoV-2 protein interaction study mapped many potential for repurposing drugs, including sex hormones (Gordon et al., 2020). That sex hormones can modulate inflammatory responses, lessen the cytokine storm or impede viral entry, has added to the suggestion that exogenous hormones could be administered as therapies, either prophylactically or as treatment adjuncts, to reduce COVID19 disease severity. Re-purposing of existing and already approved therapies is particularly exciting given there is little time to develop new ones.

In the USA, two trials are underway testing whether symptom severity can be reduced with either a short course of estradiol, administered by transdermal patch, in adult men and older women with COVID19 (NCT04359329) or oral progesterone in men (NCT04365127). In Mexico, a trial is investigating the effect of a combined estrogen and progesterone patch (NCT04539626) on clinical response and mortality in non-severe COVID19 patients. A trial in Iran is also testing the effect of injectable estradiol and testosterone on recovery in male and female COVID19 patients with respiratory, heart or kidney failure (IRCT20150716023235N15).

Trials exploring anti-androgen therapies are also underway, including in Sweden (NCT04475601), the USA (NCT04509999, NCT04374279) and Brazil (NCT04446429) with a view to reducing disease severity in older (>50 years) male and female patients, or males presenting with male-pattern baldness, by inhibiting the expression of androgen regulated proteins, such as TMPRSS2. Other trials are investigating the effect of decreasing TMPRSS2 action using TMPRSS2 inhibitors (see **Table 1**).

In Italy, a Phase II randomized trial is planned to assess the efficacy of intravenous oxytocin in patients affected by COVID19 (NCT04386447). Oxytocin known for its role in augmenting uterine contractions in labor (Arrowsmith, 2020), has also been shown to limit excessive pro-inflammatory and oxidative stress

TABLE 1 | Clinical trial identifiers, drug class and targets.

Drug class	Target	Action/effect	Trial identifier	Sponsor/location
ER agonist	Estrogen Receptor	Increase estrogen and its effects	NCT04359329	Stony Brook University Hospital, NY, USA
ER modulator	Estrogen Receptor	Decreases estrogen production Increases testosterone production	NCT04389580*	Kafrelsheikh University Egypt
P4 hormone	Progesterone Receptor	Increase progesterone and its effects	NCT04365127	Cedars Sinai Medical Center, CA, USA
E2/P4 combined	Estrogen receptor and progesterone receptor		NCT04539626	Mexico
Anti-Androgens	Androgen Receptor	Decrease androgens/androgen signaling	NCT04374279 NCT04475601 NCT04509999 NCT04446429	Johns Hopkins, MD, USA Sweden USA Brazil
LHRH antagonist	GnRH	Decrease androgens	NCT04397718	Los Angeles, Brooklyn, Manhattan, Seattle, USA
TMPRSS2 inhibitor	TMPRSS2	Decrease TMPRSS2 action	NCT04353284 NCT04338906* NCT04374019 NCT04321096 NCT04355052* NCT04352400 NCT04355026 NCT04273763* NCT04340349*	Yale, USA Heinrich-Heine University, Germany University of Kentucky, KY, USA University of Aarhus, Denmark Sheba Medical Center, Israel University Hospital Padova, Italy General and Teaching Hospital Celje, Slovenia Wenzhou Medical University, China Instituto Nacional de Rehabilitacion, Mexico
Aldosterone antagonist	Androgen receptor	Decrease androgen signaling	NCT04345887	Istanbul University, Turkey

**Denotes trial in combination with other treatment.*

reactions during infection by decreasing interleukin levels (Wang et al., 2015), as well as aiding nitric oxide signaling which promotes vasodilation (Thibonnier et al., 1999). Hence, oxytocin could also be used as prospective therapy for limiting COVID19 severity (Soumier and Sirigu, 2020).

Vaccines

Passive antibody therapy for COVID19 has already been discussed (Abraham, 2020). Many vaccines are in development, in the hope of providing protection against SARS-CoV-2. From all the above it is clear that sex will be important in the immune response to such vaccines. Women will mount stronger antibody and T-cell responses and suffer worse adverse reactions. Thus, the dosage they may need of any vaccine will be less than for men. Earlier studies of the influenza vaccines have reported that the same magnitude of protective immunity is achieved by half the dose in women compared to men (Klein, 2012). If vaccine against SARS-CoV-2 is in short supply initially, would it be ethical to give smaller shots to women?

CONCLUSION

Our main conclusion is that the sex-based differences in outcomes of COVID19 infection, tentatively reported at the beginning of the pandemic, have been reinforced by all subsequent studies. In addition, our understanding of the possible contributors to this is increasing but it is likely more exciting discoveries remain to be made., especially around the intersection of physiology, immunology and environmental factors.

We note that, generally, more men are enrolled in clinical trials and research in animals is often focused on males to avoid the cyclic fluctuations in hormones. This poses a significant barrier in understanding the sex-based differences in infection severity. The disparity in the effects of COVID19 observed between the sexes, and recent data in other physiological systems and pathologies, highlights the need to include both males and females in future research. There is clearly much more to be understood about sex-based differences. Understanding the mechanisms behind them may help to find appropriate and sex specific therapies for COVID19 and other sexually dimorphic pathologies (Bischof et al., 2020; Bunders and Altfeld, 2020).

AUTHOR NOTES

Biological sex affects areas of physiology and pathophysiology, beyond the obvious. Importantly these sex-based differences impact on both symptoms of disease and effectiveness of medication. In the light of the COVID19 pandemic, an appreciation of how the physiological and immunological differences between female and male responses to viral diseases is crucial, and it is to this that this review contributes. Compared to males, females mount stronger immune responses to combat and clear viral loads, but also have different immunological landscape during infection. Sex-dependent steroid hormones link mechanisms between sex and response to viral infection. For example, estrogens promote the production of anti-inflammatory cytokines, and having two X chromosomes can increase activity of immune genes carried on the chromosome, due to incomplete X-inactivation. The host viral receptor is angiotensin converting enzyme 2 (ACE2). Sex differences in ACE2's expression in lungs and viral handling, are being actively investigated to better understand the underlying mechanisms. These differences have manifested worldwide in fewer deaths and severe COVID19 complication in females compared to males, despite roughly equal infection rates. They have also led to active clinical trials of using sex hormone treatments, such as estradiol patches, to help mitigate the effects of COVID19.

AUTHOR CONTRIBUTIONS

SW conceived the study. SW and SA wrote and edited the article. Both authors contributed to the article and approved the submitted version.

FUNDING

We are grateful to Harris- Wellbeing Centre for Women's Health Research for supporting this work.

REFERENCES

Abate, B. B., Kassie, A. M., Kassaw, M. W., Aragie, T. G., and Masresha, S. A. (2020). Sex difference in coronavirus disease (COVID-19): a systematic review and meta-analysis. *BMJ Open* 10:e040129. doi: 10.1136/bmjopen-2020-040129

Abraham, J. (2020). Passive antibody therapy in COVID-19. *Nat. Rev. Immunol.* 20, 401–403. doi: 10.1038/s41577-020-0365-7

Acosta-Elias, J., and Espinosa-Tanguma, R. (2020). The folate concentration and/or folic acid metabolites in plasma as factor for COVID-19 infection. *Front. Pharmacol.* 11:1062. doi: 10.3389/fphar.2020.01062

Amadori, A., Zamarchi, R., De Silvestro, G., Forza, G., Cavatton, G., Danieli, G. A., et al. (1995). Genetic control of the CD4/CD8 T-cell ratio in humans. *Nat. Med.* 1, 1279–1283. doi: 10.1038/nm1295-1279

Arrowsmith, S. (2020). Oxytocin and vasopressin signalling and myometrial contraction. *Curr. Opin. Physiol.* 13, 62–70. doi: 10.1016/j.cophys.2019.10.006

Asselta, R., Paraboschi, E. M., Mantovani, A., and Duga, S. (2020). ACE2 and TMPRSS2 variants and expression as candidates to sex and country differences in COVID-19 severity in Italy. *Aging* 12, 10087–10098. doi: 10.18632/aging.103415

Bader, M., Alenina, N., Young, D., Santos, R. A. S., and Touyz, R. M. (2018). The meaning of Mas. *Hypertension* 72, 1072–1075. doi: 10.1161/hypertensionaha.118.10918

Baratchian, M., Mcmanus, J. M., Berk, M., Nakamura, F., Mukhopadhyay, S., Xu, W., et al. (2020). Sex, androgens and regulation of pulmonary AR, TMPRSS2 and ACE2. *bioRxiv* [Preprint], doi: 10.1101/2020.04.21.051201

Belouzard, S., Chu, V. C., and Whittaker, G. R. (2009). Activation of the Sars coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *Proc. Natl. Acad. Sci. U.S.A.* 106, 5871–5876. doi: 10.1073/pnas.0809524106

Bischof, E., Wolfe, J., and Klein, S. L. (2020). Clinical trials for COVID-19 should include sex as a variable. *J. Clin. Invest.* 130, 3350–3352. doi: 10.1172/jci139306

Brooks, D. J., and Saad, L. (2020). Double whammy: why the underrepresentation of women among workplace and political decision makers matters in pandemic times. *Polit. Gend.* 16, 1–13. doi: 10.2307/j.ctv301fkq.6

Bunders, M. J., and Altfeld, M. (2020). Implications of sex differences in immunity for SARS-CoV-2 pathogenesis and design of therapeutic interventions. *Immunity* 53, 487–495. doi: 10.1016/j.immuni.2020.08.003

Chakravarty, D., Nair, S. S., Hammouda, N., Ratnani, P., Gharib, Y., Wagaskar, V., et al. (2020). Sex differences in SARS-CoV-2 infection rates and the potential link to prostate cancer. *Commun. Biol.* 3:374.

Chanana, N., Palmo, T., Sharma, K., Kumar, R., Graham, B. B., and Pasha, Q. (2020). Sex-derived attributes contributing to SARS-CoV-2 mortality. *Am. J. Physiol. Endocrinol. Metab.* 319, E562–E567.

Channappanavar, R., Fett, C., Mack, M., Ten Eyck, P. P., Meyerholz, D. K., and Perlman, S. (2017). Sex-based differences in susceptibility to severe acute respiratory syndrome Coronavirus Infection. *J. Immunol.* 198, 4046–4053. doi: 10.4049/jimmunol.1601896

Chen, H., Guo, J., Wang, C., Luo, F., Yu, X., Zhang, W., et al. (2020). Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *Lancet* 395, 809–815. doi: 10.1016/s0140-6736(20)30360-3

Chen, L., Li, Q., Zheng, D., Jiang, H., Wei, Y., Zou, L., et al. (2020). Clinical characteristics of pregnant women with COVID-19 in Wuhan, China. *N. Engl. J. Med.* 382:e100. doi: 10.1056/nejmc2009226

Collin, J., Byström, E., Carnahan, A., and Ahrne, M. (2020). Public health agency of Sweden's brief report: pregnant and postpartum women with severe acute respiratory syndrome coronavirus 2 infection in intensive care in Sweden. *Acta Obstet. Gynecol. Scand.* 99, 819–822. doi: 10.1111/aogs.13901

Costeira, R., Lee, K. A., Murray, B., Christiansen, C., Castillo-Fernandez, J., Ni Lochlainn, M., et al. (2020). Estrogen and COVID-19 symptoms: associations in women from the COVID symptom study. *medRxiv* [Preprint], doi: 10.1101/2020.07.30.20164921v3

Coutard, B., Valle, C., De Lamballerie, X., Canard, B., Seidah, N. G., and Decroly, E. (2020). The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Res.* 176:104742. doi: 10.1016/j.antiviral.2020.104742

Dalpiaz, P. L., Lamas, A. Z., Caliman, I. F., Ribeiro, R. F. Jr., and Abreu, G. R. (2015). Sex hormones promote opposite effects on ACE and ACE2 activity, hypertrophy and cardiac contractility in spontaneously hypertensive rats. *PLoS One* 10:e0127515. doi: 10.1371/journal.pone.0127515

Ding, T., Zhang, J., Wang, T., Cui, P., Chen, Z., Jiang, J., et al. (2020). Potential influence of menstrual status and sex hormones on female SARS-CoV-2 infection: a cross-sectional study from multicentre in Wuhan, China. *Clin. Infect. Dis.* 2020:ciaa1022.

Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., et al. (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ. Res.* 87, E1–E9.

Dubey, P., Reddy, S. Y., Manuel, S., and Dwivedi, A. K. (2020). Maternal and neonatal characteristics and outcomes among COVID-19 infected women: an updated systematic review and meta-analysis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 252, 490–501. doi: 10.1016/j.ejogrb.2020.07.034

Foo, Y. Z., Nakagawa, S., Rhodes, G., and Simmons, L. W. (2017). The effects of sex hormones on immune function: a meta-analysis. *Biol. Rev. Camb. Philos. Soc.* 92, 551–571. doi: 10.1111/brv.12243

Forsyth, K. S., and Anguera, M. C. (2021). Time to get ill: the intersection of viral infections, sex, and the X chromosome. *Curr. Opin. Physiol.* 19, 62–72. doi: 10.1016/j.cophys.2020.09.015

Furuhashi, M., Moniwa, N., Takizawa, H., Ura, N., and Shimamoto, K. (2020). Potential differential effects of renin-angiotensin system inhibitors on SARS-CoV-2 infection and lung injury in COVID-19. *Hypertens. Res.* 43, 837–840. doi: 10.1038/s41440-020-0478-1

Gadi, N., Wu, S. C., Spihlman, A. P., and Moulton, V. R. (2020). What's sex got to do with COVID-19? gender-based differences in the host immune response to Coronaviruses. *Front. Immunol.* 11:2147. doi: 10.3389/fimmu.2020.02147

Gargaglioni, L. H., and Marques, D. A. (2020). Let's talk about sex in the context of COVID-19. *J. Appl. Physiol.* 128, 1533–1538. doi: 10.1152/japplphysiol.00335.2020

Gomez, C. R., Nomellini, V., and Kovacs, E. J. (2019). "Sex hormones and immunosenescence," in *Handbook of Immunosenescence: Basic Understanding and Clinical Implications*, eds T. Fulop, C. Franceschi, K. Hirokawa, and G. Pawelec (Cham: Springer International Publishing).

Gordon, D. E., Jang, G. M., Bouhaddou, M., Xu, J., Obernier, K., White, K. M., et al. (2020). A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* 583, 459–468.

Goren, A., Vaño-Galván, S., Wambier, C. G., Mccoy, J., Gomez-Zubiaur, A., Moreno-Arrones, O. M., et al. (2020). A preliminary observation: male pattern hair loss among hospitalized COVID-19 patients in Spain - A potential clue to the role of androgens in COVID-19 severity. *J. Cosmet. Dermatol.* 19, 1545–1547. doi: 10.1111/jocd.13443

Gupte, M., Thatcher, S. E., Boustany-Kari, C. M., Shoemaker, R., Yiannikouris, F., Zhang, X., et al. (2012). Angiotensin converting enzyme 2 contributes to sex differences in the development of obesity hypertension in C57BL/6 mice. *Arterioscler. Thromb. Vasc. Biol.* 32, 1392–1399. doi: 10.1161/atvbaha.112.248559

Hall, O. J., Limjunyawong, N., Vermillion, M. S., Robinson, D. P., Wohlgemuth, N., Pekosz, A., et al. (2016). Progesterone-based therapy protects against influenza by promoting lung repair and recovery in females. *PloS Pathog.* 12:e1005840. doi: 10.1371/journal.pone.01005840

Hamming, I., Cooper, M. E., Haagmans, B. L., Hooper, N. M., Korstanje, R., Osterhaus, A. D., et al. (2007). The emerging role of ACE2 in physiology and disease. *J. Pathol.* 212, 1–11. doi: 10.1002/path.2162

Hamming, I., Timens, W., Bulthuis, M. L., Lely, A. T., Navis, G., and Van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for Sars coronavirus. A first step in understanding Sars pathogenesis. *J. Pathol.* 203, 631–637. doi: 10.1002/path.1570

Heurich, A., Hofmann-Winkler, H., Gierer, S., Liepold, T., Jahn, O., and Pöhlmann, S. (2014). TMPRSS2 and Adam17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. *J. Virol.* 88, 1293–1307. doi: 10.1128/jvi.02202-13

Hillmer, A. M., Hanneken, S., Ritzmann, S., Becker, T., Freudenberg, J., Brockschmidt, F. F., et al. (2005). Genetic variation in the human androgen receptor gene is the major determinant of common early-onset androgenetic alopecia. *Am. J. Hum. Genet.* 77, 140–148. doi: 10.1086/431425

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271–280.e8.

Hsu, M. F., Kuo, C. J., Chang, K. T., Chang, H. C., Chou, C. C., Ko, T. P., et al. (2005). Mechanism of the maturation process of SARS-CoV 3CL protease. *J. Biol. Chem.* 280, 31257–31266. doi: 10.1074/jbc.m502577200

Jakovac, H. (2020). Sex differences in COVID-19 course and outcome: progesterone should not be neglected. *J. Appl. Physiol.* 129, 1007–1008. doi: 10.1152/japplphysiol.00740.2020

Jin, J. M., Bai, P., He, W., Wu, F., Liu, X. F., Han, D. M., et al. (2020). Gender differences in patients with COVID-19: focus on severity and mortality. *Front. Public Health* 8:152. doi: 10.3389/fpubh.2020.00152

Klein, S. L. (2012). Sex influences immune responses to viruses, and efficacy of prophylaxis and treatments for viral diseases. *Bioessays* 34, 1050–1059. doi: 10.1002/bies.201200099

Klein, S. L., and Flanagan, K. L. (2016). Sex differences in immune responses. *Nat. Rev. Immunol.* 16, 626–638. doi: 10.1038/nri.2016.90

Klein, S. L., Jedlicka, A., and Pekosz, A. (2010). The Xs and Y of immune responses to viral vaccines. *Lancet Infect. Dis.* 10, 338–349. doi: 10.1016/s1473-3099(10)70049-9

Klein, S. L., Pekosz, A., Park, H. S., Ursin, R. L., Shapiro, J. R., Benner, S. E., et al. (2020). Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population. *J. Clin. Invest.* 130, 6141–6150. doi: 10.1172/jci142004

Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., et al. (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in Sars coronavirus-induced lung injury. *Nat. Med.* 11, 875–879. doi: 10.1038/nm1267

Lazzerini, P. E., Boutjdir, M., and Capecchi, P. L. (2020a). COVID-19, arrhythmic risk, and inflammation: mind the Gap! *Circulation* 142, 7–9. doi: 10.1161/circulationaha.120.047293

Lazzerini, P. E., Laghi-Pasini, F., Acampa, M., Boutjdir, M., and Leopoldo Capecchi, P. (2020b). IL-6 (Interleukin 6) blockade and heart rate corrected QT interval prolongation in COVID-19. *Circ. Arrhythm Electrophysiol.* 13:e008791.

Levy, A., Yagil, Y., Bursztyn, M., Barkalifa, R., Scharf, S., and Yagil, C. (2008). ACE2 expression and activity are enhanced during pregnancy. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295, R1953–R1961.

Li, W., Moore, M. J., Vasilieva, N., Sui, J., Wong, S. K., Berne, M. A., et al. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the Sars coronavirus. *Nature* 426, 450–454.

Lu, L., Zhong, W., Bian, Z., Li, Z., Zhang, K., Liang, B., et al. (2020). A comparison of mortality-related risk factors of COVID-19, SARS, and MERS: a systematic review and meta-analysis. *J. Infect.* 81, e18–e25.

Mauvais-Jarvis, F., Klein, S. L., and Levin, E. R. (2020). Estradiol, Progesterone, Immunomodulation, and COVID-19 Outcomes. *Endocrinology* 161:bqaa127.

Melo Junior, A. F., Dalpiaz, P. L. M., Escouto, L. D. S., Sousa, G. J., Aires, R., Oliveira, N. D., et al. (2020). Involvement of sex hormones, oxidative stress, ACE and ACE2 activity in the impairment of renal function and remodelling in SHR. *Life Sci.* 257:118138. doi: 10.1016/j.lfs.2020.118138

Pagano, M. T., Peruzzu, D., Ruggieri, A., Ortona, E., and Gagliardi, M. C. (2020). Vitamin D and Sex Differences in COVID-19. *Front. Endocrinol.* 11:567824. doi: 10.3389/fendo.2020.567824

Pettirosso, E., Giles, M., Cole, S., and Rees, M. (2020). COVID-19 and pregnancy: a review of clinical characteristics, obstetric outcomes and vertical transmission. *Aust. N. Z. J. Obstet. Gynaecol.* 60, 640–659. doi: 10.1111/ajo.13204

Pontecorvi, G., Bellenghi, M., Ortona, E., and Carè, A. (2020). microRNAs as new possible actors in gender disparities of COVID-19 pandemic. *Acta Physiol.* 230:e13538.

Povlsen, A. L., Grimm, D., Wehland, M., Infanger, M., and Krüger, M. (2020). The vasoactive Mas receptor in essential hypertension. *J. Clin. Med.* 9:267. doi: 10.3390/jcm9010267

Pradhan, A., and Olsson, P. E. (2020). Sex differences in severity and mortality from COVID-19: are males more vulnerable? *Biol. Sex Differ.* 11:53.

Racicot, K., Kwon, J. Y., Aldo, P., Silasi, M., and Mor, G. (2014). Understanding the complexity of the immune system during pregnancy. *Am. J. Reprod. Immunol.* 72, 107–116. doi: 10.1111/aji.12289

Raparelli, V., Palmieri, L., Canevelli, M., Pricci, F., Unim, B., Lo Noce, C., et al. (2020). Sex differences in clinical phenotype and transitions of care among individuals dying of COVID-19 in Italy. *Biol. Sex Differ.* 11, 57.

Reckelhoff, J. F. (2018). Sex differences in regulation of blood pressure. *Adv. Exp. Med. Biol.* 1065, 139–151. doi: 10.1007/978-3-319-77932-4_9

Reynolds, H. R., Adhikari, S., Pulgarin, C., Troxel, A. B., Iturrate, E., Johnson, S. B., et al. (2020). Renin-angiotensin-aldosterone system inhibitors and risk of COVID-19. *N. Engl. J. Med.* 382, 2441–2448.

Robinson, D. P., and Klein, S. L. (2012). Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. *Horm. Behav.* 62, 263–271. doi: 10.1016/j.yhbeh.2012.02.023

Robinson, D. P., Lorenzo, M. E., Jian, W., and Klein, S. L. (2011). Elevated 17β-estradiol protects females from influenza A virus pathogenesis by suppressing inflammatory responses. *PLoS Pathog.* 7:e1002149. doi: 10.1371/journal.pone.1002149

Salah, H. M., and Mehta, J. L. (2020). Hypothesis: sex-related differences in ACE2 activity may contribute to higher mortality in men versus women with COVID-19. *J. Cardiovasc. Pharmacol. Ther.* 2020:1074248420967792.

Samavati, L., and Uhal, B. D. (2020). ACE2, much more than just a receptor for SARS-COV-2. *Front. Cell Infect. Microbiol.* 10:317. doi: 10.3389/fcimb.2020.00317

Schurz, H., Salie, M., Tromp, G., Hoal, E. G., Kinnear, C. J., and Möller, M. (2019). The X chromosome and sex-specific effects in infectious disease susceptibility. *Hum. Genom.* 13:2.

Scully, E. P., Haverfield, J., Ursin, R. L., Tannenbaum, C., and Klein, S. L. (2020). Considering how biological sex impacts immune responses and COVID-19 outcomes. *Nat. Rev. Immunol.* 20, 442–447. doi: 10.1038/s41577-020-0348-8

Serseg, T., Benarous, K., and Yousfi, M. (2020). Hispidin and Lepidine E: two natural compounds and folic acid as potential inhibitors of 2019-novel coronavirus Main Protease (2019-nCovmpro), molecular docking and SAR study. *Curr. Comput. Aided Drug Des.* doi: 10.2174/1573409916666200422075440

Shang, J., Wan, Y., Luo, C., Ye, G., Geng, Q., Auerbach, A., et al. (2020). Cell entry mechanisms of SARS-CoV-2. *Proc. Natl. Acad. Sci. U.S.A.* 117, 11727–11734.

Siston, A. M., Rasmussen, S. A., Honein, M. A., Fry, A. M., Seib, K., Callaghan, W. M., et al. (2010). Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. *JAMA* 303, 1517–1525. doi: 10.1001/jama.2010.479

Song, H., Seddighzadeh, B., Cooperberg, M. R., and Huang, F. W. (2020). Expression of ACE2, the SARS-CoV-2 receptor, and TMPRSS2 in prostate epithelial cells. *Eur. Urol.* 78, 296–298. doi: 10.1016/j.eururo.2020.04.065

Soumier, A., and Sirigu, A. (2020). Oxytocin as a potential defence against COVID-19? *Med. Hypothes.* 140:109785. doi: 10.1016/j.mehy.2020.109785

Souyris, M., Cenac, C., Azar, P., Daviaud, D., Canivet, A., Grunenwald, S., et al. (2018). TLR7 escapes X chromosome inactivation in immune cells. *Sci. Immunol.* 3:eaa8855.

Speyer, C. L., Rancilio, N. J., Mcclintock, S. D., Crawford, J. D., Gao, H., Sarma, J. V., et al. (2005). Regulatory effects of estrogen on acute lung inflammation in mice. *Am. J. Physiol. Cell Physiol.* 288, C881–C890.

Stelzig, K. E., Canepa-Escaro, F., Schiliro, M., Berdnikovs, S., Prakash, Y. S., and Chiarella, S. E. (2020). Estrogen regulates the expression of SARS-CoV-2 receptor ACE2 in differentiated airway epithelial cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* 318, L1280–L1281.

Sullivan, J. C. (2008). Sex and the renin-angiotensin system: inequality between the sexes in response to RAS stimulation and inhibition. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R1220–R1226.

Tai, W., He, L., Zhang, X., Pu, J., Voronin, D., Jiang, S., et al. (2020). Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. *Cell Mol. Immunol.* 17, 613–620. doi: 10.1038/s41423-020-0400-4

Takahashi, T., Ellingson, M. K., Wong, P., Israelow, B., Lucas, C., Klein, J., et al. (2020). Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature* 588, 315–320.

Tang, Y., Liu, J., Zhang, D., Xu, Z., Ji, J., and Wen, C. (2020). Cytokine storm in COVID-19: the current evidence and treatment strategies. *Front. Immunol.* 11:1708. doi: 10.3389/fimmu.2020.01708

Thibonnier, M., Conarty, D. M., Preston, J. A., Plesnicher, C. L., Dweik, R. A., and Erzurum, S. C. (1999). Human vascular endothelial cells express oxytocin receptors. *Endocrinology* 140, 1301–1309. doi: 10.1210/endo.140.3.6546

Tipnis, S. R., Hooper, N. M., Hyde, R., Karran, E., Christie, G., and Turner, A. J. (2000). A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* 275, 33238–33243.

Turner, A. J. (2015). ACE2 cell biology, regulation, and physiological functions. *Protect. Arm Renin Angioten. Syst.* 2015, 185–189. doi: 10.1016/b978-0-12-801364-9.00025-0

Ursin, R. L., Shapiro, J. R., and Klein, S. L. (2020). Sex-biased immune responses following SARS-CoV-2 Infection. *Trends Microbiol.* 28, 952–954. doi: 10.1016/j.tim.2020.10.002

Verdecchia, P., Cavallini, C., Spanevello, A., and Angeli, F. (2020). The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur. J. Intern. Med.* 76, 14–20. doi: 10.1016/j.ejim.2020.04.037

Wang, J., Syrett, C. M., Kramer, M. C., Basu, A., Atchison, M. L., and Anguera, M. C. (2016). Unusual maintenance of X chromosome inactivation predisposes female lymphocytes for increased expression from the inactive X. *Proc. Natl. Acad. Sci. U.S.A.* 113, E2029–E2038.

Wang, P., Yang, H. P., Tian, S., Wang, L., Wang, S. C., Zhang, F., et al. (2015). Oxytocin-secreting system: A major part of the neuroendocrine center regulating immunologic activity. *J. Neuroimmunol.* 289, 152–161. doi: 10.1016/j.jneuroim.2015.11.001

White, M. C., Fleeman, R., and Arnold, A. C. (2019). Sex differences in the metabolic effects of the renin-angiotensin system. *Biol. Sex Differ.* 10:31.

Williamson, E. J., Walker, A. J., Bhaskaran, K., Bacon, S., Bates, C., Morton, C. E., et al. (2020). Factors associated with COVID-19-related death using Opensafely. *Nature* 584, 430–436.

Wong, S. F., Chow, K. M., Leung, T. N., Ng, W. F., Ng, T. K., Shek, C. C., et al. (2004). Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. *Am. J. Obstet. Gynecol.* 191, 292–297.

Xie, X., Chen, J., Wang, X., Zhang, F., and Liu, Y. (2006). Age- and gender-related difference of ACE2 expression in rat lung. *Life Sci.* 78, 2166–2171. doi: 10.1016/j.lfs.2005.09.038

Young, M. J., Clyne, C. D., and Chapman, K. E. (2020). Endocrine aspects of ACE2 regulation: Raas, steroid hormones and SARS-CoV-2. *J. Endocrinol.* 247, R45–R62.

Zou, X., Chen, K., Zou, J., Han, P., Hao, J., and Han, Z. (2020). Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front. Med.* 14, 185–192. doi: 10.1007/s11684-020-0754-0

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

HYPOTHESIS AND THEORY
published: 11 February 2021
doi: 10.3389/fphys.2020.624625

Is There an Effect of Fetal Mesenchymal Stem Cells in the Mother–Fetus Dyad in COVID-19 Pregnancies and Vertical Transmission?

Athina Samara[1,2]* *and Eric Herlenius*[1,2†]

[1] Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden, [2] Astrid Lindgren Children's Hospital Karolinska University Hospital, Stockholm, Sweden

OPEN ACCESS

Edited by:
Adriana Castello Costa Girardi,
University of São Paulo, Brazil

Reviewed by:
David Zurakowski,
Harvard Medical School,
United States
Venkata Garikipati,
The Ohio State University,
United States

***Correspondence:**
Athina Samara
athina.samara@ki.se

†ORCID:
Eric Herlenius
orcid.org/0000-0002-6859-0620

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: 31 October 2020
Accepted: 30 December 2020
Published: 11 February 2021

Citation:
Samara A and Herlenius E (2021) Is There an Effect of Fetal Mesenchymal Stem Cells in the Mother–Fetus Dyad in COVID-19 Pregnancies and Vertical Transmission? Front. Physiol. 11:624625. doi: 10.3389/fphys.2020.624625

Because of the polysystemic nature of coronavirus disease 2019 (COVID-19), during the present pandemic, there have been serious concerns regarding pregnancy, vertical transmission, and intrapartum risk. The majority of pregnant patients with COVID-19 infection present with mild or asymptomatic course of the disease. Some cases were hospitalized, and few needed intensive care unit admission, or mechanical ventilation. There have also been scarce case reports where neonates required mechanical ventilation post COVID-19 pregnancies. Without approved therapies other than dexamethasone, advanced mesenchymal cell therapy is one immunomodulatory therapeutic approach that is currently explored and might hold great promise. We suggest that the circulating fetal stem cells might have an immune-protective effect to mothers and contribute to the often mild and even asymptomatic post-COVID-19 pregnancies. Thus, COVID-19 pregnancies come forth as a paradigm to be further and more comprehensively approached, to understand both the mechanism and action of circulating stem cells in immunoprotection and hypoxia in microcirculation.

Keywords: COVID-19, SARS-CoV-2, neonate, pregnancy, vertical, mesenchymal, MSCs

INTRODUCTION

Maternal–fetal transmission of viral diseases may occur transvaginally or through the hematogenic, i.e., the transplacental transmission pathway. In the latter, the virus circulating in the maternal blood vessels may reach and enter the placenta across chorionic villous and non-villous structures fetal blood vessels and be transmitted to the fetus. This mechanism of vertical transmission was not reported after the infection of pregnant women with the coronaviruses, severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) and Middle East respiratory syndrome coronavirus (MERS-CoV). Despite the fact that pregnant women may be infected by these coronaviruses other

than severe pneumonia, they may face complications such as early pregnancy loss or even death (Schwartz and Graham, 2020).

The uterine enlargement during pregnancy is known to bring major changes in maternal physiology. These can be both mechanical, such as reduced functional residual volumes and diaphragm elevation, and cellular, such as altered cellular immunity. The maternal immune system may tolerate fetal antigens suppressing cell-mediated immunity while retaining normal humoral immunity, changes known to occur locally at the maternal–fetal interface, which could affect systemic immune responses to infection (Jamieson et al., 2006). This may render pregnant women more vulnerable to viral infections, especially in cases where the infections may have an effect on the cardiorespiratory system during pregnancy and could enhance progression to respiratory failure (Dashraath et al., 2020). The ability to increase ventilation is reduced, when pregnant. Therefore, there is an increased risk of inadequate response to environmental stressors such as upper/lower respiratory tract infections and hypoxic and hypercapnic respiratory failure (Lapinsky et al., 2014). During the SARS epidemic, up to 35% of the infected pregnant patients required mechanical ventilation, and mortality rates reached 18%; in the case of MERS, the numbers reached 41 and 25%, respectively (Dashraath et al., 2020; Liu et al., 2020; Schwartz and Graham, 2020).

SARS–CORONAVIRUS 2 IN PREGNANCY

Coronavirus disease 2019 (COVID-19) is a polysystemic disease, aggravated by compromised immune response, increased body mass index, and other comorbidities. Thus, there have been serious concerns regarding the pregnancy and the vertical transmission and intrapartum risk (Ferrazzi et al., 2020; Gupta et al., 2020).

The majority of pregnant patients with COVID-19 infection present with a mild or asymptomatic course of the disease (Ferrazzi et al., 2020). Some cases were hospitalized, and few needed intensive care unit admission or mechanical ventilation; some patients received oxygen support, whereas others were treated with antibiotics, antivirals, systemic corticosteroids, and other treatment combinations (Breslin et al., 2020; Chen H. et al., 2020; Dashraath et al., 2020; Friedman et al., 2020; Hecht et al., 2020; Iqbal et al., 2020; Liu et al., 2020; Oncel et al., 2020; Savasi et al., 2020; Takemoto et al., 2020), while several clinical trials are ongoing to evaluate the choice of treatment in pregnant or breastfeeding COVID-19 patients (Dashraath et al., 2020; Pastick et al., 2020). There have also been some case reports where neonates required mechanical ventilation post-COVID-19 pregnancies (Alwardi et al., 2020; Amaral et al., 2020; Gale et al., 2020; Gregorio-Hernández et al., 2020; Kirtsman et al., 2020; Oncel et al., 2020; Savasi et al., 2020). The studies that concern maternal–fetal transmission of virus have some limitations. First, analyses of SARS coronavirus 2 (SARS-CoV-2) in newborns are often delayed; thus, there is an increased risk of extrauterine transmission. Second, the majority of the present case reports include the analysis of late vaginal smears (Zeng L. et al., 2020), instead of the sampling of amniotic fluid. The sampling of amniotic fluid could have provided an indication that vertical transmission might have occurred. A few studies investigated SARS-CoV-2-positive placental sampling to document direct viral involvement or vertical transmission (Alamar et al., 2020; Schwartz and Morotti, 2020; Smithgall et al., 2020; Taglauer et al., 2020). Although there have been case reports of histomorphologic evidence of maternal/fetal vascular malperfusions (Smithgall et al., 2020), there is still no concrete proof that SARS-CoV-2 placental invasion may lead to fetal pathology.

Different studies have shown that prevalence of COVID-19 test positivity among small studies of neonates vary, from less than 1% and up to 5% (Ashraf et al., 2020; Dumitriu et al., 2020; Khoury et al., 2020; Remaeus et al., 2020; Verma et al., 2020; Walker et al., 2020). Meanwhile, several study groups are actively investigating the subject of SARS-CoV-2 vertical transmission of infection, focusing on sample collection as early as possible and ideally within the first 12 h after birth. One of them is the periCOVID (COVID-19 Clinical Research Coalition, 2021) study, set up in the United Kingdom under the umbrella of Public Health England, also recruiting in Africa. The primary objective of the periCOVID surveillance study is to assess the risk of COVID-19 vertical transmission and identify and determine the routes, collecting samples from breast milk, placenta, and cord blood at birth for reverse transcriptase–polymerase chain reaction (RT-PCR) and sequencing followed by sequential sampling of maternal and neonatal urine and feces and blood samples for serology.

Some case reports and small studies have indicated that maternal–fetal transmission might occur (**Table 1**). They report different combinations of positive RT-PCR testing of neonatal nasopharyngeal swabs at birth followed with negative serology and later seroconversion of the mother (Alzamora et al., 2020), negative RT-PCR results of nasopharyngeal swab (Dong et al., 2020; He et al., 2020; Zeng H. et al., 2020) and positive neonatal blood serology immediately after birth, and several other combinations of COVID-19 diagnostic results. Unfortunately, neither amniotic fluid and placenta nor cord blood was tested in most cases. Immunoglobulin M (IgM) against SARS-CoV-2 proteins has been detected in some newborn case reports. Thus, as IgM does not cross the placental barrier, it raises the possibility of vertical transmission of the virus leading to IgM production by the fetus. However, this is not conclusive evidence and may also be due to placental alterations allowing the passage of IgM, or false-positive testing.

As expected, SARS-CoV-2 RT-PCR-positive diagnosed mothers with IgG antibodies against SARS-CoV-2 transfer these to the fetus (Gao J. et al., 2020). However, there have also been some reports where IgM were detected in the fetus. These include case reports and small studies from China [2 preterm (Dong et al., 2020; Wu et al., 2020d), 1 term (Zhou et al., 2020), 2 term, 10 term (Gao J. et al., 2020), 2 preterm-1 term (He et al., 2020)], the United States [one term (Edlow et al., 2020)], Italy [one preterm (Fenizia et al., 2020), one term (Cavaliere et al., 2020)], and Sweden (three term Herlenius et al. at Karolinska University Hospital, Stockholm, in progress)

TABLE 1 | List of publications providing information on laboratory tests of mothers and neonates who tested positive with COVID-19.

Author and publication date	Pregnant women (*N*)	Neonates (*N*)	Mother								Neonate												Pregnancy			
			Naso pharyngeal swab		Serum		Breast milk		Vaginal swab		Nasopharyngeal swab or Deep Trachea		Blood Sample		Gastric Juice		Anal swab/Stool		Urine Sample		Umbilical Cord blood		Amniotic fluid		Placental Tissue	
			+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Wang et al., 2020	1	1	1					1			1											1				1
Dong et al., 2020	1	1	1		1			1		1		1	1													
Zeng L. et al., 2020	6	6			5							6	5													
Nie et al., 2020	27	28	27								1	25										1				1
Carosso et al., 2020	1	1	1		1					1		1	1				1				1					1
Zamaniyan et al., 2020	1	1	1							1	1											1	1			
Baud et al., 2020	1	1	1									1		1											1	
Buonsenso et al., 2020	2	2	2									2	1	1							1	1			1	1
Hosier et al., 2020	1	1	1		1																1				1	
Govind et al., 2020	9	9	9								1	8														
Piersigilli et al., 2020	1	1	1								1															
Penfield et al., 2020	32	32	32									11													3	8
Patanè et al., 2020	2	2	2								2														2	
Costa et al., 2020	1	1	1				1														2				2	
Schoenmakers et al., 2020	1	1	1					1				1													1	
Kirtsman et al., 2020	1	1	1				1		1		1		1												1	
McDevitt et al., 2020	8	8	1	7							8															
Ferraiolo et al., 2020	1	1	1		1							1													1	
Hu et al., 2020	7	7	7								1	6		7				7		7						
Gao J. et al., 2020	64	64	24									24	11								1					
Nayak et al., 2020	141	131	141								3	128														
Pulinx et al., 2020	1	2	1		1																		2		2	

(Continued)

TABLE 1 | Continued

Author and publication date	Pregnant women (*N*)	Neonates (*N*)	Mother								Neonate										Pregnancy			
			Naso pharyngeal swab		Serum		Breast milk		Vaginal swab		Nasopharyngeal swab or Deep Trachea		Blood Sample		Gastric Juice		Anal swab/Stool		Urine Sample		Umbilical Cord blood		Amniotic fluid		Placental Tissue	
			+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Vivanti et al., 2020	1	1	1		1				1		1		1				1						1		1	
Rivera-Hernandez et al., 2020	1	1	1								1															
Peng et al., 2020	1	1		1	1							1	1													
Richtmann et al., 2020	5	5	5																				2		2	3
Farsi et al., 2020	1	3	1								1	2													1	
Sisman et al., 2020	1	1	1								1														1	
Khoury et al., 2020	241	236	241								6	230														
Hecht et al., 2020	19	19	19								1														2	
Savasi et al., 2020	77	57									4															
Lorenz et al., 2020	1	1	1								1						1									
Sun et al., 2020	3	3	3								3															
Algarroba et al., 2020	1	1										1													1	
Kulkarni et al., 2020	1	1		1	1						1										1				1	
Oncel et al., 2020	125	125						6			4	116		3				2						4		5
Toner et al., 2020	1	1	1		1							1		1							1			1		
Taglauer et al., 2020	15	15									12														15	
Hinojosa-Velasco et al., 2020	1	1	1				1				1															
Vendola et al., 2020	2	2	1	1	2							2	2								2		1			
Mongula et al., 2020	1	1	1					1				1													1	
Vashukova et al., 2020	6	6	6									6													1	5
Facchetti et al., 2020	1	1									1														1	
Anand et al., 2021	69	65									7															

(Continued)

TABLE 1 | Continued

Author and publication date	Pregnant women (*N*)	Neonates (*N*)	Mother								Neonate												Pregnancy			
			Naso pharyngeal swab		Serum		Breast milk		Vaginal swab		Nasopharyngeal swab or Deep Trachea		Blood Sample		Gastric Juice		Anal swab/Stool		Urine Sample		Umbilical Cord blood		Amniotic fluid		Placental Tissue	
			+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Gao W. et al., 2020	1	1	1		1							1	1												1	
Menter et al., 2020	5	5	5									5													2	3
Sagheb et al., 2020	2	2	1								2															
Zhou et al., 2020	16	16	1								1		1													
Cavaliere et al., 2020	1	1	1								1		1													
Fenizia et al., 2020	30	30	30		19		1		1		2										12				2	28
Bachani et al., 2020	348		57								5															
Mattar et al., 2020	16	5	16		5																5					5
Adhikari et al., 2020	252	188	252								6	182														
Hsu et al., 2021	1	1	1									1													1	
Alamar et al., 2020	1	1	1								1														1	
Stonoga et al., 2020	1	1	1								1										1		1		1	
Rodrigues et al., 2021	1	1	1								1															
Parsa et al., 2020	1	1	1								1												1			
Di Mascio et al., 2020	251		251								1															
Sileo et al., 2020	1	1	1									1									1					
Pessoa et al., 2020	1	1									1															
Alwardi et al., 2020	1	3	1								3															
Hcini et al., 2020	507	127	137		46/76						4	108														
He et al., 2020	22	22											11													
Debelenko et al., 2020	75	75	75									75									1				1	
Zaigham et al., 2020	1	1	1		1						1		1												1	
Shende et al., 2020	1	1	1																				1		1	
Bandyopadhyay et al., 2020	1	1	1								1		1													
Edlow et al., 2020	127	1	64								0		1	76							1					88

that also exhibited IgG against SARS-CoV-2 nucleocapsid and S-protein antibodies 0–2 days after birth.

SARS-COV-2 COMPROMISED IMMUNITY

Because of the unprecedented participation of volunteers, supported by huge private and governmental investments, both phase III clinical trials of the vaccines developed by BioNTech/Pfizer and Moderna concluded in November 2020. At the time of writing, Pfizer's vaccine was approved for emergency use in the European Union, United Kingdom, Canada, and the United States, whereas Moderna's vaccine was also approved for use in the United States. However, pregnant women were excluded from the vaccine clinical trials; thus, the vaccines were not authorized for use during pregnancy.

Vaccination plans take into account several factors, among which are age and comorbidities. Not only progression in COVID-19 patients but also percentages of asymptomatic prevalence vary considerably. A proof-of-principle example of this comes from a study from Stockholm, Sweden, where among patients presenting in labor at Karolinska University Hospital from March 25 to July 24, 2020, 65% of those diagnosed as RT-PCR-positive were asymptomatic (Ahlberg et al., 2020); a similar small study between February and March 2020 in Stockholm showed that 31% of the children reporting no symptoms were seropositive (Herlenius et al., 2020).

However, the highest COVID-19-related morbidity is observed in the group of old-age patients with co-morbidities. Because of their age and medical history, both the immune and the tissue regenerative capacity of these patients are compromised, resulting in this subgroup being affected the most by coronavirus.

Old-aged and other critically ill patients suffer the effects of the aberrant systemic inflammatory response known as the cytokine release syndrome, or the infamous "COVID-19 cytokine storm." This cytokine storm clinically presents with a sharp rise of cytokines within a short time and may, among others, cause lung injury, which in turn may progress into acute lung injury or its more severe form, acute respiratory distress syndrome (ARDS). The exact pathology and the mechanism of lung injury proceeding to respiratory failure and ARDS in COVID-19 patients are not yet fully understood; yet, it leads to low oxygen saturation levels. Overproduction of proinflammatory cytokines is one of the parameters and a major cause of mortality in COVID-19 (Chen N. et al., 2020; Huang et al., 2020; Lai et al., 2020).

Coronavirus disease 2019 disease progression presents with distinct symptoms, suggesting diversified host immune responses, with one of the villains being the dysfunctional interferon system. A recent study showed that excessive inflammatory response in severe and critical patients was associated with persistent viremia associated with the expression of the nuclear transcription factor nuclear factor-κB; patients presented with type I interferon deficiency, increased production, and altered signaling of tumor necrosis factor-α and interleukin 6 (IL-6; Hadjadj et al., 2020). Such data suggest that combined therapeutic approaches, which could alleviate severe COVID-19 and hasten recovery of the patients who are critically ill, are vital.

One of the main challenges when treating COVID-19 is that the documented exacerbated anti-SARS-CoV-2 immune response can cause severe disease if it remains uncontrolled. The cytokine storm is this inflammatory cascade associated with this exaggerated response of innate immunity, which might cause a late or ineffective response of adaptive immunity (Ragab et al., 2020). It seems to lead to dysfunction of the microcirculation that becomes the villain of SARS-CoV-2 infection (Colantuoni et al., 2020). Thus, a number of therapies aim to target the immune system, but dexamethasone is so far the only drug that can ameliorate disease outcome also in intubated patients (RECOVERY Collaborative Group et al., 2020). Dexamethasone has a long half-life, acts on glucocorticoid receptors, and reduces inflammation through a broad-pathway approach that has been associated, among others, with immunosuppression, hospital-acquired infections, and neuromuscular weakness, even with short courses. Monoclonal antibody therapies of Eli Lilly and Regeneron have also been granted emergency use approval in the United States, for use in mild and moderate cases. IL-6 antagonists have not yet been proven successful (Scherger et al., 2020).

The attenuated deficient immune system is the major risk factor in COVID-19. However, pregnant women also have an attenuated immune system, due to the fetal allograft, but they do not display increased vulnerability under COVID-19. But it might be that there is, currently explored, one immunomodulatory therapeutic approach that might hold great promise toward answering this question. That is advanced mesenchymal cell therapies, i.e., the application of stem cells to treat patients with COVID-19 (Saldanha-Araujo et al., 2020).

MESENCHYMAL STEM CELLS

Exogenous mesenchymal stem cells (MSCs) have been used for decades to treat other diseases caused by viruses. Examples include the acute lung injury caused by influenza virus the immunological compromises caused by the human immunodeficiency virus and the chronic hepatitis caused by hepatitis B virus (Thanunchai et al., 2015). MSCs are a type of highly proliferative adult stem cells with multilineage differentiation capacity. Initially isolated from the bone marrow, they subsequently identified in other tissues, such as the dental pulp, umbilical cord and placenta, adipose tissue and even periosteum and skeletal muscle. They can be found in various autologous and allogenic sources.

Several lines of evidence indicate that one of the main therapeutic mechanisms of MSC administration is via immunomodulation. MSC-mediated immunomodulation operates through a cohort of cell contact-dependent mechanisms including gap junctions and soluble factors (Wu et al., 2017; de Witte et al., 2018; Naji et al., 2019). The main advantage of the use of MSCs for clinical research lies with their

hypoimmunogenicity, and they are thus known as "immune-privileged cells." They do not express human leukocyte antigen (HLA) class II molecules or costimulatory molecules such as CD40, CD40L, CD80, and CD86 and express low levels of HLA class I molecules. These characteristics permits the MSCs to escape the cytotoxic effects of lymphocytic T cells, B cells, and natural killer cells (Rasmusson, 2006; Stagg, 2007; Weiss, 2014; Can and Coskun, 2020; Li et al., 2020). Furthermore, they are able to detect injury signals in their microenvironment and signal regeneration (Stappenbeck and Miyoshi, 2009; Le Blanc and Mougiakakos, 2012; Qin and Zhao, 2020).

Not only MSC-secreted cytokine-mediated effects but also apoptotic, metabolically inactivated, or even fragmented MSCs were shown to exert an immunomodulatory effect, but the roles of regulatory T cells and monocytes in the equation remain under investigation (see review Weiss and Dahlke, 2019). The MSCs' immunomodulatory properties affect proliferation, activation, and function of various immune cells (Harrell et al., 2019) and may thus alter the innate and adoptive immune responses (Li et al., 2016). The underlying cellular and molecular mechanisms of the long-term effects of MSC-mediation are yet not fully clarified, but regenerative and immunomodulatory effects have been observed in various diseases and tissue types (Aguiar et al., 2020; Qin and Zhao, 2020; Sharma et al., 2020; Tao and Chen, 2020). Although studies describe MSCs as short-lived (Eggenhofer et al., 2012), unable to cross the lung capillary network after intravenous infusion (Fischer et al., 2009), there have been preclinical reports that survival of exogenous MSCs could be detected on site up to 4 months after their direct transplantation; however, few of these cells developed the tissue phenotype of the resident cells (Muñoz et al., 2018).

Mesenchymal stem cells may also use a connexin-43-dependent mechanism to aid compromised cells via mitochondrial transfer, increasing their survival (Islam et al., 2012). Older and recent studies have documented that MSC transplantation and transdifferentiation (Dilger et al., 2020) is dependent on gap junctional cell coupling and the intricate interplay and changes in expression levels of connexin family members. Pannexins might also be involved (Swayne et al., 2020) and thus MSCs might modulate the inflammasome via gap junctional dependent mechanisms.

Mesenchymal stem cells can also suppress chronic inflammation and promote tissue regeneration via the secretion of exosomes, which may in turn regulate macrophage polarization (Ti et al., 2015). Their extracellularly secreted vesicles (termed EVs, which includes both exosomes and microvesicles) are exploited as a cell-free therapeutic tool due to their paracrine and/or endocrine effects (Meirelles Lda et al., 2009). Their mode of action involves either binding to extracellular receptors of targeted cells, merging with the membrane and secreting EV contents, or entering the target cell as endocytic vesicles. MSC-derived EV therapies come with further clinical advantages as they pass across small blood capillaries due because of their small size; they have low chances of tumor formation as they are non-proliferative, and they are immune privileged as they are HLA-I and HLA-II negative.

COVID-19 AND MSCs

Because of these immunomodulatory properties of MSCs, 66 stem cell therapy clinical protocols against SARS-CoV-2/COVID-19 have been registered, and six trials have now been completed (details are available at www.clinicaltrials.gov). The use of stem cells as possible therapeutic approaches against COVID-19, including not only MSCs but also MSC-derived exosomes, as therapeutic opportunities for COVID-19 has gained momentum (Jacob et al., 2020; Jayaramayya et al., 2020). As mentioned, most intravenously injected MSCs seem to get trapped in the lung. However, their role in the injured lung may be anti-inflammatory and antiviral and aiding in tissue repair utilizing cell-to-cell contacts and without engraftment into the damaged tissue (Galleu et al., 2017; Armitage et al., 2018), But in addition, and more importantly, *in vivo* MSCs survive in a hypoxic niche where oxygen tensions are oftenless than 10%.

Many of the protocols submitted to treat COVID-19 involve MSCs derived from the umbilical cord and Wharton jelly. It is known that fetal MSCs are more potent in comparison to those derived from adult sources, but there are ethical considerations regarding fetal tissue as a cellular source. However, perinatal tissues are easily accessible for isolation, highly abundant, and not posing any major ethical concerns. More importantly, a number of clinical trials documented no adverse events after the infusion of MSCs; thus, these cells are considered safe for clinical use (Lalu et al., 2012). MSCs from perinatal tissues have stronger immunomodulatory properties than adult bone marrow-derived MSCs (BM-MSCs; Li et al., 2014). Therefore, MSCs isolated from umbilical cord (UC-MSC) and the fetal part of the placenta (PL-MSCs) share many similarities with BM-MSCs. Compared to adult BM-MSCs, UC-MSCs are superior in colony-forming capacity and differentiation potential; PL-MSCs have a lower colony-forming capacity but similar or less differentiation potential (Beeravolu et al., 2017).

DISCUSSION

Mesenchymal stem cell-induced modulation of immune response in COVID-19 patients seems to be a feasible therapeutic treatment. Currently (December 21, 2020), results from seven single case studies and five small studies (7, 9, 12, 16, and 27 patients, respectively) have been published, where MSCs were employed as therapy on COVID-19 patients (Feng et al., 2020; Liang et al., 2020; Meng et al., 2020; Scherger et al., 2020; Sengupta et al., 2020; Shu et al., 2020; Soler Rich et al., 2020; Tao et al., 2020; Yilmaz et al., 2020; Zengin et al., 2020; Zhang et al., 2020). The phase I clinical trial with the 27 patients (Wu et al., 2020c) with COVID-19 followed the first case study (Wu et al., 2020a) of infusion of MSC-like cells [called human embryonic stem cell-derived immunity- and matrix-regulatory cells (Wu et al., 2020b)] that show higher immunomodulatory potential than standard adult MSCs. These results, although from few patients, suggest that intravenous infusion of MSCs in COVID-19 patients does not cause severe side effects. In addition, MSC transplantation or

MSC-derived exosome transplantation is associated with reduced inflammation, also in critically and severely ill COVID-19 patients (n = 70). Interestingly, a case study showed that MSC transplantation, also ameliorated and treated the central nervous system infection, and the authors propose simultaneous systemic and intrathecal administration, as MSCs can thus cross the blood–brain barrier (Yilmaz et al., 2020). Moreover, the outcome after stem cell administration included improvement in lung function. Therefore, transplantation of MSCs seems to attenuate the inflammatory response and possibly promote tissue repair and regeneration, leading to improved outcome of COVID-19 patients (Leng et al., 2020). It seems that due to the MSC-induced immunosuppression, several proinflammatory cytokines and chemokines were reduced in the serum, including IL-6 and C-reactive protein (CRP), while lymphocyte count returned to normal levels faster (Shu et al., 2020). We hypothesize that the anti-inflammatory MSC effect attenuated the recruitment of both macrophages and mononuclear cells to the inflamed lung tissue, further inducing more regulatory dendritic cells; these combined with increased IL-10 and vascular endothelial growth factor might have promoted lung repair. The effect of secreted exosomes has also been documented on antigen presentation functions, differentiation, and maturation of dendritic cells, neutrophils, and other immune cells (for details see recent reviews Abraham and Krasnodembskaya, 2020; Li et al., 2020).

These results suggest that the MSCs may help to reverse the outcome of the COVID-19 cytokine storm. Therefore, we hypothesize that the circulating fetal stem cells might have an immune-protective effect to mothers and contribute to the mild and even asymptomatic COVID-19 pregnancies.

The mechanisms of action, the life span, and the effect of circulating stem cells have been under investigation for decades. Several reports documented the presence of large numbers of fetal cells in healthy or wounded tissue (Bianchi et al., 1996; Khosrotehrani et al., 2004; Nguyen Huu et al., 2007; Zeng et al., 2010). One such study demonstrated that years after the pregnancy,presumably fetal male cells were located in pathological lung and thymus surgical specimens, and the authors speculated they were either recruited from bone marrow or had been proliferated locally (O'Donoghue et al., 2008). Moreover, the results of a gene expression analysis of fetal cells located in the murine maternal lung during pregnancy support this view (Pritchard et al., 2012). These findings back our hypothesis that the presence of placental and fetal MSCs may have an effect on prepartum and post partum maternal health.

Coronavirus disease 2019 pregnancies come forth as a paradigm to be further and more comprehensively investigated. This to understand both the mechanism and action of circulating stem cells in immune modulation and protection as well as their role in hypoxia. Non-invasive methods to monitor human pregnancies and animal models would help answer crucial aspects of both the COVID-19 disease and a possible and feasible therapeutic approaches and non-invasive treatment. When and how early fetal circulating stem cells are recruited to the lungs and what factors signal cell migration toward the maternal circulation might be answered with such studies. Moreover, this type of approaches might reveal the onset of an immunoprotective cell migration as a response to placental vascular malperfusions because of increased uterine and intervillous blood flow. Moreover, the cell migration mechanisms themselves could provide hints on blood–brain approachability for future studies and therapies.

Administration of MSCs in COVID-19 patients seems to be a promising tool in the treatment by reducing hyperinflammation (Gentile et al., 2020). MSCs from COVID-19 patients of different disease severity should be isolated and analyzed. Adipose tissue-derived stem cells (ADSCs), and their exosomes, are considered superior regarding non-invasiveness, accessibility, and abundance when compared to other sources (Jin et al., 2013). They can also be maintained and expanded in culture for long without losing their differentiation capacity, expanded thus to the large quantities needed for cell therapy purposes. ADSCs maintain their differentiation potential to differentiate, and they have low immunogenicity combined with modulatory effects, with less than 1% of them expressing human leukocyte antigen – DR isotype (HLA-DR), rendering them suitable for clinical allogeneic transplantation (Puissant et al., 2005; Strem et al., 2005; Dominici et al., 2006; McIntosh, 2011).

The route of administration and MSC preconditioning with cytokines or hypoxia should be further explored and fine-tuned. Both the COVID-19 pregnancies and the outcome of the clinical trials that investigate the possible therapeutic role of MSC transplantation will provide solid evidence of how MSCs influence the indicators of proinflammatory cytokines and further elucidate the impairment of the interferon pathway in COVID-19 patients.

Thus, studying the effect of maternal and fetal MSCS in COVID-19 pregnancies may act as a proof-of-principle approach, pave the understanding of the role of fetal circulating stem cells in the mother–fetus dyad, and elucidate the use of MSC infusion for therapy beyond COVID-19.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

AS conceptualized the study, wrote first draft, and revised the manuscript. EH revised the manuscript. Both authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Swedish Research Council, the Stockholm County Council (ALF projects 2019-0400 and 2019-0974), the Karolinska Institutet, and VINNOVA, and grants from the Swedish Brain (2019-0087) and Swedish National Heart and Lung (2018-0212) Foundations. The funders of the study played no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

REFERENCES

Abraham, A., and Krasnodembskaya, A. (2020). Mesenchymal stem cell-derived extracellular vesicles for the treatment of acute respiratory distress syndrome. *Stem Cells Transl. Med.* 9, 28–38. doi: 10.1002/sctm.19-0205

Adhikari, E. H., Moreno, W., Zofkie, A. C., MacDonald, L., McIntire, D. D., Collins, R. R. J., et al. (2020). Pregnancy outcomes among women with and without severe acute respiratory syndrome coronavirus 2 infection. *JAMA Netw. Open* 3:e2029256. doi: 10.1001/jamanetworkopen.2020.29256

Aguiar, F. S., Melo, A. S., Araújo, A. M. S., Cardoso, A. P., de Souza, S. A. L., Lopes-Pacheco, M., et al. (2020). Autologous bone marrow-derived mononuclear cell therapy in three patients with severe asthma. *Stem Cell Res. Ther.* 11:167. doi: 10.1186/s13287-020-01675-x

Ahlberg, M., Neovius, M., Saltvedt, S., Söderling, J., Pettersson, K., Brandkvist, C., et al. (2020). Association of SARS-CoV-2 test status and pregnancy outcomes. *JAMA* 324, 1782–1785. doi: 10.1001/jama.2020.19124

Alamar, I., Abu-Arja, M. H., Heyman, T., Roberts, D. J., Desai, N., Narula, P., et al. (2020). A possible case of vertical transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a newborn with positive placental in situ hybridization of SARS-CoV-2 RNA. *J. Pediatric. Infect. Dis. Soc.* 9, 636–639. doi: 10.1093/jpids/piaa109

Algarroba, G. N., Hanna, N. N., Rekawek, P., Vahanian, S. A., Khullar, P., Palaia, T., et al. (2020). Confirmatory evidence of the visualization of severe acute respiratory syndrome coronavirus 2 invading the human placenta using electron microscopy. *Am. J. Obstet. Gynecol.* 223, 953–954. doi: 10.1016/j.ajog.2020.08.106

Alwardi, T. H., Ramdas, V., Al Yahmadi, M., Al Aisari, S., Bhandari, S., Saif Al Hashami, H., et al. (2020). Is vertical transmission of SARS-CoV-2 infection possible in preterm triplet pregnancy? A case series. *Pediatr. Infect. Dis. J.* 39, e456–e458. doi: 10.1097/INF.0000000000002926

Alzamora, M. C., Paredes, T., Caceres, D., Webb, C. M., Valdez, L. M., and La Rosa, M. (2020). Severe COVID-19 during pregnancy and possible vertical transmission. *Am. J. Perinatol.* 37, 861–865. doi: 10.1055/s-0040-1710050

Amaral, W. N. D., Moraes, C. L., Rodrigues, A. P. D. S., Noll, M., Arruda, J. T., and Mendonça, C. R. (2020). Maternal coronavirus infections and neonates born to mothers with SARS-CoV-2: a systematic review. *Healthcare* 8:511. doi: 10.3390/healthcare8040511

Anand, P., Yadav, A., Debata, P., Bachani, S., Gupta, N., and Gera, R. (2021). Clinical profile, viral load, management and outcome of neonates born to COVID 19 positive mothers: a tertiary care centre experience from India. *Eur. J. Pediatr.* 180, 547–559. doi: 10.1007/s00431-020-03800-7

Armitage, J., Tan, D. B. A., Troedson, R., Young, P., Lam, K. V., Shaw, K., et al. (2018). Mesenchymal stromal cell infusion modulates systemic immunological responses in stable COPD patients: a phase I pilot study. *Eur. Respir. J.* 51:1702369. doi: 10.1183/13993003.02369-2017

Ashraf, M. A., Keshavarz, P., Hosseinpour, P., Erfani, A., Roshanshad, A., Pourdast, A., et al. (2020). Coronavirus disease 2019 (COVID-19): a systematic review of pregnancy and the possibility of vertical transmission. *J. Reprod. Infertil.* 21, 157–168.

Bachani, S., Arora, R., Dabral, A., Marwah, S., Anand, P., Reddy, K. S., et al. (2020). . Clinical profile, viral load, maternal-fetal outcomes of pregnancy with COVID-19: 4-week retrospective, tertiary care single-centre descriptive study. *J. Obstet. Gynaecol. Can.* doi: 10.1016/j.jogc.2020.09.021 Online ahead of print

Bandyopadhyay, T., Sharma, A., Kumari, P., Maria, A., and Choudhary, R. (2020). . Possible early vertical transmission of COVID-19 from an infected pregnant female to her neonate: a case report. *J. Trop. Pediatr.* doi: 10.1093/tropej/fmaa094 Online ahead of print

Baud, D., Greub, G., Favre, G., Gengler, C., Jaton, K., Dubruc, E., et al. (2020). Second-trimester miscarriage in a pregnant woman with SARS-CoV-2 infection. *JAMA* 323, 2198–2200. doi: 10.1001/jama.2020.7233

Beeravolu, N., McKee, C., Alamri, A., Mikhael, S., Brown, C., Perez-Cruet, M., et al. (2017). Isolation and characterization of mesenchymal stromal cells from human umbilical cord and fetal placenta. *J. Vis. Exp.* 3:55224. doi: 10.3791/55224

Bianchi, D. W., Zickwolf, G. K., Weil, G. J., Sylvester, S., and DeMaria, M. A. (1996). Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc. Natl. Acad. Sci. U.S.A.* 93, 705–708. doi: 10.1073/pnas.93.2.705

Breslin, N., Baptiste, C., Miller, R., Fuchs, K., Goffman, D., Gyamfi-Bannerman, C., et al. (2020). Coronavirus disease 2019 in pregnancy: early lessons. *Am. J. Obstet. Gynecol. MFM.* 2:100111. doi: 10.1016/j.ajogmf.2020.100111

Buonsenso, D., Costa, S., Sanguinetti, M., Cattani, P., Posteraro, B., Marchetti, S., et al. (2020). Neonatal Late onset infection with severe acute respiratory syndrome coronavirus 2. *Am. J. Perinatol.* 37, 869–872. doi: 10.1055/s-0040-1710541

Can, A., and Coskun, H. (2020). The rationale of using mesenchymal stem cells in patients with COVID-19-related acute respiratory distress syndrome: what to expect. *Stem Cells Transl Med.* 9, 1287–1302. doi: 10.1002/sctm.20-0164

Carosso, A., Cosma, S., Borella, F., Marozio, L., Coscia, A., Ghisetti, V., et al. (2020). Pre-labor anorectal swab for SARS-CoV-2 in COVID-19 pregnant patients: is it time to think about it? *Eur. J. Obstet. Gynecol. Reprod. Biol.* 249, 98–99. doi: 10.1016/j.ejogrb.2020.04.023

Cavaliere, A. F., Marchi, L., Aquilini, D., Brunelli, T., and Vasarri, P. L. (2020). Passive immunity in newborn from SARS-CoV-2-infected mother. *J. Med. Virol.* doi: 10.1002/jmv.26609 Online ahead of print

Chen, H., Guo, J., Wang, C., Luo, F., Yu, X., Zhang, W., et al. (2020). Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *Lancet* 395, 809–815. doi: 10.1016/S0140-6736(20)30360-3

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395, 507–513. doi: 10.1016/S0140-6736(20)30211-7

Colantuoni, A., Martini, R., Caprari, P., Ballestri, M., Capecchi, P. L., Gnasso, A., et al. (2020). COVID-19 sepsis and microcirculation dysfunction. *Front. Physiol.* 11:747. doi: 10.3389/fphys.2020.00747

Costa, S., Posteraro, B., Marchetti, S., Tamburrini, E., Carducci, B., Lanzone, A., et al. (2020). Excretion of SARS-CoV-2 in human breast milk. *Clin. Microbiol. Infect.* 26, 1430–1432. doi: 10.1016/j.cmi.2020.05.027

COVID-19 Clinical Research Coalition (2021). Available online at: https://www.pericovid.com/ (accessed January 31, 2021).

Dashraath, P., Wong, J. L. J., Lim, M. X. K., Lim, L. M., Li, S., Biswas, A., et al. (2020). Coronavirus disease 2019 (COVID-19) pandemic and pregnancy. *Am. J. Obstet. Gynecol.* 222, 521–531. doi: 10.1016/j.ajog.2020.03.021

de Witte, S. F. H., Luk, F., Sierra Parraga, J. M., Gargesha, M., Merino, A., Korevaar, S. S., et al. (2018). Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC By monocytic cells. *Stem Cells* 36, 602–615. doi: 10.1002/stem.2779

Debelenko, L., Katsyv, I., Chong, A. M., Peruyero, L., Szabolcs, M., and Uhlemann, A. C. (2020). Trophoblast damage with acute and chronic intervillositis: disruption of the placental barrier by severe acute respiratory syndrome coronavirus 2. *Hum Pathol.* 109, 69–79. doi: 10.1016/j.humpath.2020.12.004

Di Mascio, D., Sen, C., Saccone, G., Galindo, A., Grünebaum, A., Yoshimatsu, J., et al. (2020). Risk factors associated with adverse fetal outcomes in pregnancies affected by Coronavirus disease 2019 (COVID-19): a secondary analysis of the WAPM study on COVID-19. *J. Perinat. Med.* 48, 950–958. doi: 10.1515/jpm-2020-0355

Dilger, N., Neehus, A. L., Grieger, K., Hoffmann, A., Menssen, M., and Ngezahayo, A. (2020). Gap junction dependent cell communication is modulated during transdifferentiation of mesenchymal stem/stromal cells towards neuron-like cells. *Front. Cell Dev. Biol.* 8:869. doi: 10.3389/fcell.2020.00869

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., et al. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. *Cytotherapy* 8, 315–317. doi: 10.1080/14653240600855905

Dong, L., Tian, J., He, S., Zhu, C., Wang, J., Liu, C., et al. (2020). Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. *JAMA* 323, 1846–1848. doi: 10.1001/jama.2020.4621

Dumitriu, D., Emeruwa, U. N., Hanft, E., Liao, G. V., Ludwig, E., Walzer, L., et al. (2020). Outcomes of neonates born to mothers with severe acute respiratory syndrome coronavirus 2 infection at a large medical center in New York City. *JAMA Pediatr.* 12:e204298. doi: 10.1001/jamapediatrics.2020.4298

Edlow, A. G., Li, J. Z., Collier, A. Y., Atyeo, C., James, K. E., Boatin, A. A., et al. (2020). Assessment of Maternal and neonatal SARS-CoV-2 viral load, transplacental antibody transfer, and placental pathology in pregnancies during

the COVID-19 pandemic. *JAMA Netw. Open* 3:e2030455. doi: 10.1001/jamanetworkopen.2020.30455

Eggenhofer, E., Benseler, V., Kroemer, A., Popp, F. C., Geissler, E. K., Schlitt, H. J., et al. (2012). Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. *Front. Immunol.* 3:297. doi: 10.3389/fimmu.2012.00297

Facchetti, F., Bugatti, M., Drera, E., Tripodo, C., Sartori, E., Cancila, V., et al. (2020). SARS-CoV2 vertical transmission with adverse effects on the newborn revealed through integrated immunohistochemical, electron microscopy and molecular analyses of Placenta. *EBioMedicine* 59:102951. doi: 10.1016/j.ebiom.2020.102951

Farsi, Z., Derakhsh, N. T., Bassirnia, M., Ahmadi, L., Shiva, M., and Yousefzadegan, S. (2020). Coronavirus disease-2019 infection in neonates of an infected pregnant mother with triplets. *Iran. J. Neonatol.* 11, 120–122. doi: 10.22038/ijn.2020.49218.1856

Feng, Y., Huang, J., Wu, J., Xu, Y., Chen, B., Jiang, L., et al. (2020). Safety and feasibility of umbilical cord mesenchymal stem cells in patients with COVID-19 pneumonia: a pilot study. *Cell Prolif.* 53:e12947. doi: 10.1111/cpr.12947

Fenizia, C., Biasin, M., Cetin, I., Vergani, P., Mileto, D., Spinillo, A., et al. (2020). Analysis of SARS-CoV-2 vertical transmission during pregnancy. *Nat. Commun.* 11:5128. doi: 10.1038/s41467-020-18933-4

Ferraiolo, A., Barra, F., Kratochwila, C., Paudice, M., Vellone, V. G., Godano, E., et al. (2020). Report of positive placental swabs for SARS-CoV-2 in an asymptomatic pregnant woman with COVID-19. *Medicina* 56:306. doi: 10.3390/medicina56060306

Ferrazzi, E., Beretta, P., Bianchi, S., Cetin, I., Guarnerio, P., Locatelli, A., et al. (2020). SARS-CoV-2 infection testing at delivery: a clinical and epidemiological priority. *J. Matern. Fetal Neonatal Med.* 13, 1–3. doi: 10.1080/14767058.2020.1788532

Fischer, U. M., Harting, M. T., Jimenez, F., Monzon-Posadas, W. O., Xue, H., Savitz, S. I., et al. (2009). Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. *Stem Cells Dev.* 18, 683–692. doi: 10.1089/scd.2008.0253

Friedman, D. M., Kim, M., Costedoat-Chalumeau, N., Clancy, R., Copel, J., Phoon, C. K., et al. (2020). Electrocardiographic QT intervals in infants exposed to hydroxychloroquine throughout gestation. *Circ. Arrhythm. Electrophysiol.* 13:e008686. doi: 10.1161/CIRCEP.120.008686

Gale, C., Quigley, M. A., Placzek, A., Knight, M., Ladhani, S., Draper, E. S., et al. (2020). Characteristics and outcomes of neonatal SARS-CoV-2 infection in the UK: a prospective national cohort study using active surveillance. *Lancet Child Adolesc. Health* doi: 10.1016/S2352-4642(20)30342-4 Online ahead of print

Galleu, A., Riffo-Vasquez, Y., Trento, C., Lomas, C., Dolcetti, L., Cheung, T. S., von Bonin, M., et al. (2017). Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. *Sci. Transl. Med.* 9:eaam7828. doi: 10.1126/scitranslmed.aam7828

Gao, J., Li, W., Hu, X., Wei, Y., Wu, J., Luo, X., et al. (2020). Disappearance of SARS-CoV-2 antibodies in infants born to women with COVID-19, Wuhan, China. *Emerg. Infect. Dis.* 26, 2491–2494. doi: 10.3201/eid2610.202328

Gao, W., Deng, Z., Zeng, L., Yang, Y., Gong, H., Liu, J., et al. (2020). A newborn with normal IgM and elevated IgG antibodies born to an asymptomatic infection mother with COVID-19. *Aging* 12, 16672–16674. doi: 10.18632/aging.103346

Gentile, P., Sterodimas, A., Pizzicannella, J., Calabrese, C., and Garcovich, S. (2020). Research progress on mesenchymal stem cells (MSCs), adipose-derived mesenchymal stem cells (AD-MSCs), drugs, and vaccines in inhibiting COVID-19 disease. *Aging Dis.* 11, 1191–1201. doi: 10.14336/AD.2020.0711

Govind, A., Essien, S., Karthikeyan, A., Fakokunde, A., Janga, D., Yoong, W., et al. (2020). Re: novel coronavirus COVID-19 in late pregnancy: outcomes of first nine cases in an inner city London hospital. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 251, 272–274. doi: 10.1016/j.ejogrb.2020.05.004

Gregorio-Hernández, R., Escobar-Izquierdo, A. B., Cobas-Pazos, J., and Martínez-Gimeno, A. (2020). Point-of-care lung ultrasound in three neonates with COVID-19. *Eur. J. Pediatr.* 179, 1279–1285. doi: 10.1007/s00431-020-03706-4

Gupta, A., Madhavan, M. V., Sehgal, K., Nair, N., Mahajan, S., Sehrawat, T. S., et al. (2020). Extrapulmonary manifestations of COVID-19. *Nat. Med.* 26, 1017–1032. doi: 10.1038/s41591-020-0968-3

Hadjadj, J., Yatim, N., Barnabei, L., Corneau, A., Boussier, J., Smith, N., et al. (2020). Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science* 369, 718–724. doi: 10.1126/science.abc6027

Harrell, C. R., Sadikot, R., Pascual, J., Fellabaum, C., Jankovic, M. G., Jovicic, N., et al. (2019). Mesenchymal stem cell-based therapy of inflammatory lung diseases: current understanding and future perspectives. *Stem Cells Int.* 2019:4236973. doi: 10.1155/2019/4236973

Hcini, N., Maamri, F., Picone, O., Carod, J. F., Lambert, V., Mathieu, M., et al. (2020). Maternal, fetal and neonatal outcomes of large series of SARS-CoV-2 positive pregnancies in peripartum period: a single-center prospective comparative study. *Eur J Obstet Gynecol Reprod Biol.* 257, 11–18. doi: 10.1016/j.ejogrb.2020.11.068

He, Z., Fang, Y., Zuo, Q., Huang, X., Lei, Y., Ren, X., et al. (2020). Vertical transmission and kidney damage in newborns whose mothers had coronavirus disease 2019 during pregnancy. *Int. J. Antimicrob. Agents* 9:106260. doi: 10.1016/j.ijantimicag.2020.106260

Hecht, J. L., Quade, B., Deshpande, V., Mino-Kenudson, M., Ting, D. T., Desai, N., et al. (2020). SARS-CoV-2 can infect the placenta and is not associated with specific placental histopathology: a series of 19 placentas from COVID-19-positive mothers. *Mod. Pathol.* 33, 2092–2103. doi: 10.1038/s41379-020-0639-4

Herlenius, E., Åkerstedt, S. O., Herrting, O., Ottosson, L., Ramadan, L., and Hildenwall, H. (2020). *SARS-CoV-2 Exposure, Symptoms and Antibody Response in Children in Stockholm during the COVID-19 pandemic. Abstract Presented at "State of the Art Covid-19" on 15-16 December*. Available online at: https://www.sls.se/state-of-the-art-covid-19/abstracts/Abstract-book/ (accessed October 30, 2020).

Hinojosa-Velasco, A., de Oca, P. V. B., García-Sosa, L. E., Mendoza-Durán, J. G., Pérez-Méndez, M. J., Dávila-González, E., et al. (2020). A case report of newborn infant with severe COVID-19 in Mexico: detection of SARS-CoV-2 in human breast milk and stool. *Int. J. Infect. Dis.* 100, 21–24. doi: 10.1016/j.ijid.2020.08.055

Hosier, H., Farhadian, S., Morotti, R., Deshmukh, U., Lu-Culligan, A., Campbell, K. H., et al. (2020). First case of placental infection with SARS-CoV- 2. *medRxiv [Preprint]* doi: 10.1101/2020.04.30.20083907

Hsu, A. L., Guan, M., Johannesen, E., Stephens, A. J., Khaleel, N., Kagan, N., et al. (2021). Placental SARS-CoV-2 in a pregnant woman with mild COVID-19 disease. *J. Med. Virol.* 93, 1038–1044. doi: 10.1002/jmv.26386

Hu, X., Gao, J., Luo, X., Feng, L., Liu, W., Chen, J., et al. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vertical transmission in neonates born to mothers with coronavirus disease 2019 (COVID-19) pneumonia. *Obstet. Gynecol.* 136, 65–67. doi: 10.1097/AOG.0000000000003926

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Iqbal, S. N., Overcash, R., Mokhtari, N., Saeed, H., Gold, S., Auguste, T., et al. (2020). An Uncomplicated delivery in a patient with Covid-19 in the United States. *N. Engl. J. Med.* 382:e34. doi: 10.1056/NEJMc2007605

Islam, M. N., Das, S. R., Emin, M. T., Wei, M., Sun, L., Westphalen, K., et al. (2012). Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat. Med.* 18, 759–765. doi: 10.1038/nm.2736

Jacob, F., Pather, S. R., Huang, W. K., Zhang, F., Wong, S. Z. H., Zhou, H. et al. (2020). Human pluripotent stem cell-derived neural cells and brain organoids reveal SARS-CoV-2 neurotropism predominates in choroid plexus epithelium. *Cell Stem Cell.* 27:937.e9–950.e9. doi: 10.1016/j.stem.2020.09.016

Jamieson, D. J., Theiler, R. N., and Rasmussen, S. A. (2006). Emerging infections and pregnancy. *Emerg. Infect. Dis.* 12, 1638–1643. doi: 10.3201/eid1211.060152

Jayaramayya, K., Mahalaxmi, I., Subramaniam, M. D., Raj, N., Dayem, A. A., Lim, K.M. et al. (2020). Immunomodulatory effect of mesenchymal stem cells and mesenchymal stem-cell-derived exosomes for COVID-19 treatment. *BMB Rep.* 53, 400–412. doi: 10.5483/BMBRep.2020.53.8.121

Jin, H. J., Bae, Y. K., Kim, M., Kwon, S. J., Jeon, H. B., Choi, S. J., et al. (2013). Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. *Int. J. Mol. Sci.* 14, 17986–18001. doi: 10.3390/ijms140917986

Khosrotehrani, K., Johnson, K. L., Cha, D. H., Salomon, R. N., and Bianchi, D. W. (2004). Transfer of fetal cells with multilineage potential to maternal tissue. *JAMA.* 292, 75–80. doi: 10.1001/jama.292.1.75

Khoury, R., Bernstein, P. S., Debolt, C., Stone, J., Sutton, D. M., Simpson, L. L., et al. (2020). Characteristics and outcomes of 241 births to women with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection at five New York city medical centers. *Obstet. Gynecol.* 136, 273–282. doi: 10.1097/AOG.0000000000004025

Kirtsman, M., Diambomba, Y., Poutanen, S. M., Malinowski, A. K., Vlachodimitropoulou, E., Parks, W. T., et al. (2020). Probable congenital SARS-CoV-2 infection in a neonate born to a woman with active SARS-CoV-2 infection. *CMAJ* 192, E647–E650. doi: 10.1503/cmaj.200821

Kulkarni, R., Rajput, U., Dawre, R., Valvi, C., Nagpal, R., Magdum, N., et al. (2020). Early-onset symptomatic neonatal COVID-19 infection with high probability of vertical transmission. *Infection* doi: 10.1007/s15010-020-01493-6 Online ahead of print.

Lai, C. C., Shih, T. P., Ko, W. C., Tang, H. J., and Hsueh, P. R. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. *Int. J. Antimicrob. Agents.* 55:105924. doi: 10.1016/j.ijantimicag.2020.105924

Lalu, M. M., McIntyre, L., Pugliese, C., Fergusson, D., Winston, B. W., Marshall, J. C., et al. (2012). Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One* 7:e47559. doi: 10.1371/journal.pone.0047559

Lapinsky, S. E., Tram, C., Mehta, S., and Maxwell, C. V. (2014). Restrictive lung disease in pregnancy. *Chest* 145, 394–398. doi: 10.1378/chest.13-0587

Le Blanc, K., and Mougiakakos, D. (2012). Multipotent mesenchymal stromal cells and the innate immune system. *Nat. Rev. Immunol.* 12, 383–396. doi: 10.1038/nri3209

Leng, Z., Zhu, R., Hou, W., Feng, Y., Yang, Y., Han, Q., et al. (2020). Transplantation of ACE2^{-} mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. *Aging Dis.* 11, 216–228. doi: 10.14336/AD.2020.0228

Li, X., Bai, J., Ji, X., Li, R., Xuan, Y., and Wang, Y. (2014). Comprehensive characterization of four different populations of human mesenchymal stem cells as regards their immune properties, proliferation and differentiation. *Int. J. Mol. Med.* 34, 695–704. doi: 10.3892/ijmm.2014.1821

Li, Y., Xu, J., Shi, W., Chen, C., Shao, Y., Zhu, L., et al. (2016). Mesenchymal stromal cell treatment prevents H9N2 avian influenza virus-induced acute lung injury in mice. *Stem Cell Res Ther.* 7:159. doi: 10.1186/s13287-016-0395-z

Li, Z., Niu, S., Guo, B., Gao, T., Wang, L., Wang, Y., et al. (2020). Stem cell therapy for COVID-19, ARDS and pulmonary fibrosis. *Cell Prolif.* 53:e12939. doi: 10.1111/cpr.12939

Liang, B., Chen, J., Li, T., Wu, H., Yang, W., Li, Y., et al. (2020). Clinical remission of a critically ill COVID-19 patient treated by human umbilical cord mesenchymal stem cells: a case report. *Medicine* 99:e21429. doi: 10.1097/MD.0000000000021429

Liu, D., Li, L., Wu, X., Zheng, D., Wang, J., Yang, L., et al. (2020). Pregnancy and perinatal outcomes of women with coronavirus disease (COVID-19) pneumonia: a preliminary analysis. *AJR Am. J. Roentgenol.* 215, 127–132. doi: 10.2214/AJR.20.23072

Lorenz, N., Treptow, A., Schmidt, S., Hofmann, R., Raumer-Engler, M., Heubner, G., et al. (2020). Neonatal early-onset infection with SARS-CoV-2 in a newborn presenting with encephalitic symptoms. *Pediatr. Infect. Dis. J.* 39:e212. doi: 10.1097/INF.0000000000002735

Mattar, C. N., Kalimuddin, S., Sadarangani, S. P., Tagore, S., Thain, S., Thoon, K. C., et al. (2020). Pregnancy outcomes in COVID-19: a prospective cohort study in Singapore. *Ann. Acad. Med. Singap.* 49, 857–869.

McDevitt, K. E. M., Ganjoo, N., Mlangeni, D., and Pathak, S. (2020). Outcome of universal screening of neonates for COVID-19 from asymptomatic mothers. *J. Infect.* 81, 452–482. doi: 10.1016/j.jinf.2020.06.037

McIntosh, K. R. (2011). Evaluation of cellular and humoral immune responses to allogeneic adipose-derived stem/stromal cells. *Methods Mol. Biol.* 702, 133–150. doi: 10.1007/978-1-61737-960-4_11

Meirelles Lda, S., Fontes, A. M., Covas, D. T., and Caplan, A. I. (2009). Mechanisms involved in the therapeutic properties of mesenchymal stem cells. *Cytokine Growth Factor Rev.* 20, 419–427. doi: 10.1016/j.cytogfr.2009.10.002

Meng, F., Xu, R., Wang, S., Xu, Z., Zhang, C., Li, Y., et al. (2020). Human umbilical cord-derived mesenchymal stem cell therapy in patients with COVID-19: a phase 1 clinical trial. *Signal Transduct Target Ther.* 5:172. doi: 10.1038/s41392-020-00286-5

Menter, T., Mertz, K. D., Jiang, S., Chen, H., Monod, C., Tzankov, A., et al. (2020). Placental pathology findings during and after SARS-CoV-2 infection: features of villitis and malperfusion. *Pathobiology* 88, 69–77. doi: 10.1159/000511324

Mongula, J. E., Frenken, M. W. E., van Lijnschoten, G., Arents, N. L. A., de Wit-Zuurendonk, L. D., Schimmel-de Kok, A. P. A., et al. (2020). COVID-19 during pregnancy: non-reassuring fetal heart rate, placental pathology and coagulopathy. *Ultrasound. Obstet. Gynecol.* 56, 773–776. doi: 10.1002/uog.22189

Muñoz, M. F., Argüelles, S., Guzman-Chozas, M., Guillén-Sanz, R., Franco, J. M., Pintor-Toro, J. A., et al. (2018). Cell tracking, survival, and differentiation capacity of adipose-derived stem cells after engraftment in rat tissue. *J. Cell Physiol.* 233, 6317–6328. doi: 10.1002/jcp.26439

Naji, A., Favier, B., Deschaseaux, F., Rouas-Freiss, N., Eitoku, M., and Suganuma, N. (2019). Mesenchymal stem/stromal cell function in modulating cell death. *Stem Cell Res Ther.* 10:56. doi: 10.1186/s13287-019-1158-4

Nayak, A. H., Kapote, D. S., Fonseca, M., Chavan, N., Mayekar, R., Sarmalkar, M., et al. (2020). Impact of the coronavirus infection in pregnancy: a preliminary study of 141 patients. *J. Obstet. Gynaecol. India* 70, 256–261. doi: 10.1007/s13224-020-01335-3

Nguyen Huu, S., Oster, M., Uzan, S., Chareyre, F., Aractingi, S., and Khosrotehrani, K. (2007). Maternal neoangiogenesis during pregnancy partly derives from fetal endothelial progenitor cells. *Proc. Natl. Acad. Sci. U.S.A.* 104, 1871–1876. doi: 10.1073/pnas.0606490104

Nie, R., Wang, S.-S., Yang, Q., Fan, C.-F., Liu, Y.-L., He, W.-C., et al. (2020). Clinical features and the maternal and neonatal outcomes of pregnant women with coronavirus disease 2019. *medRxiv [Preprint]* doi: 10.1101/2020.03.22.20041061

O'Donoghue, K., Sultan, H. A., Al-Allaf, F. A., Anderson, J. R., Wyatt-Ashmead, J., and Fisk, N. M. (2008). Microchimeric fetal cells cluster at sites of tissue injury in lung decades after pregnancy. *Reprod. Biomed. Online* 16, 382–390. doi: 10.1016/s1472-6483(10)60600-1

Oncel, M. Y., Akın, I. M., Kanburoglu, M. K., Tayman, C., Coskun, S., Narter, F., et al. (2020). A multicenter study on epidemiological and clinical characteristics of 125 newborns born to women infected with COVID-19 by Turkish Neonatal Society. *Eur. J. Pediatr.* 10, 1–10. doi: 10.1007/s00431-020-03767-5

Parsa, Y., Shokri, N., Jahedbozorgan, T., Naeiji, Z., Zadehmodares, S., and Moridi, A. (2020). Possible vertical transmission of COVID-19 to the newborn; a case report. *Arch. Acad. Emerg. Med.* 9:e5.

Pastick, K. A., Nicol, M. R., Smyth, E., Zash, R., Boulware, D. R., Rajasingham, R., et al. (2020). A systematic review of treatment and outcomes of pregnant women with COVID-19-a call for clinical trials. *Open Forum. Infect. Dis.* 7:ofaa350. doi: 10.1093/ofid/ofaa350

Patanè, L., Morotti, D., Giunta, M. R., Sigismondi, C., Piccoli, M. G., Frigerio, L., et al. (2020). Vertical transmission of coronavirus disease 2019: severe acute respiratory syndrome coronavirus 2 RNA on the fetal side of the placenta in pregnancies with coronavirus disease 2019-positive mothers and neonates at birth. *Am. J. Obstet. Gynecol. MFM.* 2:100145. doi: 10.1016/j.ajogmf.2020.100145

Penfield, C. A., Brubaker, S. G., Limaye, M. A., Lighter, J., Ratner, A. J., Thomas, K. M., et al. (2020). Detection of severe acute respiratory syndrome coronavirus 2 in placental and fetal membrane samples. *Am. J. Obstet. Gynecol. MFM.* 2:100133. doi: 10.1016/j.ajogmf.2020.100133

Peng, J., Li, R., Yin, H., Tang, F., Xie, H., Li, M., et al. (2020). A case report of a pregnant woman infected with coronavirus disease 2019 pneumonia. *Medicine* 99:e21335. doi: 10.1097/MD.0000000000021335

Pessoa, F. S., Vale, M. S. D., Marques, P. F., Figueira, S. D. S., Salgado, I. A. D. S. C., and Mochel, R. S. W. C. (2020). Probable vertical transmission identified within six hours of life. *Rev. Assoc. Med. Bras.* 66, 1621–1624. doi: 10.1590/1806-9282.66.12.1621

Piersigilli, F., Carkeek, K., Hocq, C., van Grambezen, B., Hubinont, C., Chatzis, O., et al. (2020). COVID-19 in a 26-week preterm neonate. *Lancet Child Adolesc. Health.* 4, 476–478. doi: 10.1016/S2352-4642(20)30140-1

Pritchard, S., Wick, H. C., Slonim, D. K., Johnson, K. L., and Bianchi, D. W. (2012). Comprehensive analysis of genes expressed by rare microchimeric fetal cells in the maternal mouse lung. *Biol. Reprod.* 87:42. doi: 10.1095/biolreprod.112.101147

Puissant, B., Barreau, C., Bourin, P., Clavel, C., Corre, J., Bousquet, C., et al. (2005). Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells. *Br. J. Haematol.* 129, 118–129. doi: 10.1111/j.1365-2141.2005.05409.x

Pulinx, B., Kieffer, D., Michiels, I., Petermans, S., Strybol, D., Delvaux, S., et al. (2020). Vertical transmission of SARS-CoV-2 infection and preterm birth. *Eur. J. Clin. Microbiol. Infect. Dis.* 39, 2441–2445. doi: 10.1007/s10096-020-03964-y

Qin, H., and Zhao, A. (2020). Mesenchymal stem cell therapy for acute respiratory distress syndrome: from basic to clinics. *Protein Cell.* 11, 707–722. doi: 10.1007/s13238-020-00738-2

Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R., and Salem, R. (2020). The COVID-19 cytokine storm; what we know so far. *Front. Immunol.* 11:1446. doi: 10.3389/fimmu.2020.01446

Rasmusson, I. (2006). Immune modulation by mesenchymal stem cells. *Exp Cell Res.* 312, 2169–2179. doi: 10.1016/j.yexcr.2006.03.019

RECOVERY Collaborative Group, Horby, P., Lim, W. S., Emberson, J. R., Mafham, M., Bell, J. L., et al. (2020). Dexamethasone in hospitalized patients with Covid-19 - preliminary report. *N. Engl. J. Med.* doi: 10.1056/NEJMoa2021436 Online ahead of print

Remaeus, K., Savchenko, J., Brismar Wendel, S., Brusell Gidlöf, S., Graner, S., Jones, E., et al. (2020). Characteristics and short-term obstetric outcomes in a case series of 67 women test-positive for SARS-CoV-2 in Stockholm, Sweden. *Acta Obstet. Gynecol. Scand.* 99, 1626–1631. doi: 10.1111/aogs.14006

Richtmann, R., Torloni, M. R., Oyamada Otani, A. R., Levi, J. E., Crema Tobara, M., and de Almeida Silva, C. (2020). Fetal deaths in pregnancies with SARS-CoV-2 infection in Brazil: a case series. *Case Rep. Womens Health.* 27:e00243. doi: 10.1016/j.crwh.2020.e00243

Rivera-Hernandez, P., Nair, J., Islam, S., Davidson, L., Chang, A., and Elberson, V. (2020). Coronavirus disease 2019 in a premature infant: vertical transmission and antibody response or lack thereof. *AJP Rep.* 10, e224–e227. doi: 10.1055/s-0040-1715176

Rodrigues, M. L., Gasparinho, G., Sepúlveda, F., and Matos, T. (2021). Signs suggestive of congenital SARS-CoV-2 infection with intrauterine fetal death: a case report. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 256, 508–509. doi: 10.1016/j.ejogrb.2020.11.042

Sagheb, S., Lamsehchi, A., Jafary, M., Atef-Yekta, R., and Sadeghi, K. (2020). Two seriously ill neonates born to mothers with COVID-19 pneumonia- a case report. *Ital. J. Pediatr.* 46:137. doi: 10.1186/s13052-020-00897-2

Saldanha-Araujo, F., Melgaço Garcez, E., Silva-Carvalho, A. E., and Carvalho, J. L. (2020). Mesenchymal stem cells: a new piece in the puzzle of COVID-19 treatment. *Front. Immunol.* 11:1563. doi: 10.3389/fimmu.2020.01563

Savasi, V. M., Parisi, F., Patanè, L., Ferrazzi, E., Frigerio, L., Pellegrino, A., et al. (2020). Clinical findings and disease severity in hospitalized pregnant women with coronavirus disease 2019 (COVID-19). *Obstet. Gynecol.* 136, 252–258. doi: 10.1097/AOG.0000000000003979

Scherger, S., Henao-Martínez, A., Franco-Paredes, C., and Shapiro, L. (2020). Rethinking interleukin-6 blockade for treatment of COVID-19. *Med. Hypotheses.* 144:110053. doi: 10.1016/j.mehy.2020.110053

Schoenmakers, S., Snijder, P., Verdijk, R. M., Kuiken, T., Kamphuis, S. S. M., Koopman, L. P., et al. (2020). SARS-CoV-2 placental infection and inflammation leading to fetal distress and neonatal multi-organ failure in an asymptomatic woman. *medRxiv [Preprint]* doi: 10.1101/2020.06.08.20110437

Schwartz, D. A., and Graham, A. L. (2020). Potential maternal and infant outcomes from (Wuhan) coronavirus 2019-nCoV infecting pregnant women: lessons from SARS, MERS, and other human coronavirus infections. *Viruses* 12:194. doi: 10.3390/v12020194

Schwartz, D. A., and Morotti, D. (2020). Placental pathology of COVID-19 with and without fetal and neonatal infection: trophoblast necrosis and chronic histiocytic intervillositis as risk factors for transplacental transmission of SARS-CoV-2. *Viruses* 12:1308. doi: 10.3390/v12111308

Sengupta, V., Sengupta, S., Lazo, A., Woods, P., Nolan, A., and Bremer, N. (2020). Exosomes derived from bone marrow mesenchymal stem cells as treatment for severe COVID-19. *Stem Cells Dev.* 29, 747–754. doi: 10.1089/scd.2020.0080

Sharma, A. K., Sane, H. M., Kulkarni, P. P., Gokulchandran, N., Biju, H., and Badhe, P. B. (2020). Autologous bone marrow mononuclear cell transplantation in patients with chronic traumatic brain injury- a clinical study. *Cell Regen.* 9:3. doi: 10.1186/s13619-020-00043-7

Shende, P., Gaikwad, P., Gandhewar, M., Ukey, P., Bhide, A., Patel, V., et al. (2020). Persistence of SARS-CoV-2 in the first trimester placenta leading to transplacental transmission and fetal demise from an asymptomatic mother. *Hum. Reprod.* doi: 10.1093/humrep/deaa367 Online ahead of print

Shu, L., Niu, C., Li, R., Huang, T., Wang, Y., Huang, M., et al. (2020). Treatment of severe COVID-19 with human umbilical cord mesenchymal stem cells. *Stem Cell Res. Ther.* 11:361. doi: 10.1186/s13287-020-01875-5

Sileo, F. G., Tramontano, A. L., Leone, C., Meacci, M., Gennari, W., Ternelli, G., et al. (2020). Pregnant woman infected by coronavirus disease (COVID-19) and calcifications of the fetal bowel and gallbladder: a case report. *Minerva Ginecol.* doi: 10.23736/S0026-4784.20.04717-6 Online ahead of print

Sisman, J., Jaleel, M. A., Moreno, W., Rajaram, V., Collins, R. R. J., Savani, R. C., et al. (2020). Intrauterine transmission of SARS-COV-2 infection in a preterm infant. *Pediatr. Infect. Dis. J.* 39, e265–e267. doi: 10.1097/INF.0000000000002815

Smithgall, M. C., Liu-Jarin, X., Hamele-Bena, D., Cimic, A., Mourad, M., Debelenko, L., et al. (2020). Third-trimester placentas of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive women: histomorphology, including viral immunohistochemistry and in-situ hybridization. *Histopathology* 77, 994–999. doi: 10.1111/his.14215

Soler Rich, R., Rius Tarruella, J., and Melgosa Camarero, M. T. (2020). Expanded Mesenchymal Stem Cells: a novel therapeutic approach of SARS-CoV-2 pneumonia (COVID-19). Concepts regarding a first case in Spain. *Med. Clin.* 155, 318–319. doi: 10.1016/j.medcli.2020.06.018

Stagg, J. (2007). Immune regulation by mesenchymal stem cells: two sides to the coin. *Tissue Antigens.* 69, 1–9. doi: 10.1111/j.1399-0039.2006.00739.x

Stappenbeck, T. S., and Miyoshi, H. (2009). The role of stromal stem cells in tissue regeneration and wound repair. *Science* 324, 1666–1669. doi: 10.1126/science.1172687

Stonoga, E. T. S., de Almeida Lanzoni, L., Rebutini, P. Z., Permegiani, de Oliveira, A. L., Chiste, J. A., et al. (2020). Intrauterine Transmission of SARS-CoV-2. *Emerg. Infect. Dis.* 27, 638–641. doi: 10.3201/eid2702.203824

Strem, B. M., Hicok, K. C., Zhu, M., Wulur, I., Alfonso, Z., Schreiber, R. E., et al. (2005). Multipotential differentiation of adipose tissue-derived stem cells. *Keio. J. Med.* 54, 132–141. doi: 10.2302/kjm.54.132

Sun, M., Xu, G., Yang, Y., Tao, Y., Pian-Smith, M., Madhavan, V., et al. (2020). Evidence of mother-to-newborn infection with COVID-19. *Br. J. Anaesth.* 125, e245–e247. doi: 10.1016/j.bja.2020.04.066

Swayne, L. A., Johnstone, S. R., Ng, C. S., Sanchez-Arias, J. C., Good, M. E., Penuela, S., et al. (2020). Consideration of Pannexin 1 channels in COVID-19 pathology and treatment. *Am. J. Physiol. Lung Cell Mol. Physiol.* 319, L121–L125. doi: 10.1152/ajplung.00146.2020

Taglauer, E., Benarroch, Y., Rop, K., Barnett, E., Sabharwal, V., Yarrington, C., et al. (2020). Consistent localization of SARS-CoV-2 spike glycoprotein and ACE2 over TMPRSS2 predominance in placental villi of 15 COVID-19 positive maternal-fetal dyads. *Placenta* 100, 69–74. doi: 10.1016/j.placenta.2020.08.015

Takemoto, M., Menezes, M. O., Andreucci, C. B., Knobel, R., Sousa, L., Katz, L., et al. (2020). Clinical characteristics and risk factors for mortality in obstetric patients with severe COVID-19 in Brazil: a surveillance database analysis. *BJOG.* 127, 1618–1626. doi: 10.1111/1471-0528.16470

Tao, J., Nie, Y., Wu, H., Cheng, L., Qiu, Y., Fu, J., et al. (2020). Umbilical cord blood-derived mesenchymal stem cells in treating a critically ill COVID-19 patient. *J. Infect. Dev. Ctries.* 14, 1138–1145. doi: 10.3855/jidc.13081

Tao, Y. C., and Chen, E. Q. (2020). Clinical application of stem cell in patients with end-stage liver disease: progress and challenges. *Ann. Transl. Med.* 8:564. doi: 10.21037/atm.2020.03.153

Thanunchai, M., Hongeng, S., and Thitithanyanont, A. (2015). Mesenchymal stromal cells and viral infection. *Stem. Cells Int.* 2015:860950. doi: 10.1155/2015/860950

Ti, D., Hao, H., Tong, C., Liu, J., Dong, L., Zheng, J., et al. (2015). LPS-preconditioned mesenchymal stromal cells modify macrophage polarization for resolution of chronic inflammation via exosome-shuttled let-7b. *J. Transl. Med.* 13:308. doi: 10.1186/s12967-015-0642-6

Toner, L. E., Gelber, S. E., Pena, J. A., Fox, N. S., and Rebarber, A. (2020). A case report to assess passive immunity in a COVID positive pregnant patient. *Am. J. Perinatol.* 37, 1280–1282. doi: 10.1055/s-0040-1715643

Vashukova, M. A., Tsinzerling, V. A., Semenova, N. Y., Lugovskaya, N. A., Narkevich, T. A., and Sukhanova, Y. V. (2020). Is perinatal COVID-19 possible: first results. *J. Infectol.* 12, 51–55. doi: 10.22625/2072-6732-2020-12-3-51-55

Vendola, N., Stampini, V., Amadori, R., Gerbino, M., Curatolo, A., and Surico, D. (2020). Vertical transmission of antibodies in infants born from mothers with positive serology to COVID-19 pneumonia. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 253, 331–332. doi: 10.1016/j.ejogrb.2020.08.023

Verma, S., Bradshaw, C., Auyeung, N. S. F., Lumba, R., Farkas, J. S., Sweeney, N. B., et al. (2020). Outcomes of maternal-newborn dyads after maternal SARS-CoV-2. *Pediatrics* 146:e2020005637. doi: 10.1542/peds.2020-005637

Vivanti, A. J., Vauloup-Fellous, C., Prevot, S., Zupan, V., Suffee, C., Do Cao, J., et al. (2020). Transplacental transmission of SARS-CoV-2 infection. *Nat. Commun.* 11:3572. doi: 10.1038/s41467-020-17436-6

Walker, K. F., O'Donoghue, K., Grace, N., Dorling, J., Comeau, J. L., Li, W., et al. (2020). Maternal transmission of SARS-COV-2 to the neonate, and possible routes for such transmission: a systematic review and critical analysis. *BJOG* 127, 1324–1336. doi: 10.1111/1471-0528.16362

Wang, S., Guo, L., Chen, L., Liu, W., Cao, Y., Zhang, J., et al. (2020). A case report of neonatal 2019 coronavirus disease in China. *Clin. Infect. Dis.* 71, 853–857. doi: 10.1093/cid/ciaa225

Weiss, A. R. R., and Dahlke, M. H. (2019). Immunomodulation by mesenchymal stem cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. *Front. Immunol.* 10:1191. doi: 10.3389/fimmu.2019.01191

Weiss, D. J. (2014). Concise review: current status of stem cells and regenerative medicine in lung biology and diseases. *Stem Cells* 32, 16–25. doi: 10.1002/stem.1506

Wu, J., Hu, Z., Wang, L., Tan, Y., Hou, W., Li, Z., et al. (2020a). First case of COVID-19 infused with hESC derived immunity- and matrix-regulatory cells. *Cell Prolif.* 53:e12943. doi: 10.1111/cpr.12943

Wu, J., Song, D., Li, Z., Guo, B., Xiao, Y., Liu, W., et al. (2020b). Immunity-and-matrix-regulatory cells derived from human embryonic stem cells safely and effectively treat mouse lung injury and fibrosis. *Cell Res.* 30, 794–809. doi: 10.1038/s41422-020-0354-1

Wu, J., Zhou, X., Tan, Y., Wang, L., Li, T., Li, Z., et al. (2020c). Phase 1 trial for treatment of COVID-19 patients with pulmonary fibrosis using hESC-IMRCs. *Cell Prolif.* 53:e12944. doi: 10.1111/cpr.12944

Wu, Y. T., Liu, J., Xu, J. J., Chen, Y. F., Yang, W., Chen, Y., et al. (2020d). Neonatal outcome in 29 pregnant women with COVID-19: a retrospective study in Wuhan, China. *PLoS Med.* 17:e1003195. doi: 10.1371/journal.pmed.1003195

Wu, Y., Hoogduijn, M. J., Baan, C. C., Korevaar, S. S., de Kuiper, R., Yan, L., et al. (2017). Adipose tissue-derived mesenchymal stem cells have a heterogenic cytokine secretion profile. *Stem Cells Int.* 2017:4960831. doi: 10.1155/2017/4960831

Yilmaz, R., Adas, G., Cukurova, Z., Kart Yasar, K., Isiksacan, N., Oztel, O. N., et al. (2020). Mesenchymal stem cells treatment in COVID-19 patient with multi-organ involvement. *Bratisl. Lek. Listy.* 121, 847–852. doi: 10.4149/BLL_2020_139

Zaigham, M., Holmberg, A., Karlberg, M. L., Lindsjö, O. K., Jokubkiene, L., Sandblom, J., et al. (2020). *Intrauterine Transmission of SARS-CoV-2 - A Confirmed Case. Abstract Presented at "State of the Art Covid-19" on 15-16 December 2020.* Available online at: https://www.sls.se/state-of-the-art-covid-19/abstracts/Abstract-book/ (accessed October 30, 2020).

Zamaniyan, M., Ebadi, A., Aghajanpoor, S., Rahmani, Z., Haghshenas, M., and Azizi, S. (2020). Preterm delivery, maternal death, and vertical transmission in a pregnant woman with COVID-19 infection. *Prenat Diagn.* 40, 1759–1761. doi: 10.1002/pd.5713

Zeng, H., Xu, C., Fan, J., Tang, Y., Deng, Q., Zhang, W., et al. (2020). Antibodies in infants born to mothers with COVID-19 pneumonia. *JAMA* 323, 1848–1849. doi: 10.1001/jama.2020.4861

Zeng, L., Xia, S., Yuan, W., Yan, K., Xiao, F., Shao, J., et al. (2020). Neonatal early-onset infection with SARS-CoV-2 in 33 neonates born to mothers with COVID-19 in Wuhan, China. *JAMA Pediatr.* 174, 722–725. doi: 10.1001/jamapediatrics.2020.0878

Zeng, X. X., Tan, K. H., Yeo, A., Sasajala, P., Tan, X., Xiao, Z. C., et al. (2010). Pregnancy-associated progenitor cells differentiate and mature into neurons in the maternal brain. *Stem Cells Dev.* 19, 1819–1830. doi: 10.1089/scd.2010.0046

Zengin, R., Beyaz, O., Koc, E. S., Akinci, I. O., Kocagoz, S., Sagcan, G., et al. (2020). Mesenchymal stem cell treatment in a critically ill COVID-19 patient: a case report. *Stem Cell Investig.* 7:17. doi: 10.21037/sci-2020-024

Zhang, Y., Ding, J., Ren, S., Wang, W., Yang, Y., Li, S., et al. (2020). Intravenous infusion of human umbilical cord Wharton's jelly-derived mesenchymal stem cells as a potential treatment for patients with COVID-19 pneumonia. *Stem Cell Res. Ther.* 11:207. doi: 10.1186/s13287-020-01725-4

Zhou, J., Wang, Y., Zhao, J., Gu, L., Yang, C., Wang, J., et al. (2020). . The metabolic and immunological characteristics of pregnant women with COVID-19 and their neonates. *Eur J Clin. Microbiol. Infect. Dis.* doi: 10.1007/s10096-020-04033-0 Online ahead of print

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 12 February 2021
doi: 10.3389/fphys.2021.612319

SARS-COV-2 and Ocular Surface: From Physiology to Pathology, a Route to Understand Transmission and Disease

*Dalton de Freitas Santoro[1], Luciene Barbosa de Sousa[1], Niels O. S. Câmara[2,3], Denise de Freitas[1] and Lauro Augusto de Oliveira[1]**

[1] Department of Ophthalmology and Visual Science, Federal University of São Paulo, São Paulo, Brazil, [2] Department of Immunology, Institute of Biomedical Sciences IV, University of São Paulo, São Paulo, Brazil, [3] Nephrology Division, Federal University of São Paulo, São Paulo, Brazil

Coronaviruses gained public attention during the severe acute respiratory syndrome (SARS) outbreak in East Asia in 2003 and spread of Middle Eastern respiratory syndrome (MERS) in 2012. Direct human-to-human contact and droplet are the main methods of transmission. Viral stability in aerosols on different surfaces supports evidence on indirect viral acquisition from fomites through the mucous membranes of the mouth, nose, and eyes. Given the pandemic circumstances, the level of evidence in COVID-19 and ophthalmology regarding eye infection, conjunctival transmission, and viral shedding through tears is insufficient. Presently, conjunctival transmission of coronaviruses has not been confirmed and remains controversial. Considering the physiology of the lacrimal system and ocular surface, the eyes are considered an immunoprotective site, with several antiviral molecules and anti-inflammatory proteins. Nevertheless, they represent an interface with the exterior world and face daily putative aggressors. Understanding the host's ocular surface immunological and protective environment is crucial to clarify the potential of the conjunctiva as an entry route for SARS-CoV-2 and as part of this viral infection. We will discuss hypothetical ocular surface transmission mechanisms and related counterarguments addressed to both angiotensin-converting enzyme 2 receptors found on the conjunctival and corneal epithelia and lactoferrin, lysozyme, lipocalin and secretory IgA levels in the tear film. Hopefully, we will promote better understanding of this organ in COVID-19 infection and the potential transmission route that can be helpful in setting recommendations on best practices and protective guidelines to mitigate the disease spread.

Keywords: COVID-19, SARS-COV-2, ocular surface, ACE2 receptor, conjunctiva, IgA, tear film, lactoferrin

OPEN ACCESS

Edited by:
Adriana Castello Costa Girardi, University of São Paulo, Brazil

Reviewed by:
Keila Monteiro De Carvalho, State University of Campinas, Brazil
Friedrich Paulsen, University of Erlangen Nuremberg, Germany

***Correspondence:**
Lauro Augusto de Oliveira
laoliveira@unifesp.br; laopadilha@gmail.com
orcid.org/0000-0002-7384-9342

Specialty section:
This article was submitted to Clinical and Translational Physiology, a section of the journal Frontiers in Physiology

Received: *30 September 2020*
Accepted: *22 January 2021*
Published: *12 February 2021*

Citation:
de Freitas Santoro D, de Sousa LB, Câmara NOS, de Freitas D and de Oliveira LA (2021) SARS-COV-2 and Ocular Surface: From Physiology to Pathology, a Route to Understand Transmission and Disease. Front. Physiol. 12:612319. doi: 10.3389/fphys.2021.612319

INTRODUCTION

In December 2019, a new type of respiratory disease emerged in China, specifically in Wuhan province, with several reports of new daily cases showing that the new disease was rapidly spreading. On January 30, 2020, the World Health Organization (WHO) declared the new disease, named coronavirus disease 2019 (COVID-19), a public health emergency of international interest and,

on March 11, 2020, a pandemic. Three months after the first case and declaration of a pandemic condition, the world saw the power of this new virus, named SARS-CoV-2, belonging to an already known family of coronaviruses. Although SARS-CoV-2 has similarities to SARS-CoV, the causative agent of SARS in 2003, and MERS-CoV, the causative agent of MERS in 2012, it seems to be more effective in its transmission. To date, the number of infected individuals worldwide is 56.684.638, and the total number of deaths is 1.356.365 (COVID-19 dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU), 2020).

Therefore, understanding the aspects related to the transmission of the virus becomes crucial to contain the rapid spread of the disease and propose adequate safety protocols for the protection of health professionals and the entire population against COVID-19. The coronavirus family is known for transmission through contact with infected individuals, through the inhalation of droplets and aerosols expelled by them. However, since the transmission of SARS-CoV-2 is much higher, given its rapid dissemination to all continents, new and unclear forms of transmission need to be investigated. Likewise, understanding the peculiarities of the new virus and host invasion mechanisms are of paramount importance.

On January 22, 2020, a respiratory disease specialist from the Joint Commission of the WHO on a mission in Wuhan declared that he contracted COVID-19, even though he wore an N95 mask and gloves but did not wear goggles, starting the disease with symptoms of conjunctivitis, increasing the risk of ocular transmission of SARS-CoV-2 (Dai, 2020). The ocular surface, being exposed to aerosols and droplets, could be the route of transmission of the new coronavirus through direct penetration of the virus into the epithelial cells of the conjunctiva and cornea. Additionally, through the nasolacrimal drainage system, the virus could be drained by the tear film through the nasolacrimal duct to the upper airways, initiating transmission.

This review aimed to discuss hypothetical ocular surface transmission mechanisms based on known pathogenic requirements by the virus to penetrate host cells and related counterarguments addressed to angiotensin-converting enzyme 2 (ACE2) receptors found on the conjunctival and corneal epithelia as well as to lactoferrin, secretory IgA, lysozyme and lipocalin levels in the tear film.

CORONAVIRUS FAMILY

The coronavirus family has three subtypes already identified: alpha, beta, and gamma. Alpha and beta subtypes are causative agents of diseases in mammals, while gamma subtype affects birds (Fenner et al., 1974). Two alpha coronaviruses are related to human diseases: human coronavirus 229E and human coronavirus NL63. From the beta subtype, human coronavirus HKU1, human coronavirus OC43 (the most common of all human coronaviruses), SARS-CoV, MERS-CoV, and SARS-CoV-2 are known to be the causative agents of human diseases (Fenner et al., 1974).

A common anatomical characteristic of this family of viruses is the lipid membrane that envelops and confers its structure. Besides that, all coronaviruses have structural proteins, non-structural proteins, and accessory membrane proteins. The spike protein is attached to the envelope of the virus and represents the key element to bind with the host receptor. They are single-stranded RNA viruses, and their name is derived from the resemblance to a crown that the spike proteins confer on the surface of the virus (Wertheim et al., 2013).

Understanding the diseases caused by this family of viruses to animals might be helpful in elucidating the behavior and mutation ability of those that affect humans. For example, in rats, there are two distinct, well-defined biotypes: a variant that causes mainly gastrointestinal diseases and another that affects multiple organs. In cats, feline coronaviruses (FeCoVs) are less aggressive and present tropism to the apical epithelium of the intestine. However, 5% of the cases will develop peritonitis, a serious disease with increased mortality. FeCoVs are believed to mutate, becoming feline infectious peritonitis viruses (FIPVs) that cause vasculitis. Ocular findings from cats contaminated with FIPV resemble cases of vasculitis, such as granulomatous uveitis, choroiditis, and retinal detachment. High culture growth rate (90%), including viable virus identification, from conjunctival samples suggest the risk of ocular transmission (Willcox et al., 2020). Thus, the possibility of ocular involvement in humans and viral transmission through this route must be considered.

Among all coronaviruses that affect humans, HCoV NL63 caused conjunctivitis in children in 17% of cases (Seah and Agrawal, 2020). SARS was the first pandemic of the twenty-first century, in 2004, and no ocular involvement was reported (LeDuc and Barry, 2004); however, Loon et al. (2004) demonstrated the presence of viral particles by conjunctival polymerase chain reaction (PCR) even without associated conjunctivitis. During the MERS outbreak, there were also no reports of ocular involvement (Loon et al., 2004).

Genetic studies have shown that SARS-CoV-2 has 90% genetic similarity to the bat's coronavirus (CoV RATG13), suggesting that they might be the natural reservoir for these viruses and that COVID-19 is possibly a zoonosis (Zhou P. et al., 2020). Mackenzie and Smith (2020) and Guo et al. (2020) found 79% genetic similarity between SARS-CoV-2 and SARS-CoV. Lu et al. (2020) found 50% similarity between MERS-CoV and SARS-CoV-2. Thus, knowledge acquired from previous epidemics are guiding current investigations to elucidate SARS-CoV-2 potential similarity in pathophysiological mechanisms of penetration into host cells and its increased transmissibility.

RENIN-ANGIOTENSIN SYSTEM AND OCULAR SURFACE AREA

SARS-CoV-2 needs the ACE2 receptor to be able to invade host cells, like SARS-CoV (Hoffmann et al., 2020; Lan et al., 2020). The renin-angiotensin system (RAS) is an important modulator of the volume of body fluid and directly involved in the regulation of systemic blood pressure. Angiotensin is produced by the liver and converted into angiotensin 1 by renin. Subsequently, angiotensin 1 is converted into angiotensin 2 by ACE. Angiotensin 2 binds to various ACE receptors

(subtypes 1 and 2) distributed in various tissues. There is also local RAS tissue that can locally produce various components of the renin-angiotensin system but has specific functions in each organ. The RAS tissue has already been identified in the heart, lung, reproductive system, central nervous system, gastrointestinal system, breast, pancreas, and adrenal and eye tissues (Yaguchi et al., 2012).

The ocular RAS components have been identified in the cornea, conjunctiva, sclera, ciliary body, retina, aqueous humor, vitreous humor, and iris (Yaguchi et al., 2012; Giese and Speth, 2014; Vaajanen et al., 2015). Danser et al. (1989) demonstrated the inability of the renin-angiotensin plasma enzymes to penetrate into the eye, and Wagner et al. (1996) demonstrated the local production of the RAS components in ocular tissues and the importance of this system in the control of ocular physiology (Danser et al., 1989; Wagner et al., 1996). Ocular RAS has been linked to several eye diseases: diabetic retinopathy, glaucoma, age-related macular degeneration, uveitis, cataracts, and dry eye syndrome (Holappa et al., 2017).

As SARS-CoV-2 requires ACE2 receptors to penetrate host cells, the presence of ocular RAS raise the hypothesis of COVID-19 transmission through the ocular surface route.

OCULAR SURFACE ROUTE TO SARS-COV-2

The ocular surface, formed by epithelial cells of the cornea and conjunctiva, is exposed to the environment and, therefore, can be contaminated by droplets and aerosols of individuals infected with SARS-CoV-2 or even by contact with contaminated fomites. Additionally, the ocular surface is connected to the upper airways via the nasolacrimal duct and although less likely could receive viruses ascending through this path (Willcox et al., 2020).

Interestingly, during the influenza virus epidemic of 1919, Maxcy hypothesized that the ocular surface, which presents a surface area larger than that of the nostrils and oral cavity, would be more prone to infection by contaminated particles (Maxcy, 1919). Moreover, after comparing the ocular surface area not exposed during blinking and the area not exposed during mouth closure, Coroneo MT found that the surface of the conjunctiva and cornea was noticeably more exposed (Coroneo, 2021). However, it is also important to note that both the oral cavity and nostrils act as a continuous aerosol aspiration pump during inspiration, whereas the ocular surface has no such function and instead requires particles to be directly introduced through aerosols sprayed against it.

SARS-CoV-2 requires the presence of ACE2 receptors to penetrate host cells, which occurs through the binding of viral spike protein to the host's ACE2 receptor. It has also been demonstrated that a protease on the host cell surface, TMPRSS2, also known as Furin protein, promotes the priming of the S subunit of the spike protein in S1, which is related to the binding of the ACE receptor, and S2, which is related to the fusion of the virus lipid membrane with the cell membrane (Hoffmann et al., 2020). Without TMPRSS2, the virus can penetrate host cells via endocytosis but in a much less efficient way (Matsuyama et al., 2005; **Figure 1**).

A few studies already demonstrated ACE2 receptors in the cornea and conjunctiva, which in theory accomplish

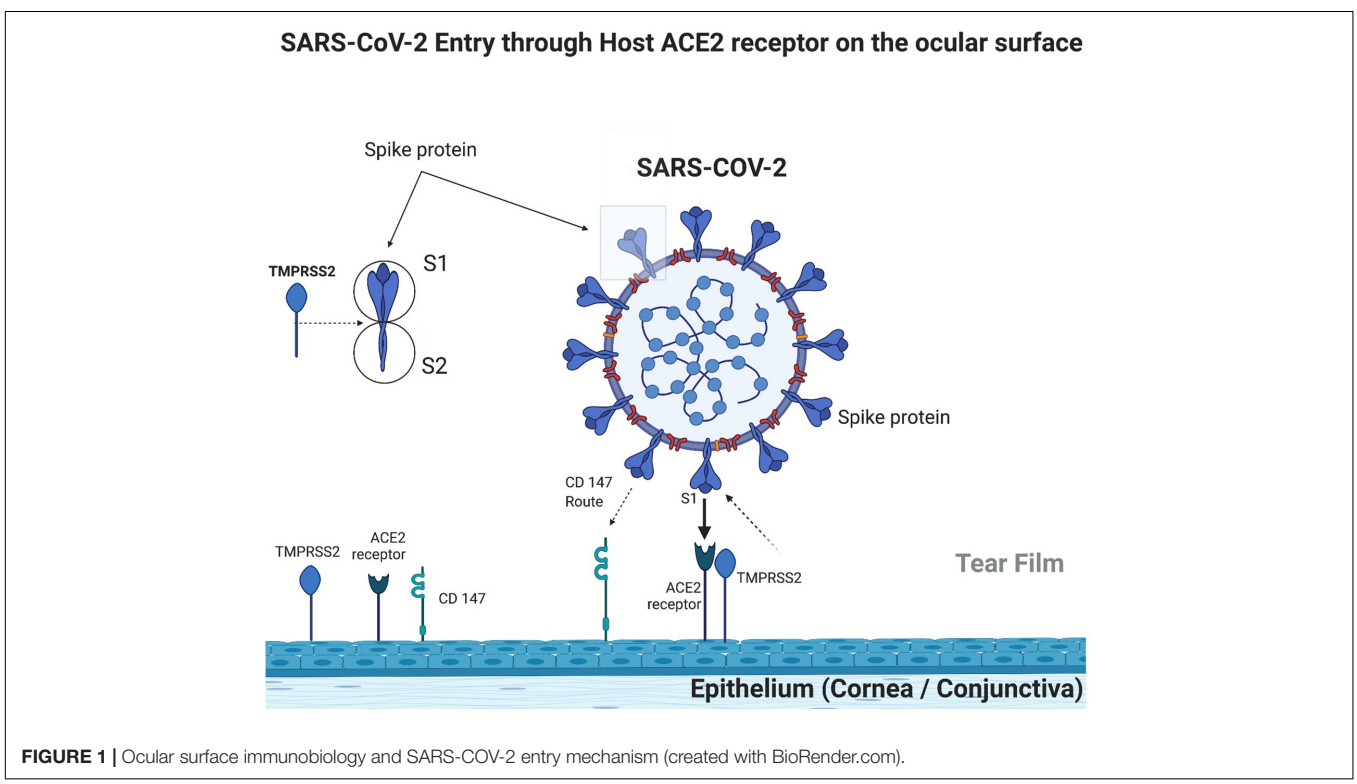


FIGURE 1 | Ocular surface immunobiology and SARS-COV-2 entry mechanism (created with BioRender.com).

SARS-CoV-2 requirement to invade host cells in the ocular surface. Zhou L. et al. (2020) demonstrated the positivity of ACE2 receptors in the corneal epithelium and endothelium, although absent in the corneal stroma. They showed a more expressive positivity in the limbus than in the superficial cells of the cornea (Zhou L. et al., 2020). Such findings were also observed by Ma et al. (2020). Collin et al. (2021) also found positive ACE2 receptors in the corneal epithelium, with higher expression in the limbus. Zhou L. et al. (2020) demonstrated the positivity of ACE2 receptors in the conjunctiva, mainly in the superficial cells, but absent in goblet cells and rarely found below the epithelium, in the substantia propria. Ma et al. (2020) found greater expression of ACE2 receptors in the conjunctiva than in the cornea, suggesting greater probability of SARS-CoV-2 penetration in the conjunctiva rather than in the cornea. Likewise, Leonardi et al. (2020) also found positivity for ACE2 receptors in the conjunctiva in low expression but greater than in the cornea.

As cellular invasion is facilitated by the protease TMPRSS 2 (Furin), its expression in ocular tissues is important to evaluate the ocular surface as a route of infection by SARS-CoV-2. Zhou L. et al. (2020) found strong positivity of the Furin protease in the corneal epithelium and endothelium; however, unlike ACE2 receptors, it was also found in the corneal stroma and all limbus layers. Collin et al. (2021) also demonstrated positivity of Furin protease in the central and peripheral corneal epithelium and the limbus.

Zhou L. et al. (2020) found strong positivity of Furin protease in conjunctival epithelial cells and weak positivity in the stroma, being absent in goblet cells. Conversely, Ma et al. (2020) found extremely low expression of Furin protease in the conjunctiva in relation to the cornea, suggesting that invasion more likely occurs in the corneal epithelium rather than in the conjunctiva. Leonardi et al. (2020) found expression of TMPRSS2 protease only in the conjunctiva but not in the cornea. Collin et al. (2021) found positivity with high expression in the superficial and basal conjunctival epithelium.

Again, both the cornea and conjunctiva with TMPRSS protease (Furin) expression show the favorable elements for the invasion by SARS-CoV-2, thus making this transmission route possible.

Although there are similarities between SARS-CoV-2 and SARS-CoV, it is known that SARS-CoV-2 binds more avidly to ACE2 receptors. So far, this suggests the role of other factors involved in SARS-CoV-2 adhesion efficiency. Wang et al. (2020) demonstrated that SARS-CoV-2 invades host cells through a new route, using CD147, which is a transmembrane glycoprotein from the immunoglobulin family. Leonardi et al. (2020) found positivity in intermediate CD147 levels in both the cornea and conjunctiva. Thus, SARS-CoV-2 could invade the ocular surface through this transmembrane glycoprotein (CD147) route (**Figure 1**).

COVID-19 AND OCULAR MANIFESTATIONS

It is interesting that the ocular surface accomplishes known requirements for SARS-CoV-2 invasion but local viral detection and ocular manifestations in patients diagnosed with COVID-19 are rare. This may not be a preferred site of viral entry and neither for its replication. Several studies have searched for viral particles in the conjunctiva (conjunctival swabs) and tear film (Schirmer strips, microcapillary tubes, and tear washing) using molecular techniques and mostly reported low and variable positivity. Sarma et al. (2020) conducted a meta-analysis and reported that the positivity of the conjunctiva or tear samples was 1.95% using molecular techniques (PCR). The authors discussed that the low positivity might be related to the date of sampling, at a time when the viral replication in the conjunctiva or tear may be absent, especially in more severe disease. Another issue discussed in a previous study regarding low positivity testing is the small amount of material obtained from both the conjunctiva and tear film. Worse yet, this small amount is usually diluted in buffer solution before running the molecular test. Zhang et al. (2020) found only one patient with conjunctival PCR positive for SARS-CoV-2 in 72 patients with confirmed diagnosis of COVID-19 by nasopharyngeal swab, who presented symptoms of conjunctivitis, suggesting that the positivity is greater when there is ocular involvement. Seah et al. (2020) conducted a viral shedding study using conjunctival swab in 17 patients with confirmed diagnosis of COVID-19 (total of 64 samples over the first, second, and third weeks after symptom onset). None of the patients had ocular symptoms, except one who developed hyperemia and chemosis during follow-up, and all analyzed samples were negative. Xie et al. (2020) analyzed conjunctival swab from 33 patients one week after systemic symptom onset, and none of the patients in this series presented ocular manifestations, and in two patients, the conjunctival sample showed a positive result. Wu et al. (2020) reported that 2 of 11 patients with ocular manifestations and confirmed diagnosis of COVID-19 by nasopharyngeal swab presented positive PCR in the conjunctival swab, suggesting that, even in patients with ocular alterations, the positivity is low.

Regarding ocular clinical findings, Sarma et al. (2020) found follicular conjunctivitis, which is common to other viral infections, in 3.17% of all patients confirmed with COVID-19 and 0.7% of these patients presented ocular manifestation as the first symptom. Other symptoms reported in this meta-analysis were conjunctival congestion (3.8%), foreign body sensation (19%), excessive tearing (13.3%), eye pain (5.7%) and increased conjunctival secretion (3.8%). To date, there are no studies on visual loss in any patient diagnosed with COVID-19 (Sarma et al., 2020).

Torres-Costa et al. hypothetically suggested that SARS-CoV-2 may have a neurotropism common to the coronavirus family and therefore affect ocular neurological structures (Torres-Costa et al., 2020). In fact, Marinho et al. (2020) found retinal changes in 12 patients with confirmed diagnosis of COVID-19 (nasopharyngeal PCR and antibody detection) using optical coherence tomography to analyze the retinal layers. All patients showed hyper-refringent lesions at the level of the ganglion cells and inner plexiform layer, mainly in the papillary-macular bundle, but without visual alteration, which suggests the possibility of neurological involvement (Marinho et al., 2020).

Colavita et al. (2020) found positive samples of conjunctival PCR in the first patient diagnosed with COVID-19 in Italy, presenting bilateral follicular conjunctivitis since the onset of

systemic symptoms. Interestingly, the PCR from the conjunctival samples showed positive results until 21 days after disease onset, with a progressive decrease in the viral concentration over time. Moreover, to prove viable viruses with ocular transmission, the material collected from the first positive sample was inoculated into Vero E6 cells, and there was viral replication confirmed by PCR, suggesting the potential risk of infection through the eye.

Regarding ophthalmologists' exposure and their potential role as spreaders, Coroneo MT determined that a proximity of approximately 38 cm between ophthalmologists and patients during slit lamp exam would place them at risk for transmission given that viral particles could easily travel through aerosols expelled during breathing and speech at distances of 30 cm (Coroneo, 2021). Rokohl et al. (2020) also expressed their concern regarding the need for ophthalmologic offices to adopt protective measures, such as protective barriers in the slit lamp and mandatory disinfection of all ophthalmic equipment coming into direct contact with patients at the end of each medical appointment, to mitigate SARS-COV-2 spread.

OCULAR SURFACE IMMUNOLOGICAL AND PROTECTIVE ENVIRONMENT AGAINST VIRAL INFECTION

A healthy human tear film provides oxygen and nutrients and contains a series of proteins that together provide a protective barrier against the invasion of various pathogens. In this scenario, a few components stand out and will be discussed: lactoferrin, immunoglobulin A (IgA), lysozyme, and lipocalin. We will also discuss in this section the tear film clearance associated to blinking rate and amount of ACE2 receptors in the ocular surface compared to other organs.

Lactoferrin, first described by Masson et al. (1966), gained notorious importance for its anti-inflammatory, antibacterial, and antiviral actions. It is a mammalian glycoprotein secreted by the exocrine glands and neutrophils. One of its immunoprotective property is associated with iron chelation, which results in hololactoferrin. Iron has an important role in cellular oxidation-reduction reactions and oxygen transport. It is also essential in DNA replication and energy generation. Thus, it is crucial for the microorganism's survival and replication. Lactoferrin acts as an anti-inflammatory agent by sequestering iron molecules (Kell et al., 2020).

Lactoferrin's antiviral action has already been proven in relation to human immunodeficiency virus (HIV), rotavirus, herpes simplex virus, and human papillomavirus (Kell et al., 2020). A few mechanisms are involved in its antiviral response: direct competition to receptors used by viruses for cell invasion; direct linkage to some viral particles; and ability to bind to heparan sulfate, which serves as the first viral anchorage site in cell membranes and facilitates cell invasion by pathogens. Lang et al. (2011) demonstrated that lactoferrin markedly inhibits the invasion of SARS-CoV pseudovirus (Lang et al., 2011) probably by binding to heparan sulfate, which serves as a link to sites of low anchoring of the SARS-CoV to the ACE2 receptor (Lang et al., 2011). As SARS-CoV and SARS-CoV-2 present an increased genetic similarity (75%) and use ACE2 receptors, it seems reasonable that heparan sulfate could work as the first SARS-CoV-2 cell membrane contact. These corroborates that lactoferrin, which is normally found in the tear film, presents an important antiviral mechanism acting as a barrier to the invasion of this virus through the eye.

Regarding IgA, this immunoglobulin is largely distributed in the mucous membranes in the body and is the most abundant immunoglobulin in the human tear film. Lacrimal IgA is mainly produced by the lacrimal gland, and its concentration rapidly increases in the presence of pathogens that attack the ocular surface. Its protective action has already been proven against viruses by preventing its binding to host's cell receptor, against bacteria by preventing its attachment and colonization, and against amoebae (O'Sullivan and Montgomery, 2015). The IgA antibody binds to SARS-CoV-2 spike protein, blocking its interaction with the host's ACE2 receptor, thus preventing the virus from entering human cells. IgA seroconversion in patients with COVID-19 can already be detected after 2 days of infection, and after the first month of infection, the seroconversion positivity is approximately 100% (Yu et al., 2020). With this background, IgA in the tear film is another important defense mechanism against aggression to the ocular surface, making it less likely that SARS-CoV-2 will invade the eye. Yu et al. (2020) demonstrated the highest serum positivity of IgA antibody in patients with severe COVID-19, suggesting the possibility of an immune-mediated disease through deposition of IgA and vasculitis.

Lysozyme, discovered by Alexander Flemming in 1922 (Tan and Tatsumura, 2015), is produced by the accessory tear glands and has already proven its activity against bacteria (Masschalck and Michiels, 2003), viruses (Lee-Huang et al., 1999), and fungi (Samaranayake et al., 2001). Ferrari et al. (1959) reported the effects of lysozyme against several viruses known at that time as herpes simplex and herpes zoster (Ferrari et al., 1959). Lee-Huang et al. (1999) demonstrated the inhibition of HIV replication by lysozyme. The effectiveness of this enzyme against SARS-CoV-2 has not yet been demonstrated, and further studies are needed to elucidate whether it can also act as a barrier to the entrance of SARS-CoV-2 through the ocular surface. However, hypothetically, it is likely that its presence in the tear film has some effect on blocking the entrance of SARS-CoV-2 through the cornea and conjunctiva.

Lipocalins are a family of diverse, low molecular weight proteins that predominantly function extracellularly. Tear lipocalin, first reported by Erickson in 1956 (Erickson, 1956), is present in large quantities in tear film, although not a tear-specific protein. Flower (1996) reported a few common characteristics of these proteins, including (1) their ability to bind to smaller, mainly hydrophobic, molecules, (2) their ability bind to specific receptors on the surface of cells, and (3) the formation of macro-molecular complexes (Flower, 1996). Tear lipocalin is a multitasking protein whose functions and receptors are yet to be fully elucidated. Dartt reported a few already identified functions, which include participating in the regulation of the immune system, modulation of cell growth, and metabolism of apolipoprotein-D (Dartt, 2011). Moreover, lipocalin possesses

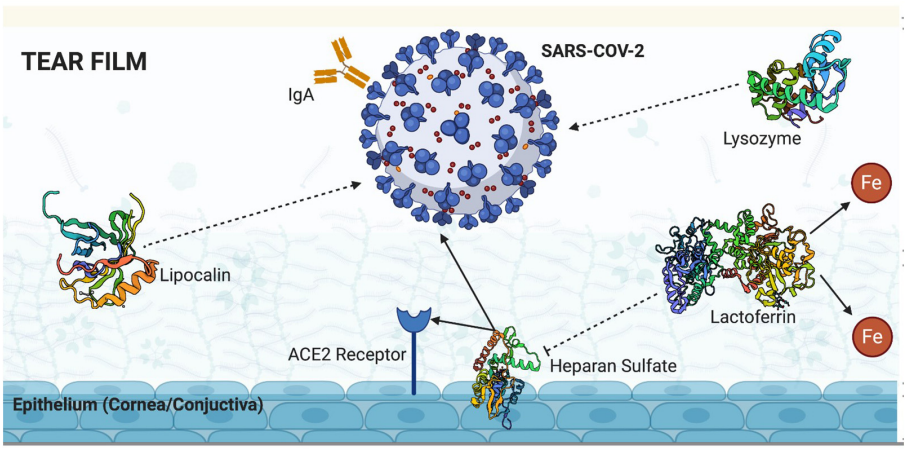


FIGURE 2 | Ocular surface immunological and protective environment against virus infection (created with BioRender.com).

antimicrobial activity that interferes with microbial siderophores, thereby decreasing the absorption of iron essential for their replication. Furthermore, it has a proven antibacterial and antifungal activity, and a potential antiviral action as well (Fluckinger et al., 2004). Nonetheless, further studies are needed to determine whether lipocalin can also interfere with SARS-COV-2 invasion of the ocular surface (**Figure 2**).

The act of blinking also plays a role as a protective mechanism against the invasion of pathogens in the ocular surface. Tsubota et al. reported that the human blink rate under relaxed conditions is up to 22 blinks/min (Tsubota and Nakamori, 1993). Blinking provides a continuous washing mechanism for microorganisms on the ocular surface, clearing them by the lacrimal duct (McClellan, 1997). Thus, a normal blink rate also decreases invasion of SARS-CoV-2 through the ocular surface. The retention time of the particles that penetrate the tear film has been related to its turnover rate, which depends on several factors, such as blink rate per minute, tear volume, climatic conditions (e.g., air humidity, environmental temperature, and wind), the use of contact lenses, and exposure time to digital screens. Local conditions (e.g., Meibomian gland dysfunction), systemic conditions (e.g., rheumatological diseases) affecting tear production, and systemic medications leading to decreased tear production can also interfere with tear film turnover and the retention time of viral particles within the tear (Pearce et al., 2011; Rolando et al., 2018). As a counterargument, since SARS-CoV-2 is mostly transmitted through the airways, the act of blinking itself could work as a pump propelling the viruses that are on the surface of the cornea and conjunctiva to the upper airways, through the nasolacrimal duct, thus allowing its transmission through the eye.

The tear film is drained through the tear punctum by continuous blinking, with the drainage occurring through the lower tear punctum. Tear drainage occurs through absorption mostly by the nasolacrimal drainage system (including tear ducts and the nasolacrimal gland until it reaches the upper airways) and to a lesser extent by the cornea and conjunctiva. However, a certain amount of tear film is also absorbed by the nasolacrimal duct tissue. Paulsen et al. (1998) described the entire drainage system of the tear film through the lacrimal canaliculi, lacrimal sac, and nasolacrimal ducts using histological, immunohistochemical, and electron microscopic assays. The authors demonstrated the presence of isolated goblet cells integrated into the epithelial cells of the lacrimal canaliculi and the presence of grouped goblet cells in the lacrimal sac, forming mucous glands (Paulsen et al., 1998). Goblet cells are known to produce mucin, a glycoprotein that not only lubricates and hydrates the mucosa of the tear drainage system but also functions as a barrier against harmful agents (Willcox et al., 2017). Paulsen et al. (1998) also demonstrated the presence of IgA throughout the tear drainage system, an allied antibody in the mucosal defense system that acts as a protective factor against pathogen invasion (Paulsen et al., 1998). Therefore, although the tear drainage system through the lacrimal canaliculi, lacrimal sac, and nasolacrimal duct can be contaminated by viral particles present in the tear film, a relevant mucosal defense system, mainly consisting of mucin and IgA that act as barriers preventing invasion through the epithelium of this drainage system, does exist. In addition, Ayub et al. (2003) demonstrated that the cavernous body of the lacrimal sac and lacrimal duct interferes in the tear drainage system through an autonomous response that is integrated to a complex innervation network with the cornea. Thus, an injured cornea (e.g., foreign body, inflammation, and infection) would increase both tear production and its outflow, consequently and actively draining harmful materials and protecting the ocular surface (Ayub et al., 2003).

Another factor that also deserves attention while investigating the ocular surface as a possible transmission route for SARS-CoV-2 is the lower ACE2 receptor expression on the cornea and conjunctiva epithelia in relation to other tissues, such as the lung and heart (Willcox et al., 2020). Thus, by lowering

the requirements necessary for viral invasion, the ocular surface becomes less prone as a preferred route.

Miner et al. (2020) have just published a paper reporting another corneal immunological protection against viral infections. The authors demonstrated that type III interferon (IFN-l) and its receptor (IFNlR1) restrict herpes simplex virus 1 and Zika virus growth in the human cornea. Interestingly, they also demonstrated that human corneal tissue did not support SARS-COV-2 infection, even after blockade of the type III IFN receptor (Miner et al., 2020). Thus, supporting the fact that the ocular surface is less prone to viral invasion and replication.

LESSONS ON ADENOVIRAL CONJUNCTIVITIS AND SARS- AND MERS-ASSOCIATED CONJUNCTIVITIS

Knowledge on the pathophysiological mechanisms involved in other ocular viral infections, specially conjunctivitis, could contribute to a better understanding of the potentially pathological interaction between SARS-CoV-2 and the ocular surface. Adenovirus conjunctivitis (DNA virus) is the most common viral infection in the ocular surface. Conjunctivitis caused by adenovirus is usually a self-limiting condition. Clinical features can vary from sporadic manifestations, such as pharyngoconjunctival fever (most prevalent strains 3, 4, and 7) to epidemic outbreaks, such as epidemic keratoconjunctivitis (most prevalent strains 8, 19, 37, and 54) (Chigbu and Labib, 2018). Classically, but non-specifically, follicular conjunctival reaction, conjunctival congestion, foreign body sensation, excessive tearing, and preauricular adenomegaly are present. Interestingly, adenoviruses use several cellular receptors to bind to host cells (CAR, CD 46, and sialic acid interact with the fine-knob protein of the adenovirus) in the infection onset. Different adenovirus strains use distinct host's cell receptors. The known mechanisms of cell invasion are the clathrin-mediated endocytosis, micropinocytosis, and caveolin-mediated pathway, and depending on some types of cells, the virus can use more than one pathway to penetrate the host cell (Pennington et al., 2019). The most used receptor by the majority of strains is the coxsackievirus-adenovirus receptor (except subtype B and some members of subtype D). After cellular invasion, adenoviruses integrate their replication machinery to the host cell DNA, consequently replicating and infecting more cells. Adenovirus infection generates an immune response in both the cornea and conjunctiva. Superficial corneal epithelial cells produce cytokines, which stimulate stromal keratocytes to release lymphocytes recruiting interleukins. The levels of lymphocytes, natural killer cells, and monocytes significantly increase in the conjunctiva during the acute phase of infection. Conjunctival epithelial cells and goblet cells produce mucins (MUC1, MUC4, MUC-5, and MUC16) and cytokines known to decrease adenoviral invasion (Pennington et al., 2019). Thus, adenoviruses' pathophysiological mechanisms of ocular surface invasion are different from what is already known as SARS-COV-2 pathophysiological requirements to invade the ocular surface. Ocular manifestations of SARS-COV-2, although rare, when compared to those of adenoviruses are similarly non-specific (follicular conjunctival reaction, conjunctival congestion, foreign body sensation, and tearing).

Regarding knowledge on pathophysiological mechanisms of SARS-CoV and MERS-CoV pandemic, although they use the same ACE2 receptors that SARS-CoV-2 uses, we could not find reports on clinical manifestations of ocular surface involvement during the SARS and MERS pandemic even though the presence of viral particles has been demonstrated by conjunctival PCR during previous outbreaks in patients without conjunctivitis. However, SARS and MERS outbreaks have been helpful in allowing tailored investigation on host cell receptor requirements for COVID-19 invasion in different human tissues.

CONCLUSION REMARKS

The ocular surface gathers the essential and yet described elements for SARS-CoV-2 invasion through the eye. Molecular approaches have found viral particles in the tear film and conjunctiva. Associated ocular surface manifestations of conjunctivitis are non-specific and relatively rare during the current outbreak. Despite accomplishing required pathophysiological conditions for SARS-CoV-2 invasion, we discussed relevant protective mechanisms that make the eye a less likely route of infection and transmission of COVID-19. One cannot assure that the eye/ocular surface is a privileged organ strong enough against SARS-CoV-2, considering the conjunction of protective mechanisms discussed in this review. The level of evidence in COVID-19 and ophthalmology regarding eye infection, conjunctival transmission, and viral shedding through tears is still insufficient. Research on novel cell membrane molecules and receptors and other local immunoprotective mechanisms to clarify the potential of the conjunctiva acting as an entry route for SARS-CoV-2 are undertaken. Presently, conjunctival transmission of coronaviruses has not been confirmed and remains controversial. Nevertheless, recommendations on the best practices and protective guidelines to mitigate this disease propagation remain necessary, including intense and judicious care in the disinfection of all ophthalmic diagnostic equipment in direct contact with the ocular surface.

AUTHOR CONTRIBUTIONS

DFS: research design, data acquisition and/or research execution, and manuscript preparation. LS and DF: data analysis and/or interpretation and manuscript preparation. NC and LO: research design, data analysis and/or interpretation, and manuscript preparation. All authors contributed to the article and approved the submitted version.

FUNDING

NC is a recipient of a FAPESP Grant (2017/05264-7) and CNPq fellowship. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

REFERENCES

Ayub, M., Thale, A. B., Hedderich, J., Tillmann, B. N., and Paulsen, F. P. (2003). The cavernous body of the human efferent tear ducts contributes to regulation of tear outflow. *Invest. Ophthalmol. Vis. Sci.* 44, 4900–4907. doi: 10.1167/iovs.03-0493

Chigbu, D. I., and Labib, B. A. (2018). Pathogenesis and management of adenoviral keratoconjunctivitis. *Infect. Drug Resist.* 11, 981–993. doi: 10.2147/idr.s162669

Colavita, F., Lapa, D., Carletti, F., Lalle, E., Bordi, L., Marsella, P., et al. (2020). SARS-CoV-2 isolation from ocular secretions of a patient with COVID-19 in Italy with prolonged viral RNA detection. *Ann. Intern. Med.* 173, 242–243. doi: 10.7326/m20-1176

Collin, J., Queen, R., Zerti, D., Dorgau, B., Georgiou, M., Djidrovski, I., et al. (2021). Co-expression of SARS-CoV-2 entry genes in the superficial adult human conjunctival, limbal and corneal epithelium suggests an additional route of entry via the ocular surface. *Ocul. Surf.* 19, 190–200. doi: 10.1016/j.jtos.2020.05.013

Coroneo, M. T. (2021). The eye as the discrete but defensible portal of coronavirus infection. *Ocul. Surf.* 19, 176–182. doi: 10.1016/j.jtos.2020.05.011

COVID-19 dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU) (2020). *COVID-19 DATA IN MOTION*. Available online at: https://coronavirus.jhu.edu/map.html (accessed November, 18 2020).

Dai, X. (2020). *Peking University Hospital Wang Guangfa disclosed treatment status on Weibo and suspected infection without wearing goggles*. Xinjing Newpaper. Available online at: http://www.bjnews.com.cn/news/2020/01/23/678189. (accessed January 24, 2020).

Danser, A. H. J., Van Den Dorpel, M. A., Deinum, J., Derkx, F. H., Franken, A. A., Peperkamp, E., et al. (1989). Renin, prorenin, and immunoreactive renin in vitreous fluid from eyes with and without diabetic retinopathy. *J. Clin. Endocrinol. Metab.* 68, 160–167. doi: 10.1210/jcem-68-1-160

Dartt, D. A. (2011). Tear lipocalin: structure and function. *Ocul. Surf.* 9, 126–138. doi: 10.1016/s1542-0124(11)70022-2

Erickson, O. (1956). Albumins in lacrimal protein patterns of Erickson. *Stanford Med. Bull.* 14, 124–125.

Fenner, F., Ginsberg, H. S., Maurin, J., and Valenta, V. (1974). International committee on taxonomy of viruses. *Intervirology* 3, 193–198.

Ferrari, R., Callerio, C., and Podio, G. (1959). Antiviral activity of lysozyme. *Nature* 183, 548. doi: 10.1038/183548a0

Flower, D. R. (1996). The lipocalin protein family: structure and function. *Biochem. J.* 318, 1–14. doi: 10.1042/bj3180001

Fluckinger, M., Haas, H., Merschak, P., Glasgow, B. J., and Redl, B. (2004). Human tear lipocalin exhibits antimicrobial activity by scavenging microbial siderophores. *Antimicrob. Agents Chemother.* 48, 3367–3372. doi: 10.1128/AAC.48.9.3367-3372.2004

Giese, M. J., and Speth, R. C. (2014). The ocular renin–angiotensin system: a therapeutic target for the treatment of ocular disease. *Pharmacol. Ther.* 142, 11–32. doi: 10.1016/j.pharmthera.2013.11.002

Guo, Y. R., Cao, Q. D., Hong, Z. S., Tan, Y. Y., Chen, S. D., Jin, H. J., et al. (2020). The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. *Mil. Med. Res.* 7:11.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181: 271–280.e8.

Holappa, M., Vapaatalo, H., and Vaajanen, A. (2017). Many faces of renin-angiotensin system – focus on eye. *Open Ophthalmol. J.* 11, 122–142. doi: 10.2174/1874364101711010122

Kell, D. B., Heyden, E. L., and Pretorius, E. (2020). The biology of lactoferrin, an iron-binding protein that can help defend against viruses and bacteria. *Front. Immunol.* 11:1221. doi: 10.3389/fimmu.2020.01221

Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., et al. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 581, 215–220.

Lang, J., Yang, N., Deng, J., Liu, K., Yang, P., Zhang, G., et al. (2011). Inhibition of SARS Pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans. *PLoS One* 6:e23710. doi: 10.1371/journal.pone.0023710

LeDuc, J. W., and Barry, M. A. (2004). SARS, the first Pandemic of the 21st Century. *Emerg. Infect. Dis.* 10:e26. doi: 10.3201/eid1011.040797_02

Lee-Huang, S., Huang, P. L., Sun, Y., Huang, P. L., Kung, H. F., Blithe, D. L., et al. (1999). Lysozyme and RNases as anti-HIV components in beta-core preparations of human chorionic gonadotropin. *Proc. Natl. Acad. Sci. U.S.A.* 96, 2678–2681. doi: 10.1073/pnas.96.6.2678

Leonardi, A., Rosani, U., and Brun, P. (2020). Ocular surface expression of SARS-CoV-2 receptors. *Ocul. Immunol. Inflamm.* 28, 735–738. doi: 10.1080/09273948.2020.1772314

Loon, S. C., Teoh, S. C. B., Oon, L. L. E., Se-Thoe, S. Y., Ling, A. E., Leo, Y. S., et al. (2004). The severe acute respiratory syndrome coronavirus in tears. *Br. J. Ophthalmol.* 88, 861–863. doi: 10.1136/bjo.2003.035931

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., et al. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 395, 565–574. doi: 10.1016/s0140-6736(20)30251-8

Ma, D., Chen, C. B., Jhanji, V., Xu, C., Yuan, X. L., Liang, J. J., et al. (2020). Expression of SARS-CoV-2 receptor ACE2 and TMPRSS2 in human primary conjunctival and pterygium cell lines and in mouse cornea. *Eye* 34, 1212–1219. doi: 10.1038/s41433-020-0939-4

Mackenzie, J. S., and Smith, D. W. (2020). COVID-19: a novel zoonotic disease caused by a coronavirus from China: what we know and what we don't. *Microbiol. Aust.* 41:MA20013.

Marinho, P. M., Marcos, A. A. A., Romano, A. C., Nascimento, H., and Belfort, R. (2020). Retinal findings in patients with COVID-19. *Lancet* 395:1610.

Masschalck, B., and Michiels, C. W. (2003). Antimicrobial properties of lysozyme in relation to foodborne vegetative bacteria. *Crit. Rev. Microbiol.* 29, 191–214. doi: 10.1080/713610448

Masson, P. L., Heremans, J. F., and Dive, C. H. (1966). An iron-binding protein common to many external secretions. *Clin. Chim. Acta.* 14, 735–739. doi: 10.1016/0009-8981(66)90004-0

Matsuyama, S., Ujike, M., Morikawa, S., Tashiro, M., and Taguchi, F. (2005). Protease-mediated enhancement of severe acute respiratory syndrome coronavirus infection. *Proc. Natl. Acad. Sci. U.S.A.* 102, 12543–12547.

Maxcy, K. F. (1919). The transmission of infection through the eye. *J. Am. Med. Assoc.* 72, 636–639. doi: 10.1001/jama.1919.02610090020005

McClellan, K. A. (1997). Mucosal defense of the outer eye. *Surv. Ophthalmol.* 42, 233–246. doi: 10.1016/s0039-6257(97)00090-8

Miner, J. J., Platt, D. J., Ghaznavi, C. M., Chandra, P., Santeford, A., Menos, A. M., et al. (2020). HSV-1 and Zika Virus but Not SARS-CoV-2 replicate in the human cornea and are restricted by corneal type III interferon. *Cell Rep.* 3:108339. doi: 10.1016/j.celrep.2020.108339

O'Sullivan, N. L., and Montgomery, P. C. (2015). Ocular mucosal immunity. *Mucosal. Immunol.* 2, 1873–1897. doi: 10.1016/b978-0-12-415847-4.00099-9

Paulsen, F., Thale, A., Kohla, G., Schauer, R., Rochels, R., Parwaresch, R., et al. (1998). Functional anatomy of human lacrimal duct epithelium. *Anat. Embryol.* 198, 1–12. doi: 10.1007/s004290050160

Pearce, E. I., Dorman, M., Wilkinson, B. C., and Oliver, K. M. (2011). Effect of blink frequency on tear turnover rate. *Invest. Ophthalmol. Vis. Sci.* 52, 3726.

Pennington, M. R., Saha, A., Painter, D. F., Gavazzi, C., Ismail, A. M., Zhou, X., et al. (2019). Disparate entry of adenoviruses dictates differential innate immune responses on the ocular surface. *Microorganisms* 7, 1–24.

Rokohl, A. C., Loreck, N., Wawer Matos, P. A., Mor, J. M., Zwingelberg, S., Grajewski, R. S., et al. (2020). . Die Rolle der Augenheilkunde in der COVID-19 Pandemie. *Ophthalmologe* 117, 642–647. doi: 10.1007/s00347-020-01148-9

Rolando, M., Cantera, E., Mencucci, R., Rubino, P., and Aragona, P. (2018). The correct diagnosis and therapeutic management of tear dysfunction: recommendations of the P.I.C.A.S.S.O. board. *Int. Ophthalmol.* 38, 875–895. doi: 10.1007/s10792-017-0524-4

Samaranayake, Y. H., Samaranayake, L. P., Pow, E. H., Beena, V. T., and Yeung, K. W. (2001). Antifungal effects of lysozyme and lactoferrin against genetically similar, sequential Candida albicans isolates from a human immunodeficiency virus-infected southern Chinese cohort. *J. Clin. Microbiol.* 39, 3296–3302. doi: 10.1128/jcm.39.9.3296-3302.2001

Sarma, P., Kaur, H., Kaur, H., Bhattacharyya, J., Prajapat, M., Shekhar, N., et al. (2020). *Ocular Manifestations and Tear or Conjunctival Swab PCR Positivity for 2019-nCoV in Patients with COVID-19: A Systematic Review and Meta-Analysis.* Available at: https://ssrn.com/abstract=3566161 (Accessed March 30, 2020).

Seah, I., and Agrawal, R. (2020). Can the coronavirus disease 2019 (COVID-19) Affect the eyes? A review of coronaviruses and ocular implications in humans

and animals. *Ocul. Immunol. Inflamm.* 28, 391–395. doi: 10.1080/09273948.2020.1738501

Seah, I. Y. J., Anderson, D. E., Kang, A. E. Z., Wang, L., Rao, P., Young, B. E., et al. (2020). Assessing viral shedding and infectivity of tears in coronavirus disease 2019 (COVID-19) patients. *Ophthalmology* 127, 977–979. doi: 10.1016/j.ophtha.2020.03.026

Tan, S. Y., and Tatsumura, Y. (2015). Alexander fleming (1881-1955): discoverer of penicillin. *Singapore Med. J.* 56, 366–367. doi: 10.11622/smedj.2015105

Torres-Costa, S., Lima-Fontes, M., Falcão-Reis, F., and Falcão, M. (2020). SARS-COV-2 in ophthalmology: current evidence and standards for clinical practice. *Acta Med. Port.* 33, 593–600.

Tsubota, K., and Nakamori, K. (1993). Dry eyes and video display terminals. *N. Engl. J. Med.* 328:584. doi: 10.1056/nejm199302253280817

Vaajanen, A., Kalesnykas, G., Vapaatalo, H., and Uusitalo, H. (2015). The expression of Mas-receptor of the renin–angiotensin system in the human eye. *Graefes Arch. Clin. Exp. Ophthalmol.* 253, 1053–1059. doi: 10.1007/s00417-015-2952-z

Wagner, J., Jan Danser, A. H., Derkx, F. H., de Jong, T. V., Paul, M., Mullins, J. J., et al. (1996). Demonstration of renin mRNA, angiotensinogen mRNA, and angiotensin converting enzyme mRNA expression in the human eye: evidence for an intraocular renin-angiotensin system. *Br. J. Ophthalmol.* 80, 159–163. doi: 10.1136/bjo.80.2.159

Wang, K., Chen, W., Zhou, Y.-S., Lian, J.-Q., Zhang, Z., Du, P., et al. (2020). SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. *bioRxiv* [Preprint]. doi: 10.1101/2020.03.14.988345

Wertheim, J. O., Chu, D. K. W., Peiris, J. S. M., Kosakovsky Pond, S. L., and Poon, L. L. M. (2013). A case for the ancient origin of coronaviruses. *J. Virol.* 87, 7039–7045.

Willcox, M. D. P., Argüeso, P., Georgiev, G. A., Holopainen, J. M., Laurie, G. W., Millar, T. J., et al. (2017). TFOS DEWS II Tear Film Report. *Ocul. Surf.* 15, 366–403.

Willcox, M. D. P., Walsh, K., Nichols, J. J., Morgan, P. B., and Jones, L. W. (2020). The ocular surface, coronaviruses and COVID-19. *Clin. Exp. Optom.* 103, 418–424. doi: 10.1111/cxo.13088

Wu, P., Duan, F., Luo, C., Liu, Q., Qu, X., Liang, L., et al. (2020). Characteristics of ocular findings of patients with coronavirus disease 2019 (COVID-19) in Hubei Province. China. *JAMA Ophthalmol.* 138, 575–578. doi: 10.1001/jamaophthalmol.2020.1291

Xie, H.-T., Jiang, S.-Y., Xu, K.-K., Liu, X., Xu, B., Wang, L., et al. (2020). SARS-CoV-2 in the ocular surface of COVID-19 patients. *Eye Vis.* 7, 7–9.

Yaguchi, S., Ogawa, Y., Shimmura, S., Hatou, S., Nakamura, S., Inaba, T., et al. (2012). Presence and physiologic function of the renin-angiotensin system in mouse lacrimal gland. *Invest. Ophthalmol. Vis. Sci.* 53, 5416–5425.

Yu, H. Q., Sun, B. Q., Fang, Z. F., Zhao, J. C., Liu, X. Y., Li, Y. M., et al. (2020). Distinct features of SARS-CoV-2-specific IgA response in COVID-19 patients. *Eur. Respir. J.* 56:2001526.

Zhang, X., Chen, X., Chen, L., Deng, C., Zou, X., Liu, W., et al. (2020). The evidence of SARS-CoV-2 infection on ocular surface. *Ocul. Surf.* 18, 360–362. doi: 10.1016/j.jtos.2020.03.010

Zhou, L., Xu, Z., Castiglione, G. M., Soiberman, U. S., Eberhart, C. G., and Duh, E. J. (2020). ACE2 and TMPRSS2 are expressed on the human ocular surface, suggesting susceptibility to SARS-CoV-2 infection. *bioRxiv* [Preprint]. doi: 10.1101/2020.05.09.086165

Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579, 270–273. doi: 10.1038/s41586-020-2012-7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a shared affiliation, though no other collaboration, with one of the authors NC at the time of review.

frontiers in Physiology

ORIGINAL RESEARCH
published: 15 February 2021
doi: 10.3389/fphys.2021.617657

Estimating COVID-19 Pneumonia Extent and Severity From Chest Computed Tomography

Alysson Roncally Silva Carvalho[1,2,3], Alan Guimarães[2], Thiego de Souza Oliveira Garcia[4], Gabriel Madeira Werberich[5], Victor Fraga Ceotto[6], Fernando Augusto Bozza[7,8], Rosana Souza Rodrigues[5,7], Joana Sofia F. Pinto[9], Willian Rebouças Schmitt[9], Walter Araujo Zin[3] and Manuela França[9,10]*

[1] Cardiovascular R&D Centre (UnIC), Department of Surgery and Physiology, Faculty of Medicine, University of Porto, Porto, Portugal, [2] Laboratory of Pulmonary Engineering, Biomedical Engineering Program, Alberto Luiz Coimbra Institute of Post-Graduation and Research in Engineering, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [3] Laboratory of Respiration Physiology, Carlos Chagas Filho Institute of Biophysics, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [4] Medical Faculty, Universidade Estácio de Sá, Rio de Janeiro, Brazil, [5] Department of Radiology, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [6] Hospital Niteroi D'Or, Niterói, Brazil, [7] D'Or Institute for Research and Education, Rio de Janeiro, Brazil, [8] National Institute of Infectious Disease, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [9] Radiology Department, Complexo Hospitalar Universitário do Porto, Porto, Portugal, [10] Instituto de Ciências Biomeìdicas Abel Salazar, Porto University, Porto, Portugal

OPEN ACCESS

Edited by:
Ravi Nistala, University of Missouri, United States

Reviewed by:
Teresa Pitts, University of Louisville, United States
Gabriele Valli, Azienda Ospedaliera San Giovanni Addolorata, Italy

***Correspondence:**
Alysson Roncally Silva Carvalho
roncally.carvalho@gmail.com; acarvalho@med.up.pt

Specialty section:
This article was submitted to Respiratory Physiology, a section of the journal Frontiers in Physiology

Received: *15 October 2020*
Accepted: *28 January 2021*
Published: *15 February 2021*

Citation:
Carvalho ARS, Guimarães A, Garcia TSO, Madeira Werberich G, Ceotto VF, Bozza FA, Rodrigues RS, Pinto JSF, Schmitt WR, Zin WA and França M (2021) Estimating COVID-19 Pneumonia Extent and Severity From Chest Computed Tomography. Front. Physiol. 12:617657. doi: 10.3389/fphys.2021.617657

Background: COVID-19 pneumonia extension is assessed by computed tomography (CT) with the ratio between the volume of abnormal pulmonary opacities (PO) and CT-estimated lung volume (CT_{LV}). CT-estimated lung weight (CT_{LW}) also correlates with pneumonia severity. However, both CT_{LV} and CT_{LW} depend on demographic and anthropometric variables.

Purposes: To estimate the extent and severity of COVID-19 pneumonia adjusting the volume and weight of abnormal PO to the predicted CT_{LV} (pCT_{LV}) and CT_{LW} (pCT_{LW}), respectively, and to evaluate their possible association with clinical and radiological outcomes.

Methods: Chest CT from 103 COVID-19 and 86 healthy subjects were examined retrospectively. In controls, predictive equations for estimating pCT_{LV} and pCT_{LW} were assessed. COVID-19 pneumonia extent and severity were then defined as the ratio between the volume and the weight of abnormal PO expressed as a percentage of the pCT_{LV} and pCT_{LW}, respectively. A ROC analysis was used to test differential diagnosis ability of the proposed method in COVID-19 and controls. The degree of pneumonia extent and severity was assessed with Z-scores relative to the average volume and weight of PO in controls. Accordingly, COVID-19 patients were classified as with limited, moderate and diffuse pneumonia extent and as with mild, moderate and severe pneumonia severity.

Results: In controls, CT_{LV} could be predicted by sex and height (adjusted $R^2 = 0.57$; $P < 0.001$) while CT_{LW} by age, sex, and height (adjusted $R^2 = 0.6$; $P < 0.001$). The cutoff of 20% (AUC = 0.91, 95%CI 0.88–0.93) for pneumonia extent and of 50% (AUC = 0.91, 95%CI 0.89–0.92) for pneumonia severity were obtained. Pneumonia extent were better correlated when expressed as a percentage of the pCT_{LV} and

pCT_{LW} (r = 0.85, $P < 0.001$), respectively. COVID-19 patients with diffuse and severe pneumonia at admission presented significantly higher CRP concentration, intra-hospital mortality, ICU stay and ventilatory support necessity, than those with moderate and limited/mild pneumonia. Moreover, pneumonia severity, but not extent, was positively and moderately correlated with age (r = 0.46) and CRP concentration (r = 0.44).

Conclusion: The proposed estimation of COVID-19 pneumonia extent and severity might be useful for clinical and radiological patient stratification.

Keywords: computed tomography, COVID-19, deep learning, CT-estimated lung volume, CT-estimated lung weight

KEY POINTS

- COVID-19 pneumonia extent and severity can be less biased computed when adjusted to the predicted CT_{LV} and CT_{LW}, respectively.
- COVID-19 patients classified as having diffuse and more severe pneumonia had worse clinical and radiological outcomes.
- COVID-19 pneumonia extent better correlates with its severity when both were adjusted to the predicted CT_{LV} and CT_{LW}.

INTRODUCTION

Chest computed tomography (CT) has been used widely to assess 2019 coronavirus disease (COVID-19) pneumonia and is key for the detection of abnormal parenchymal opacities (PO) and the evaluation of disease extent and severity (Hope et al., 2020). The extent of COVID-19 pneumonia is often determined on chest CT images by computing the volume of abnormal PO adjusted to the CT–estimated lung volume (CT_{LV}) (Colombi et al., 2020; Yang et al., 2020).

During pneumonia progression, regardless of its etiology, total lung volume is reduced (Patroniti et al., 2005). However, total lung weight can slightly increase in less severe pneumonia, while a significant increase in the lung weight is expected in more severe pneumonia. For instance, if a CT scan exhibits PO, but the total lung weight is in the normal range, atelectasis would be a likely explanation for this parenchymal opacity (Brismar et al., 1985). However, if a similar PO is associated with an increase in lung weight, consolidation resulting from a more relevant lung injury due to hemorrhage, contusion, or edema from capillary leakage might be raised (Puybasset et al., 2000a; Patroniti et al., 2005; Gattinoni et al., 2006). Thus, lung volume reduction at the expense of alveolar flooding by edema/hemorrhage seems to imply a more pronounced increase in lung weight, which could be a marker of pneumonia severity (Reske et al., 2011).

In fact, CT–estimated lung weight (CT_{LW}) has also been used for assessing COVID-19 pneumonia severity; greater pneumonia severity is likely associated with greater elastance and intrapulmonary shunting, and CT_{LW} as great as 1.5 kg have been reported (Gattinoni et al., 2020; Rello et al., 2020) in more severe COVID-19 patients.

Despite the CT_{LV} and CT_{LW} are associated with pneumonia extent and severity, both also depend on anthropometric and demographic variables; they should be greater in males than in females and increase with subject height (Whimster and Macfarlane, 1974; Mull, 1984; Cressoni et al., 2013; Protti et al., 2014). Accordingly, CT_{LV} and CT_{LW} dependencies on demographic and anthropometric variables likely influence the estimation of disease extent and severity.

To overcome this limitation, we proposed that pneumonia extent should be computed from chest CT-estimates volume of abnormal PO adjusted to the predicted lung volume (pCT_{LV}). Similarly, pneumonia severity might be estimated from CT-estimates weight of the same abnormal PO adjusted to the predicted CT-estimates lung weight (pCT_{LW}). This approach involves applying predictive equations for the extrapolation of total lung volume and tissue contents to establish a threshold to assess the extent and severity of COVID-19 pneumonia. We hypothesize that this approach would prove helpful in clinical practice by yielding a less biased non-invasive diagnostic indicator of patient risk stratification.

MATERIALS AND METHODS

COVID-19 Patients

This retrospective study was conducted with 103 consecutive patients with COVID-19 pneumonia (69 males, 34 females), confirmed by reverse-transcription polymerase chain reaction of nasopharyngeal swab samples, who were admitted to three hospitals (Hospital Copa Star and Barra D'Or, Rio de Janeiro, Brazil and Hospital de Santo António, CHUP, Porto, Portugal) in April–July 2020 and underwent chest CT examination, and for whom demographic and anthropometric data were available.

Control Subjects

Chest CT images from 86 subjects (24 males, 62 females) that underwent a helical chest CT scan for clinical purposes, and whose images were considered non-pathological by radiologists,

Abbreviations: ANN, Artificial neural network; AUC, Area under the ROC curve; COVID-19, Coronavirus disease 2019; CP/LO, Crazy paving/linear opacities; CT, Computed tomography; CTLV, Computed tomography–estimated lung volume; CTLW, Computed tomography–estimated lung weight; GGO, Ground-grass opacities; pCTLV, Predicted computed tomography–estimated lung volume; pCTLW, Predicted computed tomography–estimated lung weight; PO, Pulmonary opacities; ROC, Receiver operating characteristic; ROI, Region of interest.

were retrospectively included in this study. Exclusion criteria were age <18 years; use of contrast agents; situs inversus; previous pulmonary lobectomy/segmentectomy; and pulmonary diseases (as pneumonia, interstitial pneumopathies, pulmonary fibrosis, emphysema, chronic obstructive pulmonary disease, pulmonary tuberculosis, ARDS, lung nodules, lung cancer, pneumothorax, pleural effusion, mesothelioma).

The hospitals' research ethics committees approved the study, which complied with current national and international standards (CHUP, 075-DEFI/076-CE; IDO'r, CAAE 29496920.8.0000.5262). Since the study was retrospective, the institutional review board of all hospitals waived the necessity for collecting informed consent from patients.

The collection of morphometric data occurred in two phases: sex and age were recorded before CT execution, while height and weight were declared later by each subject, without the possibility to directly measure them. **Figure 1** shows the flow of subjects' enrollment and CT scan selection.

Chest Computed Tomography Acquisition

CT scans were performed on a 64-channel multislice (Brilliance 40 scanner, Philips Medical Systems, Cleveland, OH, United States; and General Electrics Lightspeed VCT, Chicago Illinois, United States), a 128-channel multislice dual-source CT system (Somatom Definition Flash, Siemens, Forchheim, Germany), or a 16-channel multislice (Emotion 16 CT, Siemens, Erlangen, Germany). The acquisitions were gathered with patients in the supine position at end-inspiratory holds, with 120 kV and 120–300 mA, slice thickness ranging from 1 to 2 mm with 50% superposition, and 512 × 512, 768 × 768, or 1,024 × 1,024 voxels matrix. Reconstruction algorithms were C(1), FC13(5), FC86(1), L(79), B50f(35), B60f(1), B70s(12), I50f/2(1), LUNG(51), SOFT(3), depending on the CT manufacture.

Image Processing and CT_{LV} and CT_{LW} Calculation

The lung parenchyma and airways were segmented from the chest CT images (Fedorov et al., 2012), and the resulting images were exported to an in-house program (Quantitative Lung Image, QUALI) written in MATLAB® (MathWorks®, Natick, MA, United States). The images were rescaled for comparison across cases as described previously (Staring et al., 2016).

The CT_{LV} (sum of air and tissue volumes) was calculated as:

$$\begin{aligned} CT_{LV}(mL) &= \text{pixel size}^2 \times \text{slice thickness} \\ &\times \text{total number of pixels for the whole lung.} \end{aligned} \quad (1)$$

The CT_{LW} was calculated as:

$$\begin{aligned} CT_{LW}(g) &= [(HU - HU_{Air})/(HU_{Aorta} - HU_{Air})] \\ &\times \text{voxel volume} \times 1.04\ g/mL, \end{aligned} \quad (2)$$

where 1.04 mg/mL is the lung tissue density and HU is the voxel density on the HU scale (Staring et al., 2016).

CT_{LV} and CT_{LW} Prediction

The pCT_{LV} and pCT_{LW} were calculated using images from the control group and a multiple linear regression model, taking subjects' age, sex, and height as initial predictors in accordance with Eqs 3 and 4, respectively:

$$pCT_{LV}(mL) = A \times age(y) + B \times sex + C \times height(m) + D \quad (3)$$

and

$$pCT_{LW}(g) = A' \times age(y) + B' \times sex + C' \times height(m) + D' \quad (4)$$

where A, A′, B, B′, C, C′ and D, D′ are the coefficients to be determined. Adjustment for sex was performed by including a dummy-coded sex variable (male = 0).

Visual Classification of Radiological Patterns in COVID-19 CT Scans

Two chest radiologists blinded to patient identification, clinical data, and outcomes, independently selected up to four ROIs per COVID-19 patient visually classified as well-aerated, ground-glass opacities (GGO), crazy paving/linear opacities (CP/LOs), and consolidation. The ROI consisted of a circle with a fixed radius of 4 mm with a spanning area of about 30 voxels in each CT section (Carvalho et al., 2020).

Development of the Supervised Neural Network Architecture

A complete description of the supervised artificial neural network (ANN) development was described in Carvalho et al. (2020). Briefly, a density histogram calculated from ROIs and the respective quantiles (2.5, 25, 50, 75, and 97.5%) were used to train a supervised ANN. The unweighted Cohen's kappa test between the ANN classification and their respective ROI classification attributed by the radiologists was used to assess ANN classifier.

Detailed ANN performance can also be obtained in Carvalho et al. (2020). To determine the degree of PI, the ANN identified abnormal parenchymal opacities and two radiologists blinded to patient identification, clinical data, and outcomes independently validated the results.

Determination of Pneumonia Extent in COVID-19

Pneumonia extent was calculated as the cumulative volumetric sum of GGO, CP/LOs, and consolidation, also referred as abnormal PO, adjusted to the pCT_{LV}.

A receiver operating characteristic (ROC) curve was used to test the differential diagnosis ability of the volume of PO, expressed as a percentage of the pCT_{LV}, in controls and COVID-19 patients. The area under the ROC curve (AUC) was assessed and the threshold sensitivity, specificity, accuracy, positive and negative predictive values, *F*-score, and Matthews correlation coefficient were computed.

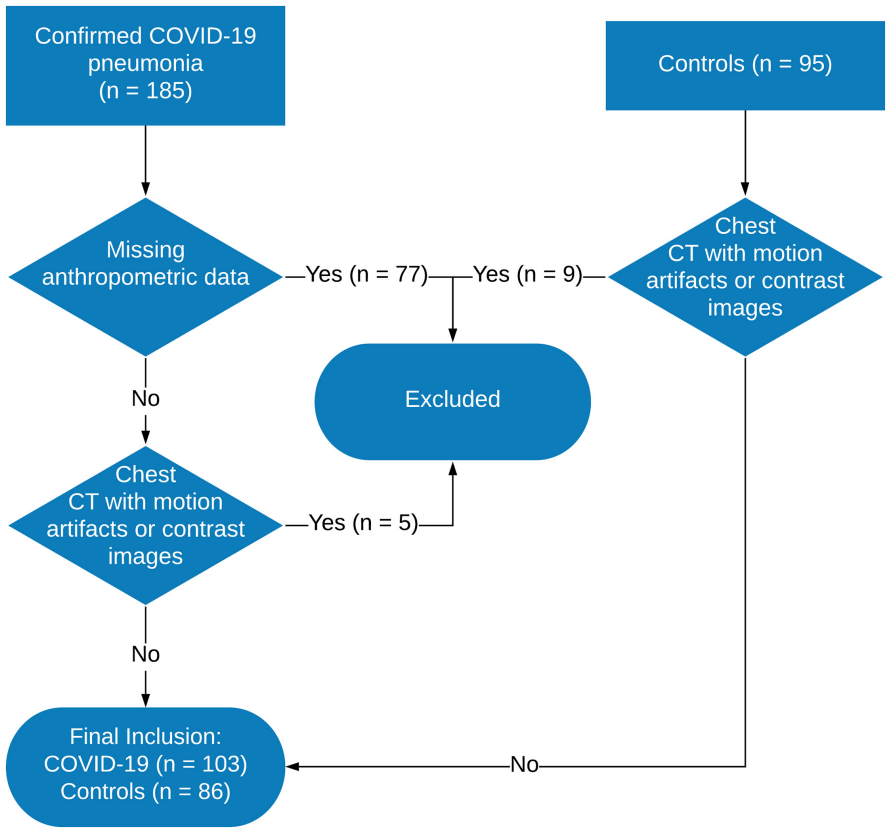


FIGURE 1 | Flowchart of patient enrollment and computed tomography image selection. CT, computed tomography; COVID-19, 2019 coronavirus disease; RT-PCR, reverse-transcription polymerase chain reaction.

To evaluate the degree of pneumonia extent in patients with COVID-19, we used the *Z*-score in relation to the average volume of lung PO in the control group adjusted to the pCT_{LV} and expressed as standard deviation units. *Z*-scores for patients with COVID-19 that exceeded the control values were deemed positive and those below this value were deemed negative. Then, patients with COVID-19 were classified as having limited (pneumonia extent < ROC threshold), moderate (ROC threshold ≤ pneumonia extent < *Z*-score 3), or extensive (pneumonia extent ≥ *Z* score 3) pneumonia.

Determination of Pneumonia Severity in COVID-19

Pneumonia severity was calculated as the weight of the same PO identified for pneumonia extent quantification adjusted to the pCT_{LW}.

The same procedure described above for computation of pneumonia extent was adopted to determine the threshold of normality and as well as the degree of the pneumonia severity in COVID-19 patients. Thus, patients with COVID-19 were classified as presenting mild (pneumonia severity < ROC threshold), moderate (ROC threshold ≤ pneumonia severity < *Z* score 3), or severe (pneumonia severity ≥ *Z* score 3) pneumonia severity.

Clinical and Laboratory Data, Definitions, and Outcomes

Clinical and laboratory findings of each patient were recorded at admission. CT was performed within 12 h after the clinical evaluation and laboratory findings.

Serum C-reactive protein concentration (CRP) collected at the admission was used as a marker of systemic inflammation. ICU stay, as well as ventilatory support, and the intra-hospital mortality were assumed as clinical outcomes. The volume and weight of consolidation, CP/LO, and ground glass opacities were computed by the ANN and used as radiological outcomes.

Statistical Analysis

The normality of the data was examined using the Kolmogorov–Smirnov test with Lilliefors' correction, and the homogeneity of variance was assessed using the Levene median test. As both conditions were satisfied, all data are presented as means and standard deviations.

The relationship between patient's height, weight, age, sex and CT_{LV} and CT_{LW} was assessed by multiple linear regression. The Bland-Altman graphic method was used to evaluate the concordance between the measured and predicted CT_{LV} and CT_{LW} in controls and COVID-19 patients.

A student *t*-test was used to compare measured and predicted CT_{LV} and CT_{LW} in controls and COVID-19 subjects. The one-way ANOVA test followed by Bonferroni *post hoc* test was used to assess statistical differences among patients with limited, moderate, and diffuse pneumonia extent, as well as among those with mild, moderate, and severe COVID-19 pneumonia severity, and control subjects.

The correlation of pneumonia extent and severity as well as between pneumonia extent and severity with clinical and demographic data was assessed using Pearson correlation analysis (very weak, r = 0.00–0.19; weak, r = 0.20–0.39; moderate, r = 0.40–0.59; strong, r = 0.60–0.79; very strong, $r \geq 0.80$). $P < 0.05$ were considered to be significant. All statistical analyses were performed using MATLAB® software (MathWorks®).

RESULTS

The ANN architecture with a single hidden layer of 60 neurons showed the best agreement in the confusion test matrix among the other architectures tested with an overall agreement of 86% being 100% for well-aerated, 76% for GGO, 72% for CP/LO, and 100% for consolidation.

No improvement in the performance of the ANN classifier was observed with the addition of a second neuron layer. The classifier performance was much better for well-aerated and consolidation with an AUC of 1.00 and 0.99. The performance for GGO and CP/LO, despite lower, was quite acceptable with an AUC of 0.94 and 0.91, respectively. The best validation performance occurred at epoch 6.

In control subjects, most ANN-classified parenchymal opacities represented small bronchi, peribronchial vessels, and pleural or diaphragm interfaces likely related to partial volume effects and were color-coded as yellow, orange and gray (**Figure 2A**). In COVID-19 patients (**Figures 2B–D**), parenchymal opacities, were therefore interpreted, in addition to the peribronchial vessels, as GGO (yellow), CP/LO (orange) and consolidation (gray) broadly spread over the lung parenchyma in COVID-19 with disuse pneumonia extent (**Figure 2D**).

Lung volume was related significantly to sex (P = 0.0002) and height ($P < 0.0001$), but not age. Thus, the pCT_{LV} (R^2 = 0.57, adjusted R^2 = 0.56, F-statistic = 55.5 and $P < 0.0001$) could be predicted as:

$$pCT_{LV}(mL) = 4808.1 \times height(m) - 3602.5 \quad (5)$$

For males, 800.6 mL should be added to the pCT_{LV}. A residual standard error of 634.3 mL on 83 degrees of freedom was obtained with this regression model.

Lung weight was related significantly to age (P = 0.011), sex (P = 0.015), and height ($P < 0.0001$; R^2 = 0.60, adjusted R^2 = 0.58, F-statistic = 40.6 and $P < 0.0001$), being calculated as:

$$pCT_{LW}(g) = -1.8 \times age + 795.2 \times height(m) - 573.8 \quad (6)$$

For males, 83 g should be added to the pCT_{LW}. A residual standard error of 103.8 g on 82 degrees of freedom.

The ROC curve indicated that the optimal threshold for pneumonia extent was 20% (sensitivity = 0.80, specificity = 0.85, AUC = 0.91 95%CI 0.88-0.93, accuracy = 0.82, F score = 0.85, Matthews' correlation coefficient = 0.65; **Figures 3A,B**). Accordingly, COVID-19 patients were classified based as with diffuse ($\geq Z$-score of 3, in 55 patients), moderate (from 20% to Z-score of 3, in 31 patients) and limited pneumonia (<20%, in 17 patients).

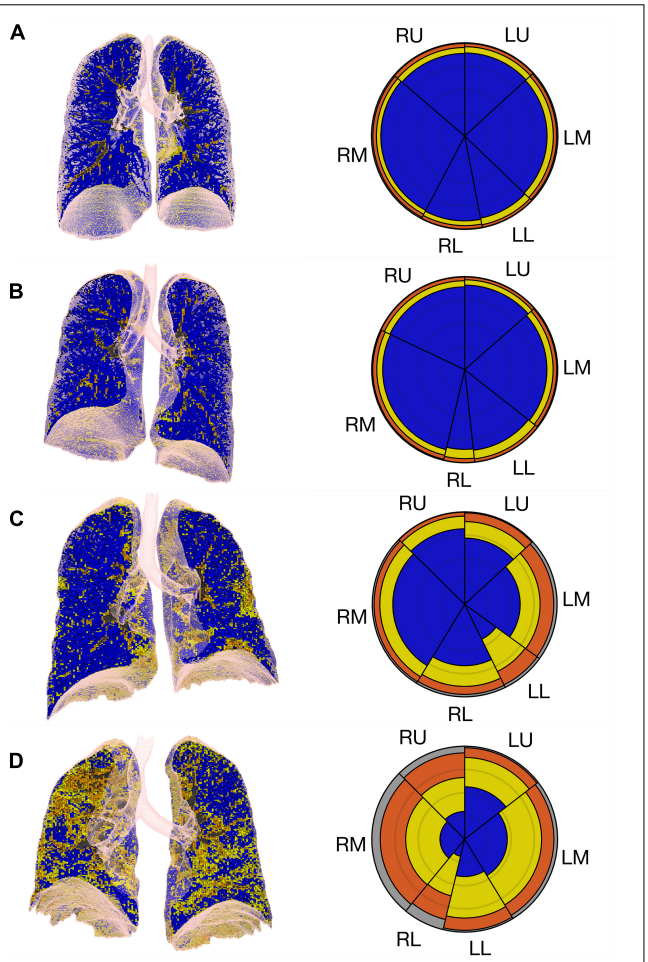


FIGURE 2 | Summary glyphs (right column) derived from three-dimensional imaging data (left column) for a representative control case (row **A**) and cases of COVID-19 with limited and mild (row **B**), moderate (row **C**), and diffuse and severe (row **D**) pneumonia extent and severity. R, right lung; L, left lung; U, upper lung; M, middle lung; L, lower lung. Ground-glass opacities (GGO) are colored in yellow, crazy-paving/reticular opacities in orange and consolidation in gray.

The optimal threshold for pneumonia severity was 50% (sensitivity = 0.86, specificity = 0.88, AUC = 0.91 95%CI 0.89–0.92, accuracy = 0.87, F-score = 0.88, Matthews' correlation coefficient = 0.75, **Figures 3C,D**). COVID-19 patients were classified as with severe PI ($\geq Z$-score of 3, in 61 patients), moderate (from 20% to Z-score of 3, in 28 patients) and mild pneumonia severity (<20%, in 14 patients).

Figure 4 depicts the relationship and the Bland-Altman bias plot between CT_{LV} and pCT_{LV} in COVID-19 patients and controls (**Figures 4A,B**) and between CT_{LW} and pCT_{LW}

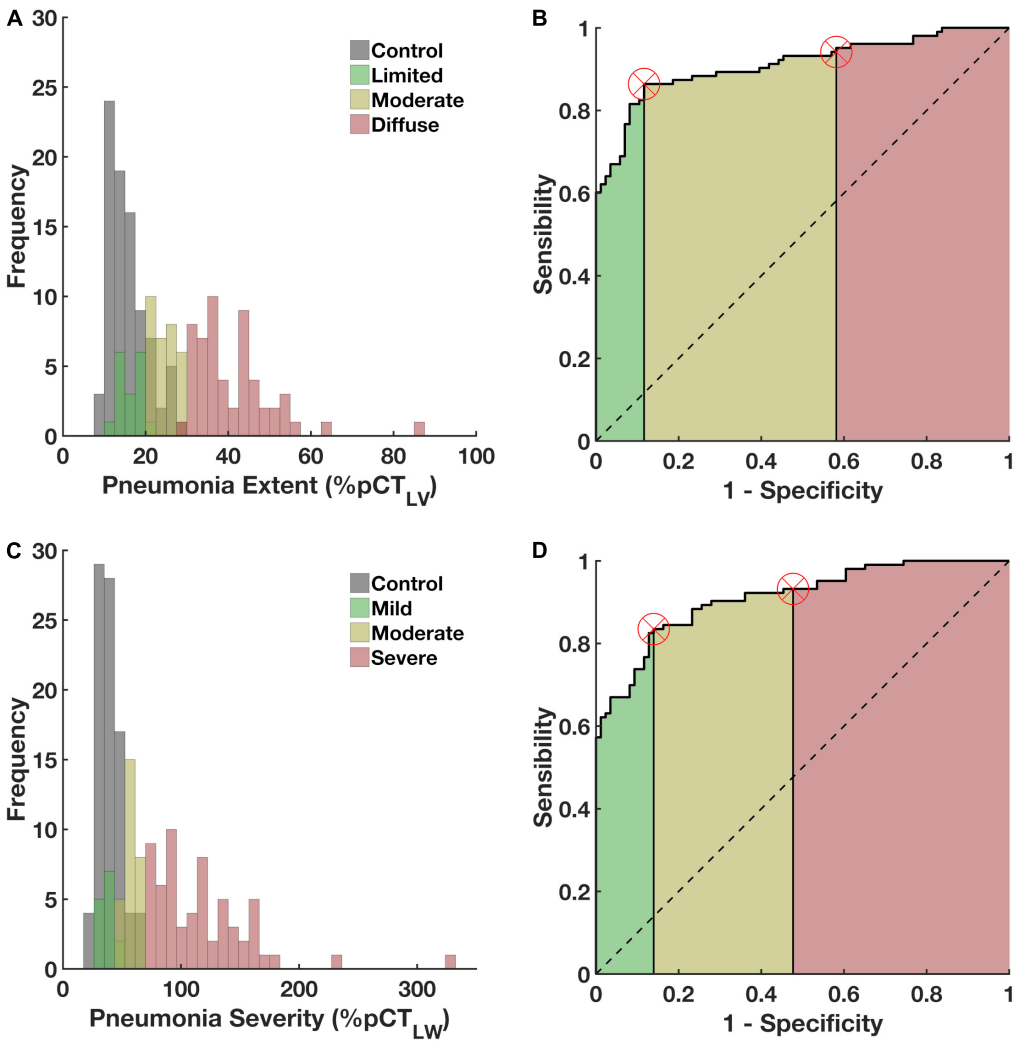


FIGURE 3 | Left panels **(A,C)** Histograms of the frequency of occurrence of pneumonia extent and severity in patients with COVID-19 (green, yellow and red colored) and the volume and weight associated to parenchymal opacities in controls (gray). **Figure 3** right panels **(B,D)** ROC curves, with the areas under the curves (AUCs) hatched in green, yellow and red. Vertical lines mark the normality cut-off (equivalent to 20% of the predicted total lung volume represented by pulmonary opacities and 50% of the predicted lung weight represented by pulmonary opacities) and the *Z* score of 3 (equivalent to 30% and 60%, respectively, and used to classify COVID-19 patients as having diffuse and severe pneumonia). Values falling between the normality cut-off and the *Z* score of 3 represent moderate pulmonary involvement. The use of parenchymal opacity adjusted to the predicted lung volume as an indicator of pneumonia extent presented 0.80 sensitivity, 0.86 specificity, an AUC of 0.91, accuracy of 0.82, 0.90 positive predictive value, 0.73 negative predictive value, *F* score of 0.85, and Matthews correlation coefficient of 0.65. Pneumonia severity presented 0.86 sensitivity, 0.88 specificity, an AUC of 0.91, accuracy of 0.87, 0.90 positive predictive value, 0.84 negative predictive value, *F* score of 0.88, and Matthews correlation coefficient of 0.75.

(**Figures 4C,D**). Note that the agreements between measured and predicted values were quite reduced in patients with more diffuse and severe COVID-19 pneumonia, whereas almost no bias was observed in controls.

The COVID-19 pneumonia extent was significantly lesser when adjusted to the pCT_{LV} than when adjusted to the CT_{LV} (40.8 ± 19.9% vs. 32.1 ± 12.6%, $P < 0.001$; **Figure 5A**). Noteworthy, pneumonia severity was significantly higher when adjusted to the pCT_{LW} (62.5 ± 17.8% vs. 90.3 ± 47%, $P < 0.001$; **Figure 5C**) specially in moderate and severe COVID-19 patients. No significant variations were observed in the control group (**Figures 5B,D**). The greatest difference these indicators was observed in patients classified as with more severe disease (**Figure 5**).

The relationship between pneumonia extent and severity was improved when both were adjusted to pCT_{LV} and pCT_{LW}, respectively (**Figure 6D**). The Pearson correlation coefficient between pneumonia extent expressed as%CT_{LV} and the pneumonia severity expressed in grams was just moderate ($r = 0.54$, **Figure 6A**), whereas that between the pneumonia extent expressed as a percentage of the pCT_{LV} and the pneumonia severity expressed as a percentage of the pCT_{LW} was very strong ($r = 0.88$; **Figure 6D**). In addition, a considerable reduction in the overall dispersion was observed.

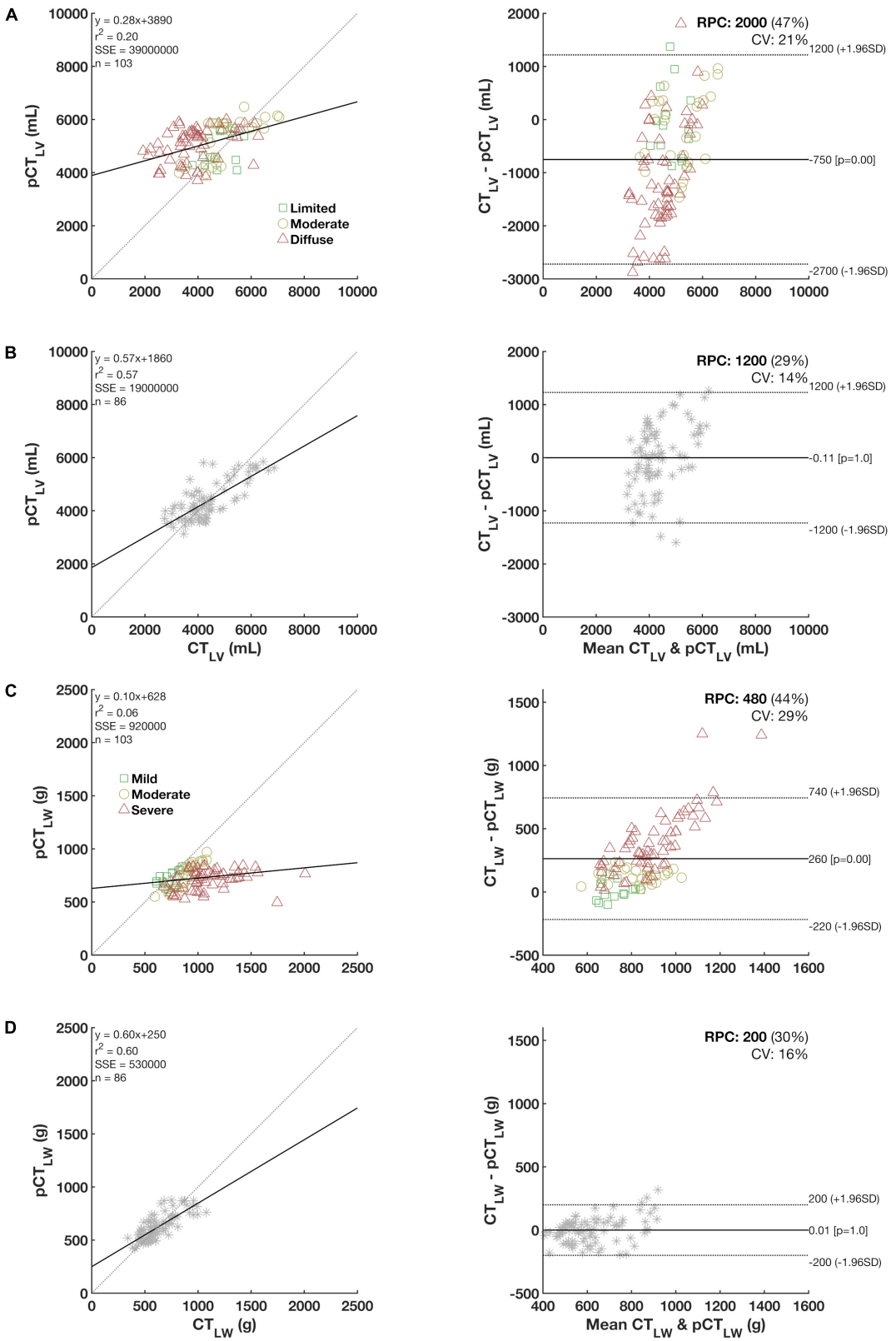


FIGURE 4 | Left panels: The relationship between predicted (pCT_{LV}) and measured lung volumes (CT_{LW}) and weight in COVID-19 patients **(A,C)** and controls **(B,D)** with each respective Bland-Altman bias plot (right panels). Note that in controls, no significant bias is observed whereas the bias tended to increase in more diffuse and severe COVID-19 pneumonia patients (colored in red).

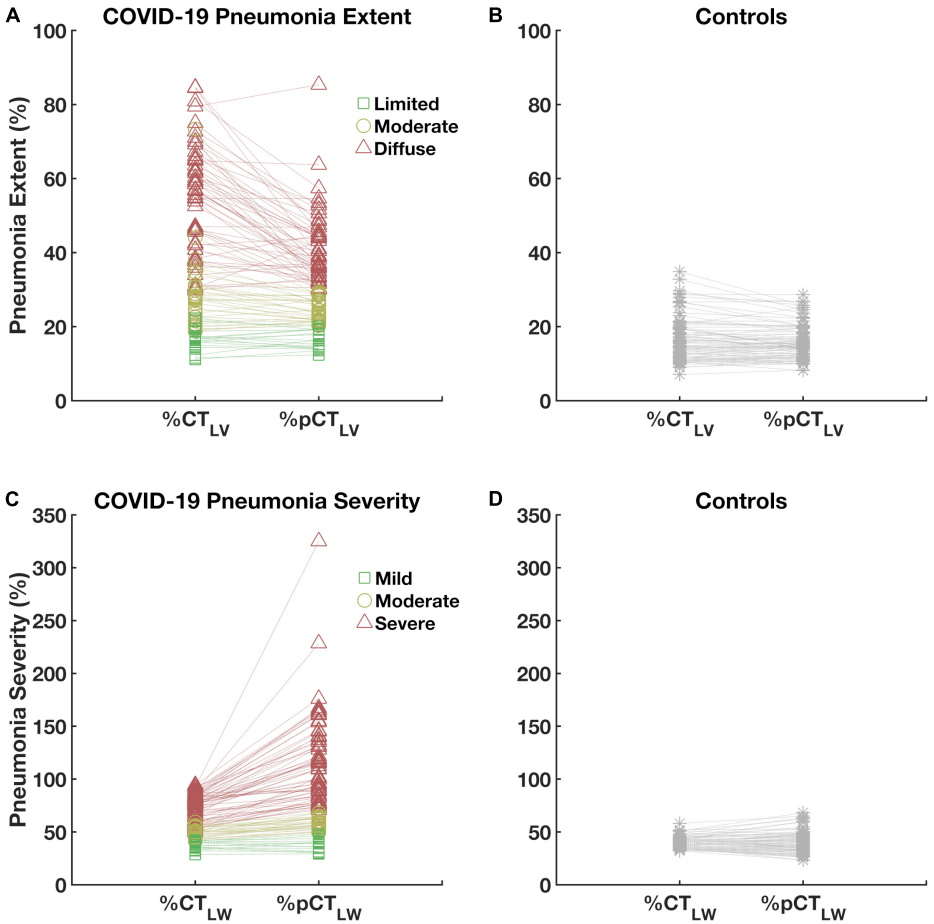


FIGURE 5 | Paired plot of pneumonia extent (upper panels, **A,B**) and severity (lower panels, **C,D**) expressed as percentages of computed tomography-estimated lung volume (%CTLV) and weight (%CTLW) and predicted computed tomography-estimated lung volume (%pCT_{LV}) and weight (%pCT_{LW}) in patients (left panels, **A,C**) and controls (right panels, **B,D**). Note that the difference in the estimation of pneumonia extent and severity from non-normalized and normalized are higher in diffuse and severe COVID-19 cases (triangles).

Relative to patients with moderate and limited pneumonia extent and moderate and mild pneumonia severity, patients with COVID-19 diffuse and severe pneumonia were older and had higher CRP concentrations at admission. Additionally, a positive and moderate correlation between pneumonia severity ($r = 0.46$) and age and CRP concentration ($r = 0.44$) was observed. Moreover, COVID-19 most diffuse and severe pneumonia required ICU stay and ventilatory support, presenting higher intra-hospital mortality. Moreover, they exhibited significantly reduced CT_{LV}s and increased CT_{LW}s. As expected, the volume of GGO, CP/LO, and consolidation significantly increased in patients with diffuse and severe pneumonia extent and severity (**Tables 1**, **2**).

DISCUSSION

In COVID-19 pneumonia, lung terminal structures, such as the interlobular septum and alveolar wall, can be involved and cause extensive edema and lymphocyte infiltration in the lung interstitium (Kim et al., 2002). Although early alveolar exudation is not prominent, the disease progresses rapidly (Li et al., 2020). Thus, the quantification of COVID-19 pneumonia extent and severity from chest CT images might be of clinical interest for risk stratification and can have some prognostic value (Colombi et al., 2020; Li et al., 2020).

In the present study, we propose a methodology to estimate COVID-19 pneumonia extent and severity and verified the possible associations between those indicators with clinical and radiological outcomes.

Our results suggested that COVID-19 pneumonia extent can be less biased calculated as the ratio between the volume of lung parenchyma opacities from chest CT images adjusted to the predicted CT_{LV} (**Figure 4**). Accordingly, an increase in the volume of abnormal opacities in the lung parenchyma would indicate an increase in the extension of pneumonia without the bias associated with the fact that larger pneumonia would also reduce the total lung volume (Patroniti et al., 2005).

As complementary information, pneumonia severity was assessed by calculating the ratio between the weight of abnormal

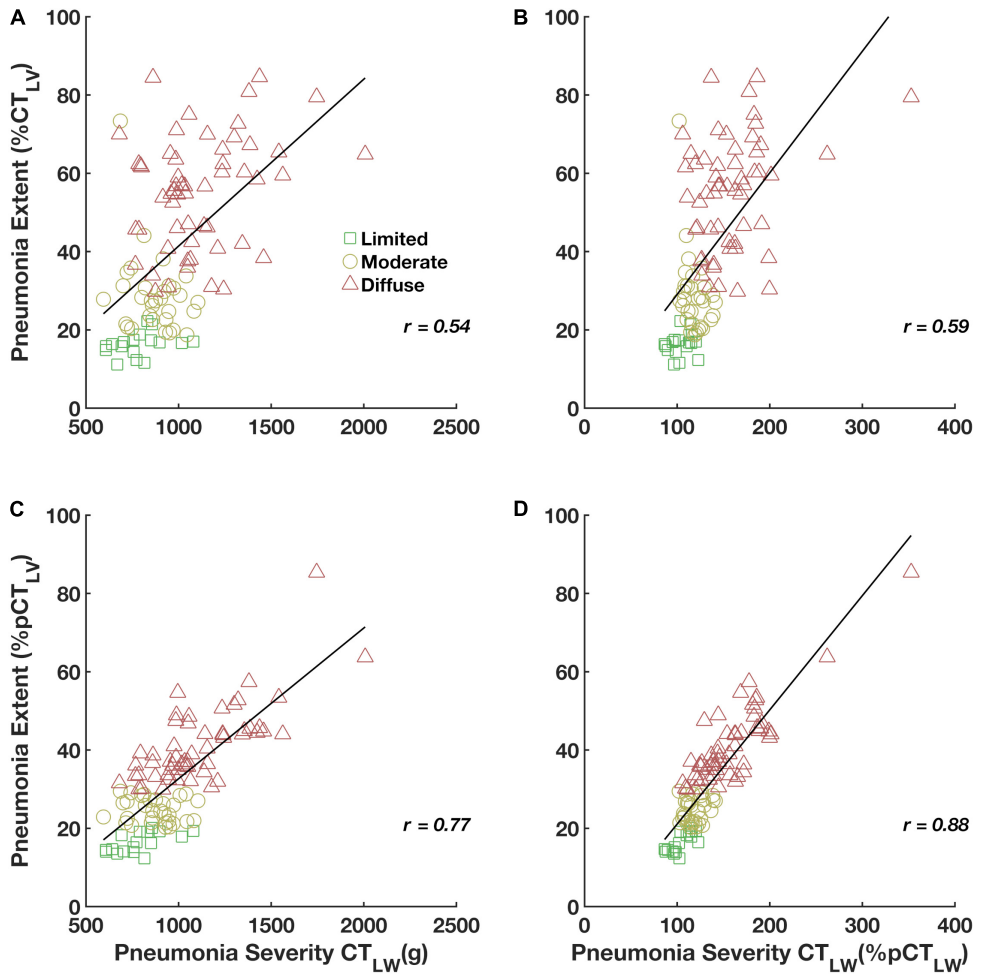


FIGURE 6 | Pearson's correlations of pneumonia extent expressed as%CT_{LV} with the CT_{LW} in grams **(A)** and expressed as%pCT_{LW} (pneumonia severity, **B**), and of pneumonia extent expressed as%pCT_{LV} with the CT_{LW} in grams **(C)** and expressed as%pCT_{LW} (pneumonia severity, **D**).

parenchymal opacities and the predicted CT_{LW}. In fact, an interesting application of the CT_{LW} estimation was the potential to distinguish pulmonary opacification in the context of inflammatory alveolar flooding from compression atelectasis (Reske et al., 2011). Therefore, patients with pulmonary opacification on CT images but with normal lung weight likely present more atelectasis due to hypoventilation, the use of anesthetics, and high inspired oxygen fractions. Alternatively, increased lung weight suggests consolidation due to significant lung injury, for example, hemorrhage or edema caused by capillary leakage (Patroniti et al., 2005; Reske et al., 2011).

The differential diagnostic ability of the proposed method to determine pneumonia extent and severity in COVID-19 pneumonia was assessed by the ROC analysis with almost the same AUC, sensitivity, specificity and accuracy (**Figure 3**). Thus, 55 COVID-19 patients were classified as having diffuse pneumonia extent and 61 as presenting severe pneumonia. Those patients were older and seemed to be more toxemic (higher CRP concentrations at admission) and more frequently required ICU and ventilatory support and presented higher intra-hospital mortality. Moreover, COVID-19 patients with diffuse and severe pneumonia also presented significant volumes of GGO, CP/LO and consolidation (**Tables 1**, **2**).

Patients with diffuse and severe COVID-19 pneumonia almost certainly present denser lung parenchyma, likely because pulmonary opacifications represent a more exuberant inflammatory component with more alveolar flooding and cell infiltration than seen with atelectasis or ventilatory defects.

Interestingly, pneumonia extent expressed as%CT_{LV} was only moderately associated with the CT_{LW} while pneumonia extent expressed as%pCT_{LV} correlated very strongly with the CT_{LW} adjusted to the pCT_{LW} (pneumonia severity), with a considerable reduction in the overall dispersion. These results emphasize the importance of adjustment to predicted values to achieve the unbiased use of pneumonia extent as an indicator of disease severity in patients with COVID-19 pneumonia (**Figure 6**).

Several predictive equations have been used for the normalization of pulmonary function data (Roberts et al., 1991; Roca et al., 1998). However, few reference values for CT_{LV} and CT_{LW} in healthy subjects have been reported (Reske et al., 2011;

TABLE 1 | Demographic, anthropometric, clinical, and computed tomography data on patients classified with limited, moderate and diffuse COVID-19 pneumonia extent and controls.

	COVID-19 pneumonia extent *N* = 103			Controls *N* = 86	*P*-values
	Diffuse *N* = 55	Moderate *N* = 31	Limited *N* = 17		
Demographic and anthropometrics					
Age (y)	66 ± 14[b]	59 ± 15	50 ± 15	60 ± 20	0.01[b]
Gender (female)	16	11	7	62	–
Body height (m)	1.7 ± 0.1[d]	1.7 ± 0.1[d]	1.7 ± 0.1[d]	1.6 ± 0.1	<0.001[d]
Body weight (kg)	79.8 ± 14.3[d]	83.4 ± 18.8[d]	78.9 ± 20.3[d]	66.6 ± 13.3	<0.01[d]
BMI (kg/m^2)	28.2 ± 4.4[d]	28.1 ± 5.3	26.4 ± 4.8	25.7 ± 4.3	0.01[d]
Laboratory data					
White blood count (×10^3/μL)	6.5 ± 3.1	5.5 ± 3.3	5.3 ± 1.2		
Lymphocytes count (×10^3/μL)	1.2 ± 1.3	1.1 ± 0.4	1.2 ± 0.5		
Lactate dehydrogenase (U/L)	312.8 ± 128.4	300.7 ± 137.5	227.6 ± 99.1		
CRP (mg/L)	54.2[a,b] ± 63.3	6.7 ± 8.7	5.1 ± 5.8		<0.002[a,b]
GOT (U/L)	38.7 ± 24.0	36.9 ± 21.8	22.1 ± 36.9		
GPT (U/L)	36.5 ± 22.9	37.9 ± 31.4	33.5 ± 33.5		
Creatinine (mg/dL)	0.98 ± 0.48	1.3 ± 1.7	0.84 ± 0.26		
Clinical outcomes					
Symptoms onset (days)	8.8 ± 5.6	6.7 ± 5.4	8.0 ± 7.8	–	
ICU (%)	17	11	4	–	
i-MV (%)	9	6	2	–	
In-hospital mortality (%)	9	3	0	–	
CT data					
CT_{LV} (mL)	3,916 ± 990[a,b]	4,639 ± 1,041	5,199 ± 869	4,350 ± 958	<0.006[a,b]
pCT_{LV} (mL)	5,052 ± 654[d]	5,156 ± 763[d]	5,133 ± 686[d]	4,350 ± 725	<0.001[d]
CT_{LW} (g)	1,113 ± 260[a,b,d]	872 ± 127[c,d]	785 ± 132[d]	621 ± 161	<0.008[a,b,c,d]
pCT_{LW} (g)	706 ± 92[d]	738 ± 114[d]	752 ± 79[d]	621 ± 124	<0.001[d]
Pneumonia extent (%CT_{LV})	55 ± 14[a,b,d]	29 ± 10[c,d]	17 ± 3[d]	16 ± 6	<0.001[a,b,c,d]
Pneumonia extent (%pCT_{LV})	41 ± 10[a,b,d]	25 ± 3[c,d]	16 ± 3	16 ± 5	<0.001[a,b,c,d,]
GGO (mL)	1,363 ± 424[a,b,d]	906 ± 151[c,d]	571 ± 143	507 ± 230	<0.001[a,b,c,d]
CP/LO (mL)	527 ± 266[a,b,d]	281 ± 98[d]	219 ± 86	136 ± 36	<0.001[a,b,d]
Consolidation (mL)	192 ± 157[a,b,d]	72 ± 25	54 ± 19	43 ± 16	<0.001[a,b,d]

[a]*Severe vs. moderate,* [b]*Severe vs. mild,* [c]*Moderate vs. mild,* [d]*COVID-19 vs. controls.*
Data are shown as means ± standard deviations.
BMI, body mass index; CRP, C-reactive protein; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ICU, intensive care unit; i-MV, invasive mechanical ventilation, CT, computed tomography; CT_{LV}, computed tomography–calculated total lung volume; pCT_{LV}, predicted computed tomography–estimated total lung volume; CT_{LW}, computed tomography–calculated total lung weight; pCT_{LW}, predicted computed tomography–estimated total lung weight; GGO, volume related to ground-glass opacities; CP/LO, volume related to crazy paving and linear opacities; consolidation, volume related to consolidation.

Cressoni et al., 2013). Most predictive equations used for pulmonary function data are obtained from persons of both sexes and of various ages, heights, and ethnicities. However, many of them used seated individuals, which clearly increases all static lung volumes compared with those obtained while subjects are supine, the most common position for CT examination (Reske et al., 2011; Cressoni et al., 2013). That was the main motivation for the assessment of such predictive equations in our control group.

In the present study, age ranged widely (from 22 to 81 years) in the control group, but female sex predominated. Despite this limitation, our predictive equations yielded acceptable adjusted R^2 values for CT_{LV} and CT_{LW} prediction (**Figure 4**) and are in accordance with the literature (Whimster and Macfarlane, 1974; Gattinoni et al., 2006; Puybasset et al., 2000b; Reske et al., 2011; Cressoni et al., 2013). Further studies with more heterogeneous populations would be of great value for the development of new and more representative predictive equations.

Study Limitation

One important limitation of the methodology proposed here is the need for data on subjects' height, which is not always included in clinical records. We recommend the routine annotation of subjects' height, which would be of great benefit for further studies and data normalization. However, it is important to note that the impact of the normalization by predicted CT_{LV} or CT_{LW} was always greater in estimating the extent, but not the severity, of COVID-19 pneumonia (**Figure 6**). This can be explained, albeit partially, by the negative contribution of age in lung weight estimation. In fact, it is noted that patients with more diffuse and severe COVID-19 pneumonia were older than the others, including controls.

TABLE 2 | Demographic, anthropometric, clinical, and computed tomography data on patients classified with limited, moderate and diffuse COVID-19 pneumonia severity and controls.

	COVID-19 pneumonia severity *N* = 103			Controls *N* = 86	*P*-values
	Severe *N* = 61	Moderate *N* = 28	Mild *N* = 14		
Demographic and anthropometrics					
Age (y)	67 ± 13[a,b]	55 ± 16	48 ± 15	60 ± 20	0.018[a] 0.001[b]
Gender (male/female)	17/44	9/19	8/6	24/62	–
Body height (m)	1.7 ± 0.1[d]	1.7 ± 0.1[d]	1.7 ± 0.05[d]	1.6 ± 0.1	<0.009[d]
Body weight (kg)	81 ± 14.4[d]	85 ± 21[d]	73 ± 15.7	66.6 ± 13.3	<0.003[d]
BMI (kg/m^2)	28.4 ± 4.4[d]	28 ± 5.4	25.4 ± 4.5	25.7 ± 4.3	0.003[d]
Laboratory data					
White blood count (× 10^3/μL)	6.6 ± 3.0	5.5 ± 3.3	4.8 ± 1.1	–	
Lymphocytes count (× 10^3/μL)	1.2 ± 1.3	1.2 ± 0.4	1.2 ± 0.6	–	
Lactate dehydrogenase (U/L)	315.3 ± 126.5[b]	304.7 ± 139.1	194.4 ± 59.5	–	0.028[b]
CRP (mg/L)	51.0 ± 61.2[a,b]	6.4 ± 8.4	3.8 ± 5.1	–	<0.006[a,b]
GOT (U/L)	39.8 ± 22.7	35.6 ± 22.1	34.0 ± 25.0	–	
GPT (U/L)	38.7 ± 23.4	34.5 ± 30.2	37.4 ± 37.6	–	
Creatinine (mg/dL)	1.0 ± 0.5	1.3 ± 1.7	0.8 ± 0.3	–	
Clinical outcomes					
Symptoms onset (days)	8.7 ± 5.5	7.2 ± 6.6	8.0 ± 7.8	–	–
ICU (%)	34	28	21	–	–
i-MV (%)	20	18	8	–	–
In-hospital mortality (%)	10	0	0	–	–
CT data					
CT_{LV} (mL)	3,909 ± 1,008[a,b,d]	5,060 ± 993[d]	4,816 ± 553	4,350 ± 958	<0.04[a,b,d]
pCT_{LV} (mL)	5,072 ± 627[d]	5,267 ± 800[d]	4,863 ± 588	4,350 ± 725	<0.001[d]
CT_{LW} (g)	1,089 ± 260[a,b,d]	891 ± 116[d]	729 ± 77	621 ± 161	<0.001[a,b,d]
pCT_{LW} (g)	706 ± 90[d]	761 ± 114[d]	720 ± 68[d]	621 ± 124	<0.013[d]
Pneumonia severity (%CT_{LW})	75 ± 12[a,b,d]	49 ± 5[c,d]	37 ± 4	39 ± 5	<0.001[a,b,c,d]
Pneumonia severity (%pCT_{LW})	117 ± 42[a,b,d]	58 ± 5[d]	38 ± 6	39 ± 10	<0.007[a,b,d]
GGO (mL)	1,314 ± 422[a,b,d]	891 ± 190[d]	545 ± 136	507 ± 230	<0.001[a,b,d] 0.003[c]
CP/LO (mL)	517 ± 251[a,b,d]	264 ± 88[d]	181 ± 57	136 ± 36	<0.001[a,b,d]
Consolidation (mL)	183 ± 150[a,b,d]	68 ± 21	45 ± 12	43 ± 16	<0.001[a,b,d]

[a]Severe vs. moderate, [b]Severe vs. mild, [c]Moderate vs. mild, [d]COVID-19 vs. controls.
Data are shown as means ± standard deviations.
BMI, body mass index; CRP, C-reactive protein; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ICU, intensive care unit; i-MV, invasive mechanical ventilation, CT, computed tomography; CT_{LV}, computed tomography–calculated total lung volume; pCT_{LV}, predicted computed tomography–estimated total lung volume; CT_{LW}, computed tomography–calculated total lung weight; pCT_{LW}, predicted computed tomography–estimated total lung weight; GGO, volume related to ground-glass opacities; CP/LO, volume related to crazy paving and linear opacities; consolidation, volume related to consolidation.

In summary, the method proposed in the present study might be of clinical interest for the determination of COVID-19 pneumonia extent and severity and might be useful for clinical and radiological patient stratification. Further studies are necessary to assess the validation of proposed pneumonia extent and severity indicators at the clinical scenario. Indeed, the association between the extent and severity of COVID-19 pneumonia with the clinical outcomes or even inflammatory markers still need to be better assessed.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Hospital Copa Star and Barra D'Or, Rio de Janeiro Brazil and Hospital de Santo António, CHUP, Porto, Portugal. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AC: image processing and analysis of results, statistical evaluation, theoretical development of the neural network and the computation method of voxel to voxel analysis,

writing of the text, and submission of the article. AG: image processing and segmentation, statistical evaluation, neural network implementation. TG: image segmentation, capture and organization of clinical data, and draft review. GM: determination of image regions of interest, capture and organization of clinical data, and draft review. VC: determination of image regions of interest and image segmentation. FB: results discussion and draft review. RR: capture and organization of clinical data, results discussion, and draft review. JP: capture and organization of clinical data. WS: determination of image regions of interest and draft review. WZ: draft and final review. MF: capture and organization of clinical data and draft review. All authors approved the final version of the manuscript.

FUNDING

This research was supported by the Brazilian Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant No. 302839/2017-8) and the Rio de Janeiro State Research Supporting Foundation (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, Grant No. E-26/203.001/2018).

REFERENCES

Brismar, B., Hedenstierna, G., Lundquist, H., Strandberg, Å, Svensson, L., and Tokics, L. (1985). Pulmonary densities during anesthesia with muscular relaxation–a proposal of atelectasis. *Anesthesiology* 62, 422–428. doi: 10.1097/00000542-198504000-00009

Carvalho, A. R. S., Guimarães, A., Werberich, G. M., de Castro, S. N., Pinto, J. S. F., Schmitt, W. R., et al. (2020). COVID-19 chest computed tomography to stratify severity and disease extension by artificial neural network computer-aided diagnosis. *Front. Med.* 7:577609. doi: 10.3389/fmed.2020.577609

Colombi, D., Bodini, F. C., Petrini, M., Maffi, G., Morelli, N., Milanese, G., et al. (2020). Well-aerated lung on admitting chest CT to predict adverse outcome in COVID-19 pneumonia. *Radiology* 296, E86–E96. doi: 10.1148/radiol.2020201433

Cressoni, M., Gallazzi, E., Chiurazzi, C., Marino, A., Brioni, M., Menga, F., et al. (2013). Limits of normality of quantitative thoracic CT analysis. *Crit. Care* 17:R93. doi: 10.1186/cc12738

Fedorov, A., Beichel, R., Kalpathy-Cramer, J., Finet, J., Fillion-Robin, J.-C., Pujol, S., et al. (2012). 3D Slicer as an image computing platform for the quantitative imaging network. *Magn. Reson. Imaging* 30, 1323–1341. doi: 10.1016/j.mri.2012.05.001

Gattinoni, L., Caironi, P., Cressoni, M., Chiumello, D., Ranieri, V. M., Quintel, M., et al. (2006). Lung recruitment in patients with the acute respiratory distress syndrome. *N. Engl. J. Med.* 354, 1775–1786. doi: 10.1056/nejmoa052052

Gattinoni, L., Coppola, S., Cressoni, M., Busana, M., Rossi, S., and Chiumello, D. (2020). Covid-19 does not lead to a "Typical" acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 201, 1299–1300. doi: 10.1164/rccm.202003-0817le

Hope, M. D., Raptis, C. A., Shah, A., Hammer, M. M., and Henry, T. S. (2020). A role for CT in COVID-19? What data really tell us so far. *Lancet* 395, 1189–1190. doi: 10.1016/s0140-6736(20)30728-5

Kim, E. A., Lee, K. S., Primack, S. L., Yoon, H. K., Byun, H. S., Kim, T. S., et al. (2002). Viral pneumonias in adults: radiologic and pathologic findings1. *Radiographics* 22, S137–S149. doi: 10.1148/radiographics.22.suppl_1.g02oc15s137

Li, K., Fang, Y., Li, W., Pan, C., Qin, P., Zhong, Y., et al. (2020). CT image visual quantitative evaluation and clinical classification of coronavirus disease (COVID-19). *Eur. Radiol.* 30, 4407–4416. doi: 10.1007/s00330-020-06817-6

Mull, R. T. (1984). Mass estimates by computed tomography: physical density from CT numbers. *AJR* 143, 1101–1104. doi: 10.2214/ajr.143.5.1101

Patroniti, N., Bellani, G., Maggioni, E., Manfio, A., Marcora, B., and Pesenti, A. (2005). Measurement of pulmonary edema in patients with acute respiratory distress syndrome. *Crit. Care Med.* 33, 2547–2554. doi: 10.1097/01.ccm.0000186747.43540.25

Protti, A., Iapichino, G. E., Milesi, M., Melis, V., Pugni, P., Comini, B., et al. (2014). Validation of computed tomography for measuring lung weight. *Intensive Care Med. Exp.* 2:31. doi: 10.1186/s40635-014-0031-0

Puybasset, L., Cluzel, P., Gusman, P., Grenier, P., Preteux, F., and Rouby, J.-J. (2000a). Regional distribution of gas and tissue in acute respiratory distress syndrome. I. Consequences for lung morphology. CT Scan ARDS Study Group. *Intensive Care Med.* 26, 857–869. doi: 10.1007/s001340051274

Puybasset, L., Gusman, P., Muller, J. C., Cluzel, P., Coriat, P., and Rouby, J.-J. (2000b). Regional distribution of gas and tissue in acute respiratory distress syndrome. III. Consequences for the effects of positive end-expiratory pressure. CT scan ARDS study group. Adult respiratory distress syndrome. *Intensive Care Med.* 26, 1215–1227. doi: 10.1007/s001340051340

Rello, J., Storti, E., Belliato, M., and Serrano, R. (2020). Clinical phenotypes of SARS-CoV-2: implications for clinicians and researchers. *Eur. Respir. J.* 12:2001028. doi: 10.1183/13993003.01028-2020

Reske, A. W., Reske, A. P., Heine, T., Spieth, P. M., Rau, A., Seiwerts, M., et al. (2011). Computed tomographic assessment of lung weights in trauma patients with early posttraumatic lung dysfunction. *Critical Care* 15:R71. doi: 10.1186/cc10060

Roberts, C. M., MacRae, K. D., Winning, A. J., Adams, L., and Seed, W. A. (1991). Reference values and prediction equations for normal lung function in a non-smoking white urban population. *Thorax* 46, 643–650. doi: 10.1136/thx.46.9.643

Roca, J., Burgos, F., Barberà, J. A., Sunyer, J., Rodriguez-Roisin, R., Castellsagué, J., et al. (1998). Prediction equations for plethysmographic lung volumes. *Respir. Med.* 92, 454–460. doi: 10.1016/s0954-6111(98)90291-8

Staring, M., Bakker, M. E., Stolk, J., Shamonin, D. P., Reiber, J. H. C., and Stoel, B. C. (2016). Towards local progression estimation of pulmonary emphysema using CT. *Med. Phys.* 41, 21905–21914. doi: 10.1118/1.4851535

Whimster, W. F., and Macfarlane, A. J. (1974). Normal lung weights in a white population. *Am. Rev. Respir. Dis.* 110:478.

Yang, S., Jiang, L., Cao, Z., Wang, L., Cao, J., Feng, R., et al. (2020). Deep learning for detecting corona virus disease 2019 (COVID-19) on high-resolution computed tomography: a pilot study. *Ann. Transl. Med.* 8:450. doi: 10.21037/atm.2020.03.132

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

MINI REVIEW
published: 17 February 2021
doi: 10.3389/fphys.2021.624185

In vitro and *In silico* Models to Study SARS-CoV-2 Infection: Integrating Experimental and Computational Tools to Mimic "COVID-19 Cardiomyocyte"

Rafael Dariolli, Chiara Campana, Amy Gutierrez and Eric A. Sobie*

Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, United States

The rapid dissemination of SARS-CoV-2 has made COVID-19 a tremendous social, economic, and health burden. Despite the efforts to understand the virus and treat the disease, many questions remain unanswered about COVID-19 mechanisms of infection and progression. Severe Acute Respiratory Syndrome (SARS) infection can affect several organs in the body including the heart, which can result in thromboembolism, myocardial injury, acute coronary syndromes, and arrhythmias. Numerous cardiac adverse events, from cardiomyocyte death to secondary effects caused by exaggerated immunological response against the virus, have been clinically reported. In addition to the disease itself, repurposing of treatments by using "off label" drugs can also contribute to cardiotoxicity. Over the past several decades, animal models and more recently, stem cell-derived cardiomyocytes have been proposed for studying diseases and testing treatments *in vitro*. In addition, mechanistic *in silico* models have been widely used for disease and drug studies. In these models, several characteristics such as gender, electrolyte imbalance, and comorbidities can be implemented to study pathophysiology of cardiac diseases and to predict cardiotoxicity of drug treatments. In this Mini Review, we (1) present the state of the art of *in vitro* and *in silico* cardiomyocyte modeling currently in use to study COVID-19, (2) review *in vitro* and *in silico* models that can be adopted to mimic the effects of SARS-CoV-2 infection on cardiac function, and (3) provide a perspective on how to combine some of these models to mimic "*COVID-19 cardiomyocytes environment.*"

Keywords: COVID-19, SARS-CoV-2, cardiomyocytes, hiPSC-CMs, modeling, pluripotent stem cells

OPEN ACCESS

Edited by:
Deborah Schechtman, University of São Paulo, Brazil

Reviewed by:
Kevin E. Healy, University of California, Berkeley, United States
Aslak Tveito, Simula Research Laboratory, Norway

****Correspondence:***
Rafael Dariolli
rafael.dariolli@mssm.edu

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *30 October 2020*
Accepted: *25 January 2021*
Published: *17 February 2021*

Citation:
Dariolli R, Campana C, Gutierrez A and Sobie EA (2021) In vitro and In silico Models to Study SARS-CoV-2 Infection: Integrating Experimental and Computational Tools to Mimic "COVID-19 Cardiomyocyte". Front. Physiol. 12:624185. doi: 10.3389/fphys.2021.624185

INTRODUCTION

Since the first reported case in Wuhan, China on December 31st, 2019, the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) has precipitated the coronavirus disease 2019 (COVID-19) pandemic, a global socio-economic and health burden (Bialek et al., 2020; Guan et al., 2020). As of January 08th, 2021, the total number of confirmed cases reported is approximately 86 million with more than 1.8 million deaths registered (WHO, 2020). The disease can affect most of the population with factors such as age, gender, race, socioeconomic status affecting prognosis (Gebhard et al., 2020; Golestaneh et al., 2020; Sharma G. et al., 2020; Zhou F. et al., 2020).

Additionally, pre-existing cardiovascular diseases (CVDs) such as hypertension, diabetes, and heart failure are prevalent in cohorts of patients with the most serious forms of COVID-19 (Goyal et al., 2020; Grasselli et al., 2020; Guan et al., 2020; Huang C. et al., 2020; Wu and McGoogan, 2020).

At the cellular level, SARS-CoV-2 infects its target by engaging with the angiotensin-converting enzyme 2 (ACE2) receptor, followed by cleavage of the viral spike (S) protein by the host serine protease TMPRSS2. Once in the cytoplasm, the viral RNA is replicated and released via exocytosis causing cellular damage (Hoffmann et al., 2020). In fact, human RNA sequencing has shown that the expression of both ACE2 and TMPRSS2 can be found in multiple organs (Chen et al., 2020), suggesting that SARS-CoV-2 might infect several target tissues.

Similar to the viruses responsible for the 2003 SARS outbreak and the 2012 MERS outbreak (Yu et al., 2006; Alhogbani, 2016), SARS-CoV-2 can trigger cardiovascular illnesses such as thromboembolism, myocardial injury, acute coronary syndromes, and arrhythmias (Clerkin et al., 2020; Madjid et al., 2020; Zheng et al., 2020). The heart is composed of many cell types including cardiomyocytes, endothelial cells, pericytes, epithelial cells, fibroblasts, smooth muscle cells, and immune cells (Pinto et al., 2016; Zhou and Pu, 2016; Chen et al., 2020) with all cell types contributing in some way to the overall cardiac function (Borg et al., 1996). Given the central role of cardiomyocytes in force generation and the minimal regenerative capacity of these cells (Cohn et al., 2000), avoiding cardiomyocyte damage and loss is of paramount importance to survival.

In the context of COVID-19, patients with poor prognosis who require hospitalization tend to exhibit a high prevalence of CVDs before viral infection. To understand the cardiovascular manifestations of COVID-19 and its treatments, experimental and mathematical cardiomyocyte models are likely to be of value. In this Mini Review, we: (1) present the state of the art of *in vitro* and *in silico* models of cardiomyocytes currently in use to study COVID-19, (2) review *in vitro* and *in silico* models that can be adopted to mimic the effects of SARS-CoV-2 infection on cardiac function, and (3) propose a perspective on how to create robust models that resemble a "*COVID-19 cardiomyocyte environment*" though the combination of *in vitro* and *in silico* strategies.

MODELING THE "COVID-19 CARDIOMYOCYTE" *IN VITRO*

In some of the first autopsies of deceased COVID-19 patients, electron microscopy identified viral particles compatible with the *Coronaviridae* family in multiple cardiac cell types, including cardiomyocytes, endothelial cells, macrophages, neutrophils, and fibroblasts (Dolhnikoff et al., 2020; Fox et al., 2020a,b; Lax et al., 2020; Lindner et al., 2020). Despite this preliminary evidence, the mechanism of direct infection of human adult cardiomyocytes is still not completely elucidated.

A recent single-cell sequencing of adult hearts demonstrated that expression of ACE2 receptors is higher in pericytes than in cardiomyocytes (Chen et al., 2020). Additionally, neither pericytes nor cardiomyocytes seem to significantly express the protease TMPRSS2 (Litviňuková et al., 2020), strongly suggesting that, if SARS-CoV-2 does in fact enter cardiomyocytes, it may do so through a pathway other than ACE2/TMPRSS2 (Pérez-Bermejo et al., 2020; Yang and Shen, 2020). Further, the high expression of ACE2 receptors in endothelial cells suggests that they represent a likely source of SARS-CoV-2 infection. In fact, the infection of endothelial cells by SARS-CoV-2 results in blood vessel inflammation (endotheliitis) in multiple organs, including the heart (Varga et al., 2020).

In this context, the use of *in vitro* models has been proposed in an effort to overcome the limitations related to the use of human tissues *post-mortem* (Yang et al., 2020). Human induced pluripotent stem-cell derived cardiomyocytes (hiPSC-CMs) can be directly infected by SARS-CoV-2 (Bojkova et al., 2020; Marchiano et al., 2020; Sharma A. et al., 2020; Yang et al., 2020). These infected cells display impairment of their spontaneous beating behavior (Bojkova et al., 2020; Marchiano et al., 2020; Sharma A. et al., 2020). Additionally, an excessive increase of caspase-3 cleavage, which drives cells to an apoptotic program, has been reported in these cells (Bojkova et al., 2020; Sharma A. et al., 2020).

Recent reports have suggested that hiPSC-CMs are infected via an alternative route involving the ACE2 receptor and cathepsin-dependent endolysosomes (Bojkova et al., 2020; Marchiano et al., 2020; Pérez-Bermejo et al., 2020), rather than through TMPRSS2 protease cleavage (Hoffmann et al., 2020). In fact, hiPSC-CMs display low expression of TMPRSS2 while cathepsins-L and -B, cysteine proteases which are also able to mediate priming of the viral S-protein (Hoffmann et al., 2020), are highly expressed in these cells (Bojkova et al., 2020). Furthermore, the block of cathepsins by chemical inhibition results in significant reduction of viral particles in hiPSC-CMs (Bojkova et al., 2020; Pérez-Bermejo et al., 2020).

In addition, SARS-CoV-2 exposure induces significant transcriptional changes resulting in the disruption of the contractile apparatus of hiPSC-CMs (Pérez-Bermejo et al., 2020). These cytopathic effects progressively affect hiPSC-CM electrophysiological and contractile properties as recently demonstrated. Microelectrode array measurements of hiPSC-CMs infected with SARS-CoV-2 documented a significant increase in their field potential duration (Marchiano et al., 2020), an *in vitro* surrogate for arrhythmogenicity. Similarly, infected three-dimensional engineered heart tissues displayed progressive impairment of contractility suggesting a disruption of the contractile apparatus following infection with SARS-CoV-2, which may contribute to whole-organ dysfunction (Huang L. et al., 2020; Marchiano et al., 2020).

Despite the exciting results describing the direct infection of cardiomyocytes by SARS-CoV-2, increasing clinical evidence points toward the indirect effects of the infection accounting for the most prevalent and severe cases that exhibit cardiac repercussions (Clerkin et al., 2020; Zheng et al., 2020). The field currently lacks robust models that can clarify these aspects of COVID-19 at the cardiomyocyte level.

Rising evidence shows that COVID-19 patients with worse prognosis present cardiac damage that correlates with the concentration of pro-inflammatory molecules (Akhmerov and Marbán, 2020; Ruan et al., 2020; Zhou F. et al., 2020). Indeed, severe symptoms, mainly related to the

hyperinflammation and deficit of oxygenation, have been described in the most aggressive cases (Iannaccone et al., 2020; Lax et al., 2020). The inability to promptly defeat a viral infection can elicit a *cytokine storm,* in which pro-inflammatory molecules including Interleukin-1β (IL-1β), Interleukin-6 (IL-6), and Tumor necrosis factor (TNF-α) are released in pathogenic concentrations causing systemic hyperinflammation (Iannaccone et al., 2020).

Pro-inflammatory molecules can directly cause adverse consequences in cardiomyocytes including arrhythmias (Long, 2001; El Khoury et al., 2014; Aromolaran et al., 2018; Keck et al., 2019), cellular hypertrophy (Long, 2001; Carreño et al., 2006; Smeets et al., 2008), and cell death (Wang et al., 2016). Elevated levels of IL-1β can trigger cardiac arrhythmias through the impairment of expression of proteins that control calcium handling, ultimately affecting cardiomyocyte's contraction (McTiernan et al., 1997; El Khoury et al., 2014). Similarly, neonatal cardiomyocytes (NCs) exposed to IL-6 in culture display augmentation of cell size suggesting cellular hypertrophy (Hirota et al., 1995). Interestingly, NCs co-cultured with fibroblasts overexpressing IL-6 are driven to apoptosis (Wang et al., 2016). Furthermore, IL-6 converts cardiac fibroblasts into myofibroblasts which produce collagen contributing to the formation of fibrosis (Wang et al., 2016). Pathological levels of IL-6 cause down-regulation of hERG channel (ether-a-go-go-related gene) expression, resulting in increased risk of action potential duration (APD) prolongation and arrhythmias (Aromolaran et al., 2018).

TNF-α is another pro-inflammatory molecule that triggers cardiac arrythmias and induces cardiomyocytes' hypertrophy and death (Nakamura et al., 1998; Carreño et al., 2006; Shen et al., 2018). Rat NCs treated with TNF-α exhibit abnormal size (Nakamura et al., 1998). Further, pathological levels of TNF-α can enhance mitochondrial fragmentation, promoting cell death (Shen et al., 2018), and a slow and sustained increase in hypertrophic markers through the NF-Kβ pathway (Smeets et al., 2008).

The *cytokine storm* caused by SARS-CoV-2 triggers an acute respiratory distress syndrome (ARDS) resulting in severe outcomes such as oxygen deprivation (hypoxia) and electrolyte disturbance (e.g., hypokalemia), factors that cause cardiomyocyte distress (Bhatia et al., 2012; Coperchini et al., 2020; Xu et al., 2020). Moreover, it appears that this hypoxia may induce release of additional cytokines, potentially leading to further myocyte dysfunction. In isolated rat NCs subjected to hypoxia (5% O_2), production and release of IL-6 are enhanced (Yamauchi-Takihara et al., 1995; Wang et al., 2016). In addition, conditioned media from rat NCs cultivated at 1% O_2 exhibit higher levels of TNF-α, IL-1β, IL-6, and transforming growth factor beta (TGF-β) compared to cells cultivated in normoxia (Shi et al., 2017).

Electrolyte imbalance and fever are two other typical conditions implicated in COVID-19 patients. Several models of hypokalemia and hyperthermia have indicated that slight changes in the cellular environment can dramatically impair cardiomyocytes' stability (El-Battrawy et al., 2016; Weiss et al., 2017; Tazmini et al., 2020). Hypokalemia is a systemic decrease in the concentration of K^+ ions that can produce APD prolongation and arrhythmias in cardiomyocytes (Weiss et al., 2017), including hiPSC-CMs (Kuusela et al., 2017). In addition, arrhythmias associated with hyperthermia have also been reported in both healthy and ill individuals (Saura et al., 2002; Pasquié et al., 2004; Burrell et al., 2007), and cellular studies have reported reductions in important cardiac ion channels caused by hyperthermia (El-Battrawy et al., 2016).

Taken together, the previous reports provide substantial information on how to model several outcomes that account for the cardiac deterioration observed in many COVID-19 patients. Studies that use patient-derived hiPSC-CMs carrying inherited diseases can also be found in the literature (Granéli et al., 2019; Hoes et al., 2019; Jimenez-Tellez and Greenway, 2019). Several of these models can be adopted to evaluate additive effects of COVID-19 and pre-existing comorbidities such as heart failure, cardiomyopathies, diabetes. The most representative *in vitro* models that mimic COVID-19 outcomes, as well as some of the significant hiPSC lines derived from patients with pre-existing comorbidities are described in **Table 1**.

MODELING THE "COVID-19 CARDIOMYOCYTE" *IN SILICO*

Besides the use of *in vitro* strategies, many authors have been reporting *in silico* models to study COVID-19. Most of them are concerned with predictions of mortality and risk factors (Scheiner et al., 2020; Wicik et al., 2020; Yadaw et al., 2020), disease infection and spread (Ivorra et al., 2020; Zeb et al., 2020), and drug-target interactions (Ciliberto and Cardone, 2020; Iqbal Choudhary and Shaikh, 2020; Muthuramalingam et al., 2020; Zhou Y. et al., 2020). Regarding the cardiac repercussions of COVID-19 and its potential treatments, mechanistic approaches based on dynamical models have been proposed to predict effectiveness of treatments (Iqbal Choudhary and Shaikh, 2020; Peterson, 2020), and to measure the toxicity of repurposed drugs (Sutanto and Heijman, 2020; Varshneya et al., 2020).

Notably, several drugs currently under testing have been previously reported to cause toxicity (Chary et al., 2020; Saleh et al., 2020; Smith et al., 2020; Zhang et al., 2020). Particular attention needs to be paid to drugs that can substantially increase arrhythmia risk or increase the risk of heart failure (Michaud et al., 2020), as addressed in a few recent studies. For example, *Sutanto and Heijman* used a canine ventricular cardiomyocyte model to simulate action potentials (APs) of cardiomyocytes treated with chloroquine (CQ) and azithromycin (AZM). The authors demonstrated that β-adrenergic stimulation is protective against CQ- and AZM-induced proarrhythmia by preventing APD prolongation and afterdepolarizations (Sutanto and Heijman, 2020). Meanwhile, our group has combined pharmacokinetics (PK) and electrophysiology modeling of human ventricular cardiomyocytes to predict the risk of potential cardiac adverse events caused by CQ, AZM, lopinavir (LP), and ritonavir (RT). Our simulations showed treatment with clinically relevant doses of CQ/AZM was more dangerous than treatment with LP/RT, and that females with pre-existing heart failure were at the highest risk of developing ventricular arrhythmia

TABLE 1 | *In vitro* models to be used to mimic "COVID-19 cardiomyocytes."

Outcome	Stimuli	Treatment	Specie	Cell type	References
Inflammation/ Hypertrophy	IL-1β	1 ng/mL, 12–16 h (adult cells) and 30 pg/mL 24–32 h	mouse	neonatal ventricular myocytes and adult ventricular myocytes	El Khoury et al., 2014
	TNF-α	1 ng/mL, 12–16 h and 30 pg/mL 24–32 h			
	TNF-α	50 ng/mL, 2, 12, 24, and 48 h	rat	Neonatal cardiomyocytes	Smeets et al., 2008
	TNF-α	1, 10, and 100 ng/mL (centered in 10 ng/mL), from 1 hour to 3 days.	rat	Neonatal cardiomyocytes	Nakamura et al., 1998
	TNF-α	5, 10, and 20 ng/mL, 6 or 48 h	rat	H9C2 rat cardiomyocytes	Shen et al., 2018
	IL-6	5, 10, and 20 ng/mL, 6 or 48 h			
	IL-6	2 μg/mL for 72 h	mouse	neonatal cardiomyocytes	Hirota et al., 1995
	IL-6	20 ng/mL for 40 min	guinea-pig	adult ventricular myocytes	Aromolaran et al., 2018
Hypoxia	Gases concentration	95% N_2 – 5% CO_2 (different regimens of time)	rat	neonatal ventricular cardiomyocytes	Yamauchi-Takihara et al., 1995
	Gases concentration	not described	rat	neonatal ventricular cardiomyocytes	Wang et al., 2016
	Gases concentration and culture medium	nitrogen equilibrated DMEM and 1% O_2 and 5% CO_2 for 2, 4, 6, 8, 10, and 12 h	mouse	neonatal cardiomyocytes	Shi et al., 2017
	Gases concentration	1% O_2 (adjusted by N_2 replacement in different regimens of time)	human/chimpanzee	iPSC-CMs	Ward and Gilad, 2019
	Gases concentration	1% O_2 (adjusted by N_2 replacement)	human	iPSC-CMs	Plant et al., 2020
Electrolyte imbalance (hypokalemia)	K^+ concentration in the medium	5.33, 4, 3, 2 and 1 mM of K^+ (adjusted by adding KCl into a K^+ free medium)	human	iPSC-CMs	Kuusela et al., 2017
	K^+ concentration in buffer solution	from 5 to 2.7 mmol/L rapidly reduction of K^+ superfusion	rat	atrial and ventricular adult myocytes	Tazmini et al., 2020
Hyperthermia	Temperature increase	36 vs. 40°C	human	iPSC-CMs	El-Battrawy et al., 2016
HCM	MYH7	missense mutation (Arginine442Glycine)	human	iPSC-CMs	Han et al., 2014
	MYBPC3	c.2373dupG mutation	human	iPSC-CMs	Birket et al., 2015
	MYBPC3	heterozygous c.1358-1359insC	human	iPSC-CMs	Prondzynski et al., 2017
DCM	phospholamban (PLN)	R14del mutation	human	iPSC-CMs	Stillitano et al., 2016
	LMNA gene	R225X, Q354X, and T518fs patient mutation	human	iPSC-CMs	Lee et al., 2017

from drug treatments (Varshneya et al., 2020). These studies (Sutanto and Heijman, 2020; Varshneya et al., 2020) suggests that future work needs to address how pre-existing diseases and COVID-19 clinical presentation (e.g., hyperinflammation, fever, ion imbalance) may affect arrhythmia susceptibility.

The use of *in silico* models to simulate electrophysiological perturbations and to predict disease severity and treatment efficacy is a mature field of research (Lancaster and Sobie, 2016; Passini et al., 2017; Jæger et al., 2019a,b; Li et al., 2019; Gando et al., 2020). Dynamic models of cardiomyocyte's electrophysiology are particularly useful for simulating between-patient variability (Muszkiewicz et al., 2016), allowing the study of phenotypic minorities, such as high-risk COVID-19 patients (Varshneya et al., 2020). This variability among individuals is hard to replicate in other model types (e.g., *in vivo*, *in vitro*). Further, *in silico* approaches can provide a quick illustration of how multiple factors in combinations (e.g., *cytokine storm* plus pre-existing diseases plus drug-treatments), can exacerbate positive or negative outcomes.

Over the past decades numerous *in silico* models that resemble electrophysiological properties of cardiomyocytes have been proposed based on experimental data from different species (Krogh-Madsen et al., 2016; Mayourian et al., 2018). These models can be used to highlight physio- and pathophysiological characteristics of ion channels and cellular compartments, as well as their intricate relationships, in order to gain a mechanistic understanding of a variety of illnesses and drug-treatments (Pandit et al., 2003; Sarkar and Sobie, 2011; Petkova-Kirova et al., 2012; Atkinson et al., 2016; Devenyi and Sobie, 2016; Das et al., 2017; Paci et al., 2018; Varshneya et al., 2018;

Gong et al., 2020; Sutanto et al., 2020). However, only few models that mimic inflammation (Petkova-Kirova et al., 2012) and hyperthermia (Atkinson et al., 2016) can be found in the literature, posing barriers to the modeling of COVID-19 secondary effects on cardiomyocytes. Further, many of these models are based on animal experiments, limiting translation of their findings.

It is worth mentioning that most of the models previously cited are based on ordinary differential equations (ODEs) and describe the action potential and calcium transient of an isolated cardiomyocyte. Therefore, these models cannot assess how interactions among cardiomyocytes as well as with fibroblasts and other cell types contribute to the overall cardiac function. These shortcomings can be addressed by bi-(2D) and tridimensional (3D) models (e.g., fiber, tissue), where electrical coupling and the resultant tissue-level behavior can be simulated (Lines et al., 2002; Jæger et al., 2019a; Hwang et al., 2020). These models can be used to investigate mechanisms of cardiac arrhythmia, such as cardiac reentry that can be induced by Early After Depolarizations (EADs). Complex models are promising and can be particularly beneficial considering correlations with experimental data obtained from 3D hiPSC-CM models (e.g., field potential assessments by MEA in hiPSC-CM monolayers or 3D structures) (Kügler, 2020). However, these spatial models are more computationally expensive and are less frequently used than single-cell models (Jæger et al., 2019a). The most significant *in silico* models able to partially simulate COVID-19 effect in cardiomyocytes are listed in **Table 2**.

DISCUSSION/PERSPECTIVE

Despite the rapid dissemination of high-quality science during the COVID-19 pandemic, crucial gaps of knowledge remain open. Here, we (1) reviewed the most up to date protocols

TABLE 2 | *In silico* models to be used to mimic "COVID-19 cardiomyocytes."

Outcome	Stimuli	Treatment/Simulation	Specie	Cell type	References
Drug-treatment (β-adrenergic signaling)	healthy cells under sympathetic stimulation	CQ, AZM	dog	ventricular cardiomyocytes	Sutanto and Heijman, 2020
Drug-treatment, heart failure, gender	healthy cells and heart failure cells from male and female	CQ, AZM, LP, RT	human	endocardial ventricular myocytes	Varshneya et al., 2020
Drug-treatment	healthy cells	Several drugs	human	ventricular cardiomyocytes	Lancaster and Sobie, 2016
Drug-treatment	healthy cells	several drugs	human	ventricular cardiomyocytes	Passini et al., 2017
Drug-treatment	healthy cells/dynamic hERG submodels	several drugs	human	ventricular cardiomyocytes	Li et al., 2019
Genetic disease (Q1475P Na_v 1.5 mutation)	healthy cells modified by Markov model for fast and late Na^+ current	$Na_v1.5$ mutation	human	endocardial ventricular myocytes	Gando et al., 2020
Comorbidity (diabetes type-I)	streptozotocin-induced, type-I diabetes in rats	baseline model vs. diabetes model	rat	right ventricle cardiomyocytes	Pandit et al., 2003
Ion current changes	75% block of I_{Kr}	baseline vs. I_{Kr} blocked cells	dog and human	ventricular cardiomyocytes	Sarkar and Sobie, 2011
Arrhythmogenic susceptibility	changes in the conductances of I_{Kr} and I_{Ks}	baseline vs. I_{Kr} and I_{Ks} modified cells	several	ventricular cardiomyocytes	Varshneya et al., 2018
β-adrenergic signaling/activity	healthy cells under sympathetic	human model parametrization for β-adrenergic system	human	epicardial ventricular cardiomyocytes	Gong et al., 2020
Inflammation/Hypertrophy	TNF-α overexpression in the heart	Parameterization using cardiomyocytes isolated from hearts overexpressing TNF-α	mouse	apical ventricular cardiomyocytes	Petkova-Kirova et al., 2012
Hyperthermia (fever)	Fever	baseline vs. adjusted model for fever based on malaria	human	atrial and ventricular cardiomyocytes	Atkinson et al., 2016
Drug-treatments, model validation, ion current changes	healthy cells vs. modifications: physiological and cardiotoxic spectrum	Tetrodotoxin, nifedipine, 3R4S-Chromanol 293B, E4031	human	Atrial and ventricular hiPSC-CMs	Paci et al., 2013
Ion imbalances, heart failure, hiPSC-CM/adult cardiomyocytes predictions	healthy cells vs. a variety of conditions and species cross predictions and validations	ion channel blocks, ion buffer composition changes, pacing rates, heart failure	human, guinea pig, rabbit	iPSC-CMs (human) and adult cardiomyocytes (different species)	Gong and Sobie, 2018
hiPSC-CM/adult cardiac microtissues, Drug-treatments	healthy microtissues from hiPSC-CMs	Cisapride and verapamil treatments (different doses)	human	iPSC-CMs (human)/adult myocytes	Tveito et al., 2018
hiPSC-CM/adult cardiac microtissues, Drug-treatments	healthy microtissues from hiPSC-CMs	Cisapride, verapamil, lidocaine, nifedipine, flecainide (many dose)	human	iPSC-CMs (human)/adult myocytes	Jæger et al., 2020

used to study COVID-19 effects in cardiomyocytes, and (2) reviewed several *in vitro* and *in silico* models of inflammation, ischemia/hypoxia, hyperthermia, hypokalemia, and hypertrophy, with great relevance cardiovascular effects of COVID-19 that are not due to direct infection of cardiomyocytes by SARS-CoV-2.

In this scenario, hiPSC-CMs emerge as a promising platform for modeling COVID-19. However, these cells display limited maturation and biological heterogeneity, partly due to a lack of consensus protocols for their generation and characterization, negatively contributing to their clinical translation (Lundy et al., 2013; Robertson et al., 2013; Koivumäki et al., 2018; Gintant et al., 2019; Hoang et al., 2019; Ribeiro et al., 2019). Several strategies have been proposed to overcome hiPSC-CMs maturation obstacles. Nonetheless, most of them only result in limited improvement, especially in the case of 2D models (Talkhabi et al., 2016; Sun and Nunes, 2017). In parallel, 3D models such as cardiac spheroids (Polonchuk et al., 2017; Mattapally et al., 2018) and "*engineered heart tissues*" (EHTs) (Nunes et al., 2013; Stoehr et al., 2014) have shown promising results toward obtaining mature cells and a phenotype that more closely resembles adult tissue. However, the specialized expertise required for 3D technologies and the expense of these assays remains a challenge that limits the use of these approaches for research groups that require scalable or high throughput implementation (Zuppinger, 2019).

Additionally, *in silico* models of hiPSC-CMs' electrophysiology became a reality (Paci et al., 2013, 2018; Gong and Sobie, 2018; Tveito et al., 2018; Jæger et al., 2020), allowing the simulation of disease effects and drug toxicity (Gong and Sobie, 2018; Jæger et al., 2020). Similarly to the case of experimental models, there are peculiarities and limitations for modeling cardiomyocytes *in silico* (Gong et al., 2017). However, *in silico* models have the flexibility of being easily adapted to new experimental data, such as the ones obtained from hiPSC-CMs, allowing for more accurate quantitative predictions (Lei et al., 2017; Jæger et al., 2020; Paci et al., 2020). Furthermore, the most recent mechanistic models permit an improved translation of electrophysiological findings from hiPSC-CMs to human adult myocytes at both single-cell and tissue level (Gong and Sobie, 2018; Tveito et al., 2018). Overall, these strategies consider proportional changes in proteins expression throughout maturation without significant changes in the cell's function. Therefore, regression models, among other strategies can be used to parameterize ion current densities and correlate hiPSC-CM to adult cardiomyocyte models (Gong and Sobie, 2018; Tveito et al., 2018).

Currently, universal protocols for the generation and characterization of hiPSC-CMs are not available. Depending on their application, strengths and weaknesses exist for both 2D and 3D models (Zuppinger, 2019). Especially in the context of a pandemic, strengths, and weaknesses should be pondered to allow for fast and meaningful experimental research. The best models of choice in this scenario are the ones that can generate accurate results but in a timely fashion. Independently of the model of choice, experiments need to be conducted in well-controlled environment, replicated for different cell lines, and always accompanied by negative controls (non-treated, healthy). Analogously, *in silico* models should be chosen to best match the experimental approach. The interpretation of results needs to be cautious, always considering the intrinsic limitations of each model.

Thus, the integration of experimental data obtained from hiPSC-CMs (single-cell, 2D, and 3D models) with appropriate *in silico* models that can quantitatively predict functional cardiac outcomes in adult cells is of paramount importance. hiPSC-CMs from healthy donors or patients with pre-established comorbidities can be utilized to investigate *in vitro* the reaction of cardiomyocytes to several conditions precipitated by the systemic effects of SARS-CoV-2 infection. Many of these models were discussed in this mini review. The results obtained from experiments with hiPSC-CMs will provide valuable information that can be integrated into *in silico* models and used to predict disease progression and the effects of treatment.

In conclusion, we presented a perspective on how to combine *in vitro* and *in silico* approaches to generate human-based platforms to study COVID-19 repercussions on the cardiomyocyte's function. The use of robust and precise models and their integration in mechanistic platforms may contribute substantially to understanding the impact of COVID-19 and COVID-19 drug treatments on the heart, constituting an additional source of guidance to help clinicians in the front line.

AUTHOR CONTRIBUTIONS

RD conceived the manuscript and wrote the initial draft. CC, AG, and ES helped to edit the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Research on cardiomyocytes and drug-induced arrhythmias in Dr. Sobie's laboratory was funded by the National Heart, Lung, and Blood Institute projects U01 HL 136297 and R44HL139248.

ACKNOWLEDGMENTS

We thank Ananya Pavuluri, Itziar Irurzun Arana, Meera Varshneya, Taylor Pullinger, and Angelita Dona for the discussions during this time of pandemic, that were all very insightful for conceiving this manuscript.

REFERENCES

Akhmerov, A., and Marbán, E. (2020). COVID-19 and the heart. *Circ. Res.* 126, 1443–1455. doi: 10.1161/CIRCRESAHA.120.317055

Alhogbani, T. (2016). Acute myocarditis associated with novel Middle East respiratory syndrome coronavirus. *Ann. Saudi Med.* 36, 78–80. doi: 10.5144/0256-4947.2016.78

Aromolaran, A. S., Srivastava, U., Alí, A., Chahine, M., Lazaro, D., El-Sherif, N., et al. (2018). Interleukin-6 inhibition of hERG underlies risk for acquired long

QT in cardiac and systemic inflammation. *PLoS One* 13:e0208321. doi: 10.1371/journal.pone.0208321

Atkinson, L., Basharat, A., Benson, A. P., Colman, M. A., Holden, A. V., Kagugube, G. W., et al. (2016). Computational modelling of cardiac electrophysiological changes in malarial fever. *Comput. Cardiol. (2010).* 43, 1097–1100. doi: 10.22489/cinc.2016.315-270

Bhatia, M., Zemans, R. L., and Jeyaseelan, S. (2012). Role of chemokines in the pathogenesis of acute lung injury. *Am. J. Respir. Cell Mol. Biol.* 46, 566–572. doi: 10.1165/rcmb.2011-0392TR

Bialek, S., Boundy, E., Bowen, V., Chow, N., Cohn, A., Dowling, N., et al. (2020). Severe outcomes among patients with coronavirus disease 2019 (COVID-19) — United States, february 12–march 16, 2020. *MMWR. Morb. Mortal. Wkly. Rep.* 69, 343–346. doi: 10.15585/mmwr.mm6912e2

Birket, M. J., Ribeiro, M. C., Kosmidis, G., Ward, D., Leitoguinho, A. R., van de Pol, V., et al. (2015). Contractile defect caused by mutation in MYBPC3 revealed under conditions optimized for human PSC-Cardiomyocyte function. *Cell Rep.* 13, 733–745. doi: 10.1016/j.celrep.2015.09.025

Bojkova, D., Wagner, J. U. G., Shumliakivska, M., Aslan, G. S., Saleem, U., Hansen, A., et al. (2020). SARS-CoV-2 infects and induces cytotoxic effects in human cardiomyocytes. *Cardiovasc. Res.* 116, 2207–2215. doi: 10.1093/cvr/cvaa267

Borg, T. K., Rubin, K., Carver, W., Samarel, A., and Terracio, L. (1996). The cell biology of the cardiac interstitium. *Trends Cardiovasc. Med.* 6, 65–70. doi: 10.1016/1050-1738(96)00005-9

Burrell, C., Reddy, S., Haywood, G., and Cunningham, R. (2007). Cardiac arrest associated with febrile illness due to U.K. acquired Cyclospora cayetanensis. *J. Infect.* 54, e13–e15. doi: 10.1016/j.jinf.2006.03.020

Carreño, J. E., Apablaza, F., Ocaranza, M. P., and Jalil, J. E. (2006). Cardiac hypertrophy: molecular and cellular events. *Rev. Española Cardiol. (English Ed.* 59, 473–486. doi: 10.1016/s1885-5857(06)60796-2

Chary, M. A., Barbuto, A. F., Izadmehr, S., Hayes, B. D., and Burns, M. M. (2020). COVID-19: therapeutics and their toxicities. *J. Med. Toxicol.* 16, 284–294. doi: 10.1007/s13181-020-00777-5

Chen, L., Li, X., Chen, M., Feng, Y., and Xiong, C. (2020). The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. *Cardiovasc. Res.* 116, 1097–1100. doi: 10.1093/cvr/cvaa078

Ciliberto, G., and Cardone, L. (2020). Boosting the arsenal against COVID-19 through computational drug repurposing. *Drug Discov. Today* 25, 946–948. doi: 10.1016/j.drudis.2020.04.005

Clerkin, K. J., Fried, J. A., Raikhelkar, J., Sayer, G., Griffin, J. M., Masoumi, A., et al. (2020). COVID-19 and cardiovascular disease. *Circulation* 141, 1648–1655. doi: 10.1161/CIRCULATIONAHA.120.046941

Cohn, J. N., Ferrari, R., and Sharpe, N. (2000). Cardiac remodeling—concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. *J. Am. Coll. Cardiol.* 35, 569–582. doi: 10.1016/S0735-1097(99)00630-0

Coperchini, F., Chiovato, L., Croce, L., Magri, F., and Rotondi, M. (2020). The cytokine storm in COVID-19: an overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine Growth Factor Rev.* 53, 25–32. doi: 10.1016/j.cytogfr.2020.05.003

Das, P. N., Kumar, A., Bairagi, N., and Chatterjee, S. (2017). Restoring calcium homeostasis in diabetic cardiomyocytes: an investigation through mathematical modelling. *Mol. Biosyst.* 13, 2056–2068. doi: 10.1039/c7mb00264e

Devenyi, R. A., and Sobie, E. A. (2016). There and back again: Iterating between population-based modeling and experiments reveals surprising regulation of calcium transients in rat cardiac myocytes. *J. Mol. Cell. Cardiol.* 96, 38–48. doi: 10.1016/j.yjmcc.2015.07.016

Dolhnikoff, M., Ferreira Ferranti, J., de Almeida Monteiro, R. A., Duarte-Neto, A. N., Soares Gomes-Gouvêa, M., Viu Degaspare, N., et al. (2020). SARS-CoV-2 in cardiac tissue of a child with COVID-19-related multisystem inflammatory syndrome. *Lancet Child Adolesc. Heal.* 4, 790–794. doi: 10.1016/S2352-4642(20)30257-1

El Khoury, N., Mathieu, S., and Fiset, C. (2014). Interleukin-1β reduces L-type Ca2+ current through protein kinase C∈ activation in mouse heart. *J. Biol. Chem.* 289, 21896–21908. doi: 10.1074/jbc.M114.549642

El-Battrawy, I., Lang, S., Zhao, Z., Akin, I., Yücel, G., Meister, S., et al. (2016). Hyperthermia influences the effects of sodium channel blocking drugs in human-induced pluripotent stem cell-derived cardiomyocytes. *PLoS One* 11:e0166143. doi: 10.1371/journal.pone.0166143

Fox, S. E., Akmatbekov, A., Harbert, J. L., Li, G., Quincy Brown, J., and Vander Heide, R. S. (2020a). Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. *Lancet Respir. Med.* 8, 681–686. doi: 10.1016/S2213-2600(20)30243-5

Fox, S. E., Li, G., Akmatbekov, A., Harbert, J. L., Lameira, F. S., Brown, J. Q., et al. (2020b). Unexpected features of cardiac pathology in COVID-19 infection. *Circulation* 142, 1123–1125. doi: 10.1161/circulationaha.120.049465

Gando, I., Campana, C., Tan, R. B., Cecchin, F., Sobie, E. A., and Coetzee, W. A. (2020). A distinct molecular mechanism by which phenytoin rescues a novel long QT 3 variant. *J. Mol. Cell. Cardiol.* 144, 1–11. doi: 10.1016/j.yjmcc.2020.04.027

Gebhard, C., Regitz-Zagrosek, V., Neuhauser, H. K., Morgan, R., and Klein, S. L. (2020). Impact of sex and gender on COVID-19 outcomes in Europe. *Biol. Sex Differ.* 11:29. doi: 10.1186/s13293-020-00304-9

Gintant, G., Burridge, P., Gepstein, L., Harding, S., Herron, T., Hong, C., et al. (2019). Use of human induced pluripotent stem cell-derived cardiomyocytes in preclinical cancer drug cardiotoxicity testing: a scientific statement from the american heart association. *Circ. Res.* 125, e75–e92. doi: 10.1161/RES.0000000000000291

Golestaneh, L., Neugarten, J., Fisher, M., Billett, H. H., Gil, M. R., Johns, T., et al. (2020). The association of race and COVID-19 mortality. *EClinicalMedicine* 25:100455. doi: 10.1016/j.eclinm.2020.100455

Gong, J. Q. X., Shim, J. V., Núñez-Acosta, E., and Sobie, E. A. (2017). I love it when a plan comes together: insight gained through convergence of competing mathematical models. *J. Mol. Cell. Cardiol.* 102, 31–33. doi: 10.1016/j.yjmcc.2016.10.015

Gong, J. Q. X., and Sobie, E. A. (2018). Population-based mechanistic modeling allows for quantitative predictions of drug responses across cell types. *npj Syst. Biol. Appl.* 4:11. doi: 10.1038/s41540-018-0047-2

Gong, J. Q. X., Susilo, M. E., Sher, A., Musante, C. J., and Sobie, E. A. (2020). Quantitative analysis of variability in an integrated model of human ventricular electrophysiology and β-adrenergic signaling. *J. Mol. Cell. Cardiol.* 143, 96–106. doi: 10.1016/j.yjmcc.2020.04.009

Goyal, P., Choi, J. J., Pinheiro, L. C., Schenck, E. J., Chen, R., Jabri, A., et al. (2020). Clinical characteristics of Covid-19 in New York City. *N. Engl. J. Med.* 382, 2372–2374. doi: 10.1056/NEJMc2010419

Granéli, C., Hicks, R., Brolén, G., Synnergren, J., and Sartipy, P. (2019). Diabetic cardiomyopathy modelling using induced pluripotent stem cell derived cardiomyocytes: recent advances and emerging models. *Stem Cell Rev. Reports* 15, 13–22. doi: 10.1007/s12015-018-9858-1

Grasselli, G., Zangrillo, A., Zanella, A., Antonelli, M., Cabrini, L., Castelli, A., et al. (2020). Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the lombardy region, Italy. *JAMA* 323:1574. doi: 10.1001/jama.2020.5394

Guan, W., Ni, Z., Hu, Y., Liang, W., Ou, C., He, J., et al. (2020). Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* 382, 1708–1720. doi: 10.1056/NEJMoa2002032

Han, L., Li, Y., Tchao, J., Kaplan, A. D., Lin, B., Li, Y., et al. (2014). Study familial hypertrophic cardiomyopathy using patient-specific induced pluripotent stem cells. *Cardiovasc. Res.* 104, 258–269. doi: 10.1093/cvr/cvu205

Hirota, H., Yoshida, K., Kishimotot, T., and Taga, T. (1995). Continuous activation of gpl30, a signal-transducing receptor component for interleukin 6-related cytokines, causes myocardial hypertrophy in mice (interleukin 6 receptor). *Proc. Natl. Acad. Sci. U.S.A.* 92, 4862–4866. doi: 10.1073/pnas.92.11.4862

Hoang, P., Jacquir, S., Lemus, S., and Ma, Z. (2019). Quantification of contractile dynamic complexities exhibited by human stem cell-derived cardiomyocytes using nonlinear dimensional analysis. *Sci. Rep.* 9:14714. doi: 10.1038/s41598-019-51197-7

Hoes, M. F., Bomer, N., and van der Meer, P. (2019). Concise review: the current state of human in vitro cardiac disease modeling: a focus on gene editing and tissue engineering. *Stem Cells Transl. Med.* 8, 66–74. doi: 10.1002/sctm.18-0052

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271.e–280.e. doi: 10.1016/j.cell.2020.02.052

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Huang, L., Zhao, P., Tang, D., Zhu, T., Han, R., Zhan, C., et al. (2020). Cardiac involvement in patients recovered from COVID-2019 identified using magnetic resonance imaging. *JACC Cardiovasc. Imaging* 13, 2330–2339. doi: 10.1016/j.jcmg.2020.05.004

Hwang, M., Lim, C.-H., Leem, C. H., and Shim, E. B. (2020). In silico models for evaluating proarrhythmic risk of drugs. *APL Bioeng.* 4:021502. doi: 10.1063/1.5132618

Iannaccone, G., Scacciavillani, R., Del Buono, M. G., Camilli, M., Ronco, C., Lavie, C. J., et al. (2020). Weathering the cytokine storm in COVID-19: therapeutic implications. *Cardiorenal. Med.* 10, 277–287. doi: 10.1159/000509483

Iqbal Choudhary, M. I., and Shaikh, M. (2020). In silico identification of potential inhibitors of key SARS-CoV-2 3CL hydrolase (Mpro) via molecular docking, MMGBSA predictive binding energy calculations, and molecular dynamics simulation. *PLoS One* 15:e0235030. doi: 10.1371/journal.pone.0235030

Ivorra, B., Ferrández, M. R., Vela-Pérez, M., and Ramos, A. M. (2020). Mathematical modeling of the spread of the coronavirus disease 2019 (COVID-19) taking into account the undetected infections. The case of China. *Commun. Nonlinear Sci. Numer. Simul.* 88:105303. doi: 10.1016/j.cnsns.2020.105303

Jæger, K. H., Charwat, V., Charrez, B., Finsberg, H., Maleckar, M. M., Wall, S., et al. (2020). Improved computational identification of drug response using optical measurements of human stem cell derived cardiomyocytes in microphysiological systems. *Front. Pharmacol.* 10:1648. doi: 10.3389/fphar.2019.01648

Jæger, K. H., Edwards, A. G., McCulloch, A., and Tveito, A. (2019a). Properties of cardiac conduction in a cell-based computational model. *PLoS Comput. Biol.* 15:e1007042. doi: 10.1371/journal.pcbi.1007042

Jæger, K. H., Wall, S., and Tveito, A. (2019b). Detecting undetectables: can conductances of action potential models be changed without appreciable change in the transmembrane potential? *Chaos* 29:073102. doi: 10.1063/1.5087629

Jimenez-Tellez, N., and Greenway, S. C. (2019). Cellular models for human cardiomyopathy: what is the best option? *World J. Cardiol.* 11, 221–235. doi: 10.4330/wjc.v11.i10.221

Keck, M., Flamant, M., Mougenot, N., Favier, S., Atassi, F., Barbier, C., et al. (2019). Cardiac inflammatory CD11b/c cells exert a protective role in hypertrophied cardiomyocyte by promoting TNFR 2 - and Orai3- dependent signaling. *Sci. Rep.* 9, 1–18. doi: 10.1038/s41598-019-42452-y

Koivumäki, J. T., Naumenko, N., Tuomainen, T., Takalo, J., Oksanen, M., Puttonen, K. A., et al. (2018). Structural immaturity of human iPSC-derived cardiomyocytes: in silico investigation of effects on function and disease modeling. *Front. Physiol.* 9:80. doi: 10.3389/fphys.2018.00080

Krogh-Madsen, T., Sobie, E. A., and Christini, D. J. (2016). Improving cardiomyocyte model fidelity and utility via dynamic electrophysiology protocols and optimization algorithms. *J. Physiol.* 594, 2525–2536. doi: 10.1113/JP270618

Kügler, P. (2020). Modelling and simulation for preclinical cardiac safety assessment of drugs with human iPSC-derived cardiomyocytes. *Jahresbericht der Dtsch. Math.* 122, 209–257. doi: 10.1365/s13291-020-00218-w

Kuusela, J., Larsson, K., Shah, D., Prajapati, C., and Aalto-Setala, K. (2017). Low extracellular potassium prolongs repolarization and evokes early after depolarization in human induced pluripotent stem cell-derived cardiomyocytes. *Biol. Open* 6, 777–784. doi: 10.1242/bio.024216

Lancaster, M. C., and Sobie, E. (2016). Improved prediction of drug-induced torsades de pointes through simulations of dynamics and machine learning algorithms. *Clin. Pharmacol. Ther.* 100, 371–379. doi: 10.1002/cpt.367

Lax, S. F., Skok, K., Zechner, P., Kessler, H. H., Kaufmann, N., Koelblinger, C., et al. (2020). Pulmonary arterial thrombosis in COVID-19 with fatal outcome: results from a prospective, single-center, clinicopathologic case series. *Ann. Intern. Med.* 173, 350–361. doi: 10.7326/m20-2566

Lee, Y., Lau, Y., Cai, Z., Lai, W., Wong, L., Tse, H., et al. (2017). Modeling treatment response for lamin A/C related dilated cardiomyopathy in human induced pluripotent stem cells. *J. Am. Heart Assoc.* 6:e005677. doi: 10.1161/JAHA.117.005677

Lei, C. L., Wang, K., Clerx, M., Johnstone, R. H., Hortigon-Vinagre, M. P., Zamora, V., et al. (2017). Tailoring mathematical models to stem-cell derived cardiomyocyte lines can improve predictions of drug-induced changes to their electrophysiology. *Front. Physiol.* 8:986. doi: 10.3389/fphys.2017.00986

Li, Z., Ridder, B. J., Han, X., Wu, W. W., Sheng, J., Tran, P. N., et al. (2019). Assessment of an *In Silico* mechanistic model for proarrhythmia risk prediction under the CiPA initiative. *Clin. Pharmacol. Ther.* 105, 466–475. doi: 10.1002/cpt.1184

Lindner, D., Fitzek, A., Bräuninger, H., Aleshcheva, G., Edler, C., Meissner, K., et al. (2020). Association of cardiac infection with SARS-CoV-2 in confirmed COVID-19 autopsy cases. *JAMA Cardiol.* 5, 1281–1285. doi: 10.1001/jamacardio.2020.3551

Lines, G. T., Buist, M. L., Grottum, P., Pullan, A. J., Sundnes, J., and Tveito, A. (2002). Mathematical models and numerical methods for the forward problem in cardiac electrophysiology. *Comput. Vis. Sci.* 5, 215–239. doi: 10.1007/s00791-003-0101-4

Litviňuková, M., Talavera-López, C., Maatz, H., Reichart, D., Worth, C. L., Lindberg, E. L., et al. (2020). Cells of the adult human heart. *Nature* 588, 466–472. doi: 10.1038/s41586-020-2797-4

Long, C. S. (2001). The role of interleukin-1 in the failing heart. *Heart Fail. Rev.* 6, 81–94.

Lundy, S. D., Zhu, W.-Z., Regnier, M., and Laflamme, M. A. (2013). Structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. *Stem Cells Dev.* 22, 1991–2002. doi: 10.1089/scd.2012.0490

Madjid, M., Safavi-Naeini, P., Solomon, S. D., and Vardeny, O. (2020). Potential effects of coronaviruses on the cardiovascular system. *JAMA Cardiol.* 5:831. doi: 10.1001/jamacardio.2020.1286

Marchiano, S., Hsiang, T.-Y., Higashi, T., Kanna, A., Reinecke, H., Yang, X., et al. (2020). SARS-CoV-2 infects human pluripotent stem cell-derived cardiomyocytes, impairing electrical and mechanical function. *bioRxiv* [Preprint]. doi: 10.1101/2020.08.30.274464

Mattapally, S., Zhu, W., Fast, V. G., Gao, L., Worley, C., Kannappan, R., et al. (2018). Spheroids of cardiomyocytes derived from human-induced pluripotent stem cells improve recovery from myocardial injury in mice. *Am. J. Physiol. Hear. Circ. Physiol.* 315, 327–339. doi: 10.1152/ajpheart.00688.2017.-The

Mayourian, J., Sobie, E. A., and Costa, K. D. (2018). An introduction to computational modeling of cardiac electrophysiology and arrhythmogenicity. *Methods Mol. Biol.* 1816, 17–35. doi: 10.1007/978-1-4939-8597-5_2

McTiernan, C. F., Lemster, B. H., Frye, C., Brooks, S., Combes, A., and Feldman, A. M. (1997). Interleukin-1β inhibits phospholamban gene expression in cultured cardiomyocytes. *Circ. Res.* 81, 493–503. doi: 10.1161/01.RES.81.4.493

Michaud, V., Dow, P., Al Rihani, S. B., Deodhar, M., Arwood, M., Cicali, B., et al. (2020). Risk of drug-induced long QT syndrome associated with the use of repurposed COVID-19 drugs: a systematic review. *medRxiv* [Preprint]. doi: 10.1101/2020.04.21.20066761

Muszkiewicz, A., Britton, O. J., Gemmell, P., Passini, E., Sánchez, C., Zhou, X., et al. (2016). Variability in cardiac electrophysiology: using experimentally-calibrated populations of models to move beyond the single virtual physiological human paradigm. *Prog. Biophys. Mol. Biol.* 120, 115–127. doi: 10.1016/j.pbiomolbio.2015.12.002

Muthuramalingam, P., Jeyasri, R., Valliammai, A., Selvaraj, A., Karthika, C., Gowrishankar, S., et al. (2020). Global multi-omics and systems pharmacological strategy unravel the multi-targeted therapeutic potential of natural bioactive molecules against COVID-19: an in silico approach. *Genomics* 112, 4486–4504. doi: 10.1016/j.ygeno.2020.08.003

Nakamura, K., Fushimi, K., Kouchi, H., Mihara, K., Miyazaki, M., Ohe, T., et al. (1998). Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-α and angiotensin II. *Circulation* 98, 794–799. doi: 10.1161/01.CIR.98.8.794

Nunes, S. S., Miklas, J. W., Liu, J., Aschar-Sobbi, R., Xiao, Y., Zhang, B., et al. (2013). Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. *Nat. Methods* 10, 781–787. doi: 10.1038/nmeth.2524

Paci, M., Hyttinen, J., Aalto-Setälä, K., and Severi, S. (2013). Computational models of ventricular-and atrial-like human induced pluripotent stem cell derived cardiomyocytes. *Ann. Biomed. Eng.* 41, 2334–2348. doi: 10.1007/s10439-013-0833-3

Paci, M., Passini, E., Klimas, A., Severi, S., Hyttinen, J., Rodriguez, B., et al. (2020). All-optical electrophysiology refines populations of in silico human iPSC-CMs for drug evaluation. *Biophys. J.* 118, 2596–2611. doi: 10.1016/j.bpj.2020.03.018

Paci, M., Pölönen, R. P., Cori, D., Penttinen, K., Aalto-Setälä, K., Severi, S., et al. (2018). Automatic optimization of an in silico model of human iPSC derived cardiomyocytes recapitulating calcium handling abnormalities. *Front. Physiol.* 9:709. doi: 10.3389/fphys.2018.00709

Pandit, S. V., Giles, W. R., and Demir, S. S. (2003). A mathematical model of the electrophysiological alterations in rat ventricular myocytes in type-I diabetes. *Biophys. J.* 84, 832–841. doi: 10.1016/S0006-3495(03)74902-9

Pasquié, J. L., Sanders, P., Hocini, M., Hsu, L. F., Scavée, C., Jais, P., et al. (2004). Fever as a precipitant of idiopathic ventricular fibrillation in patients with normal hearts. *J. Cardiovasc. Electrophysiol.* 15, 1271–1276. doi: 10.1046/j.1540-8167.2004.04388.x

Passini, E., Britton, O. J., Lu, H. R., Rohrbacher, J., Hermans, A. N., Gallacher, D. J., et al. (2017). Human in silico drug trials demonstrate higher accuracy than animal models in predicting clinical pro-arrhythmic cardiotoxicity. *Front. Physiol.* 8:668. doi: 10.3389/fphys.2017.00668

Pérez-Bermejo, J. A., Kang, S., Rockwood, S. J., Simoneau, C. R., Joy, D. A., Ramadoss, G. N., et al. (2020). SARS-CoV-2 infection of human iPSC-derived cardiac cells predicts novel cytopathic features in hearts of COVID-19 patients. *bioRxiv Prepr. Serv. Biol.* doi: 10.1101/2020.08.25.265561

Peterson, L. (2020). *COVID-19 and Flavonoids: In Silico Molecular Dynamics Docking to the Active Catalytic Site of SARS-CoV and SARS-CoV-2 Main Protease (May 12, 2020)*. doi: 10.2139/ssrn.3599426

Petkova-Kirova, P. S., London, B., Salama, G., Rasmusson, R. L., and Bondarenko, V. E. (2012). Mathematical modeling mechanisms of arrhythmias in transgenic mouse heart overexpressing TNF-α. *Am. J. Physiol. Circ. Physiol.* 302, H934–H952. doi: 10.1152/ajpheart.00493.2011

Pinto, A. R., Ilinykh, A., Ivey, M. J., Kuwabara, J. T., D'Antoni, M. L., Debuque, R., et al. (2016). Revisiting cardiac cellular composition. *Circ. Res.* 118, 400–409. doi: 10.1161/CIRCRESAHA.115.307778

Plant, L. D., Xiong, D., Romero, J., Dai, H., and Goldstein, S. A. N. (2020). Hypoxia produces pro-arrhythmic late sodium current in cardiac myocytes by SUMOylation of NaV1.5 Channels. *Cell Rep.* 30, 2225–2236.e4. doi: 10.1016/j.celrep.2020.01.025

Polonchuk, L., Chabria, M., Badi, L., Hoflack, J. C., Figtree, G., Davies, M. J., et al. (2017). Cardiac spheroids as promising in vitro models to study the human heart microenvironment. *Sci. Rep.* 7:7005. doi: 10.1038/s41598-017-06385-8

Prondzynski, M., Krämer, E., Laufer, S. D., Shibamiya, A., Pless, O., Flenner, F., et al. (2017). Evaluation of MYBPC3 trans-Splicing and gene replacement as therapeutic options in human iPSC-derived cardiomyocytes. *Mol. Ther. Nucleic. Acids* 7, 475–486. doi: 10.1016/j.omtn.2017.05.008

Ribeiro, A., Guth, B., Engwall, M., Eldridge, S., Foley, C. M., Guo, L., et al. (2019). Considerations for an in vitro, cell-based testing platform for detection of drug-induced inotropic effects in early drug development. Part 2: designing and fabricating microsystems for assaying cardiac contractility with physiological relevance using human iPSC-cardiomyocytes. *Front. Pharmacol.* 10:934. doi: 10.3389/fphar.2019.00934

Robertson, C., Tran, D. D., and George, S. C. (2013). Concise review: maturation phases of human pluripotent stem cell-derived cardiomyocytes. *Stem Cells* 31, 829–837. doi: 10.1002/stem.1331

Ruan, Q., Yang, K., Wang, W., Jiang, L., and Song, J. (2020). Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan. *China. Intensive Care Med.* 46, 846–848. doi: 10.1007/s00134-020-05991-x

Saleh, M., Gabriels, J., Chang, D., Soo Kim, B., Mansoor, A., Mahmood, E., et al. (2020). Effect of chloroquine, hydroxychloroquine, and azithromycin on the corrected QT interval in patients with SARS-CoV-2 infection. *Circ. Arrhythmia Electrophysiol.* 13, 496–504. doi: 10.1161/CIRCEP.120.008662

Sarkar, A. X., and Sobie, E. A. (2011). Quantification of repolarization reserve to understand interpatient variability in the response to proarrhythmic drugs: a computational analysis. *Hear. Rhythm* 8, 1749–1755. doi: 10.1016/j.hrthm.2011.05.023

Saura, D., García-Alberola, A., Carrillo, P., Pascual, D., Martínez-Sánchez, J., and Valdés, M. (2002). Brugada-like electrocardiographic pattern induced by fever. *PACE Pacing Clin. Electrophysiol.* 25, 856–859. doi: 10.1046/j.1460-9592.2002.t01-1-00856.x

Scheiner, S., Ukaj, N., and Hellmich, C. (2020). Mathematical modeling of COVID-19 fatality trends: Death kinetics law versus infection-to-death delay rule. *Chaos Solitons Fractals* 136:109891. doi: 10.1016/j.chaos.2020.109891

Sharma, A., Garcia, G., Wang, Y., Plummer, J. T., Morizono, K., Arumugaswami, V., et al. (2020). Human iPSC-derived cardiomyocytes are susceptible to SARS-CoV-2 infection. *Cell Reports Med.* 1:100052. doi: 10.1016/j.xcrm.2020.100052

Sharma, G., Volgman, A. S., and Michos, E. D. (2020). Sex differences in mortality from COVID-19 pandemic. *JACC Case Reports* 2, 1407–1410. doi: 10.1016/j.jaccas.2020.04.027

Shen, Y. L., Shi, Y. Z., Chen, G. G., Wang, L. L., Zheng, M. Z., Jin, H. F., et al. (2018). TNF-α induces drp1-mediated mitochondrial fragmentation during inflammatory cardiomyocyte injury. *Int. J. Mol. Med.* 41, 2317–2327. doi: 10.3892/ijmm.2018.3385

Shi, H., Zhang, X., He, Z., Wu, Z., Rao, L., and Li, Y. (2017). Metabolites of hypoxic cardiomyocytes induce the migration of cardiac fibroblasts. *Cell. Physiol. Biochem.* 41, 413–421. doi: 10.1159/000456531

Smeets, P. J. H., Teunissen, B. E. J., Planavila, A., De Vogel-Van Den Bosch, H., Willemsen, P. H. M., Van Der Vusse, G. J., et al. (2008). Inflammatory pathways are activated during cardiomyocyte hypertrophy and attenuated by peroxisome proliferator-activated receptors PPARα and PPARδ. *J. Biol. Chem.* 283, 29109–29118. doi: 10.1074/jbc.M802143200

Smith, T., Bushek, J., Leclaire, A., and Prosser, T. (2020). *COVID-19 Drug Therapy*. Amsterdam: Elsevier.

Stillitano, F., Turnbull, I. C., Karakikes, I., Nonnenmacher, M., Backeris, P., Hulot, J. S., et al. (2016). Genomic correction of familial cardiomyopathy in human engineered cardiac tissues. *Eur. Heart J.* 37, 3282–3284. doi: 10.1093/eurheartj/ehw307

Stoehr, A., Neuber, C., Baldauf, C., Vollert, I., Friedrich, F. W., Flenner, F., et al. (2014). Automated analysis of contractile force and Ca2+ transients in engineered heart tissue. *Am. J. Physiol. Heart Circ. Physiol.* 306, H1353–H1363. doi: 10.1152/ajpheart.00705.2013

Sun, X., and Nunes, S. S. (2017). Bioengineering approaches to mature human pluripotent stem cell-derived cardiomyocytes. *Front. Cell Dev. Biol.* 5:19. doi: 10.3389/fcell.2017.00019

Sutanto, H., Cluitmans, M. J. M., Dobrev, D., Volders, P. G. A., Bébarová, M., and Heijman, J. (2020). Acute effects of alcohol on cardiac electrophysiology and arrhythmogenesis: Insights from multiscale in silico analyses. *J. Mol. Cell. Cardiol.* 146, 69–83. doi: 10.1016/j.yjmcc.2020.07.007

Sutanto, H., and Heijman, J. (2020). Beta-adrenergic receptor stimulation limits the cellular proarrhythmic effects of chloroquine and azithromycin. *[Preprint]*. doi: 10.20944/preprints202007.0418.v1

Talkhabi, M., Aghdami, N., and Baharvand, H. (2016). Human cardiomyocyte generation from pluripotent stem cells: a state-of-art. *Life Sci.* 145, 98–113. doi: 10.1016/j.lfs.2015.12.023

Tazmini, K., Frisk, M., Lewalle, A., Laasmaa, M., Morotti, S., Lipsett, D. B., et al. (2020). Hypokalemia promotes arrhythmia by distinct mechanisms in atrial and ventricular myocytes. *Circ. Res.* 126, 889–906. doi: 10.1161/CIRCRESAHA.119.315641

Tveito, A., Jæger, K. H., Huebsch, N., Charrez, B., Edwards, A. G., Wall, S., et al. (2018). Inversion and computational maturation of drug response using human stem cell derived cardiomyocytes in microphysiological systems. *Sci. Rep.* 8, 1–14. doi: 10.1038/s41598-018-35858-7

Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., et al. (2020). Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 395, 1417–1418. doi: 10.1016/S0140-6736(20)30937-5

Varshneya, M., Devenyi, R. A., and Sobie, E. A. (2018). Slow delayed rectifier current protects ventricular myocytes from arrhythmic dynamics across multiple species. *Circ. Arrhythm. Electrophysiol.* 11:e006558. doi: 10.1161/CIRCEP.118.006558

Varshneya, M., Irurzun-Arana, I., Campana, C., Dariolli, R., Gutierrez, A., Pullinger, T. K., et al. (2020). Investigational treatments for COVID−19 may increase ventricular arrhythmia risk through drug interactions. *CPT Pharmacometrics Syst. Pharmacol.* doi: 10.1002/psp4.12573

Wang, J.-H., Zhao, L., Pan, X., Chen, N.-N., Chen, J., Gong, Q.-L., et al. (2016). Hypoxia-stimulated cardiac fibroblast production of IL-6 promotes myocardial fibrosis via the TGF-β1 signaling pathway. *Lab. Investig.* 96, 839–852. doi: 10.1038/labinvest.2016.65

Ward, M. C., and Gilad, Y. (2019). A generally conserved response to hypoxia in iPSC-derived cardiomyocytes from humans and chimpanzees. *Elife* 8:e42374. doi: 10.7554/eLife.42374

Weiss, J. N., Qu, Z., and Shivkumar, K. (2017). Electrophysiology of hypokalemia and hyperkalemia. *Circ. Arrhythmia Electrophysiol.* 10:675. doi: 10.1161/CIRCEP.116.004667

WHO (2020). *World Health Organization. Coronavirus disease (COVID-19) outbreak. Geneva World Heal. Organ. c2020*. Geneva: World Health Organization.

Wicik, Z., Eyileten, C., Jakubik, D., Simões, S. N., Martins, D. C., Pavão, R., et al. (2020). ACE2 interaction networks in COVID-19: a physiological framework for prediction of outcome in patients with cardiovascular risk factors. *J. Clin. Med.* 9:3743. doi: 10.3390/jcm9113743

Wu, Z., and McGoogan, J. M. (2020). Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China. *JAMA* 323:1239. doi: 10.1001/jama.2020.2648

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., et al. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med.* 8, 420–422. doi: 10.1016/S2213-2600(20)30076-X

Yadaw, A. S., Li, Y.-C., Bose, S., Iyengar, R., Bunyavanich, S., and Pandey, G. (2020). Clinical features of COVID-19 mortality: development and validation of a clinical prediction model. *Lancet Digit. Heal.* 2, e516–e525. doi: 10.1016/S2589-7500(20)30217-X

Yamauchi-Takihara, K., Ihara, Y., Ogata, A., Yoshizaki, K., Azuma, J., and Kishimoto, T. (1995). Hypoxic stress induces cardiac myocyte–derived interleukin-6. *Circulation* 91, 1520–1524. doi: 10.1161/01.CIR.91.5.1520

Yang, L., Han, Y., Nilsson-Payant, B. E., Gupta, V., Wang, P., Duan, X., et al. (2020). A human pluripotent stem cell-based platform to study SARS-CoV-2 tropism and model virus infection in human cells and organoids. *Cell Stem Cell* 27, 125–136.e7. doi: 10.1016/j.stem.2020.06.015

Yang, N., and Shen, H.-M. (2020). Targeting the endocytic pathway and autophagy process as a novel therapeutic strategy in COVID-19. *Int. J. Biol. Sci.* 16, 1724–1731. doi: 10.7150/ijbs.45498

Yu, C. M., Wong, R. S. M., Wu, E. B., Kong, S. L., Wong, J., Yip, G. W. K., et al. (2006). Cardiovascular complications of severe acute respiratory syndrome. *Postgrad. Med. J.* 82, 140–144. doi: 10.1136/pgmj.2005.037515

Zeb, A., Alzahrani, E., Erturk, V. S., and Zaman, G. (2020). Mathematical model for coronavirus disease 2019 (COVID-19) containing isolation class. *Biomed Res. Int.* 2020:3452402. doi: 10.1155/2020/3452402

Zhang, Y., Xu, Q., Sun, Z., and Zhou, L. (2020). Current targeted therapeutics against COVID-19: Based on first-line experience in China. *Pharmacol. Res.* 157:104854. doi: 10.1016/j.phrs.2020.104854

Zheng, Y.-Y., Ma, Y.-T., Zhang, J.-Y., and Xie, X. (2020). COVID-19 and the cardiovascular system. *Nat. Rev. Cardiol.* 17, 259–260. doi: 10.1038/s41569-020-0360-5

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395, 1054–1062. doi: 10.1016/S0140-6736(20)30566-3

Zhou, P., and Pu, W. T. (2016). Recounting cardiac cellular composition. *Circ. Res.* 118, 368–370. doi: 10.1161/CIRCRESAHA.116.308139

Zhou, Y., Hou, Y., Shen, J., Huang, Y., Martin, W., and Cheng, F. (2020). Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. *Cell Discov.* 6:14. doi: 10.1038/s41421-020-0153-3

Zuppinger, C. (2019). 3D cardiac cell culture: a critical review of current technologies and applications. *Front. Cardiovasc. Med.* 6:87. doi: 10.3389/fcvm.2019.00087

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 18 February 2021
doi: 10.3389/fphys.2020.615690

The Fundamentals of Respiratory Physiology to Manage the COVID-19 Pandemic: An Overview

*Edem Allado[1,2], Mathias Poussel[1,2], Simon Valentin[1,3], Antoine Kimmoun[4,5], Bruno Levy[4,5], Duc Trung Nguyen[6,7], Cécile Rumeau[1,6] and Bruno Chenuel[1,2]**

[1]EA 3450 DevAH-Développement, Adaptation et Handicap, Régulations cardio-respiratoires et de la motricité, Université de Lorraine, Nancy, France, [2]Explorations Fonctionnelles Respiratoires et de l'Aptitude à l'Exercice, Centre Universitaire de Médecine du Sport et Activité Physique Adaptée, CHRU-Nancy, Nancy, France, [3]Département de Pneumologie, CHRU-Nancy, Nancy, France, [4]Médecine Intensive et Réanimation Brabois, CHRU-Nancy, Nancy, France, [5]INSERM U1116, Université de Lorraine, Nancy, France, [6]ORL et Chirurgie Cervico-Faciale, CHRU-Nancy, Nancy, France, [7]INSERM U1254-IADI, Université de Lorraine, Nancy, France

The growing coronavirus disease (COVID-19) crisis has stressed worldwide healthcare systems probably as never before, requiring a tremendous increase of the capacity of intensive care units to handle the sharp rise of patients in critical situation. Since the dominant respiratory feature of COVID-19 is worsening arterial hypoxemia, eventually leading to acute respiratory distress syndrome (ARDS) promptly needing mechanical ventilation, a systematic recourse to intubation of every hypoxemic patient may be difficult to sustain in such peculiar context and may not be deemed appropriate for all patients. Then, it is essential that caregivers have a solid knowledge of physiological principles to properly interpret arterial oxygenation, to intubate at the satisfactory moment, to adequately manage mechanical ventilation, and, finally, to initiate ventilator weaning, as safely and as expeditiously as possible, in order to make it available for the next patient. Through the expected mechanisms of COVID-19-induced hypoxemia, as well as the notion of silent hypoxemia often evoked in COVID-19 lung injury and its potential parallelism with high altitude pulmonary edema, from the description of hemoglobin oxygen affinity in patients with severe COVID-19 to the interest of the prone positioning in order to treat severe ARDS patients, this review aims to help caregivers from any specialty to handle respiratory support following recent knowledge in the pathophysiology of respiratory SARS-CoV-2 infection.

Keywords: coronavirus disease-19, respiratory physiology, control of breathing, hypoxemia, respiratory failure

OPEN ACCESS

Edited by:
Adriana Castello Costa Girardi, University of São Paulo, Brazil

Reviewed by:
Matteo Parotto, University of Toronto, Canada
John T. Fisher, Queen's University, Canada

****Correspondence:***
Bruno Chenuel
b.chenuel@chru-nancy.fr

Specialty section:
This article was submitted to Respiratory Physiology, a section of the journal Frontiers in Physiology

Received: *09 October 2020*
Accepted: *29 December 2020*
Published: *18 February 2021*

Citation:
Allado E, Poussel M, Valentin S, Kimmoun A, Levy B, Nguyen DT, Rumeau C and Chenuel B (2021) The Fundamentals of Respiratory Physiology to Manage the COVID-19 Pandemic: An Overview. Front. Physiol. 11:615690. doi: 10.3389/fphys.2020.615690

INTRODUCTION

The growing coronavirus disease (COVID-19) crisis has stressed worldwide healthcare systems probably as never before, requiring a tremendous increase of the capacity of intensive care units to handle the sudden increase of patients in critical status. In many countries, innovative solutions have been found to change the routine hospital organization and cope with limited resources, leading to massive task-shifting with suspension of elective medical and surgical procedures and reassignment of volunteers (Aziz et al., 2020; Meschi et al., 2020; Xie et al., 2020b). If lung infection resulting from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been shown to encompass various clinical features, the most serious presentation is worsening arterial

hypoxemia, eventually leading to acute respiratory distress syndrome (ARDS) promptly needing mechanical ventilation (Guan et al., 2020; Wu and McGoogan, 2020). The systematic recourse to intubation of every patient suffering from hypoxemia may be difficult to sustain and may not be deemed appropriate for all patients. Then, it is essential that caregivers have solid knowledge of physiological principles to properly interpret arterial oxygenation, to intubate at the satisfactory moment, to adequately manage mechanical ventilation, and, finally, to begin weaning from the ventilator, as safely and as expeditiously as possible, in order to make it available for the next patient.

COVID-19-RELATED HYPOXEMIA, INTERPRETATION OF BLOOD OXYGEN LEVELS, AND THE CONCEPT OF "SILENT HYPOXEMIA"

COVID-19-Related Hypoxemia and Suspected Physiopathological Mechanisms

Hypoxemia is a defining feature of COVID-19. Viral respiratory infection has been shown to cause interstitial pneumonia, leading to a reduction in lung capacity and evolving in some patients to ARDS and respiratory failure. The typical imaging characteristics of COVID-19 pneumonia are non-specific, including peripheral ground-glass opacities with or without consolidation (Bernheim et al., 2020; Lang et al., 2020). They reflect diffuse alveolar injury associated to interstitial thickening, greatly altering gas exchange. In that context, four basic mechanisms of hypoxemia can be discussed: hypoventilation, diffusion impairment, shunt (i.e., hypoventilated areas of the lung are hyperemic), and ventilation-perfusion inequality. However, the most important cause by far is ventilation-perfusion mismatch, resulting from blood perfusing lung regions that have either limited or no ventilation [i.e., regions with low ventilation-perfusion ratios $\dot{V}_A/\dot{Q}$ ratios) or intraparenchymal shunt, respectively], as Gattinoni et al. have reported in their cohort of COVID-19 patients with ARDS (Gattinoni et al., 2020c). They observed a shunt fraction around ~0.5 [i.e., venous to arterial shunt estimated by the shunted blood flow/total blood flow ratio ($\dot{Q}_s/\dot{Q}_T$ ratio) of 50%] and a large alveolar-to-arterial oxygen gradient (P_AO_2-P_aO_2 gradient), enhanced by impaired hypoxic vasoconstriction (Gattinoni et al., 2020c). In addition, COVID-19 is often associated to coagulopathy, providing microemboli which could divert lung perfusion to regions with low $\dot{V}_A/\dot{Q}$ ratios (Altemeier et al., 1998; Connors and Levy, 2020). Two major different phenotypes of COVID-19-associated ARDS have been described and probably involve different pathophysiological mechanisms: COVID-19 pneumonia type L depicted by high compliance (i.e., low elastance), low ventilation-to-perfusion ratio, and low recruitability, and COVID-19 pneumonia type H characterized by low compliance (i.e., high elastance), high right-to-left shunt (i.e., the hypoventilated areas of the lung are hyperemic), and high recruitability, analogous to what is experienced in common acute respiratory distress (Gattinoni et al., 2020a).

Therefore, in addition to the CT scan evaluation, the response to oxygen therapy can be helpful to distinguish the two phenotypes. The delivery of raised FIO_2 would increase PaO_2 and oxygen saturation in the L phenotype when ventilation-to-perfusion ratio mismatch drives hypoxia, avoiding or delaying the recourse to intubation and mechanical ventilation with satisfactory levels of arterial oxygenation by oxygen therapy. At the opposite, when hypoxia is mainly determined by a shunt, in H phenotype, a modest enhancement in oxygen saturation is expected by the delivery of high FIO_2, often requiring earlier invasive ventilator assistance (Gattinoni et al., 2020a).

The underlying physiopathology has not been fully elucidated but partly due to the SARS-CoV-2 infecting the host recognizing the angiotensin-converting enzyme 2 (ACE-2) receptor as a specific target (Hoffmann et al., 2020; Lu et al., 2020). It is a membrane-bound aminopeptidase expressed on many human cells (respiratory tract, lung, heart, arteries, veins, kidney, and intestines; Hamming et al., 2004). More particularly, the ACE-2 receptor is located in alveolar epithelial cells and vascular endothelium, and when SARS-CoV-2 binds to it, a reduction in intracellular ACE-2 protein activity is provided, resulting in a marked immune response with hyperinflammatory syndrome and widespread endothelial dysfunction (Connors and Levy, 2020; Mehta et al., 2020; Polidoro et al., 2020; Zhang et al., 2020). Physiologically, ACE-2 is a vasodepressor, at the opposite of the homologous enzyme ACE-1 acting as a vasoconstrictor, and both proteins form the oxygen-sensitive renin-angiotensin system (Hampl et al., 2015). Histopathologically, recent works have emphasized the development of alveolar and interstitial exudative inflammation characterized by macrophage and monocyte predominance and associated to focal respiratory epithelial desquamation, hemorrhage, and type 2 pneumocyte proliferation (Tian et al., 2020; Xu et al., 2020).

Hypoxemia has been shown to be an independent prognostic factor for the severe form of COVID-19 (Wei et al., 2020) and associated with in-hospital mortality (Xie et al., 2020a).

Interpretation of Blood Oxygenation From Pulse Oximetry, Caution, and Limits

The assessment of oxygen saturation in the arterial blood by pulse oximetry should be carefully interpreted. Indeed pulse oximetry provides an estimate of the arterial oxygen saturation (SpO_2) and is not a direct measurement, as CO-oximeters are able to do (SaO_2). By definition, oxygen saturation is the percentage of hemoglobin-binding sites occupied by oxygen, varying according to the arterial PO_2, as stipulated by the oxyhemoglobin dissociation curve. The difference between the two methods is not negligible, reaching as much as ±4% (Tobin, 1990).

The peculiar sigmoidal shape of the oxyhemoglobin dissociation curve involves several important features. In the higher range of partial pressures, the upper part of the curve is flat, impeding a significant decline in oxygen saturation when PO_2 starts to drop. In contrast, the steeper portion of the dissociation curve markedly enhances the carriage of oxygen in the lungs (on-loading) and oxygen delivery to the tissues (off-loading). As lung injury progresses, leading to further impairment of gas exchange, PO_2 may fall on the

steep part of the dissociation curve (from 20 to 60 mmHg), allowing noticeable changes in the measured oxygen saturation with small changes in PO_2. In this context, the natural variability of ventilation due to physiological acts as talking, laughing, or breath holding may change the alveolar PO_2, thereby inducing similar variations in PaO_2. Then, oxygen saturation monitoring should be observed for at least several minutes. Moreover, the position of the dissociation curve itself can be modified by the patient's acid-base status. Acidemia shifts it rightward and alkalemia in the opposite way. In the early course of COVID-19 pneumonia, numerous patients begin to hyperventilate in order to compensate for their collapsing PaO_2. The hyperventilation consequently generates a respiratory alkalosis, shifting the dissociation curve to the left (increasing hemoglobin's oxygen affinity to facilitate oxygen loading) such that the predictable decrease in oxygen saturation with a falling PaO_2 will be dampened and, in some cases, prevented (Hamilton et al., 2004). In addition, with respect to the alveolar gas equation, the decreased alveolar CO_2 partial pressure ($PACO_2$) will lead to a comparable increase in alveolar oxygen partial pressure (PAO_2). These combined mechanisms are able to improve SaO_2 in hypocapnic hypoxic stimulation compared with an isocapnic or hypercapnic hypoxia. In contrast, a right shift in oxygen dissociation (decreasing hemoglobin's oxygen affinity to facilitate oxygen unloading) is expected with fever, an obvious clinical feature in COVID-19, leading to noticeable desaturation without any change in the chemosensitive drive of breathing.

Some important practical limits of pulse oximetry also need to be known. Movements of the digits (shivering patient, for example), avoiding to identify an adequate pulse signal, or bright artificial light as observed in an operating room can induce false low readings (Schnapp and Cohen, 1990; Sinex, 1999).

The pulse oximeter uses two different wavelengths to estimate oxygen saturation, generated by two light-emitting diodes, but both wavelengths of light are similarly absorbed by hemoglobin in arterial blood, capillary, venous blood, and other soft tissues. Then, it is necessary to distinguish the pulsatile signal of arterial blood flow in order to limit the signal-to-noise ratio and dispense a valid result (Sinex, 1999). Therefore, factors that are able to limit pulsatile blood flow in the digits, such as hypotension and use of vasoconstrictor agent as well as the presence of peripheral vascular disease or Raynaud's phenomenon, may worsen the signal-to-noise ratio, resulting in an inaccurate estimation of arterial oxygen saturation. Chilblains have been increasingly recognized in association with COVID-19 (Bouaziz et al., 2020; Gottlieb and Long, 2020; Tosti et al., 2020), and peripheral vascular disease has been found to be associated with the usual comorbidities in patients suffering from severe COVID-19, such as diabetes and coronary artery disease (Du et al., 2020; Wu and McGoogan, 2020). It is also important to know that pulse oximeters dispense misleading results in front of either carboxyhemoglobinemia or methemoglobinemia since they are not able to distinguish these dyshemoglobinemias from oxygenated and deoxygenated hemoglobin. If carboxyhemoglobinemia is involved in heavy smokers or individuals using grills or heaters in enclosed spaces, it has been demonstrated that methemoglobinemia can result from the use of some drugs, including chloroquine (Rizvi et al., 2012).

Other important sources of artifact need to be cited, such as nail polish and increased skin pigmentation, especially if the real oxygen saturation is diminished (Bickler et al., 2005; Sutcu Cicek et al., 2011).

Furthermore, it has been demonstrated that large SpO_2 to SaO_2 differences exist in patients in critical condition with mediocre reproducibility of SpO_2, specifically in shocked patients with low cardiac output or under high doses of vasopressor. In hemodynamically unstable patients, the detection limit of the sensor is most often exceeded (Van de Louw et al., 2001).

In addition to interpretation of blood oxygenation by pulse oximetry, to correctly assess the real efficacy of pulmonary gas exchange, it is required to know the fraction of inspired oxygen (FIO_2) in order to adequately calculate the P_AO_2-P_aO_2 gradient using the alveolar gas equation (cf. **Figure 1**). Then, if interpretation of blood oxygenation with supplemental oxygen is straightforward when a patient is breathing room air or is intubated, it is clearly problematic when a nasal cannula is used to deliver oxygen since the inspiratory fraction of oxygen is difficult to estimate. For example, depending on the effective patient's minute ventilation (more specifically tidal volume patient's demand), when a nasal cannula or a face mask is used to deliver pure oxygen flow rate at 2 l/min, FIO_2 can vary from 24 to 35% (Bazuaye et al., 1992). Therefore, the severity of hypoxemia cannot be assessed by the level of supplemental oxygen delivery. In practice, peculiar attention on the level of gas exchange impairment is recommended when high F_iO_2 is used to treat hypoxemia according to a simple target level on pulse oximetry, given the flatness of the upper portion of the dissociation curve (Bickler et al., 2017).

A synthesis is proposed in **Figure 2** in order to present a practical assessment of blood oxygenation using pulse oximetry and limitations.

Concept of "Silent Hypoxemia"

In one of the first largest studies on the clinical characteristics of coronavirus in China, shortness of breath has been reported in only 18.7% of 1,099 hospitalized patients with COVID-19 pneumonia, despite hypoxemia commonly requiring supplemental oxygen (41%) and abnormal results on CT scans (86.2%; Guan et al., 2020). Numerous reports worldwide have described a subset of patients with severe hypoxemia presenting no obvious respiratory difficulties or dyspnea, leading to abundant coverage in media with sensational headlines such as "happy hypoxia" or, more conventionally, "silent hypoxemia" (Couzin-Frankel, 2020; Levitan, 2020; Tobin et al., 2020b). However, in contrast to media's assertion, this questioning discrepancy is not really defying biology since fundamentals in respiratory physiology can account for most of it, with the specific effect of SARS-CoV-2 on control of breathing or chemoreceptors excepted.

Then, knowledge of the putative mechanisms involved in the genesis of dyspnea, basics of control of breathing, ventilatory response to hypoxia, and the role of PCO_2 is necessary to address the mystery.

Alveolar gas equation:

$$P_{A_{O_2}} = P_{I_{O_2}} - \frac{P_{A_{CO_2}}}{R} + \left[P_{A_{CO_2}} \cdot F_{I_{O_2}} \cdot \frac{1 - R}{R} \right]$$

Where $P_{A_{O2}}$ = alveolar partial pressure of oxygen, $P_{I_{O2}}$ = Inspired partial pressure of oxygen, $P_{A_{CO2}}$= alveolar partial pressure of CO_2, $F_{I_{O2}}$= inspired fraction of oxygen and R = respiratory exchange ratio (from 0.7 to 1).
This is only valid if there is no CO_2 in inspired gas.

The term in square brackets is relatively small and an estimation of $P_{A_{O2}}$ can be provided by the following equation:

$$P_{A_{O_2}} = P_{I_{O_2}} - \frac{P_{a\,CO_2}}{0.8}$$

With $P_{I_{O2}}=F_{I_{O2}}.(P_{atm}-P_{H_2O})$; P_{atm}= atmospheric pressure , P_{H_2O}= water vapour pressure
On room air ($F_{I_{O2}}$ = 0.21, or 21%), at sea level (P_{atm} = 760 mmHg) assuming 100% humidity in the alveoli (PH_2O = 47 mmHg at 37°C).

Alveolar–arterial gradient of oxygen partial pressure:

$$A\text{-}a\ (P_{O_2}) = P_{A_{O_2}} - P_{a_{O_2}}$$

FIGURE 1 | Useful toolkit to interpret oxygenation in an appropriate way.

Patient Measurements in practice	Main Conditions limiting PO accuracy
Patient at rest with quiet breathing if possible	Poor perfusion (hypotension, hypovolemic or septic shock, cardiac failure…)
Use the index or middle finger (avoid the ear lobe or toe)	Dyshemoglobinemias (Carbon monoxide poisoning, methemoglobinemia, fetal hemoglobinemia)
Remove fingernail polish if needed	Dark pigmented skin (risk of overestimation of oxygen saturation with values < 80%)
Warm cold fingers	Sickle cell anemia (vasoocclusive crises)
Accept values only if the pulse signal is strong	Severe hyperbilirubinemia (> 30 mg/dL, due to increased heme metabolism (hemolysis) or decreased bilirubin metabolism (liver disease))
Use the most common value on readings for 1 to several minutes	Some drugs (affecting oxygen's affinity for hemoglobin)

FIGURE 2 | Blood oxygenation assessment with pulse oximetry (PO).

Dyspnea and Control of Breathing

Dyspnea is a highly multidimensional subjective experience needing careful assessment. It shows tremendous variability in regards to cultural and linguistic features and affective and cognitive factors (Anonymus, 1999; Parshall et al., 2012). The neurophysiologic mechanisms that give rise to the perception of dyspnea are incompletely understood, but the sensation of dyspnea probably results from a mismatch between efferent motor commands from the central nervous system (CNS) to the respiratory system and afferent sensory inputs (e.g., expected airflow, cage movements) from the respiratory system to the CNS (Adler and Janssens, 2019). It increases as inputs from receptors increase, and the central nervous system perceives that respiratory muscles cannot match the inputs and maintain adequate ventilation (Laviolette et al., 2014).

Chemoreceptors are certainly involved in the sensation of dyspnea, rising respiratory output and subsequently activating respiratory afferences, associated to corollary discharges and direct projections from chemoreceptors to forebrain structures (notably the limbic system, also underlying the genesis of pain sensation; Banzett et al., 2000; Evans et al., 2002; Buchanan and Richerson, 2009). The insular cortex appears to play a crucial role since it has been demonstrated that insular lesions are associated with a blunted perception of dyspnea (Schon et al., 2008).

With the lung injury due to SARS-CoV-2, numerous sources of stimulation of sensory receptors may gather information and feed it to the central controller, from inflammation of the respiratory tract and lungs to hypoxemia, leading to dyspnea (Tobin, 2020). However, the experience of the subjective sensation of breathlessness is not systematic, depending on the patient and circumstances and with great similarity to pain sensation (Lansing et al., 2009).

Ventilatory Response to Hypoxia and Dyspnea

In healthy humans, the ventilatory response to partial pressure of arterial oxygen (PaO_2) is hyperbolic (Rebuck and Campbell, 1974). Reducing the partial pressure of arterial oxygen (PaO_2) from its normal value to 60 mmHg has a marginal effect on pulmonary ventilation ($\dot{V}_E$) and $PaCO_2$ (Forster and Dempsey, 1981). Nevertheless, further reducing PaO_2, from about 60 to 30 mmHg, provides a progressive increase in $\dot{V}_E$ following an exponential pattern (hyperbolic curve) and a decrease in $PaCO_2$ (Forster and Dempsey, 1981). In contrast, the relationship between ventilation and arterial oxygen saturation (SaO_2) is linear (Rebuck and Campbell, 1974). Physiologically, in human subjects, the increase in ventilation occurs primarily because of a rise of tidal volume and only a small increase in the frequency of breathing (Reynolds and Milhorn, 1973; Bender et al., 1987). If tachypnea is one of the most important clinical indicators of respiratory distress, it could be without proportion to severe hypoxemia. Moreover, in COVID-19 patients, tachypnea would be more elicited by stimulation of lung receptors (pulmonary stretch, irritant, and J receptors) due to lung inflammation than by the hypoxic stimulus and therefore would not be the cornerstone of the intubation decision (Tobin, 2020).

It has been demonstrated that the level of hypoxia corresponding to the perception of air hunger in healthy subjects matches with the sharp increase of minute ventilation but far from all the subjects have complained as a strong increase in air hunger with a fall end-tidal oxygen partial pressure below 60 mmHg (Moosavi et al., 2003). Dyspnea often occurs when PaO_2 declines below 40 mmHg (Manning and Schwartzstein, 1995). Like the large variability of the resting respiratory drive, there is a great between-subject and within-subject variability of ventilatory response to hypoxia in healthy subjects (Sahn et al., 1977; Tobin et al., 1988; Matsuzawa et al., 1989). It has been demonstrated that the ventilatory response to hypoxia is decreased by half in elderly healthy people (Kronenberg and Drage, 1973; Peterson et al., 1981). The decrease is even more pronounced in patients suffering from diabetes (Nishimura et al., 1989; Weisbrod et al., 2005), who not only presented an impaired perception of sensory input from organs but also demonstrated an increased threshold for the perception of respiratory sensations has been (O'Donnell et al., 1988). Since diabetes is among the most frequently reported comorbidities and the median age is easily over 60 years in patients infected with COVID-19, it is not so surprising to observe numerous cases of "silent hypoxemia" (Grasselli et al., 2020; Huang et al., 2020; Richardson et al., 2020).

Furthermore, hypoxia is also well known to depress ventilation at the central nervous system level, possibly masking unpleasant sensations (Berry et al., 1989).

Modulation of the Hypoxic Ventilatory Response by CO_2

In the absence of isocapnia, the ventilatory response to hypoxia is severely attenuated by hypocapnia associated with hyperventilation. This attenuation is due to an effect on the peripheral chemoreceptors (carotid body essentially) as well as to reduced drive from the central chemoreceptors (Lahiri and DeLaney, 1975; Fitzgerald and Dehghani, 1982; Moore et al., 1984). It has been demonstrated that moderate hypocapnia, corresponding to $PaCO_2$ values from 5 to 10 mmHg below eucapnia, flattened the hypoxic response, suggesting that a minimum level of CO_2 is required to generate the hypoxic ventilatory response (Jounieaux et al., 2002; Corne et al., 2003; Wilson and Teppema, 2016). In order to elicit a valuable rise in ventilation, severe hypoxia must be associated to baseline $PaCO_2$ that exceeds 39 mmHg (Moosavi et al., 2003). Since hypoventilation is uncommon with COVID-19, hypoxemia accompanied by a normal alveolar-to-arterial oxygen gradient and increase in $PaCO_2$ is highly unlikely, especially in the early phase of lung injury. In the great majority of severe cases, hypoxemia is accompanied by an increased alveolar-to-arterial oxygen gradient reflecting either ventilation-perfusion mismatch or intrapulmonary shunting and the compensatory ventilatory response to hypoxemia, leading to noticeable hypocapnia (Tobin, 2020).

Consequently, knowledge of the accompanying $PaCO_2$ is imperative to assess the severity of the respiratory failure associated to hypoxemia, another reason to claim that isolated monitoring of SaO_2 is insufficient to guide clinical decisions.

Taken together, it would not be so astonishing that many COVID-19 patients face hypoxemia and rapid respiratory failure without evidence of dyspnea.

LESSONS FROM HIGH ALTITUDE AND AVIATION PHYSIOLOGY: ARE THE SIMILARITIES BETWEEN COVID-19 ARDS AND HIGH-ALTITUDE PULMONARY EDEMA RELEVANT?

The common clinical pattern of COVID-19 lung injury is based upon a noticeable imbalance between relatively well-preserved lung compliance and a severely impaired pulmonary gas exchange, resulting in hypoxemia without corresponding signs of dyspnea or respiratory distress. Since the physiological characteristics of the hypocapnic ventilatory response to hypoxia have been extensively investigated in high altitude physiology and aviation

medicine, learnings from them could be helpful in order to better manage the COVID-19 pandemic.

Beyond the apparent similarity between the COVID-19 silent hypoxemia and the non-lethal high altitude-induced hypoxemia associated to respiratory alkalosis, even allowing climbers to exercise in ascent despite very low levels of PaO_2, some authors have advocated parallelism between COVID-19 acute respiratory distress syndrome and high-altitude pulmonary edema (HAPE), with great amplification *via* social media (Solaimanzadeh, 2020).

With the first descriptions of the clinical features of severe COVID-19 pneumonia, a debate has emerged on the development of typical ARDS or not, allowing specific and important clinical implications (Gattinoni et al., 2020b,c). Most of the patients with severe COVID-19 pneumonia meet the criteria that define internationally the ARDS [ARDS Berlin definition: acute onset of hypoxemia assessed by the PaO_2/FIO_2 ratio $\leq$300 mmHg in a ventilated patient with a positive end-expiratory pressure (PEEP) of at least 5 cmH_2O and bilateral lung infiltrates not fully explained by heart failure or volume overload (Force et al., 2012)], but unusual presentations exist (Gattinoni et al., 2020b,c). The main difference is relatively well-preserved lung mechanics with maintenance of a relatively high respiratory system compliance (close to the normal value of 50 ml/cm H_2O), in contrast to typical severe ARDS (Gattinoni et al., 2020b,c). For some authors, the hypothesis for such hypoxemia associated to compliant lungs could be a hypoxic vasoconstriction (Gattinoni et al., 2020c). HAPE and ARDS are a non-cardiogenic form of pulmonary edema characterized by diffuse bilateral opacities on chest imaging caused by an imbalance in Starling forces, thus inducing fluid accumulation in the interstitial and alveolar spaces. However, the pathogenesis of such pulmonary edema is radically different between the two entities. HAPE is related to an excessive hypoxia-mediated increase in pulmonary vascular resistance or hypoxic pulmonary vasoconstriction increasing microvascular pressure and leading to a substantial increase in pulmonary artery pressure with overperfusion of some regions of the lung, elevated pulmonary capillary hydrostatic pressure, and leakage of fluid into the alveolar space (Swenson and Bartsch, 2012). Consequently, HAPE is a life-threatening condition that is favorably influenced (often reversed) by oxygen therapy, exposure to hyperbaric environment (using portable hyperbaric chambers), or descent/evacuation to lower altitude and, finally, very unusually needs intensive care (Swenson and Bartsch, 2012; Strapazzon et al., 2020). Since hypoxic vasoconstriction is the fundamental pathogenesis mechanism in HAPE, increasing the alveolar PO_2 decreases pulmonary artery pressure, allowing the resolution of alveolar and interstitial edema and full recovery within hours to a few days of exposure. Distinctly, the underlying pathophysiological mechanisms in ARDS due to COVID-19 involve multi-organ viral-mediated inflammatory responses leading in the lung to genesis of alveolar epithelial inflammation and dysfunction of surfactant and alveolar fluid clearance, finally leading to alveolar collapse and/or filling and marked ventilation-perfusion mismatch (Gattinoni et al., 2020a). Therefore, in marked contrast to HAPE, the delivery of supplemental oxygen in COVID-19 pneumonia may increase oxygen availability but will not be able to counteract the underlying inflammation or lung injury (Luks and Swenson, 2020; Strapazzon et al., 2020). This major distinction has crucial clinical implications since drugs well known to inhibit hypoxic pulmonary vasoconstriction—acetazolamide, systemic vasodilators like calcium channel blockers, or phosphodiesterase-5 inhibitors—are not only inappropriate but also expected to worsen ventilation/perfusion mismatch by raising prefusion blood flow to poorly and/or nonventilated lung regions, exacerbating hypoxemia and provoking hypotension in COVID-19 patients (Archer et al., 2020; Brugger et al., 2020; Luks and Swenson, 2020; Strapazzon et al., 2020).

ON THE INTEREST OF PRONE POSITIONING IN COVID-19 PNEUMONIA, NOT ONLY TO IMPROVE GAS EXCHANGE BUT ALSO AS A STRATEGY TO DELAY OR AVOID MECHANICAL VENTILATION

Prone positioning, i.e., when a patient is repositioned from supine position to lie on their front, has been used for more than 45 years to improve oxygenation in patients with acute respiratory failure and more specifically with ARDS (Guerin, 2014). Historically, in the 1970s, Mellins observed that children suffering from advanced cystic fibrosis spontaneously position themselves on their hands and knees to improve their ventilation, while Bryan hypothesized that, in acute respiratory failure with consequent impairment of functional residual capacity and enhancement of dependent airway closure, the prone position might recruit and stabilize the dependent lung (Bryan, 1974; Mellins, 1974). Since then, numerous randomized controlled trials and meta-analyses have demonstrated a conclusive and important mortality reduction using prone positioning early and for a prolonged time in subjects with severe ARDS (Abroug et al., 2008; Alsaghir and Martin, 2008; Guerin et al., 2013; Beitler et al., 2014; Hu et al., 2014; Lee et al., 2014; Bloomfield et al., 2015; Munshi et al., 2017). Nowadays, prone positioning is used not only as an efficient treatment in case of life-threatening hypoxemia but also in the prevention of ventilatory-induced lung injury (VILI; Chiumello and Brioni, 2016; Guerin, 2017; Mitchell and Seckel, 2018).

The main underlying physiologic mechanism for the ensuing improvement in patients' oxygenation with prone position is the decrease in intrapulmonary shunting, but an improvement of ventilatory mechanics is also involved (Gattinoni et al., 2013; Guerin et al., 2014). Prone positioning provides reduction in intrapulmonary shunt ($\dot{Q}_s/\dot{Q}_T$), variation in lung ventilation ($\dot{V}_A$), and lung perfusion ($\dot{Q}$) distribution with improved $\dot{V}_A/\dot{Q}$ matching. By recruiting dorsal regions which have a larger number of alveolar units and by obtaining an increase in chest wall elastance, better ventilation to the perfused lung is provided, improving the ventilation/perfusion ratio and allowing a more homogeneous distribution of ventilation. This leads to a decrease in lung strain and, consecutively, reduction of VILI, reducing the risk of right heart failure (Gattinoni et al., 2013; Guerin et al., 2014; Ruste et al., 2018). The improvement of oxygenation in ARDS

patients during a prone session is observed in ~75% of the cases and sometimes intense (Guerin, 2014). The positive oxygenation response is commonly defined as an improvement in PaO_2 by 20% or an increase in the PaO_2/FIO_2 ratio by 20 mmHg (Guerin, 2014). It has been demonstrated that prone positioning reduced relative shunt fraction by about 30% and improved PaO_2/FIO_2 ratio by 34–62%, with a variable temporal response (from an immediate response to a continued response for up to 24 h; Kallet, 2015; Scholten et al., 2017).

Additional data are also important to note concerning the drainage of secretions which improves when prone, with material in the dorsal lung traveling more easily to open airways. Nevertheless, no significant reduction in the incidence of ventilator-associated pneumonia has been observed in a recent prospective study cohort of patients with severe ARDS (Ayzac et al., 2016). Major improvements in thoraco-abdominal compliance were particularly observed in patients with higher body mass index (Kallet, 2015).

During the COVID-19 pandemic, the use of prone positioning was proposed not only in ARDS patients requiring mechanical ventilation, as it is internationally recommended (Alhazzani et al., 2020; Wilson et al., 2020), but also in order to avoid or delay the recourse to intubation in the dramatic context of limited resources and capacity of intensive care units (Chad and Sampson, 2020; Elharrar et al., 2020; Sartini et al., 2020; Villarreal-Fernandez et al., 2020).

Innovative solutions have been found worldwide to cope with limited resources and to include the prone positioning in the management of patients requiring mechanical ventilation, even at the surge of the outbreak, resulting in the emergence of prone teams (Doussot et al., 2020; Kimmoun et al., 2020; Settembre et al., 2020).

In COVID-19 patients, the Surviving Sepsis campaign recommends a trial of prone positioning in mechanically ventilated patients who meet the moderate-to-severe ARDS definition (Alhazzani et al., 2020). Periods of 12–16 h are suggested, based upon evidence for non-COVID ARDS (Alhazzani et al., 2020).

In conscious non-ventilated COVID-19 patients, it is expected that the underlying mechanism leading to an improvement in oxygenation is analogous, but only few studies evaluated the benefits of the prone position and no clear recommendations have emerged (Elharrar et al., 2020; Sartini et al., 2020). Short-term improvements of oxygenation are observed in such patients, but further studies are needed to clarify the real benefit, particularly on mortality.

PHYSIOLOGICAL BASIS FOR VENTILATORY SUPPORT

If the initial message from the Chinese medical teams at the surge of the outbreak was to intubate early, the current ventilatory approach is to delay intubation if it clinically appears safe and feasible (Alhazzani et al., 2020). Currently, any therapy that could prevent intubation and mechanical ventilation (MV) or enhance MV weaning without further deterioration is welcome. Regrettably, "safe" lung-protective ventilation does not really exist; thus, ventilatory support needs to be individualized as the best compromise among respiratory mechanics, recruitability, gas exchange, and hemodynamics to minimize VILI and to ensure adequate oxygenation when arterial hypoxemia is refractory to oxygen therapy.

The spectrum of therapies and the different lung support which have been proposed to the management of ARDS with critical hypoxemia (i.e., severe ARDS, with PaO_2/FIO_2 <100 mmHg) encompass the delivery of oxygen therapy by high-flow nasal cannula (HFNC) system and non-invasive positive pressure ventilation (NIPPV). In severe COVID-19 patients, these therapies should only be used in selected patients with hypoxemic respiratory failure and who are closely observed for early detection of further deterioration (Pfeifer et al., 2020).

With oxygen flow rates that can reach 60–80 L per minute, HFNC systems can more accordingly ensure the ventilatory demands of patients with respiratory distress and respiratory failure compared to the standard nasal cannula (Suffredini and Allison, 2020). They are able to reduce dead space, raise the end-expiratory lung volume, improve compliance, and reduce the work of breathing, resulting in improvement of pulmonary gas exchange (Suffredini and Allison, 2020). There is limited data to promote or refute the use of HFNC in SARS-CoV-2 and in ARDS patients; the failure rate has been found to be relatively high (Messika et al., 2015). However, it has been proposed to be combined with prone positioning (Colla et al., 2020; Suffredini and Allison, 2020; Villarreal-Fernandez et al., 2020). Decisions to continue HFNC treatment might depend on the results of periodic clinical assessments and repeated biological measurements corroborating clinical stability or improvement (Suffredini and Allison, 2020).

The use of NIPPV with a pressure support tailored to ensure a tidal volume between 7 and 10 ml/kg and a PEEP set between 2 and 10 cm H_2O could also lessen the intrapulmonary shunt and diminish the work of breathing, but just as the HFNC, NIPPV is associated with a high risk of failure and associated risks of a delayed start of invasive mechanical ventilation (Evans, 2001). The clinical result of the use of NIPPV needs to be carefully assessed, and if, following the first few hours, no significant improvement in pulmonary gas exchange is observed, it should be ceased and invasive mechanical ventilation should be initiated (Evans, 2001). More specifically, the magnitude of oxygenation disturbance is a predictor of NIPPV failure, and a PaO_2/FIO_2 ratio <150 mmHg is described as the decisive threshold for increased mortality (Bellani et al., 2017). However, some very recent works have emphasized the interest on non-invasive strategies in COVID-19, especially in order to avoid intubation (Brusasco et al., 2020; Oranger et al., 2020; Tobin et al., 2020a).

Invasive Mechanical Ventilation

The decision to intubate mainly relies on the clinical judgment of the critical care physician but is also based upon combined features such as level of hypoxemia, respiratory distress, increased work of breathing, fatigue, and gas exchange (Tobin, 2020). In the peculiar context of the COVID-19 pandemic, the most appropriate timing for the intubation of hypoxic patients with severe lung injury is not well known and also depends on the local capacity for mechanical ventilation.

The main objective of mechanical ventilation is to lessen work and the oxygen cost of breathing, allowing oxygen stores to be redirected to vulnerable tissue beds (Tobin et al., 2012). In patients in acute respiratory distress, it has been demonstrated that the oxygen cost of breathing is enhanced to as much as 50% of total oxygen consumption (Field et al., 1984).

The basic principles of the assist-control ventilation are based upon the delivery of a breath under positive pressure provided by the ventilator, either triggered by the inspiratory effort achieved by the patient (pressure or flow triggered) or, independently, if such an effort is not performed within a preselected time period.

The main challenge for the physician then is to cycle the rhythm of the ventilator in synchrony with the patient's central respiratory rhythm while improving gas exchange. Three critical points have been identified: triggering (cycling on), post-trigger inflation, and inspiration-expiration switchover (cycling off; Tobin et al., 2012; Tobin, 2018).

The two most common modes used for mechanical ventilation are pressure-controlled ventilation (PCV), using a predetermined inflation pressure applied for a predetermined inflation time, and volume-controlled ventilation (VCV), using a predetermined volume.

With PCV, the delivered volume varies according to the properties of the respiratory system and also to the patient's effort and the inspiratory flow displays a decelerating shape; in VCV, the delivered volume is maintained constant, independently of the patient's effort, while the airway pressure is non-uniform and the inspiratory flow has a fixed shape.

It is important to note that the amount of active work performed by a patient in volume-cycled assist-control crucially relies on the sensitivity of the trigger and inspiratory flow settings. Despite optimal selected settings, it has been established that patients actively perform about a third of the work carried out by the ventilator during passive conditions (Marini et al., 1985). Pressure support can efficiently decrease the work of inspiration, but the level of inspiratory muscle unloading appears highly labile, with a coefficient of variation reaching up to 96% among patients (Jubran et al., 1995).

If mechanical ventilation is a valid life-saving intervention, it can also enhance lung injury and, through VILI, contribute to multi-organ failure in patients with ARDS (Slutsky and Ranieri, 2013). The major determinant of VILI is the genesis of non-physiologic stress (tension) and strain (deformation), which relies not only on the size of the delivered tidal volume but also on the amount of lung resting volume (Gattinoni et al., 2012).

Therefore, the most common strategy to minimize VILI is low tidal volume (V_T) ventilation. A V_T from 4 to 8 ml/kg of predicted body weight is recommended in mechanically ventilated adults with COVID-19 and ARDS (Alhazzani et al., 2020). Along with low V_T ventilation, lower airway pressure use [i.e., plateau pressure (P_{plat}) $\leq$30 cmH_2O] is a lung-protective strategy (Petrucci and De Feo, 2013).

Ventilator Weaning

Considering the side effects of mechanical ventilation and, additionally, the limitation of the intensive care resources during the COVID-19 pandemic, it is critical to get patients off the ventilator at the earliest possible time.

Since a delayed initiation of the weaning process has recurrently been observed, weaning predictor tests have been developed (Yang and Tobin, 1991; Tobin and Jubran, 2006). Among the physiological measurements that can alert a physician at initiating the weaning process, the level of rapid shallow breathing, quantified by frequency of breathing-to-V_T ratio (fb/V_T), has been shown to be the best predictor of weaning outcome (Yang and Tobin, 1991; Tobin and Jubran, 2006). Synchronized mandatory ventilation is not recommended (Brochard et al., 1994).

Several approaches are used to manage weaning: from the use of a T-tube circuit allowing bouts of spontaneous breathing trials to the gradual reduction in the level of ventilator assistance (Tobin et al., 2012). Almost invariably, weaning failure arises within the first hour of attempted spontaneous breathing (Tobin, 2018).

CONCLUSION

In COVID-19 lung injury, as observed in many other respiratory diseases, control of breathing is the cornerstone of the clinical presentation, from dyspnea to respiratory failure, not only explaining symptoms but also allowing appropriate levels of physiological compensations in order to maintain efficient spontaneous ventilation. However, when overwhelmed, a patient critically requires ventilator assistance, which also greatly involves the key elements of the control of breathing.

A clear view of COVID-19-related hypoxemia needs an appropriate interpretation of blood oxygenation from pulse oximetry, keeping in mind cautions and limits of accuracy. The role of the position of the dissociation curve associated to changes of the patient's acid-base status or hyperventilation-related hypocapnia, as well as the calculation of the P_AO_2-P_aO_2 gradient using the alveolar gas equation, is crucial to assess the real efficacy of pulmonary gas exchange. The participation of ventilatory response to hypoxia in the genesis of dyspnea and its modulation by CO_2 can help to explain that many COVID-19 patients face hypoxemia and rapid respiratory failure without evidence of dyspnea.

When mechanical ventilation is decided in critical COVID-19 patients, the usual strategies to tailor it are involved, based upon the basis of respiratory physiology to lessen work and the oxygen cost of breathing. The safe discontinuation of mechanical ventilation needs a careful assessment of physiological parameters (level of rapid shallow breathing) in order to warn a physician that a ventilated patient might be able to come off the ventilator in order to make it available for the next patient in such a peculiar context of the COVID-19 pandemic.

AUTHOR CONTRIBUTIONS

EA, MP, AK, CR, and BC contributed to conception of this work: literature search, drafting, writing, and critical review of the text. SV, DN, and BL contributed to literature search, writing, and critical review of the final document. All authors contributed to the article and approved the submitted version.

REFERENCES

Abroug, F., Ouanes-Besbes, L., Elatrous, S., and Brochard, L. (2008). The effect of prone positioning in acute respiratory distress syndrome or acute lung injury: a meta-analysis. Areas of uncertainty and recommendations for research. *Intensive Care Med.* 34, 1002–1011. doi: 10.1007/s00134-008-1062-3

Adler, D., and Janssens, J. P. (2019). The pathophysiology of respiratory failure: control of breathing, respiratory load, and muscle capacity. *Respiration* 97, 93–104. doi: 10.1159/000494063

Alhazzani, W., Moller, M. H., Arabi, Y. M., Loeb, M., Gong, M. N., Fan, E., et al. (2020). Surviving sepsis campaign: guidelines on the management of critically ill adults with coronavirus disease 2019 (COVID-19). *Intensive Care Med.* 46, 854–887. doi: 10.1007/s00134-020-06022-5

Alsaghir, A. H., and Martin, C. M. (2008). Effect of prone positioning in patients with acute respiratory distress syndrome: a meta-analysis. *Crit. Care Med.* 36, 603–609. doi: 10.1097/01.CCM.0000299739.98236.05

Altemeier, W. A., Robertson, H. T., Mckinney, S., and Glenny, R. W. (1998). Pulmonary embolization causes hypoxemia by redistributing regional blood flow without changing ventilation. *J. Appl. Physiol.* 85, 2337–2343. doi: 10.1152/jappl.1998.85.6.2337

Anonymus (1999). Dyspnea. Mechanisms, assessment, and management: a consensus statement. American Thoracic Society. *Am. J. Respir. Crit. Care Med.* 159, 321–340. doi: 10.1164/ajrccm.159.1.ats898

Archer, S. L., Sharp, W. W., and Weir, E. K. (2020). Differentiating COVID-19 pneumonia from acute respiratory distress syndrome and high altitude pulmonary edema: therapeutic implications. *Circulation* 142, 101–104. doi: 10.1161/CIRCULATIONAHA.120.047915

Ayzac, L., Girard, R., Baboi, L., Beuret, P., Rabilloud, M., Richard, J. C., et al. (2016). Ventilator-associated pneumonia in ARDS patients: the impact of prone positioning. A secondary analysis of the PROSEVA trial. *Intensive Care Med.* 42, 871–878. doi: 10.1007/s00134-015-4167-5

Aziz, S., Arabi, Y. M., Alhazzani, W., Evans, L., Citerio, G., Fischkoff, K., et al. (2020). Managing ICU surge during the COVID-19 crisis: rapid guidelines. *Intensive Care Med.* 46, 1303–1325. doi: 10.1007/s00134-020-06092-5

Banzett, R. B., Mulnier, H. E., Murphy, K., Rosen, S. D., Wise, R. J., and Adams, L. (2000). Breathlessness in humans activates insular cortex. *Neuroreport* 11, 2117–2120. doi: 10.1097/00001756-200007140-00012

Bazuaye, E. A., Stone, T. N., Corris, P. A., and Gibson, G. J. (1992). Variability of inspired oxygen concentration with nasal cannulas. *Thorax* 47, 609–611. doi: 10.1136/thx.47.8.609

Beitler, J. R., Shaefi, S., Montesi, S. B., Devlin, A., Loring, S. H., Talmor, D., et al. (2014). Prone positioning reduces mortality from acute respiratory distress syndrome in the low tidal volume era: a meta-analysis. *Intensive Care Med.* 40, 332–341. doi: 10.1007/s00134-013-3194-3

Bellani, G., Laffey, J. G., Pham, T., Madotto, F., Fan, E., Brochard, L., et al. (2017). Noninvasive ventilation of patients with acute respiratory distress syndrome. Insights from the LUNG SAFE study. *Am. J. Respir. Crit. Care Med.* 195, 67–77. doi: 10.1164/rccm.201606-1306OC

Bender, P. R., Weil, J. V., Reeves, J. T., and Moore, L. G. (1987). Breathing pattern in hypoxic exposures of varying duration. *J. Appl. Physiol.* 62, 640–645. doi: 10.1152/jappl.1987.62.2.640

Bernheim, A., Mei, X., Huang, M., Yang, Y., Fayad, Z. A., Zhang, N., et al. (2020). Chest CT findings in coronavirus disease-19 (COVID-19): relationship to duration of infection. *Radiology* 295:200463. doi: 10.1148/radiol.2020200463

Berry, D. T., Mcconnell, J. W., Phillips, B. A., Carswell, C. M., Lamb, D. G., and Prine, B. C. (1989). Isocapnic hypoxemia and neuropsychological functioning. *J. Clin. Exp. Neuropsychol.* 11, 241–251.

Bickler, P. E., Feiner, J. R., Lipnick, M. S., Batchelder, P., Macleod, D. B., and Severinghaus, J. W. (2017). Effects of acute, profound hypoxia on healthy humans: implications for safety of tests evaluating pulse oximetry or tissue oximetry performance. *Anesth. Analg.* 124, 146–153. doi: 10.1213/ANE.0000000000001421

Bickler, P. E., Feiner, J. R., and Severinghaus, J. W. (2005). Effects of skin pigmentation on pulse oximeter accuracy at low saturation. *Anesthesiology* 102, 715–719. doi: 10.1097/00000542-200504000-00004

Bloomfield, R., Noble, D. W., and Sudlow, A. (2015). Prone position for acute respiratory failure in adults. *Cochrane Database Syst. Rev.* 2015:CD008095. doi: 10.1002/14651858.CD008095.pub2

Bouaziz, J. D., Duong, T., Jachiet, M., Velter, C., Lestang, P., Cassius, C., et al. (2020). Vascular skin symptoms in COVID-19: a french observational study. *J Eur Acad Dermatol. Venereol.* 34, e451–e452. doi: 10.1111/jdv.16544

Brochard, L., Rauss, A., Benito, S., Conti, G., Mancebo, J., Rekik, N., et al. (1994). Comparison of three methods of gradual withdrawal from ventilatory support during weaning from mechanical ventilation. *Am. J. Respir. Crit. Care Med.* 150, 896–903. doi: 10.1164/ajrccm.150.4.7921460

Brugger, H., Basnyat, B., Ellerton, J., Hefti, U., Strapazzon, G., and Zafren, K. (2020). Letter to the editor: COVID-19 lung injury is different from high altitude pulmonary edema. *High Alt. Med. Biol.* 21, 204–205. doi: 10.1089/ham.2020.0061

Brusasco, C., Corradi, F., Di Domenico, A., Raggi, F., Timossi, G., Santori, G., et al. (2020). Continuous positive airway pressure in Covid-19 patients with moderate-to-severe respiratory failure. *Eur. Respir. J.* 8:2002524. doi: 10.1183/13993003.02524-2020

Bryan, A. C. (1974). Conference on the scientific basis of respiratory therapy. Pulmonary physiotherapy in the pediatric age group. Comments of a devil's advocate. *Am. Rev. Respir. Dis.* 110, 143–144. doi: 10.1164/arrd.1974.110.6P2.143

Buchanan, G. F., and Richerson, G. B. (2009). Role of chemoreceptors in mediating dyspnea. *Respir. Physiol. Neurobiol.* 167, 9–19. doi: 10.1016/j.resp.2008.12.002

Chad, T., and Sampson, C. (2020). Prone positioning in conscious patients on medical wards: a review of the evidence and its relevance to patients with COVID-19 infection. *Clin. Med.* 20, e97–e103. doi: 10.7861/clinmed.2020-0179

Chiumello, D., and Brioni, M. (2016). Severe hypoxemia: which strategy to choose. *Crit. Care* 20:132. doi: 10.1186/s13054-016-1304-7

Colla, J., Rodos, A., Seyller, H., and Weingart, S. (2020). Fighting COVID-19 hypoxia with one hand tied behind our back: blanket prohibition of high-flow oxygen and noninvasive positive end-expiratory pressure in US hospitals. *Ann. Emerg. Med.* 75, 791–792. doi: 10.1016/j.annemergmed.2020.04.015

Connors, J. M., and Levy, J. H. (2020). COVID-19 and its implications for thrombosis and anticoagulation. *Blood* 135, 2033–2040. doi: 10.1182/blood.2020006000

Corne, S., Webster, K., and Younes, M. (2003). Hypoxic respiratory response during acute stable hypocapnia. *Am. J. Respir. Crit. Care Med.* 167, 1193–1199. doi: 10.1164/rccm.2203019

Couzin-Frankel, J. (2020). The mystery of the pandemic's 'happy hypoxia. *Science* 368, 455–456. doi: 10.1126/science.368.6490.455

Doussot, A., Ciceron, F., Cerutti, E., Salomon Du Mont, L., Thines, L., Capellier, G., et al. (2020). Prone positioning for severe acute respiratory distress syndrome in COVID-19 patients by a dedicated team: a safe and pragmatic reallocation of medical and surgical work force in response to the outbreak. *Ann. Surg.* 272, e311–e315. doi: 10.1097/SLA.0000000000004265

Du, Y., Tu, L., Zhu, P., Mu, M., Wang, R., Yang, P., et al. (2020). Clinical features of 85 fatal cases of COVID-19 from Wuhan. A retrospective observational study. *Am. J. Respir. Crit. Care Med.* 201, 1372–1379. doi: 10.1164/rccm.202003-0543OC

Elharrar, X., Trigui, Y., Dols, A. M., Touchon, F., Martinez, S., Prud'homme, E., et al. (2020). Use of prone positioning in nonintubated patients with COVID-19 and hypoxemic acute respiratory failure. *JAMA* 323, 2336–2338. doi: 10.1001/jama.2020.8255

Evans, T. W. (2001). International consensus conferences in intensive care medicine: non-invasive positive pressure ventilation in acute respiratory failure. Organised jointly by the American Thoracic Society, the European Respiratory Society, the European Society of Intensive Care Medicine, and the Societe de reanimation de langue Francaise, and approved by the ATS Board of Directors, December 2000. *Intensive Care Med.* 27, 166–178. doi: 10.1007/s001340000721

Evans, K. C., Banzett, R. B., Adams, L., Mckay, L., Frackowiak, R. S., and Corfield, D. R. (2002). BOLD fMRI identifies limbic, paralimbic, and cerebellar activation during air hunger. *J. Neurophysiol.* 88, 1500–1511. doi: 10.1152/jn.2002.88.3.1500

Field, S., Sanci, S., and Grassino, A. (1984). Respiratory muscle oxygen consumption estimated by the diaphragm pressure-time index. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* 57, 44–51. doi: 10.1152/jappl.1984.57.1.44

Fitzgerald, R. S., and Dehghani, G. A. (1982). Neural responses of the cat carotid and aortic bodies to hypercapnia and hypoxia. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* 52, 596–601. doi: 10.1152/jappl.1982.52.3.596

Force, A. D. T., Ranieri, V. M., Rubenfeld, G. D., Thompson, B. T., Ferguson, N. D., Caldwell, E., et al. (2012). Acute respiratory distress syndrome: the Berlin definition. *JAMA* 307, 2526–2533. doi: 10.1001/jama.2012.5669

Forster, H. V., and Dempsey, J. A. (1981). "Ventilatory adaptations" in *Lung biology in health and disease. Regulation of breathing*. ed. T. Hornbein (New York: Dekker M), 845–904.

Gattinoni, L., Carlesso, E., and Caironi, P. (2012). Stress and strain within the lung. *Curr. Opin. Crit. Care* 18, 42–47. doi: 10.1097/MCC.0b013e32834f17d9

Gattinoni, L., Chiumello, D., Caironi, P., Busana, M., Romitti, F., Brazzi, L., et al. (2020a). COVID-19 pneumonia: different respiratory treatments for different phenotypes? *Intensive Care Med.* 46, 1099–1102. doi: 10.1007/s00134-020-06033-2

Gattinoni, L., Chiumello, D., and Rossi, S. (2020b). COVID-19 pneumonia: ARDS or not? *Crit. Care* 24:154. doi: 10.1186/s13054-020-02880-z

Gattinoni, L., Coppola, S., Cressoni, M., Busana, M., Rossi, S., and Chiumello, D. (2020c). COVID-19 does not Lead to a "typical" acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 201, 1299–1300. doi: 10.1164/rccm.202003-0817LE

Gattinoni, L., Taccone, P., Carlesso, E., and Marini, J. J. (2013). Prone position in acute respiratory distress syndrome. Rationale, indications, and limits. *Am. J. Respir. Crit. Care Med.* 188, 1286–1293. doi: 10.1164/rccm.201308-1532CI

Gottlieb, M., and Long, B. (2020). Dermatologic manifestations and complications of COVID-19. *Am. J. Emerg. Med.* 38, 1715–1721. doi: 10.1016/j.ajem.2020.06.011

Grasselli, G., Zangrillo, A., Zanella, A., Antonelli, M., Cabrini, L., Castelli, A., et al. (2020). Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the lombardy region, Italy. *JAMA* 323, 1574–1581. doi: 10.1001/jama.2020.5394

Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W. H., Ou, C. Q., He, J. X., et al. (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Engl. J. Med.* 382, 1708–1720. doi: 10.1056/NEJMoa2002032

Guerin, C. (2014). Prone ventilation in acute respiratory distress syndrome. *Eur. Respir. Rev.* 23, 249–257. doi: 10.1183/09059180.00001114

Guerin, C. (2017). Prone positioning acute respiratory distress syndrome patients. *Ann Transl Med* 5:289. doi: 10.21037/atm.2017.06.63

Guerin, C., Baboi, L., and Richard, J. C. (2014). Mechanisms of the effects of prone positioning in acute respiratory distress syndrome. *Intensive Care Med.* 40, 1634–1642. doi: 10.1007/s00134-014-3500-8

Guerin, C., Reignier, J., Richard, J. C., Beuret, P., Gacouin, A., Boulain, T., et al. (2013). Prone positioning in severe acute respiratory distress syndrome. *N. Engl. J. Med.* 368, 2159–2168. doi: 10.1056/NEJMoa1214103

Hamilton, C., Steinlechner, B., Gruber, E., Simon, P., and Wollenek, G. (2004). The oxygen dissociation curve: quantifying the shift. *Perfusion* 19, 141–144. doi: 10.1191/0267659104pf734oa

Hamming, I., Timens, W., Bulthuis, M. L., Lely, A. T., Navis, G., and Van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203, 631–637. doi: 10.1002/path.1570

Hampl, V., Herget, J., Bibova, J., Banasova, A., Huskova, Z., Vanourkova, Z., et al. (2015). Intrapulmonary activation of the angiotensin-converting enzyme type 2/angiotensin 1-7/G-protein-coupled mas receptor axis attenuates pulmonary hypertension in Ren-2 transgenic rats exposed to chronic hypoxia. *Physiol. Res.* 64, 25–38. doi: 10.33549/physiolres.932861

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271.e8–280.e8. doi: 10.1016/j.cell.2020.02.052

Hu, S. L., He, H. L., Pan, C., Liu, A. R., Liu, S. Q., Liu, L., et al. (2014). The effect of prone positioning on mortality in patients with acute respiratory distress syndrome: a meta-analysis of randomized controlled trials. *Crit. Care* 18:R109. doi: 10.1186/cc13896

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Jounieaux, V., Parreira, V. F., Aubert, G., Dury, M., Delguste, P., and Rodenstein, D. O. (2002). Effects of hypocapnic hyperventilation on the response to hypoxia in normal subjects receiving intermittent positive-pressure ventilation. *Chest* 121, 1141–1148. doi: 10.1378/chest.121.4.1141

Jubran, A., Van De Graaff, W. B., and Tobin, M. J. (1995). Variability of patient-ventilator interaction with pressure support ventilation in patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 152, 129–136. doi: 10.1164/ajrccm.152.1.7599811

Kallet, R. H. (2015). A comprehensive review of prone position in ARDS. *Respir. Care* 60, 1660–1687. doi: 10.4187/respcare.04271

Kimmoun, A., Levy, B., and Chenuel, B. (2020). Usefulness and safety of a dedicated team to prone patients with severe ARDS due to COVID-19. *Crit. Care* 24:509. doi: 10.1186/s13054-020-03128-6

Kronenberg, R. S., and Drage, C. W. (1973). Attenuation of the ventilatory and heart rate responses to hypoxia and hypercapnia with aging in normal men. *J. Clin. Invest.* 52, 1812–1819.

Lahiri, S., and Delaney, R. G. (1975). Stimulus interaction in the responses of carotid body chemoreceptor single afferent fibers. *Respir. Physiol.* 24, 249–266. doi: 10.1016/0034-5687(75)90017-1

Lang, M., Som, A., Mendoza, D. P., Flores, E. J., Reid, N., Carey, D., et al. (2020). Hypoxaemia related to COVID-19: vascular and perfusion abnormalities on dual-energy CT. *Lancet Infect. Dis.* 20, 1365–1366. doi: 10.1016/S1473-3099(20)30367-4

Lansing, R. W., Gracely, R. H., and Banzett, R. B. (2009). The multiple dimensions of dyspnea: review and hypotheses. *Respir. Physiol. Neurobiol.* 167, 53–60. doi: 10.1016/j.resp.2008.07.012

Laviolette, L., Laveneziana, P., and Faculty, E. R. S. R. S. (2014). Dyspnoea: a multidimensional and multidisciplinary approach. *Eur. Respir. J.* 43, 1750–1762. doi: 10.1183/09031936.00092613

Lee, J. M., Bae, W., Lee, Y. J., and Cho, Y. J. (2014). The efficacy and safety of prone positional ventilation in acute respiratory distress syndrome: updated study-level meta-analysis of 11 randomized controlled trials. *Crit. Care Med.* 42, 1252–1262. doi: 10.1097/CCM.0000000000000122

Levitan, R. (2020). The infection that's silently killing coronavirus patients. The New York Times, April 20.

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., et al. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 395, 565–574. doi: 10.1016/S0140-6736(20)30251-8

Luks, A. M., and Swenson, E. R. (2020). COVID-19 lung injury and high-altitude pulmonary edema. A false equation with dangerous implications. *Ann. Am. Thorac. Soc.* 17, 918–921. doi: 10.1513/AnnalsATS.202004-327FR

Manning, H. L., and Schwartzstein, R. M. (1995). Pathophysiology of dyspnea. *N. Engl. J. Med.* 333, 1547–1553. doi: 10.1056/NEJM199512073332307

Marini, J. J., Capps, J. S., and Culver, B. H. (1985). The inspiratory work of breathing during assisted mechanical ventilation. *Chest* 87, 612–618. doi: 10.1378/chest.87.5.612

Matsuzawa, Y., Fujimoto, K., Kobayashi, T., Namushi, N. R., Harada, K., Kohno, H., et al. (1989). Blunted hypoxic ventilatory drive in subjects susceptible to high-altitude pulmonary edema. *J. Appl. Physiol.* 66, 1152–1157. doi: 10.1152/jappl.1989.66.3.1152

Mehta, P., Mcauley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., Manson, J. J., et al. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 395, 1033–1034. doi: 10.1016/S0140-6736(20)30628-0

Mellins, R. B. (1974). Pulmonary physiotherapy in the pediatric age group. *Am. Rev. Respir. Dis.* 110, 137–142. doi: 10.1164/arrd.1974.110.6P2.137

Meschi, T., Rossi, S., Volpi, A., Ferrari, C., Sverzellati, N., Brianti, E., et al. (2020). Reorganization of a large academic hospital to face COVID-19 outbreak: the model of Parma, Emilia-Romagna region, Italy. *Eur. J. Clin. Investig.* 50:e13250. doi: 10.1111/eci.13250

Messika, J., Ben Ahmed, K., Gaudry, S., Miguel-Montanes, R., Rafat, C., Sztrymf, B., et al. (2015). Use of high-flow nasal cannula oxygen therapy in subjects with ARDS: a 1-year observational study. *Respir. Care* 60, 162–169. doi: 10.4187/respcare.03423

Mitchell, D. A., and Seckel, M. A. (2018). Acute respiratory distress syndrome and prone positioning. *AACN Adv. Crit. Care* 29, 415–425. doi: 10.4037/aacnacc2018161

Moore, L. G., Huang, S. Y., Mccullough, R. E., Sampson, J. B., Maher, J. T., Weil, J. V., et al. (1984). Variable inhibition by falling CO2 of hypoxic ventilatory response in humans. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* 56, 207–210. doi: 10.1152/jappl.1984.56.1.207

Moosavi, S. H., Golestanian, E., Binks, A. P., Lansing, R. W., Brown, R., and Banzett, R. B. (2003). Hypoxic and hypercapnic drives to breathe generate equivalent levels of air hunger in humans. *J. Appl. Physiol.* 94, 141–154. doi: 10.1152/japplphysiol.00594.2002

Munshi, L., Del Sorbo, L., Adhikari, N. K. J., Hodgson, C. L., Wunsch, H., Meade, M. O., et al. (2017). Prone position for acute respiratory distress syndrome. A systematic review and meta-analysis. *Ann. Am. Thorac. Soc.* 14, S280–S288. doi: 10.1513/AnnalsATS.201704-343OT

Nishimura, M., Miyamoto, K., Suzuki, A., Yamamoto, H., Tsuji, M., Kishi, F., et al. (1989). Ventilatory and heart rate responses to hypoxia and hypercapnia in patients with diabetes mellitus. *Thorax* 44, 251–257. doi: 10.1136/thx.44.4.251

O'Donnell, C. R., Friedman, L. S., Russomanno, J. H., and Rose, R. M. (1988). Diminished perception of inspiratory-resistive loads in insulin-dependent diabetics. *N. Engl. J. Med.* 319, 1369–1373. doi: 10.1056/NEJM198811243192102

Oranger, M., Gonzalez-Bermejo, J., Dacosta-Noble, P., Llontop, C., Guerder, A., Trosini-Desert, V., et al. (2020). Continuous positive airway pressure to avoid intubation in SARS-CoV-2 pneumonia: a two-period retrospective case-control study. *Eur. Respir. J.* 56:2001692. doi: 10.1183/13993003.01692-2020

Parshall, M. B., Schwartzstein, R. M., Adams, L., Banzett, R. B., Manning, H. L., Bourbeau, J., et al. (2012). An official American Thoracic Society statement: update on the mechanisms, assessment, and management of dyspnea. *Am. J. Respir. Crit. Care Med.* 185, 435–452. doi: 10.1164/rccm.201111-2042ST

Peterson, D. D., Pack, A. I., Silage, D. A., and Fishman, A. P. (1981). Effects of aging on ventilatory and occlusion pressure responses to hypoxia and hypercapnia. *Am. Rev. Respir. Dis.* 124, 387–391. doi: 10.1164/arrd.1981.124.4.387

Petrucci, N., and De Feo, C. (2013). Lung protective ventilation strategy for the acute respiratory distress syndrome. *Cochrane Database Syst. Rev.* 2013: CD003844. doi: 10.1002/14651858.CD003844.pub4

Pfeifer, M., Ewig, S., Voshaar, T., Randerath, W. J., Bauer, T., Geiseler, J., et al. (2020). Position paper for the state-of-the-art application of respiratory support in patients with COVID-19. *Respiration* 99, 521–542. doi: 10.1159/000509104

Polidoro, R. B., Hagan, R. S., De Santis Santiago, R., and Schmidt, N. W. (2020). Overview: systemic inflammatory response derived from lung injury caused by SARS-CoV-2 infection explains severe outcomes in COVID-19. *Front. Immunol.* 11:1626. doi: 10.3389/fimmu.2020.01626

Rebuck, A. S., and Campbell, E. J. (1974). A clinical method for assessing the ventilatory response to hypoxia. *Am. Rev. Respir. Dis.* 109, 345–350. doi: 10.1164/arrd.1974.109.3.345

Reynolds, W. J., and Milhorn, H. T. Jr. (1973). Transient ventilatory response to hypoxia with and without controlled alveolar PCO2. *J. Appl. Physiol.* 35, 187–196. doi: 10.1152/jappl.1973.35.2.187

Richardson, S., Hirsch, J. S., Narasimhan, M., Crawford, J. M., Mcginn, T., Davidson, K. W., et al. (2020). Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the new York City area. *JAMA* 323, 2052–2059. doi: 10.1001/jama.2020.6775

Rizvi, I., Zaman, S., Zaidi, N., Asif, M. S., and Abdali, N. (2012). Acute life-threatening methaemoglobinaemia following ingestion of chloroquine. *BMJ Case Rep.* 2012:bcr1220115383. doi: 10.1136/bcr.12.2011.5383

Ruste, M., Bitker, L., Yonis, H., Riad, Z., Louf-Durier, A., Lissonde, F., et al. (2018). Hemodynamic effects of extended prone position sessions in ARDS. *Ann. Intensive Care* 8:120. doi: 10.1186/s13613-018-0464-9

Sahn, S. A., Zwillich, C. W., Dick, N., Mccullough, R. E., Lakshminarayan, S., and Weil, J. V. (1977). Variability of ventilatory responses to hypoxia and hypercapnia. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* 43, 1019–1025. doi: 10.1152/jappl.1977.43.6.1019

Sartini, C., Tresoldi, M., Scarpellini, P., Tettamanti, A., Carcò, F., Landoni, G., et al. (2020). Respiratory parameters in patients with COVID-19 after using noninvasive ventilation in the prone position outside the intensive care unit. *JAMA* 323, 2338–2340. doi: 10.1001/jama.2020.7861

Schnapp, L. M., and Cohen, N. H. (1990). Pulse oximetry. Uses and abuses. *Chest* 98, 1244–1250.

Scholten, E. L., Beitler, J. R., Prisk, G. K., and Malhotra, A. (2017). Treatment of ARDS with prone positioning. *Chest* 151, 215–224. doi: 10.1016/j.chest.2016.06.032

Schon, D., Rosenkranz, M., Regelsberger, J., Dahme, B., Buchel, C., and Von Leupoldt, A. (2008). Reduced perception of dyspnea and pain after right insular cortex lesions. *Am. J. Respir. Crit. Care Med.* 178, 1173–1179. doi: 10.1164/rccm.200805-731OC

Settembre, N., Maurice, P., Paysant, J., Theurel, J., Claudon, L., Kimmoun, A., et al. (2020). The use of exoskeletons to help with prone positioning in the intensive care unit during COVID-19. *Ann. Phys. Rehabil. Med.* 63, 379–382. doi: 10.1016/j.rehab.2020.05.004

Sinex, J. E. (1999). Pulse oximetry: principles and limitations. *Am. J. Emerg. Med.* 17, 59–67. doi: 10.1016/S0735-6757(99)90019-0

Slutsky, A. S., and Ranieri, V. M. (2013). Ventilator-induced lung injury. *N. Engl. J. Med.* 369, 2126–2136. doi: 10.1056/NEJMra1208707

Solaimanzadeh, I. (2020). Acetazolamide, Nifedipine Phosphodiesterase inhibitors: rationale for their utilization as adjunctive countermeasures in the treatment of coronavirus disease 2019 (COVID-19). *Cureus* 12:e7343. doi: 10.7759/cureus.7343

Strapazzon, G., Hilty, M. P., Bouzat, P., Pratali, L., Brugger, H., and Rauch, S. (2020). To compare the incomparable: COVID-19 pneumonia and high-altitude disease. *Eur. Respir. J.* 55:2001362. doi: 10.1183/13993003.01362-2020

Suffredini, D. A., and Allison, M. G. (2020). A rationale for use of high flow nasal cannula for select patients with suspected or confirmed severe acute respiratory syndrome Coronavirus-2 infection. *J. Intensive Care Med.* 36, 9–17. doi: 10.1177/0885066620956630

Sutcu Cicek, H., Gumus, S., Deniz, O., Yildiz, S., Acikel, C. H., Cakir, E., et al. (2011). Effect of nail polish and henna on oxygen saturation determined by pulse oximetry in healthy young adult females. *Emerg. Med. J.* 28, 783–785. doi: 10.1136/emj.2010.096073

Swenson, E. R., and Bartsch, P. (2012). High-altitude pulmonary edema. *Compr. Physiol.* 2, 2753–2773. doi: 10.1002/cphy.c100029

Tian, S., Hu, W., Niu, L., Liu, H., Xu, H., and Xiao, S. Y. (2020). Pulmonary pathology of early-phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. *J. Thorac. Oncol.* 15, 700–704. doi: 10.1016/j.jtho.2020.02.010

Tobin, M. J. (1990). Respiratory monitoring. *JAMA* 264, 244–251. doi: 10.1001/jama.1990.03450020096034

Tobin, M. J. (2018). Physiologic basis of mechanical ventilation. *Ann. Am. Thorac. Soc.* 15, S49–S52. doi: 10.1513/AnnalsATS.201705-417KV

Tobin, M. J. (2020). Basing respiratory management of COVID-19 on physiological principles. *Am. J. Respir. Crit. Care Med.* 201, 1319–1320. doi: 10.1164/rccm.202004-1076ED

Tobin, M. J., and Jubran, A. (2006). Variable performance of weaning-predictor tests: role of Bayes' theorem and spectrum and test-referral bias. *Intensive Care Med.* 32, 2002–2012. doi: 10.1007/s00134-006-0439-4

Tobin, M. J., Jubran, A., and Laghi, F. (2020a). Noninvasive strategies in COVID-19: epistemology, randomised trials, guidelines, physiology. *Eur. Respir. J.* doi: 10.1183/13993003.04247-2020 [Epub ahead of print]

Tobin, M. J., Laghi, F., and Jubran, A. (2012). Ventilatory failure, ventilator support, and ventilator weaning. *Compr. Physiol.* 2, 2871–2921. doi: 10.1002/cphy.c110030

Tobin, M. J., Laghi, F., and Jubran, A. (2020b). Why COVID-19 silent hypoxemia is baffling to physicians. *Am. J. Respir. Crit. Care Med.* 202, 356–360. doi: 10.1164/rccm.202006-2157CP

Tobin, M. J., Mador, M. J., Guenther, S. M., Lodato, R. F., and Sackner, M. A. (1988). Variability of resting respiratory drive and timing in healthy subjects. *J. Appl. Physiol.* 65, 309–317. doi: 10.1152/jappl.1988.65.1.309

Tosti, G., Barisani, A., Queirolo, P., Pennacchioli, E., Villa, L., Lodeserto, A. M., et al. (2020). Skin signs resembling vascular acrosyndromes during the COVID-19 outbreak in Italy. *Clin. Exp. Dermatol.* 45, 757–758. doi: 10.1111/ced.14267

Van De Louw, A., Cracco, C., Cerf, C., Harf, A., Duvaldestin, P., Lemaire, F., et al. (2001). Accuracy of pulse oximetry in the intensive care unit. *Intensive Care Med.* 27, 1606–1613. doi: 10.1007/s001340101064

Villarreal-Fernandez, E., Patel, R., Golamari, R., Khalid, M., Dewaters, A., and Haouzi, P. (2020). A plea for avoiding systematic intubation in severely hypoxemic patients with COVID-19-associated respiratory failure. *Crit. Care* 24:337. doi: 10.1186/s13054-020-03063-6

Wei, Y. Y., Wang, R. R., Zhang, D. W., Tu, Y. H., Chen, C. S., Ji, S., et al. (2020). Risk factors for severe COVID-19: evidence from 167 hospitalized patients in Anhui, China. *J. Infect.* 81, e89–e92. doi: 10.1016/j.jinf.2020.04.010

Weisbrod, C. J., Eastwood, P. R., O'driscoll, G., and Green, D. J. (2005). Abnormal ventilatory responses to hypoxia in type 2 diabetes. *Diabet. Med.* 22, 563–568. doi: 10.1111/j.1464-5491.2005.01458.x

Wilson, K. C., Chotirmall, S. H., Bai, C., and Rello, J. (2020). COVID-19: Interim Guidance on Management Pending Empirical Evidence. From an American Thoracic Society-led International Task Force: A.T. Society; on behalf of the International Task Force on COVID-19. Available at: https://www.thoracic.org/covid/covid-19-guidance.pdf (Accessed May 9, 2020).

Wilson, R. J., and Teppema, L. J. (2016). Integration of central and peripheral respiratory Chemoreflexes. *Compr. Physiol.* 6, 1005–1041. doi: 10.1002/cphy.c140040

Wu, Z., and Mcgoogan, J. M. (2020). Characteristics of and important Lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. *JAMA* 323, 1239–1242. doi: 10.1001/jama.2020.2648

Xie, J., Covassin, N., Fan, Z., Singh, P., Gao, W., Li, G., et al. (2020a). Association between hypoxemia and mortality in patients with COVID-19. *Mayo Clin. Proc.* 95, 1138–1147. doi: 10.1016/j.mayocp.2020.04.006

Xie, J., Tong, Z., Guan, X., Du, B., Qiu, H., and Slutsky, A. S. (2020b). Critical care crisis and some recommendations during the COVID-19 epidemic in China. *Intensive Care Med.* 46, 837–840. doi: 10.1007/s00134-020-05979-7

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., et al. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med.* 8, 420–422. doi: 10.1056/NEJM199105233242101

Yang, K. L., and Tobin, M. J. (1991). A prospective study of indexes predicting the outcome of trials of weaning from mechanical ventilation. *N. Engl. J. Med.* 324, 1445–1450. doi: 10.1056/NEJM199105233242101

Zhang, H., Penninger, J. M., Li, Y., Zhong, N., and Slutsky, A. S. (2020). Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med.* 46, 586–590. doi: 10.1007/s00134-020-05985-9

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

MINI REVIEW
published: 19 February 2021
doi: 10.3389/fphys.2020.622466

COVID-19-Associated Neurological Manifestations: An Emerging Electroencephalographic Literature

Geoffroy Vellieux[1,2], Romain Sonneville[3,4], Sérafima Vledouts[1,2], Pierre Jaquet[3,4], Anny Rouvel-Tallec[1,2] and Marie-Pia d'Ortho[1,2]*

[1] Université de Paris, NeuroDiderot, Inserm, Paris, France, [2] Neurophysiologie clinique, Service de Physiologie – Explorations Fonctionnelles, AP-HP, Hôpital Bichat-Claude Bernard, Paris, France, [3] Médecine Intensive-Réanimation, AP-HP, Hôpital Bichat-Claude Bernard, Paris, France, [4] Université de Paris, UMR 1148, LVTS, Paris, France

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide since the end of year 2019 and is currently responsive for coronavirus infectious disease 2019 (COVID-19). The first reports considered COVID-19 as a respiratory tract disease responsible for pneumonia, but numerous studies rapidly emerged to warn the medical community of COVID-19-associated neurological manifestations, including encephalopathy at the acute phase and other postinfectious manifestations. Using standard visual analysis or spectral analysis, recent studies reported electroencephalographic (EEG) findings of COVID-19 patients with various neurological symptoms. Most EEG recordings were normal or revealed non-specific abnormalities, such as focal or generalized slowing, interictal epileptic figures, seizures, or status epilepticus. Interestingly, novel EEG abnormalities over frontal areas were also described at the acute phase. Underlying mechanisms leading to brain injury in COVID-19 are still unknown and matters of debate. These frontal EEG abnormalities could emphasize the hypothesis whereby SARS-CoV-2 enters the central nervous system (CNS) through olfactory structures and then spreads in CNS via frontal lobes. This hypothesis is reinforced by the presence of anosmia in a significant proportion of COVID-19 patients and by neuroimaging studies confirming orbitofrontal abnormalities. COVID-19 represents a new viral disease characterized by not only respiratory symptoms but also a systemic invasion associated with extra-respiratory signs. Neurological symptoms must be the focus of our attention, and functional brain evaluation with EEG is crucial, in combination with anatomical and functional brain imaging, to better understand its pathophysiology. Evolution of symptoms together with EEG patterns at the distance of the acute episode should also be scrutinized.

Keywords: SARS-CoV-2, coronavirus, COVID-19, encephalopathy, neurophysiology, EEG

OPEN ACCESS

Edited by:
Georges Leftheriotis, Université Côte d'Azur, France

Reviewed by:
Simona Mrakic-Sposta, Italian National Research Council, Italy
Marion Simonetta-Moreau, INSERM UMR 1214 ToNIC, France

****Correspondence:***
Geoffroy Vellieux
geoffroy.vellieux@aphp.fr;
geoffroyvellieux@hotmail.com

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *28 October 2020*
Accepted: *29 December 2020*
Published: *19 February 2021*

Citation:
Vellieux G, Sonneville R, Vledouts S, Jaquet P, Rouvel-Tallec A and d'Ortho M-P (2021) COVID-19-Associated Neurological Manifestations: An Emerging Electroencephalographic Literature. Front. Physiol. 11:622466. doi: 10.3389/fphys.2020.622466

INTRODUCTION

The coronavirus infectious disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, was initially recognized as a respiratory tract disease which could lead to an acute respiratory distress syndrome. However, there is growing evidence of a multi-organ involvement (Gupta et al., 2020). Several authors

reported central nervous system (CNS) manifestations, as anosmia referring to olfactory tract involvement. Other critical presentations, including meningoencephalitis, seizures, status epilepticus (SE), encephalopathy, and altered mental status were also described (Ellul et al., 2020). Neurological complications, such as encephalopathy and seizures/SE, and electroencephalographic (EEG) abnormalities, mainly diffuse slowing and epileptiform discharges, have already been described in past viral pandemics such as influenza A H1N1 (Ekstrand et al., 2010; Kedia et al., 2011; Ibrahim and Haddad, 2014). Results of EEG in patients with COVID-19 were increasingly reported. While the volume of COVID-19-related case studies is still growing, we present the spectrum of EEG findings published at the moment, allowing physicians to be cognizant of this new and emerging literature while dealing with COVID-19 patients.

METHODS

We considered all studies with EEG findings at the acute phase in COVID-19 patients with neurological manifestations. We performed an electronic research from December 1, 2019, to October 1, 2020, using the database PUBMED by Medline with the following terms (in all fields): (i) ("EEG" OR "electroencephalogram" OR "electroencephalography") AND ("COVID" OR "coronavirus" OR "SARS-CoV-2") and (ii) ("brain" OR "nervous system" OR "neurology") AND ("COVID" OR "coronavirus" OR "SARS-CoV-2"). We also scanned the reference lists of all included articles or relevant reviews for studies to be included in our work. We did not include reviews, non-English articles, unavailable full-text articles, and animal studies. After exclusion of duplicates, we screened the title/abstract or full-text reports and decided whether these met the inclusion criteria.

EEG OBSERVATIONS IN COVID-19 PATIENTS

A total of 107 studies were included. Normal EEG findings were reported in adult series (Cecchetti et al., 2020; Helms et al., 2020b; Petrescu et al., 2020) and case reports of patients who displayed various neurological conditions such as focal or generalized seizures (Elgamasy et al., 2020; Fasano et al., 2020; García-Howard et al., 2020; Lyons et al., 2020), non-epileptic seizures (Logmin et al., 2020), myoclonus (Muccioli et al., 2020b; Rábano-Suárez et al., 2020), psychotic symptoms (Lim et al., 2020), encephalopathy (Andriuta et al., 2020; Chaumont et al., 2020; Delorme et al., 2020; Paterson et al., 2020; Perrin et al., 2020), encephalitis (Paterson et al., 2020), brainstem encephalitis (Khoo et al., 2020), and encephalomyelitis (Zoghi et al., 2020). Some studies also reported non-specific abnormalities without more precise EEG features specified by authors (Chougar et al., 2020; Farley and Zuberi, 2020; Freij et al., 2020; Helms et al., 2020a; Pugin et al., 2020).

Diffuse and Focal Slowing

Diffuse slowing of the background activity or focal slowing (sometimes associated with focal sharp waves or epileptiform discharges) was the most frequently published abnormality, especially in adult series (Ayub et al., 2020; Canham et al., 2020; Cecchetti et al., 2020; Chougar et al., 2020; Galanopoulou et al., 2020; Helms et al., 2020a,b; Louis et al., 2020; Pasini et al., 2020; Pellinen et al., 2020; Petrescu et al., 2020; Pilotto et al., 2020a; Scullen et al., 2020; Sethi, 2020; Vespignani et al., 2020) (**Figure 1A**). Main results of adult series including at least 10 patients with confirmed SARS-CoV-2 infection and EEG recordings are summarized in **Table 1**. Diffuse or focal slowing was also associated in many case reports with various neurological presentations, mainly of vascular or inflammatory origin. Main vascular complications included ischemic and hemorrhagic strokes (Chaumont et al., 2020; Díaz-Pérez et al., 2020; Morassi et al., 2020; Soldatelli et al., 2020; Zahid et al., 2020), intracranial hemorrhage with cerebral venous thrombosis (Roy-Gash et al., 2020), posterior reversible encephalopathy syndrome (PRES) (Llansó and Urra, 2020; Princiotta Cariddi et al., 2020), intracranial vasculitis (Dixon et al., 2020), subarachnoid hemorrhage (Harrogate et al., 2020), acute hemorrhagic leukoencephalitis or leukoencephalomyelitis (Handa et al., 2020; Kihira et al., 2020; Svedung Wettervik et al., 2020), and acute necrotizing encephalopathy (Delamarre et al., 2020; Virhammar et al., 2020). Main inflammatory syndromes included acute disseminated encephalomyelitis (ADEM) (Parsons et al., 2020; Umapathi et al., 2020), acute leukoencephalopathy (Abenza-Abildúa et al., 2020; Anand et al., 2020; Brun et al., 2020; Huang H. et al., 2020; Kihira et al., 2020; Klironomos et al., 2020), acute leukoencephalitis (Perrin et al., 2020), meningoencephalitis without any acute lesions on brain imaging (Duong et al., 2020; El-Zein et al., 2020; Pilotto et al., 2020b), Bickerstaff encephalitis (Llorente Ayuso et al., 2020), and concomitant autoimmune encephalitis (Grimaldi et al., 2020; Panariello et al., 2020). In critically ill patients, other conditions were described including post-hypoxic injury (Fischer et al., 2020; Radmanesh et al., 2020; Radnis et al., 2020; Vellieux et al., 2020), unresponsiveness after sedation discontinuation (Espinosa et al., 2020; Vellieux et al., 2020), encephalopathy or altered mental status without any acute lesions on brain imaging (Chaumont et al., 2020; Delorme et al., 2020; Filatov et al., 2020; Gaughan et al., 2020; Jang et al., 2020; Manganelli et al., 2020; Muccioli et al., 2020a; Méndez-Guerrero et al., 2020; Romero-Sánchez et al., 2020; Shekhar et al., 2020), encephalopathy with seizures (Ashraf and Sajed, 2020; Benameur et al., 2020; Farhadian et al., 2020; Haddad et al., 2020), defined toxic/metabolic encephalopathy (Flamand et al., 2020; Radmard et al., 2020; Rasmussen et al., 2020), neuroleptic malignant syndrome (Kajani et al., 2020), after seizures or SE (Anand et al., 2020; Edén et al., 2020; Emami et al., 2020), and critical illness-associated cerebral microbleeds (De Stefano et al., 2020). EEG slowing was also observed in pediatric reports (Abdel-Mannan et al., 2020; Abel et al., 2020; Dugue et al., 2020; Panda et al., 2020).

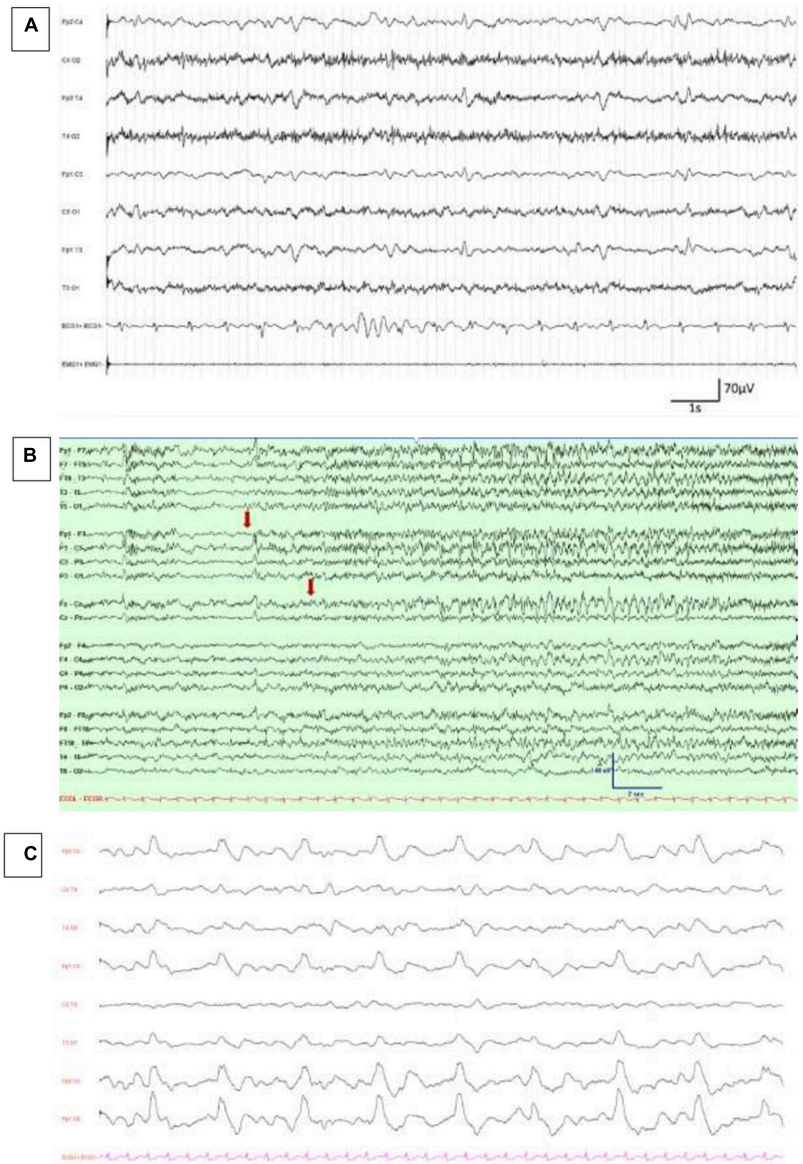


FIGURE 1 | EEG findings in COVID-19 patients. **(A)** Diffuse theta–delta slowing and continuous generalized periodic discharges, reproduced with authors' agreement from Petrescu et al. (2020). **(B)** Emergence of low-amplitude ictal fast rhythmic activity over left frontocentral and midline regions (marked with an arrow), reproduced with authors' agreement from Somani et al. (2020). **(C)** Continuous, periodic, monomorphic diphasic, delta slow waves over both frontal areas, published in Vellieux et al. (2020).

Seizures and SE

Seizures and/or SE were recorded in 10 patients out of 111 included in the series of Pellinen et al. (2020), in 2 out of 22 in the series of Louis et al. (2020), in 1 out of 37 in the series of Ayub et al. (2020), in 1 out of 15 in the series of Pasini et al. (2020), in 1 out of 27 in the series of Scullen et al. (2020), and in an unknown precise number of patients out of the 73 included in the series of Chougar et al. (2020) (**Table 1**).

Seizures and/or SE were recorded in reports of patients without any acute or chronic cortical lesions on brain imaging nor cerebrospinal fluid (CSF) abnormalities. The EEG of the patient reported by Balloy et al. (2020) revealed two widespread, but predominantly in frontal localizations, seizures that were interrupted by a moderate interictal frontal activity. Sohal and Mansur (2020) reported a patient whose 24-h EEG revealed six left temporal seizures and left temporal sharp waves. One of the two patients reported by Somani et al. (2020) displayed, on a continuous EEG (cEEG) monitoring, multiple seizures emanating from the midline and left frontocentral regions (**Figure 1B**). Hepburn et al. (2020) reported the cases of two patients whose cEEG monitoring showed, for the first one, three focal seizures arising from the right frontocentral region and,

TABLE 1 | Main results of case series including at least 10 patients admitted for COVID-19 with EEG recordings.

Series	Patients' features	Brain imaging results	CSF results	EEG settings	Ongoing psychoactive drugs	Main EEG results
Ayub et al., 2020	>Included, *n*=37	> CT-scan, *n* = 35 and MRI, *n* = 9	>CSF examination, *n*=4	>Total EEG recordings, *n* = 37	>At time of EEG or the day prior	**>Background activity**
USA	M/F, *n*=27/10	IS, *n*=3	Abnormal WBC count, *n*=2		Propofol, *n*=19	Absent PDR, *n*=34
Monocentric	Median age: 66 years	ICH, *n*=3	Abnormal protein level, *n*=1	>Types of EEG	Dexmedetomidine, *n*=13	Asymmetry, *n*=4
	Anosmia, *n*=4			Long-term monitoring EEG, *n*=23	Empiric AD, *n*=11	Generalized delta and theta slowing, *n*=34
	Intubated, *n*=28				Midazolam or lorazepam, *n*=8	Burst suppression, *n*=5
				>Period of recordings: NA	Ketamine, *n*=2	Unreactive, *n*=1
	Prior neurological history					
	Stroke, *n*=6			>EEG indication		**>Rhythmic and periodic patterns**
	Cerebral aneurysm, *n*=1			Altered mental status, *n*=24		GPDs without triphasic waves, *n*=4
	Epilepsy, *n*=1			Possible seizures, *n*=11		GPDs with triphasic waves, *n*=8
	ICH, *n*=1			Cardiac arrest, *n*=2		SIRPIDs, *n*=3
	DLB, *n*=1					GRDA, *n*=5
						LRDA, *n*=1
						>Epileptiform findings and seizures
						Burst suppression with epileptiform activity, *n*=4
						Focal sporadic discharges, *n*=1
						Multifocal sporadic discharges, *n*=6
						Generalized sporadic discharges without triphasic waves, *n*=8
						Generalized NCSE, *n*=1
Canham et al., 2020	>Included, *n*=10	>CT-scan, *n*=10	>CSF examination, *n*=6	>Total EEG recordings, *n*=11	>At time of EEG	**>Background activity**
United Kingdom	M/F, *n*=8/2	Normal, *n*=3	Abnormal WBC count, *n*=3		Levetiracetam, *n*=6	Generalized symmetrical slowing, *n*=11
Multicentric	Median age: 65 years	Small vessel disease, *n*=4	Abnormal protein level, *n*=4	>Types of EEG	Propofol, *n*=2	Anterior emphasis of slow activity, *n*=3
	Anosmia/agueusia: NA	SAH, *n*=2	Negative HSV 1&2, VZV	9 electrodes 20-30 min EEG, *n*=11	Alfentanil, *n*=2	Asymmetry, *n*=1
	Intubated: NA	Atrophy, *n*=2	and enterovirus PCR, *n*=6		Phenytoin, *n*=2	
			Negative SARS-CoV-2 PCR, *n*=2	>Period of recordings: NA	Valproate, *n*=2	**>Rhythmic and periodic patterns**
	Prior neurological history	>MRI, *n*=4			Lamotrigine, *n*=1	FIRDA, *n*=1
	SAH, *n*=1	Normal, *n*=1		>EEG indication	Gabapentin, *n*=1	
	Stroke, *n*=1	Small vessel disease, *n*=2		Altered mental status, *n*=6	Carabamazepine, *n*=1	**>Epileptiform findings and seizures, *n*=0**
	Learning difficulties, *n*=1	IS, *n*=1		Seizure, *n*=6	Lacosamide, *n*=1	
	Essential tremor, *n*=1	Atrophy, *n*=1		Delirium, *n*=2	Primidone, *n*=1	
	Epilepsy, *n*=1				Amitriptyline, *n*=1	
					Lorazepam, *n*=1	
					Citalopram, *n*=1	
					Olanzapine, *n*=1	
					Clozapine, *n*=1	
					Paliperidone, *n*=1	
					Midazolam, *n*=1	
					Remifentanyl, *n*=1	
					Morphine, *n*=1	
Cecchetti et al., 2020	>Included, *n*=18	>CT-scan and/or MRI	>CSF examination, *n*=1	>Total EEG recordings, *n*=18	NA	**>Background activity**
Italy	M/F, *n*=11/7	PRES, *n*=1	Normal WBC count, *n*=1			Normal or with mild alteration, *n*=5
Monocentric	Mean age: 67 years	Remote ICH, *n*=1	Normal protein level, *n*=1	>Types of EEG		With moderate alteration, *n*=9
	Anosmia/agueusia, *n*=0	Remote IS, *n*=1	Negative bacteriologic and	Basal EEG, *n*=18		With severe alteration, *n*=4
	Intubated: NA	Glioblastoma, *n*=1	virologic assays (including			Generalized slowing, *n*=16
		Metastasis, *n*=1	SARS-CoV-2 RT-PCR), *n*=1	>Period of recordings: NA		Anterior (bifrontal) prevalence of slow waves, *n*=10
	Prior neurological history	Traumatic SDH, *n*=1				Focal slowing, *n*=7
	NA	Anterior pontine demyelinating lesion, *n*=1		>EEG indication		
				Transient loss of consciousness, *n*=5		**>Rhythmic and periodic patterns, *n*=0**
				Seizures/spasms, *n*=5		
				Coma, *n*=5		**>Epileptiform findings and seizures**
				Delirium, *n*=3		Epileptiform discharges, *n*=2
						Seizures, *n*=0

(Continued)

TABLE 1 | Continued

Series	Patients' features	Brain imaging results	CSF results	EEG settings	Ongoing psychoactive drugs	Main EEG results
Chougar et al., 2020	>Included, *n*=73	>MRI, *n*=73	>CSF examination, *n*=39	>Total EEG recordings, *n*=40	NA	**>Background activity / Epileptiform findings and seizures**
France	M/F, *n*=48/25	No significant abnormalities, *n*=30	Abnormal WBC count, *n*=8			Pathological findings related to seizure or encephalopathy, *n*=9
Monocentric	Mean age: 56 years	Acute IS, *n*=17	Abnormal protein level, *n*=10	>Types of EEG: NA		Non-specific findings, *n*=24
	Anosmia/agueusia, *n*=4	Multiple microhemorrhages, *n*=8	Oligoclonal bands, *n*=2			
	Intubated: NA	Multifocal enhancing WM lesions, *n*=4	Negative bacteriologic and	>Period of recordings: NA		**>Rhythmic and periodic patterns: NA**
		Basal ganglia lesions, *n*=4	virologic assays (including			
	Prior neurological history	Hypoxic-ischemic lesions, *n*=3	HSV 1&2, VZV, CMV, EBV	>EEG indication: NA		
	Stroke, *n*=NA	Cytotoxic lesions of the CC, *n*=3	and SARS-CoV-2 RT-PCR), *n*=39			
		Central pontine myelinolysis, *n*=3				
		PRES, *n*=2				
		Meningeal enhancement, *n*=2				
		Neuritis, *n*=2				
		Deep venous thrombosis, *n*=1				
		Corticospinal tracts FLAIR hyperintensity, *n*=1				
		>Perfusion MRI, *n*=46				
		Seizure-related perfusion abnormalities, *n*=9				
		Recent or old vascular lesions-related perfusion abnormalities, *n*=4				
		Perfusion abnormalities unrelated to seizures or ischemia, *n*=10				
Galanopoulou et al., 2020	>Included, *n*=22	>Modality: NA (at least 1 brain MRI)	NA	>Total EEG recordings, *n*=31	>During hospital stay (at time of EEG: NA)	**>Background activity**
USA	M/F, *n*=14/8	Subcortical and mild periventricular WM signal			Sedatives, *n*=14	Bilateral slowing, *n*=22
Multicentric	Mean age: 63 years	hyperintensity, *n*=1		>Types of EEG	AD, *n*=12	Focal slowing, *n*=5
	Anosmia/agueusia: NA	SAH due to aneurysm, *n*=1		10 electrodes/8-channel EEG, *n*=20		Asymmetry, *n*=3
	Intubated, *n*=14	SDH, *n*=1		Routine EEG, *n*=4		Absent PDR, *n*=18
				cEEG, *n*=7		Slow PDR, *n*=4
	Prior neurological history					Discontinuous or burst suppression, *n*=1
	Epilepsy, *n*=4			>Period of recordings: NA		
	Neurological disorders except epilepsy, *n*=7					**>Rhythmic and periodic patterns**
				>EEG indication		Generalized or frontal RDA, *n*=3
				Altered mental status, *n*=20		Temporal LRDA, *n*=1
				Motor seizure-like event or seizure		Bifrontal sharply contoured periodic waves, *n*=1
				at presentation or confusion		
				resembling prior seizures, *n*=12		**>Epileptiform findings and seizures**
				Gaze deviation, *n*=2		Bilateral frontal sharp waves, *n*=6
				Confusion at presentation and no		Unilateral frontal sharp waves, *n*=2
				prior seizures, *n*=1		Temporal or hemispheric sharp waves, *n*=2
						Seizures, *n*=0
Helms et al., 2020a	>Included, *n*=58	>MRI, *n*=13	>CSF examination, *n*=7	>Total EEG recordings, *n*=8	>During hospital stay (at time of EEG: NA)	**>Background activity**
France	M/F: NA	Leptomeningeal enhancement, *n*=8	Normal WBC count, *n*=7		Sufentanil, *n*=58	Nonspecific changes, *n*=8
Bicentric	Median age: 63 years	Acute IS, *n*=2	Elevated protein level, *n*=1	>Types of EEG: NA	Midazolam, *n*=50	Diffuse bifrontal slowing, *n*=1
	Anosmia/agueusia: NA	Subacute IS, *n*=1	Oligoclonal bands with mirror		Propofol, *n*=27	
	Intubated, *n*=58		pattern, *n*=2	>Period of recordings: NA		**>Rhythmic and periodic patterns: NA**
		>Perfusion MRI, *n*=11	Negative SARS-CoV-2 RT-PCR,			
	Prior neurological history	Bilateral frontotemporal hypoperfusion, *n*=11	*n*=7	>EEG indication: NA		**>Epileptiform findings and seizures: NA**
	TIA, partial epilepsy, MCI, *n*=7					

(Continued)

TABLE 1 | Continued

Series	Patients' features	Brain imaging results	CSF results	EEG settings	Ongoing psychoactive drugs	Main EEG results
Helms et al., 2020b	>Included, *n*=140	>MRI, *n*=28	>CSF examination, *n*=25	>Total EEG recordings, *n*=42	>During hospital stay (at time of EEG: NA)	**>Background activity**
France	M/F, *n*=100/40	Subarachnoid spaces FLAIR and T1 contrast	Elevated WBC count, *n*=3		Midazolam, *n*=121	Normal, *n*=5
Bicentric	Median age: 62 years	enhancement, *n*=17	Elevated protein level, *n*=8	>Types of EEG: NA	Sufentanil, *n*=138	Unspecific abnormalities, with low voltage, rapid rhythm, and lack of asymmetry, *n*=26
	Anosmia/agueusia: NA	WM microhemorrhages, *n*=7	Elevated IgG levels, *n*=9		Propofol, *n*=83	
	Intubated, *n*=140	WM FLAIR hyperintensities, *n*=4, with small foci of contrast enhancement, *n*=2 and diffusion	Oligoclonal bands with mirror pattern, *n*=13	>Period of recordings: NA		Diffuse, especially bifrontal, slow activity *n*=11
	Prior neurological history	hyperintensities, *n*=2	Positive SARS-CoV-2 RT-PCR	> EEG indication		**>Rhythmic and periodic patterns: NA**
	Stroke/TIA, *n* = 9	Acute IS, *n*=2	(negative result in blood), *n*=1	Unexplained and persistent altered consciousness		
	Migraine, *n* = 5	Intraparenchymal hematoma, n=1	Negative bacterial cultures and viral	after prolonged sedation discontinuation		**> Epileptiform findings and seizures: NA**
	Mild cognitive alteration, *n*=4	Preexisting IS, n=1	research (HSV 1&2, enterovirus),	(> 3 days)		
	Partial epilepsy, *n*=2		*n* = 25	Multimodality neurological screening in		
	Trauma brain injury, *n*=2	>Perfusion MRI, *n*=26		combination with brain MRI and/or CSF		
	Aneurysm, *n*=1	Perfusion abnormalities, *n*=17		examination		
Louis et al., 2020	>Included, *n*=22	>CT-scan, *n*=18	NA	>Total EEG recordings, *n*=22	>At time of EEG	**>Background activity**
USA	M/F, *n*=14/8	Possible IS, *n*=2			Sedative drugs (including fentanyl, propofol	Continuous generalized polymorphic delta slowing, *n*=19
Monocentric	Mean age: 67 years	Acute IS, *n*=1		>Types of EEG	and/or midazolam), *n*=14	Slow PDR, *n*=9
	Anosmia/agueusia: NA	ICH, *n*=1		cEEG, *n*=19		Absent PDR, *n*=11
	Intubated, *n*=18			Routine EEG, *n*=3		Normal PDR, *n*=2
		>MRI, *n*=1				
	Prior neurological history	Acute IS, *n*=1		>Period of recordings: NA		**>Rhythmic and periodic patterns**
	Epilepsy, *n*=2					GPDs, *n*=7
	Stroke, *n*=1			>EEG indication		GPDs with triphasic morphology, *n*=5
	Headache, *n*=1			Altered mental status, *n*=17		GPDs with sharply contoured morphology, *n*=2
	Traumatic brain injury, *n*=1			Seizure-like event, *n*=5		Intermittent GRDA, *n*=11
	Spinal stenosis, *n*=1					Hemispheric LRDA, *n*=1
						>Epileptiform findings and seizures
						Epileptic abnormalities, *n*=5
						Seizures, *n*=2
Pasini et al., 2020	>Included, *n*=15	>CT-scan, *n*=8	>CSF examination, *n*=5	>Total EEG recordings, *n*=15	NA	*Subset of non post-anoxic patients, n=13*
Italy	M/F, *n*=6/9	Normal, *n*=8	Elevated protein level, *n*=1			**>Background activity**
Monocentric	Mean age: 65 years		Negative SARS-CoV-2 detection, *n*=5	>Types of EEG		Generalized slowing with theta prevalence, *n*=5
	Anosmia/agueusia: NA	>MRI, *n*=6		18 electrodes EEG, *n*=15		Generalized slowing with intrusions of theta/delta activity, *n*=4
	Intubated: NA	Mild WM T2 hyperintensity, *n*=2				Focal slowing predominantly over the frontal or central regions *n*=3
				>Period of recordings: NA		Unreactive, *n*=10
	Prior neurological history					
	Cognitive decline, *n*=2			>EEG indication		**>Rhythmic and periodic patterns**
	Limbic encephalitis, *n*=1			Confusion, *n*=11		FIRDA, *n*=1
	Frontal metastasis, *n*=1			Impairment of consciousness, *n*=4 with post-anoxic coma, *n*=2		**>Epileptiform findings and seizures**
				Aphasia, *n*=1		Epileptiform abnormalities, *n*=0
						Subset of post-anoxic comas, n=2
						Severely suppressed activity, *n*=1
						Discontinued activity compatible with post-anoxic SE, *n*=1
						Unreactive, *n*=2

(Continued)

TABLE 1 | Continued

Series	Patients' features	Brain imaging results	CSF results	EEG settings	Ongoing psychoactive drugs	Main EEG results
Pellinen et al., 2020	>Included, *n*=111	>Brain imaging, *n*=90 (with CT-scan only, *n*=75)	NA	>Total EEG recordings, *n*=118	>During EEG	**>Background activity**
USA	M/F, *n*=79/32	Acute IS, *n*=18			Sedative drugs (including propofol,	Normal, *n*=5
Multicentric	Median age: 64 years	Acute ICH, *n*=15		>Types of EEG	midazolam, pentobarbital,	Mild generalized slowing, *n*=17
	Anosmia/agueusia: NA	Cerebral edema, *n*=6		21-channel cEEG for a target of at least 24	dexmedetomidine and/or fentanyl)	Moderate generalized slowing, *n*=60
	Intubated, *n*=79	Diffuse leukoencephalopathy with		hours, *n*=111	*n*=67	Severe generalized slowing/discontinuous/ECI, *n*=29
		microhemorrhages, *n*=4		Rapid EEG system with 8-bipolar channel		Focal slowing, *n*=27
	Prior neurological history	Mixed acute ischemic and hemorrhagic lesions,		montage 0.5-12 hours, *n*=7	>Prior to EEG	
	Stroke, *n*=23	*n*=3			AD, *n*=57	**>Rhythmic and periodic patterns**
	Epilepsy, *n*=13			>Period of recordings: NA		GRDA, *n*=4
	ICH, *n*=4					LRDA, *n*=7
	Dementia, *n*=4			>EEG indication		LRDA and GRDA, *n*=2
	Developmental delay/intellectual			Persistent encephalopathy, *n*=72		GPDs, *n*=11
	disability, *n*=3			Paroxysmal activity of unclear cause, *n*=25		LPDs, *n*=3
	Brain tumor, *n*=3			Seizure exacerbation, *n*=10		
	Traumatic brain injury, *n*=2			Cardiac arrest *n*=11		**>Epileptiform findings and seizures**
	Parkinson disease, *n*=2					Focal epileptiform discharges, *n*=12
	Vascular malformation, *n*=1					Multifocal epileptiform discharges, *n*=6
	Tuberous sclerosis complex, *n*=1					Generalized epileptiform discharges, *n*=5
	Herpes encephalitis, *n*=1					Seizures, *n*=8
						NCSE, *n*=2
Petrescu et al., 2020	>Included, *n*=36	>CT-scan, *n*=14	>CSF examination, *n*=4	>Total EEG recordings, *n*=40	>At time of EEG	**>Background activity**
France	M/F, *n*=26/10	Normal, *n*=4	Normal, *n*=4		Levetiracetam, *n*=6	Normal, *n*=4
Monocentric	Mean age: 70 years	Atrophy, *n*=9		>Types of EEG	Sedations, *n*=5	Mildly altered, *n*=19
	Anosmia/agueusia: NA	IS, *n*=2		Routine 20 min EEG, *n*=40	Risperidone, *n*=4	Moderately altered, *n*=4
	Intubated, *n*=11	Calcification, *n*=2			Clobazam, *n*=2	Severely altered, *n*=8
		SDH, *n*=1		>Period of recordings: NA	Dexmedetomidine, *n*=2	Critically altered, *n*=5
	Prior neurological history	Leukoaraiosis, *n*=1			Citalopram or escitalopram, *n*=2	Focal bioccipital slowing, *n*=1
	Dementia, *n*=10	Meningioma, *n*=1		>EEG indication	Midazolam, *n*=2	Sporadic triphasic waves, *n*=1
	Stroke, *n*=3	Postoperative lesion, *n*=1		Fluctuating alertness, *n*=13	Oxazepam, *n*=2	
	SDH, *n*=2			Confusion, *n*=9	Morphine, *n*=2	**>Rhythmic and periodic patterns**
	Memory impairment, *n*=1	>MRI, *n*=11		Delayed awakening after stopping	Oxazepine, *n*=1	RDA, *n*=7 with frontal predominant, *n*=1
	Hydrocephalus, *n*=1	Atrophy, *n*=4		sedation or inadequate emerge of	Haloperidol, *n*=1	GPDs, *n*=6
	Epilepsy, *n*=1	IS, *n*=2		sedation, *n*=8	Doxylamine succinate, *n*=1	Multifocal PDs, *n*=2
	Parkinson disease, *n*=1	SDH, *n*=2		Focal neurologic symptoms, *n*=6	Lacosamide, *n*=1	
		Gliosis of CC, *n*=1		Seizures, *n*=3	Diazepam, *n*=1	**>Epileptiform findings and seizures**
		Leukoaraiosis, *n*=1		Abnormal movements, *n*=3	Valproate, *n*=1	Epileptiform discharges, *n*=0
		Leptomeningeal enhancement, *n*=1		Cardiac arrest, *n*=1	Bromazepam, *n*=1	Seizures, *n*=0
		Probable septic lesions (multiple ischemic and		Encephalopathy, *n*=1	Gabapentin, *n*=1	
		hemorrhagic lesions) related to endocarditis, *n*=1		Control follow-up, *n*=1	Paroxetine, *n*=1	
		Multiple FLAIR hyperintense lesions, *n*=1			Alprazolam, *n*=1	
					Hydroxyzine, *n*=1	
					Mianserine, *n*=1	

(Continued)

TABLE 1 | Continued

Series	Patients' features	Brain imaging results	CSF results	EEG settings	Ongoing psychoactive drugs	Main EEG results
Pilotto et al., 2020a	>Included patients, *n*=25	>MRI, *n*=25	>CSF examination, *n*=25	>Total EEG recordings, *n*=25	NA	>**Background activity**
Italy	M/F, *n*=15/10	Normal, *n*=13	Normal, *n*=8			Generalized slowing especially localized to frontal derivations, *n*=16
Multicentric	Mean age: 66 years	Multiple subcortical T2-hyperintensities, *n*=4	Elevated WBC count, *n*=9	>Types of EEG: NA		
	Anosmia/agueusia: NA	Focal cortical T2 and DWI hyperintensities, *n*=3	Elevated protein level, *n*=15			>**Rhythmic and periodic patterns: NA**
	Intubated, *n*=4	Acute necrotizing encephalopathy, *n*=2	Negative bacteriological and	>Period of recordings: NA		
		Limbic encephalitis, *n*=2	virological screening, *n*=25			>**Epileptiform findings and seizures**
	Prior neurological history	ADEM, *n*=1	Negative SARS-CoV-2 RT-PCR,	>EEG indication		Focal epileptic alterations, *n*=6
	Stroke, *n*=2	Leptomeningeal enhancement, *n*=1	*n*=14	Delirium/altered mental status, *n*=17		
	Mental retardation, *n*=1			Aphasia/dysarthria, *n*=6		
	Possible encephalitis and Behçet disease, *n*=1			Seizures, *n*=6		
Scullen et al., 2020	>Included patients, *n*=27	>CT-scan, *n*=27	NA	>Total EEG recordings, *n*=13	NA	>**Background activity**
USA	M/F, *n*=14/13	Focal hypodensities in deep structures, *n*=14				Generalized encephalopathy (i.e. irregular slowing with delta and
Monocentric	Mean age: 60 years	Diffuse hypoattenuation, *n*=6		>Types of EEG		theta frequency oscillations), *n*=11
	Anosmia/agueusia, *n*=1	Subacute IS, *n*=4		cEEG, *n*=13		
	Intubated: NA	Subcortical parenchymal hematoma, *n*=3				>**Rhythmic and periodic patterns: NA**
				>Period of recordings: NA		
						>**Epileptiform findings and seizures**
	Prior neurological history	>MRI, *n*=8		>EEG indication		
	Stroke, *n*=3	Viral encephalitis with diffuse involvement of the		Pronounced encephalopathy not explained		NCSE, *n*=1
	Pseudotumor cerebri, *n*=1	deep WM, CC and basal ganglia, *n*=NA		by previous CT alone, *n*=9		
				Pronounced encephalopathy not explained by previous combined CT and MRI, *n*=4		
Sethi, 2020	NA	NA	NA	>Total EEG recordings, *n*=20	NA	>**Background activity**
USA						Diffuse theta and delta slowing
Monocentric				>Types of EEG: NA		
						>**Rhythmic and periodic patterns: NA**
				>Period of recordings: NA		
						>**Epileptiform findings and seizures: NA**
				>EEG indication		
				Altered mental status		
Vespignani et al., 2020	>Included patients, *n*=26	>CT-scan, *n*=1	>CSF examination, *n*=2	>Total EEG recordings, *n*=26	*Subset of the 5 patients with PDs*	>**Background activity**
France		Occipital cyst, *n*=1	Normal, *n*=2		>At time of EEG	Diffuse slowing without PDs, *n*=19
Multicentric	*Subset of patients with PDs, n=5*			>Types of EEG	Propofol, *n*=2	Isoelectric, *n*=2
	M/F, *n*=4/1	>MRI, *n*=1		9 electrodes 30 min EEG, *n*=26	Fentanyl, *n*=2	
	Mean age: 67 years	Diffuse WM hyperintensities, *n*=1			Midazolam, *n*=1	>**Rhythmic and periodic patterns**
	Anosmia/agueusia: NA			>Period of recordings: NA		GPDs with frontal involvement, *n*=4
	Intubated, *n*=4					LPDs with frontal involvement, *n*=1
	Prior neurological history: NA			>EEG indication		
				Mental status changes		>**Epileptiform findings and seizures, *n*=0**
				Poor responsiveness		
				Determine the presence of SE in non-arousable patients		
				Subset of the 5 patients with PDs		
				Poor or absent responsiveness, *n*=4		
				Cardiac arrest, *n*=1		
				Confusion and lethargy, *n*=1		

AD: antiepileptic drug, ADEM: acute disseminated encephalomyelitis, CC: corpus callosum, cEEG: continuous EEG, CMV: cytomegalovirus, CSF: cerebrospinal fluid, CT: computed tomography, DLB: dementia with Lewy bodies, DWI: diffusion weighted imaging, EBV: Epstein-Barr virus, ECI: electrocerebral inactivity, EEG: electroencephalogram, FIRDA: frontal intermittent rhythmic delta activity, FLAIR: fluid-attenuated inversion recovery, GPDs: generalized periodic discharges, GRDA: generalized rhythmic delta activity, HSV: herpes simplex virus, ICH: intracranial hemorrhage, IS: ischemic stroke, LRDA: lateralized rhythmic delta activity, MCI: mild cognitive impairment, M/F: male/female, n: number, MRI: magnetic resonance imaging, NA: not available, NCSE: non convulsive SE, PCR: polymerase chain reaction, PDR: posterior dominant rhythm, PDs: periodic discharges, PRES: posterior reversible encephalopathy syndrome, RDA: rhythmic delta activity, RT-PCR: reverse transcriptase PCR, SAH: subarachnoid hemorrhage, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, SDH: subdural hematoma, SE: status epilepticus, SIRPIDs: stimulus-induced rhythmic, periodic or ictal discharges, TIA: transient ischemic attack, VZV: varicella-zoster virus, WBC: white blood cells, WM: white matter.

for the second one, left more than right frontotemporal seizures which progressed to focal SE. The EEG of the patient reported by Le Guennec et al. (2020) revealed a non-convulsive SE (NCSE) over the right frontal region. The brain MRI of this patient only showed peri-ictal diffusion abnormalities over the right orbital and mesial prefrontal cortex and right caudate nucleus. Flamand et al. (2020) reported the case of a patient who benefited from several EEG. The first two EEG findings were consistent with a bilateral frontal SE. One EEG in the series of five patients reported by Chen et al. (2020) showed a bifrontal SE, and another one revealed a generalized NCSE. Finally, the EEG of the patient reported by Rodrigo-Armenteros et al. (2020) showed a bilateral frontotemporal NCSE.

Seizures and/or SE were recorded more rarely in patients with acute CNS lesions on brain imaging and/or significant CSF abnormalities, of either vascular or inflammatory origin. Among the four patients with a PRES reported by Parauda et al. (2020), two had seizures or SE emanating from posterior regions: for the first one, a focal NCSE arising from the left posterior quadrant and, for the second one, focal seizures arising from the right posterior quadrant. The history of a 2-month-old boy was published by Schupper et al. (2020). His brain imaging revealed multiple infarctions with hemorrhagic transformations, and his cEEG showed NCSE. Zanin et al. (2020) published the case of a patient with diffuse CNS demyelinating lesions on brain and spine imaging whose EEG revealed two seizures starting from the right frontotemporal region and diffusing in the homologous contralateral hemisphere. Hussein et al. (2020) reported the case of a patient with an ADEM whose EEG revealed left hemispheric seizures and, 3 days later, brief focal right posterior seizures. Finally, Bernard-Valnet et al. (2020) reported the history of a patient with a lymphocytic meningitis on CSF analysis with normal brain MRI whose EEG showed a focal anterior NCSE.

Seizures and/or SE were recorded in patients with a prior neurological history and radiological sequelae but without any acute lesions. The EEG of the second patient, who had a prior history of skull base surgery, reported by Somani et al. (2020) showed recurrent seizures emanating from either right or left frontocentroparietal regions. Vollono et al. (2020) reported the case of a left frontocentrotemporal SE in a patient with a remote *herpes simplex virus* 1 encephalitis.

Seizures were reported on cEEG in the series of 33 patients published by Radmard et al. (2020), as frontotemporal and parasagittal seizures in two patients, but without precise imaging or CSF results available for these two patients.

Rhythmic and Periodic Discharges

Rhythmic discharges were mentioned in series, as generalized rhythmic delta activity (GRDA) (Ayub et al., 2020; Galanopoulou et al., 2020; Louis et al., 2020; Pellinen et al., 2020; Petrescu et al., 2020), lateralized rhythmic delta activity (LRDA) (Ayub et al., 2020; Galanopoulou et al., 2020; Louis et al., 2020; Pellinen et al., 2020), and frontal intermittent rhythmic delta activity (Canham et al., 2020; Pasini et al., 2020) (**Table 1**). Rhythmic patterns were also reported in a few case reports. Vandervorst et al. (2020) published the EEG of a patient with a clinical and radiological picture of encephalitis with temporal bilateral more left than right imaging abnormalities. The EEG showed short-lasting left temporal LRDA. In the series of Beach et al. (2020), one patient, with a previous history of dementia with Lewy bodies and remote traumatic brain injury, displayed GRDA with sharp contouring and bifrontal predominance, without any acute lesions on brain imaging. The EEG of the three other patients reported in the series of Chen et al. (2020) previously mentioned revealed GRDA, with unremarkable CSF analysis for the three and no acute lesions on brain imaging for one of them (unavailable for the two others). One EEG recorded among the seven patients reported by Anand et al. (2020) showed GRDA in a patient with extensive leukoencephalopathy on brain MRI and normal CSF sample.

Periodic discharges were noted in series, as generalized periodic discharges (GPDs) (Ayub et al., 2020; Galanopoulou et al., 2020; Louis et al., 2020; Pellinen et al., 2020; Petrescu et al., 2020; Vespignani et al., 2020) and lateralized periodic discharges (LPDs) (Pellinen et al., 2020; Petrescu et al., 2020; Vespignani et al., 2020) (**Table 1**). Especially, in the series of Vespignani et al. (2020), five EEGs out of 26 showed periodic discharges. Four of these five patients were under mechanical ventilation (MV), and three were sedated. One patient suffered from a cardiac arrest. EEG showed periodic (with a < 4 s interval), monomorphic biphasic, delta activity, which was diffuse with frontal predominance for four and lateralized over right frontal area for one. The second patient reported in the work of Beach al. previously mentioned presented with a left-sided acute-on-chronic subdural hematoma (SDH) due to a fall with head trauma. The EEG showed frequent runs of epileptiform GPDs (Beach et al., 2020). Young et al. (2020) reported 1–1.5 Hz LPDs and diffuse delta–theta slowing in a patient who displayed Creutzfeldt–Jakob disease in tandem with symptomatic onset of COVID-19. Conte et al. (2020) published the history of a patient who presented a severe COVID-19 pneumonia and then a PRES-like encephalopathy. She displayed focal seizures, and after seizure treatment, EEG revealed LPDs in the right posterior regions. Vellieux et al. (2020) published the EEG of two critically ill patients who displayed a severe COVID-19 pneumonia requiring MV. For the first one, the brain MRI was consistent with a hypoxic encephalopathy, and the EEG was recorded while he was sedated and under extracorporeal membrane oxygenation. For the second one, the EEG was recorded 24 h after sedation discontinuation. EEG revealed continuous, symmetric, non-reactive, generalized but mainly bifrontal, monomorphic diphasic or even triphasic, periodic (with a short interval of 1–2 s) delta slow waves (**Figure 1C**). One patient, without any acute abnormalities on brain MRI and with normal CSF analysis, reported by Delorme et al. (2020) showed GPDs. In the previously mentioned case reported by Le Guennec et al. (2020), a control follow-up EEG was recorded the day after the first EEG. It showed persistent right frontal LPDs with a short interval (0.7–1.2 s). The brain MRI performed 1 month later was normal. Finally, the previously mentioned patient reported by Flamand et al. (2020) who benefited from iterative EEG showed, on the last two recordings, a generalized periodic triphasic activity with short periods (1–1.5 s) over a worsened background activity, without concomitant metabolic disorders.

Spectral Analysis

Two studies reported quantitative analysis of EEG (qEEG) in COVID-19 patients. The study of Pastor et al. (2020) reported 20 patients with COVID-19 encephalopathy for whom standard visual analysis of EEG showed scarce abnormalities. However, compared to 31 infectious toxic encephalopathy patients and 21 post-cardiorespiratory arrest encephalopathy patients, some qEEG features were specific in COVID-19 patients, such as the distribution of EEG bands, the structure of Shannon's spectral entropy, and the hemispheric connectivity. Finally, the study of Pati et al. (2020) showed that some qEEG markers, especially an increase in both the theta power and its temporal variance during EEG reactivity, can predict a good neurological outcome in 10 critically ill COVID-19 patients.

DISCUSSION

The vast majority of these studies emphasized the absence of specificity of EEG abnormalities reported in COVID-19 patients, as generalized slowing of the background activity, focal slowing sometimes associated with sharp waves, seizures, SE, and predictable pattern of metabolic/toxic or postanoxic encephalopathy in ICU patients. Numerous EEGs in the context of COVID-19 were recorded in elderly patients and mainly in male patients, with multiple comorbidities especially chronic brain disorders, under various psychotropic drugs or in critically ill conditions. Confounding factors such as infections, metabolic disturbances, severe hypoxemia, hyperthermia, and psychotropic drugs (such as antiepileptic or sedative drugs) were frequent at the time of EEG recordings. All these confounding factors may contribute to the modification of brain activity and therefore EEG findings. Thus, based on the current literature, it seems not possible to identify a specific EEG pattern due to the suspected neuroinvasion of SARS-CoV-2 in patients who displayed neurological manifestations of COVID-19.

Most current studies with available EEG data are case reports or retrospective single-center series. All reported patients are very heterogeneous concerning prior neurological histories, illness severity, and use of psychotropic drugs. Moreover, some studies reported EEG recorded with limited montage and number of electrodes that may limit the detection of EEG abnormalities. EEG is not a systematic exam in the diagnostic workup of COVID-19 patients. All patients reported in the current literature had an EEG for an urgent clinical indication due to concerning neurological symptoms. A wider neurological multimodality screening, including EEG, of COVID-19 patients may be suggested to grow the body of knowledge on the SARS-CoV-2 infection. However, it will face many logistic difficulties and ethical and safety concerns regarding the availability of trained personnel to EEG recordings and the risk of contamination with the SARS-CoV-2.

It should be pointed out that many EEG abnormalities reported were recorded over anterior or frontal regions. Regardless of EEG montage used by clinicians and neurophysiologists, it thus seems essential to include frontal electrodes. Periodicity, morphology, and reactivity of these frontal abnormalities were not mentioned in all studies. Moreover, a few reported periodic patterns, as GPDs (Ayub et al., 2020; Beach et al., 2020; Delorme et al., 2020; Louis et al., 2020; Pellinen et al., 2020; Petrescu et al., 2020), GPDs with bifrontal predominance (Galanopoulou et al., 2020; Vellieux et al., 2020; Vespignani et al., 2020), and LPDs (Conte et al., 2020; Le Guennec et al., 2020; Pellinen et al., 2020; Petrescu et al., 2020; Vespignani et al., 2020; Young et al., 2020). In particular, these frontal periodic discharges were monomorphic and displayed a short interval, and the absence of reactivity was noted (Vellieux et al., 2020; Vespignani et al., 2020). These frontal periodic discharges may indicate an acute neurological process linked to the brain SARS-CoV-2 infection. In COVID-19 patients, the combination of the frontal localization of these EEG discharges, the frequently reported anosmia (Yazdanpanah et al., 2020), the olfactory bulb abnormalities found on brain imaging (Lin et al., 2020), and the hypometabolism within the orbitofrontal cortex on functional brain imaging (Karimi-Galougahi et al., 2020) may support the hypothesis whereby SARS-CoV-2 could invade the brain through the olfactory pathway. Then, it could spread transneuronally to other related brain areas particularly to frontal lobes, especially the orbital prefrontal cortex, which are adjacent to olfactory structures (Huang J. et al., 2020).

CONCLUSION

In the context of the SARS-CoV-2 infection, increasing EEG results were published along with clinical reports describing various neurological symptoms in patients with COVID-19. Due to the suspected neuroinvasion of SARS-CoV-2, the major issue when interpreting EEG is to determine whether the observed abnormalities reflect this viral neuroinvasion, a severe encephalopathy with systemic and brain inflammation, hypoxemia and hyperthermia, and/or many confounding factors especially due to critical illness. At this time, no study had described specific EEG abnormalities of the SARS-CoV-2 infection. The majority of currently reported EEGs showed generalized slowing, focal slowing, epileptiform discharges with seizures, and SE. However, frontal discharges, for some periodic, may integrate in the olfactory hypothesis of the CNS invasion of SARS-CoV-2. It reinforces the need to accumulate precise neurophysiological observations of COVID-19 patients worldwide and to aggregate multimodality screening of these patients also with clinical, radiological, biological, and neuropathological data.

AUTHOR CONTRIBUTIONS

GV collected the data and wrote the manuscript. RS, SV, PJ, and AR-T revised the manuscript. M-PO suggested and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING STATEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

Abdel-Mannan, O., Eyre, M., Löbel, U., Bamford, A., Eltze, C., Hameed, B., et al. (2020). Neurologic and radiographic findings associated with COVID-19 infection in children. *JAMA Neurol.* 77:e202687. doi: 10.1001/jamaneurol.2020.2687

Abel, D., Shen, M. Y., Abid, Z., Hennigan, C., Boneparth, A., Miller, E. H., et al. (2020). Encephalopathy and bilateral thalamic lesions in a child with MIS-C associated with COVID-19. *Neurology* 95, 745–748. doi: 10.1212/WNL.0000000000010652

Abenza-Abildúa, M. J., Novo-Aparicio, S., Moreno-Zabaleta, R., Algarra-Lucas, M. C., Rojo Moreno-Arcones, B., Salvador-Maya, M. Á, et al. (2020). Encephalopathy in severe SARS-CoV2 infection: inflammatory or infectious? *Int. J. Infect. Dis.* 98, 398–400. doi: 10.1016/j.ijid.2020.07.020

Anand, P., Al-Faraj, A., Sader, E., Dashkoff, J., Abdennadher, M., Murugesan, R., et al. (2020). Seizure as the presenting symptom of COVID-19: a retrospective case series. *Epilepsy Behav.* 112:107335. doi: 10.1016/j.yebeh.2020.107335

Andriuta, D., Roger, P.-A., Thibault, W., Toublanc, B., Sauzay, C., Castelain, S., et al. (2020). COVID-19 encephalopathy: detection of antibodies against SARS-CoV-2 in CSF. *J. Neurol.* 11, 1–2. doi: 10.1007/s00415-020-09975-1

Ashraf, M., and Sajed, S. (2020). Seizures related to coronavirus disease (COVID-19): case series and literature review. *Cureus* 12:e9378. doi: 10.7759/cureus.9378

Ayub, N., Cohen, J., Jing, J., Jain, A., Tesh, R., Mukerji, S. S., et al. (2020). Clinical electroencephalography findings and considerations in hospitalized patients with coronavirus SARS-CoV-2. *medRxiv* [Preprint]. doi: 10.1101/2020.07.13.20152207

Balloy, G., Leclair-Visonneau, L., Péréon, Y., Magot, A., Peyre, A., Mahé, P.-J., et al. (2020). Non-lesional status epilepticus in a patient with coronavirus disease 2019. *Clin. Neurophysiol.* 131, 2059–2061. doi: 10.1016/j.clinph.2020.05.005

Beach, S. R., Praschan, N. C., Hogan, C., Dotson, S., Merideth, F., Kontos, N., et al. (2020). Delirium in COVID-19: a case series and exploration of potential mechanisms for central nervous system involvement. *Gen. Hosp. Psychiatry* 65, 47–53. doi: 10.1016/j.genhosppsych.2020.05.008

Benameur, K., Agarwal, A., Auld, S. C., Butters, M. P., Webster, A. S., Ozturk, T., et al. (2020). Encephalopathy and Encephalitis Associated with Cerebrospinal Fluid Cytokine Alterations and Coronavirus Disease, Atlanta, Georgia, USA, 2020. *Emerg. Infect. Dis.* 26, 2016–2021. doi: 10.3201/eid2609.202122

Bernard-Valnet, R., Pizzarotti, B., Anichini, A., Demars, Y., Russo, E., Schmidhauser, M., et al. (2020). Two patients with acute meningoencephalitis concomitant with SARS-CoV-2 infection. *Eur. J. Neurol.* doi: 10.1111/ene.14298 [Epub ahead of print].

Brun, G., Hak, J.-F., Coze, S., Kaphan, E., Carvelli, J., Girard, N., et al. (2020). COVID-19-White matter and globus pallidum lesions: demyelination or small-vessel vasculitis? *Neurol. Neuroimmunol. Neuroinflamm.* 7:e777. doi: 10.1212/NXI.0000000000000777

Canham, L. J. W., Staniaszek, L. E., Mortimer, A. M., Nouri, L. F., and Kane, N. M. (2020). Electroencephalographic (EEG) features of encephalopathy in the setting of Covid-19: a case series. *Clin Neurophysiol Pract.* 5, 199–205. doi: 10.1016/j.cnp.2020.06.001

Cecchetti, G., Vabanesi, M., Chieffo, R., Fanelli, G., Minicucci, F., Agosta, F., et al. (2020). Cerebral involvement in COVID-19 is associated with metabolic and coagulation derangements: an EEG study. *J. Neurol.* doi: 10.1007/s00415-020-09958-2 [Epub ahead of print].

Chaumont, H., San-Galli, A., Martino, F., Couratier, C., Joguet, G., Carles, M., et al. (2020). Mixed central and peripheral nervous system disorders in severe SARS-CoV-2 infection. *J. Neurol.* 267, 3121–3127. doi: 10.1007/s00415-020-09986-y

Chen, W., Toprani, S., Werbaneth, K., and Falco-Walter, J. (2020). Status epilepticus and other EEG findings in patients with COVID-19: a case series. *Seizure* 81, 198–200. doi: 10.1016/j.seizure.2020.08.022

Chougar, L., Shor, N., Weiss, N., Galanaud, D., Leclercq, D., Mathon, B., et al. (2020). Retrospective observational study of brain magnetic resonance imaging findings in patients with acute SARS-CoV-2 infection and neurological manifestations. *Radiology* 297:202422. doi: 10.1148/radiol.2020202422

Conte, G., Avignone, S., Carbonara, M., Meneri, M., Ortolano, F., Cinnante, C., et al. (2020). COVID-19-associated PRES-like encephalopathy with perivascular gadolinium enhancement. *AJNR Am. J. Neuroradiol.* 41, 2206–2208. doi: 10.3174/ajnr.A6762

De Stefano, P., Nencha, U., De Stefano, L., Mégevand, P., and Seeck, M. (2020). Focal EEG changes indicating critical illness associated cerebral microbleeds in a Covid-19 patient. *Clin. Neurophysiol. Pract.* 5, 125–129. doi: 10.1016/j.cnp.2020.05.004

Delamarre, L., Gollion, C., Grouteau, G., Rousset, D., Jimena, G., Roustan, J., et al. (2020). COVID-19–associated acute necrotising encephalopathy successfully treated with steroids and polyvalent immunoglobulin with unusual IgG targeting the cerebral fibre network. *J. Neurol. Neurosurg. Psychiatry* 91, 1004–1006. doi: 10.1136/jnnp-2020-323678

Delorme, C., Paccoud, O., Kas, A., Hesters, A., Bombois, S., Shambrook, P., et al. (2020). Covid-19-related encephalopathy: a case series with brain FDG-PET/CT findings. *Eur. J. Neurol.* 27, 2651–2657. doi: 10.1111/ene.14478

Díaz-Pérez, C., Ramos, C., López-Cruz, A., Muñoz Olmedo, J., Lázaro González, J., De Vega-Ríos, E., et al. (2020). Acutely altered mental status as the main clinical presentation of multiple strokes in critically ill patients with COVID-19. *Neurol. Sci.* 41, 2681–2684. doi: 10.1007/s10072-020-04679-w

Dixon, L., Coughlan, C., Karunaratne, K., Gorgoraptis, N., Varley, J., Husselbee, J., et al. (2020). Immunosuppression for intracranial vasculitis associated with SARS-CoV-2: therapeutic implications for COVID-19 cerebrovascular pathology. *J. Neurol. Neurosurg. Psychiatry* doi: 10.1136/jnnp-2020-324291 [Epub ahead of print].

Dugue, R., Cay-Martínez, K. C., Thakur, K. T., Garcia, J. A., Chauhan, L. V., Williams, S. H., et al. (2020). Neurologic manifestations in an infant with COVID-19. *Neurology* 94, 1100–1102. doi: 10.1212/WNL.0000000000009653

Duong, L., Xu, P., and Liu, A. (2020). Meningoencephalitis without respiratory failure in a young female patient with COVID-19 infection in Downtown Los Angeles, early April 2020. *Brain Behav. Immun.* 87:33. doi: 10.1016/j.bbi.2020.04.024

Edén, A., Kanberg, N., Gostner, J., Fuchs, D., Hagberg, L., Andersson, L.-M., et al. (2020). CSF biomarkers in patients with COVID-19 and neurological symptoms: a case series. *Neurology* 96, e294–e300. doi: 10.1212/WNL.0000000000010977

Ekstrand, J. J., Herbener, A., Rawlings, J., Turney, B., Ampofo, K., Korgenski, E. K., et al. (2010). Heightened neurologic complications in children with pandemic H1N1 influenza. *Ann. Neurol.* 68, 762–766. doi: 10.1002/ana.22184

Elgamasy, S., Kamel, M. G., Ghozy, S., Khalil, A., Morra, M. E., and Islam, S. M. S. (2020). First case of focal epilepsy associated with SARS-coronavirus-2. *J. Med. Virol.* 92, 2238–2242. doi: doi:10.1002/jmv.26113

Ellul, M. A., Benjamin, L., Singh, B., Lant, S., Michael, B. D., Easton, A., et al. (2020). Neurological associations of COVID-19. *Lancet Neurol.* 19, 767–783. doi: 10.1016/S1474-4422(20)30221-0

El-Zein, R. S., Cardinali, S., Murphy, C., and Keeling, T. (2020). COVID-19-associated meningoencephalitis treated with intravenous immunoglobulin. *BMJ Case Rep.* 13:e237364. doi: 10.1136/bcr-2020-237364

Emami, A., Fadakar, N., Akbari, A., Lotfi, M., Farazdaghi, M., Javanmardi, F., et al. (2020). Seizure in patients with COVID-19. *Neurol. Sci.* doi: 10.1007/s10072-020-04731-9 [Epub ahead of print].

Espinosa, P. S., Rizvi, Z., Sharma, P., Hindi, F., and Filatov, A. (2020). Neurological complications of coronavirus disease (COVID-19): encephalopathy, mri brain and cerebrospinal fluid findings: case 2. *Cureus* 12:e7930. doi: 10.7759/cureus.7930

Farhadian, S., Glick, L. R., Vogels, C. B. F., Thomas, J., Chiarella, J., Casanovas-Massana, A., et al. (2020). Acute encephalopathy with elevated CSF inflammatory markers as the initial presentation of COVID-19. *BMC Neurol.* 20:248. doi: 10.1186/s12883-020-01812-2

Farley, M., and Zuberi, J. (2020). COVID-19 precipitating status epilepticus in a pediatric patient. *Am. J. Case Rep.* 21:e925776. doi: 10.12659/AJCR.925776

Fasano, A., Cavallieri, F., Canali, E., and Valzania, F. (2020). First motor seizure as presenting symptom of SARS-CoV-2 infection. *Neurol. Sci.* 41, 1651–1653. doi: 10.1007/s10072-020-04460-z

Filatov, A., Sharma, P., Hindi, F., and Espinosa, P. S. (2020). Neurological complications of coronavirus disease (COVID-19): encephalopathy. *Cureus* 12:e7352. doi: 10.7759/cureus.7352

Fischer, D., Threlkeld, Z. D., Bodien, Y. G., Kirsch, J. E., Huang, S. Y., Schaefer, P. W., et al. (2020). Intact brain network function in an unresponsive patient with COVID -19. *Ann. Neurol.* 88, 851–854.

Flamand, M., Perron, A., Buron, Y., and Szurhaj, W. (2020). Pay more attention to EEG in COVID-19 pandemic. *Clin. Neurophysiol.* 131, 2062–2064. doi: 10.1016/j.clinph.2020.05.011

Freij, B. J., Gebara, B. M., Tariq, R., Wang, A.-M., Gibson, J., El-Wiher, N., et al. (2020). Fatal central nervous system co-infection with SARS-CoV-2 and tuberculosis in a healthy child. *BMC Pediatr.* 20:429. doi: 10.1186/s12887-020-02308-1

Galanopoulou, A. S., Ferastraoaru, V., Correa, D. J., Cherian, K., Duberstein, S., Gursky, J., et al. (2020). EEG findings in acutely ill patients investigated for SARS-CoV-2/COVID-19: a small case series preliminary report. *Epilepsia Open* 5, 314–324. doi: 10.1002/epi4.12399

García-Howard, M., Herranz-Aguirre, M., Moreno-Galarraga, L., Urretavizcaya-Martínez, M., Alegría-Echauri, J., Gorría-Redondo, N., et al. (2020). Case report: benign infantile seizures temporally associated with COVID-19. *Front. Pediatr.* 8:507. doi: 10.3389/fped.2020.00507

Gaughan, M., Connolly, S., Direkze, S., and Kinsella, J. A. (2020). Acute new-onset symptomatic seizures in the context of mild COVID-19 infection. *J. Neurol.* doi: 10.1007/s00415-020-10214-w [Epub ahead of print].

Grimaldi, S., Lagarde, S., Harle, J.-R., Boucraut, J., and Guedj, E. (2020). Autoimmune encephalitis concomitant with SARS-CoV-2 infection: insight from 18F-FDG PET imaging and neuronal autoantibodies. *J. Nucl. Med.* 61, 1726–1729. doi: 10.2967/jnumed.120.249292

Gupta, A., Madhavan, M. V., Sehgal, K., Nair, N., Mahajan, S., Sehrawat, T. S., et al. (2020). Extrapulmonary manifestations of COVID-19. *Nat. Med.* 26, 1017–1032. doi: 10.1038/s41591-020-0968-3

Haddad, S., Tayyar, R., Risch, L., Churchill, G., Fares, E., Choe, M., et al. (2020). Encephalopathy and seizure activity in a COVID-19 well controlled HIV patient. *IDCases* 21:e00814. doi: 10.1016/j.idcr.2020.e00814

Handa, R., Nanda, S., Prasad, A., Anand, R., Zutshi, D., Dass, S. K., et al. (2020). Covid-19-associated acute haemorrhagic leukoencephalomyelitis. *Neurol. Sci.* doi: 10.1007/s10072-020-04703-z [Epub ahead of print].

Harrogate, S., Mortimer, A., Burrows, L., Fiddes, B., Thomas, I., and Rice, C. M. (2020). Non-aneurysmal subarachnoid haemorrhage in COVID-19. *Neuroradiology* doi: 10.1007/s00234-020-02535-4 [Epub ahead of print].

Helms, J., Kremer, S., Merdji, H., Clere-Jehl, R., Schenck, M., Kummerlen, C., et al. (2020a). Neurologic Features in Severe SARS-CoV-2 Infection. *N. Engl. J. Med.* 382, 2268–2270. doi: 10.1056/NEJMc2008597

Helms, J., Kremer, S., Merdji, H., Schenck, M., Severac, F., Clere-Jehl, R., et al. (2020b). Delirium and encephalopathy in severe COVID-19: a cohort analysis of ICU patients. *Crit. Care* 24:491. doi: 10.1186/s13054-020-03200-1

Hepburn, M., Mullaguri, N., George, P., Hantus, S., Punia, V., Bhimraj, A., et al. (2020). Acute symptomatic seizures in critically ill patients with COVID-19: is there an association? *Neurocrit. Care* doi: 10.1007/s12028-020-01006-1 [Epub ahead of print].

Huang, H., Eichelberger, H., Chan, M., Valdes, E., Kister, I., Krupp, L., et al. (2020). Pearls and Oy-sters: leukoencephalopathy in critically ill COVID-19 patients. *Neurology* 95, 753–757. doi: 10.1212/WNL.0000000000010636

Huang, J., Zheng, M., Tang, X., Chen, Y., Tong, A., and Zhou, L. (2020). Potential of SARS-CoV-2 to cause CNS infection: biologic fundamental and clinical experience. *Front. Neurol.* 11:659. doi: 10.3389/fneur.2020.00659

Hussein, O., Abd Elazim, A., and Torbey, M. T. (2020). Covid-19 systemic infection exacerbates pre-existing acute disseminated encephalomyelitis (ADEM). *J. Neuroimmunol.* 349:577405. doi: 10.1016/j.jneuroim.2020.577405

Ibrahim, F., and Haddad, N. (2014). New onset refractory status epilepticus in a young man with H1N1 infection. *Case Rep. Neurol. Med.* 2014:585428. doi: 10.1155/2014/585428

Jang, K., Khatri, A., and Majure, D. T. (2020). COVID-19 leading to acute encephalopathy in a patient with heart transplant. *J. Heart Lung Transplant.* 39, 853–855. doi: 10.1016/j.healun.2020.05.016

Kajani, R., Apramian, A., Vega, A., Ubhayakar, N., Xu, P., and Liu, A. (2020). Neuroleptic malignant syndrome in a COVID-19 patient. *Brain Behav. Immun.* 88, 28–29. doi: 10.1016/j.bbi.2020.05.042

Karimi-Galougahi, M., Yousefi-Koma, A., Bakhshayeshkaram, M., Raad, N., and Haseli, S. (2020). 18FDG PET/CT scan reveals hypoactive orbitofrontal cortex in anosmia of COVID-19. *Acad. Radiol.* 27, 1042–1043. doi: 10.1016/j.acra.2020.04.030

Kedia, S., Stroud, B., Parsons, J., Schreiner, T., Curtis, D. J., Bagdure, D., et al. (2011). Pediatric neurological complications of 2009 pandemic influenza A (H1N1). *Arch. Neurol.* 68, 455–462. doi: 10.1001/archneurol.2010.318

Khoo, A., McLoughlin, B., Cheema, S., Weil, R. S., Lambert, C., Manji, H., et al. (2020). Postinfectious brainstem encephalitis associated with SARS-CoV-2. *J. Neurol. Neurosurg. Psychiatry* 91, 1013–1014. doi: 10.1136/jnnp-2020-323816

Kihira, S., Delman, B. N., Belani, P., Stein, L., Aggarwal, A., Rigney, B., et al. (2020). Imaging features of acute encephalopathy in patients with COVID-19: a case series. *AJNR Am J Neuroradiol.* 41, 1804–1808. doi: 10.3174/ajnr.A6715

Klironomos, S., Tzortzakakis, A., Kits, A., Öhberg, C., Kollia, E., Ahoromazdae, A., et al. (2020). Nervous System Involvement in COVID-19: results from a retrospective consecutive neuroimaging cohort. *Radiology* 297:202791. doi: 10.1148/radiol.2020202791

Le Guennec, L., Devianne, J., Jalin, L., Cao, A., Galanaud, D., Navarro, V., et al. (2020). Orbitofrontal involvement in a neuroCOVID-19 patient. *Epilepsia* 61, e90–e94. doi: 10.1111/epi.16612

Lim, S. T., Janaway, B., Costello, H., Trip, A., and Price, G. (2020). Persistent psychotic symptoms following COVID-19 infection. *BJPsych Open* 6:e105. doi: 10.1192/bjo.2020.76

Lin, E., Lantos, J. E., Strauss, S. B., Phillips, C. D., Campion, T. R., Navi, B. B., et al. (2020). Brain imaging of patients with COVID-19: findings at an academic institution during the height of the outbreak in New York City. *AJNR Am. J. Neuroradiol.* 41, 2001–2008. doi: 10.3174/ajnr.A6793

Llansó, L., and Urra, X. (2020). Posterior reversible encephalopathy syndrome in COVID-19 disease: a case-report. *SN Compr. Clin. Med.* doi: 10.1007/s42399-020-00470-2 [Epub ahead of print].

Llorente Ayuso, L., Torres Rubio, P., Beijinho do Rosário, R. F., Giganto Arroyo, M. L., and Sierra-Hidalgo, F. (2020). Bickerstaff encephalitis after COVID-19. *J. Neurol.* doi: 10.1007/s00415-020-10201-1 [Epub ahead of print].

Logmin, K., Karam, M., Schichel, T., Harmel, J., and Wojtecki, L. (2020). Non-epileptic seizures in autonomic dysfunction as the initial symptom of COVID-19. *J. Neurol.* 267, 2490–2491. doi: 10.1007/s00415-020-09904-2

Louis, S., Dhawan, A., Newey, C., Nair, D., Jehi, L., Hantus, S., et al. (2020). Continuous electroencephalography characteristics and acute symptomatic seizures in COVID-19 patients. *Clin. Neurophysiol.* 131, 2651–2656. doi: 10.1016/j.clinph.2020.08.003

Lyons, S., O'Kelly, B., Woods, S., Rowan, C., Brady, D., Sheehan, G., et al. (2020). Seizure with CSF lymphocytosis as a presenting feature of COVID-19 in an otherwise healthy young man. *Seizure* 80, 113–114. doi: 10.1016/j.seizure.2020.06.010

Manganelli, F., Vargas, M., Iovino, A., Iacovazzo, C., Santoro, L., and Servillo, G. (2020). Brainstem involvement and respiratory failure in COVID-19. *Neurol. Sci.* 41, 1663–1665. doi: 10.1007/s10072-020-04487-2

Méndez-Guerrero, A., Laespada-García, M. I., Gómez-Grande, A., Ruiz-Ortiz, M., Blanco-Palmero, V. A., Azcarate-Diaz, F. J., et al. (2020). Acute hypokinetic-rigid syndrome following SARS-CoV-2 infection. *Neurology* 95, e2109–e2118. doi: 10.1212/WNL.0000000000010282

Morassi, M., Bagatto, D., Cobelli, M., D'Agostini, S., Gigli, G. L., Bnà, C., et al. (2020). Stroke in patients with SARS-CoV-2 infection: case series. *J. Neurol.* 267, 2185–2192. doi: 10.1007/s00415-020-09885-2

Muccioli, L., Pensato, U., Cani, I., Guerra, L., Provini, F., Bordin, G., et al. (2020a). COVID-19-related encephalopathy presenting with aphasia resolving following tocilizumab treatment. *J. Neuroimmunol.* 349:577400. doi: 10.1016/j.jneuroim.2020.577400

Muccioli, L., Rondelli, F., Ferri, L., Rossini, G., Cortelli, P., and Guarino, M. (2020b). Subcortical myoclonus in COVID-19: comprehensive evaluation of a patient. *Mov. Disord. Clin. Pract.* 7, 971–973. doi: 10.1002/mdc3.13046

Panariello, A., Bassetti, R., Radice, A., Rossotti, R., Puoti, M., Corradin, M., et al. (2020). Anti-NMDA receptor encephalitis in a psychiatric Covid-19 patient: a case report. *Brain Behav. Immun.* 87, 179–181. doi: 10.1016/j.bbi.2020.05.054

Panda, P. K., Sharawat, I. K., Panda, P., Natarajan, V., Bhakat, R., and Dawman, L. (2020). Neurological Complications of SARS-CoV-2 infection in children:

a systematic review and meta-analysis. *J. Trop. Pediatr.* doi: 10.1093/tropej/fmaa070 [Epub ahead of print].

Parauda, S. C., Gao, V., Gewirtz, A. N., Parikh, N. S., Merkler, A. E., Lantos, J., et al. (2020). Posterior reversible encephalopathy syndrome in patients with COVID-19. *J. Neurol. Sci.* 416:117019. doi: 10.1016/j.jns.2020.117019

Parsons, T., Banks, S., Bae, C., Gelber, J., Alahmadi, H., and Tichauer, M. (2020). COVID-19-associated acute disseminated encephalomyelitis (ADEM). *J. Neurol.* doi: 10.1007/s00415-020-09951-9 [Epub ahead of print].

Pasini, E., Bisulli, F., Volpi, L., Minardi, I., Tappatà, M., Muccioli, L., et al. (2020). EEG findings in COVID-19 related encephalopathy. *Clin. Neurophysiol.* 131, 2265–2267. doi: 10.1016/j.clinph.2020.07.003

Pastor, J., Vega-Zelaya, L., and Martín Abad, E. (2020). Specific EEG encephalopathy pattern in SARS-CoV-2 patients. *J. Clin. Med.* 9:1545. doi: 10.3390/jcm9051545

Paterson, R. W., Brown, R. L., Benjamin, L., Nortley, R., Wiethoff, S., Bharucha, T., et al. (2020). The emerging spectrum of COVID-19 neurology: clinical, radiological and laboratory findings. *Brain* 143, 3104–3120. doi: 10.1093/brain/awaa240

Pati, S., Toth, E., and Chaitanya, G. (2020). Quantitative EEG markers to prognosticate critically ill patients with COVID-19: a retrospective cohort study. *Clin. Neurophysiol.* 131, 1824–1826. doi: 10.1016/j.clinph.2020.06.001

Pellinen, J., Carroll, E., Friedman, D., Boffa, M., Dugan, P., Friedman, D. E., et al. (2020). Continuous EEG findings in patients with COVID-19 infection admitted to a New York academic hospital system. *Epilepsia* 61, 2097–2105. doi: 10.1111/epi.16667

Perrin, P., Collongues, N., Baloglu, S., Bedo, D., Bassand, X., Lavaux, T., et al. (2020). Cytokine release syndrome-associated encephalopathy in patients with COVID-19. *Eur. J. Neurol.* 28, 248–258. doi: 10.1111/ene.14491

Petrescu, A.-M., Taussig, D., and Bouilleret, V. (2020). Electroencephalogram (EEG) in COVID-19: a systematic retrospective study. *Neurophysiol. Clin.* 50, 155–165. doi: 10.1016/j.neucli.2020.06.001

Pilotto, A., Masciocchi, S., Volonghi, I., Crabbio, M., Magni, E., De Giuli, V., et al. (2020a). Clinical presentation and outcomes of SARS-CoV-2 related encephalitis: the ENCOVID multicentre study. *J. Infect. Dis.* 28:jiaa609. doi: 10.1093/infdis/jiaa609

Pilotto, A., Odolini, S., Masciocchi, S., Comelli, A., Volonghi, I., Gazzina, S., et al. (2020b). Steroid-responsive encephalitis in coronavirus disease 2019. *Ann. Neurol.* 88, 423–427. doi: 10.1002/ana.25783

Princiotta Cariddi, L., Tabaee Damavandi, P., Carimati, F., Banfi, P., Clemenzi, A., Marelli, M., et al. (2020). Reversible encephalopathy syndrome (PRES) in a COVID-19 patient. *J. Neurol.* doi: 10.1007/s00415-020-10001-7 [Epub ahead of print].

Pugin, D., Vargas, M.-I., Thieffry, C., Schibler, M., Grosgurin, O., Pugin, J., et al. (2020). COVID-19-related encephalopathy responsive to high doses glucocorticoids. *Neurology* 95, 543–546. doi: 10.1212/WNL.0000000000010354

Rábano-Suárez, P., Bermejo-Guerrero, L., Méndez-Guerrero, A., Parra-Serrano, J., Toledo-Alfocea, D., Sánchez-Tejerina, D., et al. (2020). Generalized myoclonus in COVID-19. *Neurology* 95, e767–e772. doi: 10.1212/WNL.0000000000009829

Radmanesh, A., Derman, A., and Ishida, K. (2020). COVID-19-associated delayed posthypoxic necrotizing leukoencephalopathy. *J. Neurol. Sci.* 415:116945. doi: 10.1016/j.jns.2020.116945

Radmard, S., Epstein, S. E., Roeder, H. J., Michalak, A. J., Shapiro, S. D., Boehme, A., et al. (2020). Inpatient neurology consultations during the onset of the SARS-CoV-2 New York city pandemic: a single center case series. *Front. Neurol.* 11:805. doi: 10.3389/fneur.2020.00805

Radnis, C., Qiu, S., Jhaveri, M., Da Silva, I., Szewka, A., and Koffman, L. (2020). Radiographic and clinical neurologic manifestations of COVID-19 related hypoxemia. *J. Neurol. Sci.* 418:117119. doi: 10.1016/j.jns.2020.117119

Rasmussen, C., Niculescu, I., Patel, S., and Krishnan, A. (2020). COVID-19 and involvement of the corpus callosum: potential effect of the cytokine storm? *AJNR Am. J. Neuroradiol.* 41, 1625–1628. doi: 10.3174/ajnr.A6680

Rodrigo-Armenteros, P., Uterga-Valiente, J. M., Zabala-Del-Arco, J., Taramundi-Argüeso, S., Erburu-Iriarte, M., Antón-Méndez, L., et al. (2020). Non-convulsive status epilepticus in a patient with COVID-19 infection. *Clin. Neurophysiol.* 131, 2588–2590. doi: 10.1016/j.clinph.2020.08.005

Romero-Sánchez, C. M., Díaz-Maroto, I., Fernández-Díaz, E., Sánchez-Larsen, Á, Layos-Romero, A., García-García, J., et al. (2020). Neurologic manifestations in hospitalized patients with COVID-19: the ALBACOVID registry. *Neurology* 95, e1060–e1070. doi: 10.1212/WNL.0000000000009937

Roy-Gash, F., De Mesmay, M., Devys, J.-M., Vespignani, H., Blanc, R., and Engrand, N. (2020). COVID-19-associated acute cerebral venous thrombosis: clinical, CT, MRI and EEG features. *Crit. Care* 24:419. doi: 10.1186/s13054-020-03131-x

Schupper, A. J., Yaeger, K. A., and Morgenstern, P. F. (2020). Neurological manifestations of pediatric multi-system inflammatory syndrome potentially associated with COVID-19. *Childs Nerv. Syst.* 36, 1579–1580. doi: 10.1007/s00381-020-04755-8

Scullen, T., Keen, J., Mathkour, M., Dumont, A. S., and Kahn, L. (2020). Coronavirus 2019 (COVID-19)-associated encephalopathies and cerebrovascular disease: the new orleans experience. *World Neurosurg.* 141, e437–e446. doi: 10.1016/j.wneu.2020.05.192

Sethi, N. K. (2020). EEG during the COVID-19 pandemic: what remains the same and what is different. *Clin. Neurophysiol.* 131:1462. doi: 10.1016/j.clinph.2020.04.007

Shekhar, R., Sheikh, A. B., Suriya, S. S., Upadhyay, S., and Zafar, A. (2020). Neurological complications among native americans with COVID-19: our experience at a tertiary care academic hospital in the U.S. *J. Stroke Cerebrovasc. Dis.* 29:105260. doi: 10.1016/j.jstrokecerebrovasdis.2020.105260

Sohal, S., and Mansur, M. (2020). COVID-19 Presenting with Seizures. *IDCases* 20:e00782. doi: 10.1016/j.idcr.2020.e00782

Soldatelli, M. D., do Amaral, L. F., Veiga, V. C., Rojas, S. S. O., Omar, S., and Marussi, V. H. R. (2020). Neurovascular and perfusion imaging findings in coronavirus disease 2019: case report and literature review. *Neuroradiol. J.* 33, 368–373. doi: 10.1177/1971400920941652

Somani, S., Pati, S., Gaston, T., Chitlangia, A., and Agnihotri, S. (2020). De novo status epilepticus in patients with COVID-19. *Ann. Clin. Trans. Neurol.* 7, 1240–1244. doi: 10.1002/acn3.51071

Svedung Wettervik, T., Kumlien, E., Rostami, E., Howells, T., von Seth, M., Velickaite, V., et al. (2020). Intracranial pressure dynamics and cerebral vasomotor reactivity in coronavirus disease 2019 patient with acute encephalitis. *Crit. Care Explor.* 2:e0197. doi: 10.1097/CCE.0000000000000197

Umapathi, T., Jason, Q. W. M., Min, Y. J., Wai, K. H. S., Yuan, M. Y., Yee, J. C. C., et al. (2020). Encephalopathy in COVID-19 patients; viral, parainfectious, or both? *eNeurologicalSci* 21:100275. doi: 10.1016/j.ensci.2020.100275

Vandervorst, F., Guldolf, K., Peeters, I., Vanderhasselt, T., Michiels, K., Berends, K. J., et al. (2020). Encephalitis associated with the SARS-CoV-2 virus: a case report. *Interdiscip. Neurosurg.* 22:100821. doi: 10.1016/j.inat.2020.100821

Vellieux, G., Rouvel-Tallec, A., Jaquet, P., Grinea, A., Sonneville, R., and d'Ortho, M.-P. (2020). COVID-19 associated encephalopathy: is there a specific EEG pattern? *Clin. Neurophysiol.* 131, 1928–1930. doi: 10.1016/j.clinph.2020.06.005

Vespignani, H., Colas, D., Lavin, B. S., Soufflet, C., Maillard, L., Pourcher, V., et al. (2020). Report on electroencephalographic findings in critically ill patients with COVID -19. *Ann. Neurol.* 88, 626–630. doi: 10.1002/ana.25814

Virhammar, J., Kumlien, E., Fällmar, D., Frithiof, R., Jackmann, S., Sköld, M. K., et al. (2020). Acute necrotizing encephalopathy with SARS-CoV-2 RNA confirmed in cerebrospinal fluid. *Neurology* 95, 445–449. doi: 10.1212/WNL.0000000000010250

Vollono, C., Rollo, E., Romozzi, M., Frisullo, G., Servidei, S., Borghetti, A., et al. (2020). Focal status epilepticus as unique clinical feature of COVID-19: a case report. *Seizure* 78, 109–112. doi: 10.1016/j.seizure.2020.04.009

Yazdanpanah, N., Saghazadeh, A., and Rezaei, N. (2020). Anosmia: a missing link in the neuroimmunology of coronavirus disease 2019 (COVID-19). *Rev. Neurosci.* doi: 10.1515/revneuro-2020-0039 [Epub ahead of print].

Young, M. J., O'Hare, M., Matiello, M., and Schmahmann, J. D. (2020). Creutzfeldt-Jakob disease in a man with COVID-19: SARS-CoV-2-accelerated neurodegeneration? *Brain Behav. Immun.* 89, 601–603. doi: 10.1016/j.bbi.2020.07.007

Zahid, M. J., Baig, A., Galvez-Jimenez, N., and Martinez, N. (2020). Hemorrhagic stroke in setting of severe COVID-19 infection requiring Extracorporeal Membrane Oxygenation (ECMO). *J. Stroke Cerebrovasc. Dis.* 29:105016. doi: 10.1016/j.jstrokecerebrovasdis.2020.105016

Zanin, L., Saraceno, G., Panciani, P. P., Renisi, G., Signorini, L., Migliorati, K., et al. (2020). SARS-CoV-2 can induce brain and spine demyelinating lesions. *Acta Neurochir.* 162, 1491–1494. doi: 10.1007/s00701-020-04374-x

Zoghi, A., Ramezani, M., Roozbeh, M., Darazam, I. A., and Sahraian, M. A. (2020). A case of possible atypical demyelinating event of the central nervous system following COVID-19. *Mult. Scler. Relat. Disord.* 44:102324. doi: 10.1016/j.msard.2020.102324

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 22 February 2021
doi: 10.3389/fphys.2021.624052

Controversial Roles of the Renin Angiotensin System and Its Modulators During the COVID-19 Pandemic

*Simon B. Gressens[1], Georges Leftheriotis[2], Jean-Claude Dussaule[3,4], Martin Flamant[5,6], Bernard I. Levy[7] and Emmanuelle Vidal-Petiot[5,6]**

[1] Department of Infectious and Tropical Diseases, Assistance Publique-Hôpitaux de Paris, Bichat-Claude Bernard University Hospital, Paris, France, [2] Laboratory of Molecular Physiology and Medicine, Université Cote d'Azur, Nice, France, [3] Sorbonne Université, INSERM, Unité des Maladies Rénales Fréquentes et Rares: des Mécanismes Moléculaires à la Médecine Personnalisée, AP-HP, Hôpital Tenon, Paris, France, [4] Faculty of Medicine, Sorbonne University, Paris, France, [5] Department of Physiology, Assistance Publique-Hôpitaux de Paris, Bichat-Claude Bernard University Hospital, Paris, France, [6] Inserm U1149, Centre for Research on Inflammation, Université de Paris, Paris, France, [7] Inserm U970, PARCC, Paris, France

OPEN ACCESS

Edited by:
Amy C. Arnold, Pennsylvania State University, United States

Reviewed by:
Mark Chappell, Wake Forest School of Medicine, United States
Ravindra K. Sharma, University of Florida, United States

***Correspondence:**
Emmanuelle Vidal-Petiot
emmanuelle.vidal-petiot@aphp.fr

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *30 October 2020*
Accepted: *12 January 2021*
Published: *22 February 2021*

Citation:
Gressens SB, Leftheriotis G, Dussaule J-C, Flamant M, Levy BI and Vidal-Petiot E (2021) Controversial Roles of the Renin Angiotensin System and Its Modulators During the COVID-19 Pandemic. Front. Physiol. 12:624052. doi: 10.3389/fphys.2021.624052

Since December 2019, the coronavirus 2019 (COVID-19) pandemic has rapidly spread and overwhelmed healthcare systems worldwide, urging physicians to understand how to manage this novel infection. Early in the pandemic, more severe forms of COVID-19 have been observed in patients with cardiovascular comorbidities, who are often treated with renin-angiotensin aldosterone system (RAAS)-blockers, such as angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), but whether these are indeed independent risk factors is unknown. The cellular receptor for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the membrane-bound angiotensin converting enzyme 2 (ACE2), as for SARS-CoV(-1). Experimental data suggest that expression of ACE2 may be increased by RAAS-blockers, raising concerns that these drugs may facilitate viral cell entry. On the other hand, ACE2 is a key counter-regulator of the RAAS, by degrading angiotensin II into angiotensin (1-7), and may thereby mediate beneficial effects in COVID-19. These considerations have raised concerns about the management of these drugs, and early comments shed vivid controversy among physicians. This review will describe the homeostatic balance between ACE-angiotensin II and ACE2-angiotensin (1-7) and summarize the pathophysiological rationale underlying the debated role of the RAAS and its modulators in the context of the pandemic. In addition, we will review available evidence investigating the impact of RAAS blockers on the course and prognosis of COVID-19 and discuss why retrospective observational studies should be interpreted with caution. These considerations highlight the importance of solid evidence-based data in order to guide physicians in the management of RAAS-interfering drugs in the general population as well as in patients with more or less severe forms of SARS-CoV-2 infection.

Keywords: SARS-CoV-2, COVID-19, renin-angiotensin-aldosterone system, RAAS blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers

INTRODUCTION

Since December 2019, the coronavirus disease 2019 (COVID-19) pandemic has rapidly spread and overwhelmed healthcare systems worldwide. Physicians and scientists urgently attempted to decipher the pathophysiology of the disease in order to define appropriate prevention of viral transmission and management of infected patients. The discovery that the viral receptor was a key enzyme of the renin-angiotensin-aldosterone system (RAAS), namely angiotensin-converting enzyme 2 (ACE2) (Chen Y et al., 2020; Hoffmann et al., 2020), shed light on the potential interactions between the novel coronavirus and the RAAS.

ACE2 is a mono-carboxypeptidase discovered by Donoghue et al. (2000), expressed in the epithelium of the respiratory tract, but also in the intestine, the central nervous system, the heart, the vessels (on endothelial cells), the kidney, and the testicle (Harmer et al., 2002; Hamming et al., 2004; Lee I. T. et al., 2020). In polarized epithelia of the lung, kidney, intestine, ACE2 is found at the apical membrane. In the lung, ACE2 is primarily found on a subset of epithelial cells (type II pneumocytes) facing the airspace, while the angiotensin-converting enzyme (ACE) is expressed on the endothelium, facing the blood. ACE2 had previously been shown to be the receptor for SARS-CoV, but the novel coronavirus has a higher affinity for ACE2 (Wrapp et al., 2020). ACE2 displays significant homology with ACE and is considered as a key counter-regulator of the RAAS, by degrading the octapeptide angiotensin (Ang) II into Ang (1-7) (Chappell, 2016). It also allows the transformation of Ang I into Ang (1-7), via the synthesis of an intermediate metabolite, Ang (1–9), although this pathway is quantitatively marginal. Ang (1-7) binds to the G protein-coupled Mas receptor and induces effects not only on the cardiovascular system, but in fact far beyond, generally "protective" because promoting vasodilation and limiting inflammation, fibrosis, coagulation, and capillary leakage (Kreutz et al., 2020). Biological effects of Ang (1-7) are indeed opposite to those induced by Ang II after binding to its type 1 (AT1) receptor (**Figure 1**). Importantly, angiotensin converting enzyme inhibitors (ACEIs) do not inhibit ACE2 (Tipnis et al., 2000). In times of emerging infectious threat, the interest toward what appears as a molecular cornerstone in the disease has grown rapidly.

Since early in the pandemic, more severe forms of COVID-19 have been observed in patients with cardiovascular comorbidities such as hypertension, diabetes and coronary heart disease (Wang D. et al., 2020; Wu Y. et al., 2020; Zhou et al., 2020b). Several authors hypothesized that that the link between cardiovascular conditions and severity of the disease might be related to the frequent use of RAAS blockers in these patients. The underlying rationale came from experimental studies in rodents suggesting that RAAS blockers may be responsible for an increased expression of ACE2 (Ferrario et al., 2005a,b; Soler et al., 2009).

While concerns about the safety of these widely used drugs rapidly spread from the medical literature (Aronson and Ferner, 2020; Diaz, 2020; Esler and Esler, 2020; Fang et al., 2020) to general and social media, other authors suggested that modulating ACE-Ang II versus ACE2-Ang (1-7) homeostasis in favor of the latter, using RAAS blockers, might actually be beneficial in patients with COVID-19 (Hanff et al., 2020; Vaduganathan et al., 2020).

Consequently, the management of RAAS-blockers, especially ACEIs and angiotensin receptor blockers (ARBs) in patients at risk of infection or infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a matter of controversy (South et al., 2020). Many observational studies have attempted to elucidate whether these drugs increased the risk to become infected with COVID-19 and/or modified the course and prognosis of the disease in infected patients while several randomized clinical trials are ongoing to establish whether RAAS blockers should be maintained, discontinued, or even introduced *de novo* in patients with COVID-19.

This review aims to analyze existing data supporting each side of the debate and to describe the available clinical evidence in order to help clinicians understand the underlying pathophysiology and manage prescription of these drugs in the context of the COVID-19 pandemic. The closely related issue of mineralocorticoid receptor antagonists has been less widely debated although it would deserve a dedicated review in itself. The potential role of mineralocorticoid receptor antagonists is beyond the scope of the present review and is only briefly mentioned in this manuscript.

RAAS BLOCKERS AND SARS-CoV-2 INFECTION: REASONS FOR CONCERN

Early concerns regarding the potential deleterious role of RAAS blockers mainly relied on animal data suggesting that ACE2 expression or activity is increased by these drugs, thereby facilitating viral cell entry. However, as reviewed below, this observation is inconsistent in various animal models, is not established in humans, and most importantly, there are no data showing an increased expression of the transmembrane ACE2 protein in the lung. In addition, cardiovascular conditions themselves, independent of RAAS blockade treatment, may also, and probably to a larger extent, influence the expression of ACE2.

ACE2 Expression and RAAS Blockers – Animal Data

Since the discovery that the ACE2-Ang (1-7)-Mas receptor pathway was a key counter-regulatory system of the ACE-Ang II-AT1 receptor pathway through the past two decades, the role, tissue expression and regulation of ACE2 have been extensively studied and described (Santos et al., 2018), but remain incompletely understood, in part due to discrepant results. In particular, the influence of the pharmacological blockade of ACE and of the AT1 receptor on ACE2 is an unsettled issue.

As reviewed in **Supplementary Tables 1A,B,** animal studies investigating the effect of ACEIs and/or ARBs on the expression of ACE2 have relied on different animal species, disease models, and readouts (mRNA versus protein level, or enzyme activity). In the early publications warning against the use of RAAS blockers, a widely cited study was that by Ferrario et al. published in 2005. The authors studied the effects of a 12-day treatment of wild-type rats with an ACEI, an ARB, or their combination on expression and activity of the components of the RAAS, in plasma

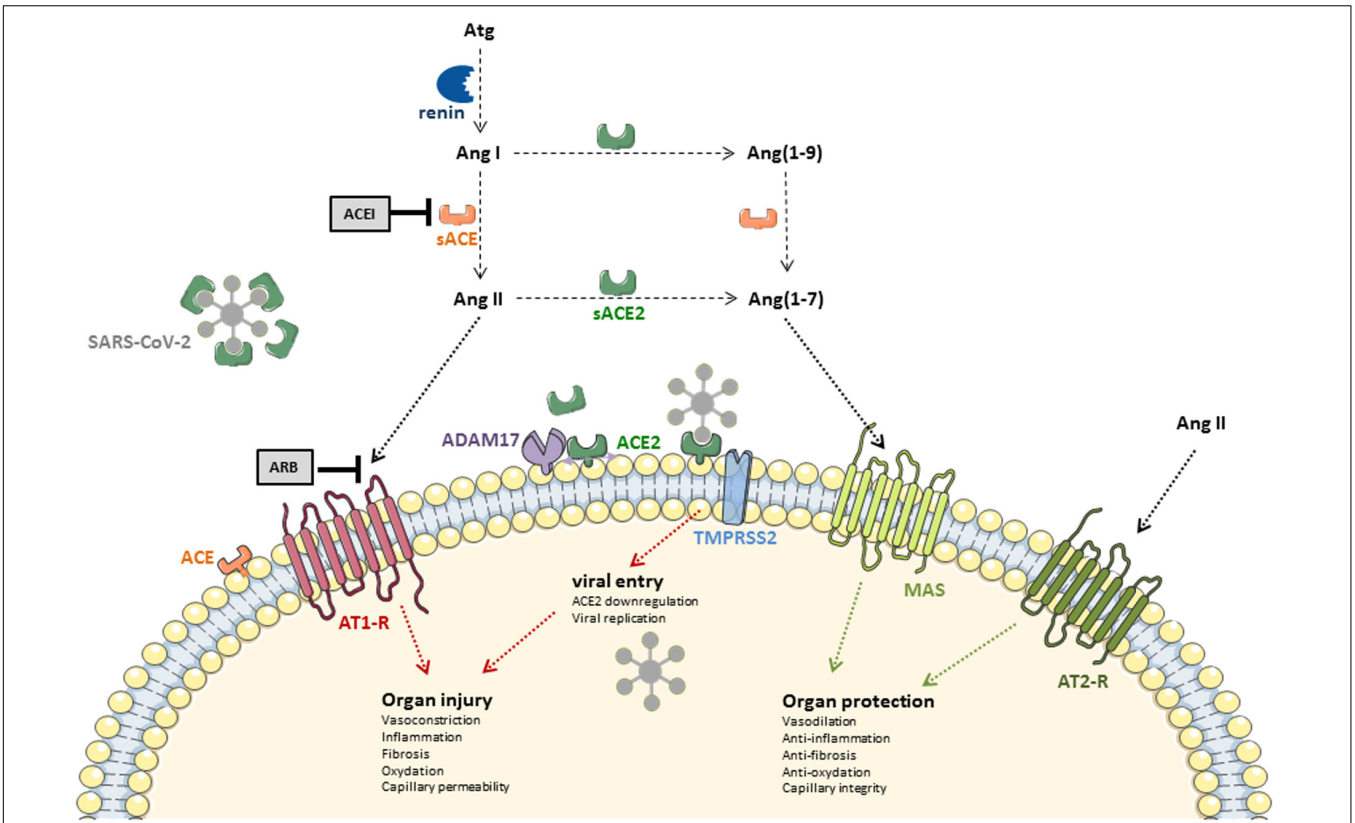


FIGURE 1 | ACE-angiotensin II/ACE2-angiotensin (1-7) homeostasis: general overview. Atg, angiotensinogen; Ang I, angiotensin I; Ang II, angiotensin II; Ang(1-9), angiotensin (1-9); Ang(1-7), angiotensin (1-7); ACEI, ACE inhibitor; ARB, Ang II receptor blocker; ACE, angiotensin converting enzyme; ACE, angiotensin converting enzyme 2; sACE, soluble ACE; sACE2, soluble ACE2; AT1-R, Ang II receptor type 1; AT2-R, Ang II receptor type 2; MAS, G protein-coupled MAS receptor; TMPRSS2, transmembrane protease serine 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ADAM17, A disintegrin and metalloproteinase 17.

and cardiac tissue. Both losartan (an ARB) and lisinopril (an ACEI) increased the level of ACE2 mRNA in the heart compared to vehicle-treated animals. Losartan, but not lisinopril, also increased ACE2 activity in cardiac membranes [assessed from the rate of conversion of Ang II to Ang (1-7)] and cardiac tissue concentrations of Ang (1-7), while both drugs increased plasma levels of Ang (1-7) (Ferrario et al., 2005a). The same team found somewhat different results in the rat kidney, where lisinopril and losartan did not modify ACE2 gene expression but increased ACE2 enzyme activity in membranes of renal cortex, as well as plasma and urine levels of Ang (1-7) (Ferrario et al., 2005b).

The putative mechanisms underlying an upregulation of ACE2 in the presence of RAAS blockers are partially elucidated. Ang II has been shown to inhibit ACE2 expression through an AT1 receptor-mediated extracellular regulated (ERK)1/2 and p38 mitogen-activated protein (MAP) kinases, both *in vitro* in different cellular models and *in vivo* (Gallagher et al., 2006, 2008a,b; Koka et al., 2008). In addition, Ang II also down-regulates ACE2 at the post-transcriptional level. It has been shown that ACE2 and the AT1 receptor interact on the cell surface and that the binding of Ang II to its AT1 receptor leads the reduction of these AT1 receptor-ACE2 complexes, and to the internalization and ubiquitination of ACE2 (Deshotels et al., 2014). Therefore, the alleged underlying mechanism for an increased expression of ACE2 in the presence of RAAS blockers results from the alleviations of these AT1-receptor mediated inhibiting pathways. According to this hypothesis, both ACEIs (by inhibiting Ang II synthesis) and ARBs (by blocking the AT1-mediated effects of Ang II) are expected to upregulate ACE2, although ARBs interfere more directly with the involved pathway and are thereby potentially expected to have a stronger effect. In line with this, studies seem to have more consistently shown an up-regulation of ACE2 protein concentration in tissues of interest when animals are treated by ARBs (Ishiyama et al., 2004; Igase et al., 2005; Agata et al., 2006; Jessup et al., 2006; Takeda et al., 2007; Soler et al., 2009; Han et al., 2010; Sukumaran et al., 2011, 2012; Zhang et al., 2014), than in studies using ACEIs, which produced more discrepant results (Hamming et al., 2008; Burchill et al., 2012; Burrell et al., 2012; Yang et al., 2013; Zhang et al., 2014; Wang G. et al., 2016), as detailed in **Supplementary Tables 1A,B**.

However, the biological actions of ACEIS and ARBs on ACE2 in healthy animals remain unclear as not all studies have found similar results, even with each separate class of drug (**Supplementary Table 1A**). Although many studies have confirmed an increase in ACE2 expression, either at the mRNA and/or protein level, or an increased activity of the enzyme

(Ocaranza et al., 2006; Soler et al., 2009; Li et al., 2015), others have shown no effect of RAAS blockers on ACE2 expression or activity (Han et al., 2010; Velkoska et al., 2010; Wösten-van Asperen et al., 2011; Wu C. et al., 2020).

More importantly, although of high relevance in the context of SARS-CoV-2 infection, data on the influence of RAAS blockers on the expression of ACE2 in the lungs are scarce. In ventilated rats with no other cause of lung injury, losartan did not influence ACE2 activity in bronchoalveolar lavage fluid (Wösten-van Asperen et al., 2011), while captopril was found to increase the expression of ACE2 in the lungs of control rats breathing spontaneously (Li et al., 2015). However, variations of ACE2 in lavage fluid may not reflect changes in tissue expression within the lung epithelium. In a recent publication, Wu C. et al. (2020) analyzed the effect of a 14-day infusion of enalapril or losartan on the expression on ACE2 in various tissues (ileum, kidney, heart, and lungs) of wild-type male C57BL/6J mice. Although plasma renin increased by over 100-fold with both drugs, confirming potent RAAS inhibition, neither enalapril nor losartan changed the abundance of ACE2 mRNA in any of the tissues, including the lungs. Likewise, in a recent publication, Wysocki et al. (2020) used kidney and lung lysates to examine the effect of captopril and telmisartan, administered for 2 weeks, on ACE2 expression in mice. Interestingly, in lysates of kidney cortex, ACE2 activity and protein did not differ significantly in captopril- or losartan-treated animals compared with vehicle-treated animals. However, in isolated kidney membranes, there was a profound decrease in kidney ACE2 protein. This decrease in membrane-bound ACE2 was associated with a significant increase in cytosolic ACE2 protein, interpreted as a possible internalization of the protein. In lung tissue, attempts to perform Western blots for ACE2 with either total lysates or isolated membranes did not yield a signal, in agreement with the low physiological expression of the enzyme in type 2 pneumocytes. Conversely, ACE2 activity was low but consistently detected and was not significantly modified by either captopril or telmisartan, both in lung total lysates and lung membranes. Overall, this study showed that ACE2 is not increased by RAAS blockers in these two organs (lung and kidney) that are potential key target sites for SARS-CoV-2 infection.

One potential explanation for these discrepant results is that ACE2 activity does not always correlate with mRNA and protein expression levels. Indeed, several authors have shown uncoupled variations between mRNA, the protein and its activity (Ferrario et al., 2005a,b; Wysocki et al., 2006; Hamming et al., 2008; Yang et al., 2013), revealing potential strong post-transcriptional and post-translational regulations of ACE2. Consequently, based on the readout, conclusions on the impact of RAAS-blockers on ACE2 vary markedly. In addition, as viral entry requires the transmembrane expression of ACE2 in the respiratory tract, any conclusions drawn from mRNA levels, or even from protein level or enzymatic activity of the circulating enzyme is highly speculative. As outlined by Wysocki et al. (2020), when assessing the relative quantities of ACE2 that can act as the SARS-CoV-2 receptor, what matters most is the abundance of full-length, membrane-bound ACE2, which was either not modified or decreased in their well-conducted study with appropriate experimental methodology. Furthermore, specific properties of each molecule within each drug-class may also explain discrepant results which may not be class-related. For instance, captopril is a member of a sulfhydryl class of ACEI that have direct antioxidant properties (Liu et al., 2006).

Other potential explanations for discrepant results between laboratories include the major experimental difficulties associated with the biochemical assessment of the various components of the RAAS, due to the poor specificity of antibodies and of synthetic substrates or inhibitors of ACE2, and to the poor availability of the most accurate assays of ACE2 activity such as high-performance liquid chromatography/mass spectrometry, as reviewed elsewhere (Chappell, 2016; Sparks et al., 2020).

Moreover, the observed effect of RAAS-blockers has been shown to differ between organs, unraveling the complexity of the communications between the local RAAS amongst different tissues. As detailed above, the same team, with similar methods and in the same animal model, found different effects of RAAS blockade in heart and kidney (Ferrario et al., 2005a,b). Effects may also vary within each organ. Burrell et al. (2012) showed that ramipril (an ACE inhibitor) restored ACE2 activity in the renal cortex, but not in the renal medulla after partial nephrectomy. In addition, many experimental studies used very high doses of RAAS blockers (Sriram and Insel, 2020), limiting their physiological relevance. Furthermore, duration (from a few days to several months) and route of administration of treatment are not homogeneous between studies, which may also explain discrepancies. This heterogeneity, combined with the lack of long-term effect evaluation of these drugs in animal models, further limits generalization to human settings. Finally, not only is the membrane bound form of ACE2 required for SARS-CoV-2 binding and infection, but other peptidases are also requisite on the host cells including furin and the transmembrane protease serine 2 (TMPRSS2), which further limits the interpretation of ACE2 expression as a direct explanation for facilitated viral entry (Hoffmann et al., 2020; Wrapp et al., 2020).

Another crucial difference between all experimental models is that although some of them specifically analyzed the impact of RAAS blockade on ACE2 in wild type animals or control conditions (**Supplementary Table 1A**), others used animal models of pathological conditions such as hypertension, heart failure, or organ injury, in order to analyze their effect in conditions for which they are commonly used in humans (**Supplementary Tables 1A,B**). However, these disease-models have complex and sometimes strong influences on ACE and ACE2 expressions and activities themselves. Indeed, animal models for diseases such as hypertension (Agata et al., 2006; Takeda et al., 2007; Yang et al., 2013) heart failure (Karram et al., 2005), or lung injury (Asperen et al., 2010; Wösten-van Asperen et al., 2011; Li et al., 2015) were associated with a decreased ACE2 protein expression in most observed tissues compared to wild type or control animals, when other models such as myocardial infarction mostly showed an increased ACE2 expression in the heart (Burrell et al., 2005; Ocaranza et al., 2006; Burchill et al., 2012). In the vast majority of these studies, RAAS blockers tended to restore a physiological level of ACE2 (**Supplementary Tables 1A,B**).

In summary, animal data on the expression of ACE2 in the presence of RAAS blockers yielded inconsistent results and

it is highly uncertain that these treatments actually increase the expression of ACE2 in target organs of SARS-CoV-2. Interpretation of experimental data is all the more complex as (1) the effect differs depending on the animal model, (2) the effects of ACEIs, ARBs, or both combined, may differ, (3) the observed modifications of ACE2 expression vary depending on the tissue examined, (4) the effect on mRNA does not always correlate with the expression on the protein and/or its activity, (5) the level on soluble ACE2, often examined in models, does not reflect the level of transmembrane ACE2 (the only relevant one for viral cell entry), (6) biochemical evaluation of components of the RAAS is technically challenging, (7) other peptidases on the host cells are also requisite for viral entry, and (8) last and most importantly, these are only animals models, and these putative effects and mechanisms need to be confirmed in humans.

Of note, mineralocorticoid receptor antagonists may increase ACE2 expression to a larger extent than ACEIs and ARBs (Keidar et al., 2005; Kreutz et al., 2020).

ACE2 Expression and RAAS Blockers – Human Data

In human, circulating ACE2 is found at very low levels in healthy subjects (Lew et al., 2008), is higher in men than in women (Soro-Paavonen et al., 2012; Ramchand et al., 2018, 2020), and increases with age (Rice et al., 2006). Plasma ACE2 has been shown to be increased in cardiovascular diseases such as heart failure (Epelman et al., 2009; Uri et al., 2016), coronary artery disease (Ortiz-Pérez et al., 2013; Ramchand et al., 2018), aortic stenosis (Ramchand et al., 2020), and atrial fibrillation (Walters et al., 2016). In addition, the level of plasma ACE2 is a biomarker of poor outcome in these conditions (Epelman et al., 2008, 2009; Ramchand et al., 2018; Ramchand and Burrell, 2020) as well as in the general population (Narula et al., 2020).

Human data on the influence of RAAS-blockers on ACE2 expression, independently of the effect of underlying diseases, is even sparser than animal data and summarized in **Supplementary Table 2**.

Although transmembrane ACE2 is the form of interest regarding viral entry into target cells, due to obvious difficulties to obtain tissue samples, the influence of RAAS blockers on protein expression of ACE2 in tissues of interest has rarely been studied in humans. In particular, human data on potential modifications of ACE2 expression in the lungs with RAAS-blockers are scarce. Very recently, using a panel of banked human tissue, Lee I. T. et al. (2020) reported that ACE2 robustly localizes within the motile cilia of airway epithelial cells. Interestingly, ACE2 expression was not increased in the nasal airway of ACEI or ARB users, and may even be slightly reduced in patients taking ACEIs. Three studies have described mRNA or protein expression of ACE2 in the kidney (Lely et al., 2004; Reich et al., 2008; Jiang et al., 2020). Neither ACE2 mRNA nor ACE2 protein expression were modified by the use of these drugs among patients with diabetes or membranous glomerulopathies (Lely et al., 2004). However, tubular ACE2 mRNA expression was found to be increased by ACEIs and ARBs in biopsies from patients with chronic allograft nephropathy or primary focal segmental glomerulosclerosis (Reich et al., 2008). In a recent study of more than 400 patients whose kidney transcriptomes were characterized by RNA-sequencing, no association between renal expression of ACE2 and RAAS-blockers was found. Interestingly, age was positively correlated with an increased mRNA expression in both kidney tissues and lung samples from the Genotype-Tissue Expression project (Jiang et al., 2020). In a study exploring the expression of ACE2 in the human intestine, ACEI users (n = 9) were shown to have increased ACE2 mRNA levels in duodenum biopsies (Vuille-dit-Bille et al., 2015) compared to non-users (n = 22), whereas ARBs were not associated with any modification in the expression of ACE2. Recently, in human myocardial samples, Stegbauer et al. (2020) showed that ACE2 protein level was increased in patients with severe aortic stenosis (but not in patients with severe mitral valve regurgitation) compared to control patients, and that ACEIs, but not ARBs, were associated with increased ACE2 expression (Stegbauer et al., 2020).

In most human studies, ACE2 expression was measured in plasma or urine samples (**Supplementary Table 2**). To our knowledge, only two studies reported an increased plasma ACE2 activity associated with RAAS blockers; Soro-Paavonen et al. (2012) showed an increase in plasma ACE2 activity among diabetic patients treated with ACEIs (a similar effect with ARBs was only observed in women), while Anguiano et al., reported that ARBs, but not ACEIs, increased plasma ACE2 activity in some subgroups of patients with chronic kidney disease (including those on dialysis). As reviewed in **Supplementary Table 2**, the reported effects of RAAS-blockers in all other studies were either an unmodified or decreased circulating ACE2. Indeed, several studies have examined the effect of RAAS blockers on plasma ACE2 in patients with heart failure, and consistently shown that the protein concentration and/or activity of the enzyme was either unchanged (Epelman et al., 2008; Uri et al., 2016; Chirinos et al., 2020), or decreased [in one cohort (Sama et al., 2020)], but never increased by these drugs. Similar results were found in chronic (Ramchand et al., 2018) or acute (Ortiz-Pérez et al., 2013) coronary artery disease, as well as in patients with aortic stenosis (Ramchand et al., 2020). A recent large study assessed the potential determinants of ACE2 levels in the general population within a subset of PURE (Prospective Urban Rural Epidemiology) participants. In 5216 subjects with hypertension, the authors found no associations between plasma ACE2 levels and ACEIs or ARBs (Narula et al., 2020).

Therefore, there is a large body of evidence suggesting that RAAS inhibitors do not upregulate plasma ACE2 in human. Of note, methodological considerations are again very important when interpreting studies reporting ACE2 activity, and may explain discrepancies. Indeed, as ACE can cleave the ACE2 fluorescent substrate commonly used to measure ACE2 activity, patients on ACEI may appear to have reduced ACE2 activities compared to controls when an ACE inhibitor is not included in the assay (Chappell, 2016).

Data obtained in urine samples are somewhat discrepant but overall do not support an increased ACE2 expression more than those obtained with plasma samples. Among diabetic patients, Liang et al. (2015) showed a decrease in urine ACE2 concentration with RAAS-blockers, when another study by Mariana et al. (2016) reported no effect of these drugs in patients

with preserved renal function. Furuhashi et al. (2015) studied the effect of different anti-hypertensive medications among 100 hypertensive patients compared to 101 healthy controls. An increased urinary ACE2 protein concentration was found with the ARB olmesartan (received by 13 patients), but no significant change was observed with any other ARB (including losartan, telmisartan, valsartan, and candesartan) or with the ACEI enalapril. Of note, a distinct biological effect of olmesartan on ACE2 has not been reported elsewhere to our knowledge.

Very importantly, the level of expression of soluble ACE2 in plasma and urine may not reflect the tissue expression of transmembrane ACE2. Plasma ACE2 originates in part from shedding of the cell surface in tissues in which ACE2 is expressed, mainly in endothelial cells. ACE2 is cleaved from the cell membrane by ADAM17 (A disintegrin and metalloproteinase 17), and the regulation of this process is still poorly elucidated. In a recent study, Ramchand et al. (2020) analyzed plasma and myocardial expression of ACE2 in 22 patients with aortic stenosis and reported that higher circulating ACE2 levels were found in patients with reduced myocardial ACE2 gene expression, suggesting that increased levels of plasma ACE2 might actually reflect downregulation of the enzyme in tissues (Ramchand et al., 2020). Of note, a similar observation of opposite trends for myocardial and plasma activities of ACE2 was reported in dogs with heart failure (Larouche-Lebel et al., 2019). Regarding ACE2 expression in urine samples, ACE2 is unlikely to be physiologically filtered through the glomerulus due its size, so that urine ACE2 probably reflects its tubular expression (mainly in the apical membrane of the proximal tubule), after an ADAM17-mediated cleavage. Altogether, biological mechanisms underlying circulating ACE2 concentration/activity remain mostly hypothetical, as a modified ACE2 plasma concentration could reflect either a dysregulation of shedding or a modified tissue ACE2 expression.

Of note, even if an upregulation of circulating ACE2 was pharmacologically induced, this could actually be expected to be beneficial against SARS-CoV-2 viral infection by the binding of ACE2 to viral particles, preventing them to reach their target cells. This mechanism is the rationale for the therapeutic use of recombinant soluble ACE in the early phase of SARS-CoV-2 infection (Batlle et al., 2020; Monteil et al., 2020).

Overall, data regarding the expression of ACE2 in patients treated with RAAS blockers is scarce, is not in favor of an upregulation, and most importantly, there are no data showing an increased expression of the transmembrane ACE2 protein in the lung or upper respiratory tract (Kreutz et al., 2020), while an increase in the circulating form of the enzyme would not necessarily be deleterious and may even be protective. In addition, the binding of the viral spike protein to transmembrane ACE2 allows attachment of SARS-CoV-2 to the surface of target cells but is not the unique necessary step for viral cell entry. Penetration of the viral particle inside the cell requires the so-called priming of the spike protein by the cellular serine protease TMPRSS2, which then allows fusion of viral and cellular membranes (Hoffmann et al., 2020). In the murine study by Wu et al., mentioned above, the abundance of TMPRSS2 mRNA was not modified in any tissue after enalapril or losartan infusion.

Therefore, there is no solid experimental evidence supporting the concern that RAAS blockers may increase the expression of the transmembrane viral receptor and thereby facilitate its entry into the cell.

Cardiovascular Comorbidities and COVID-19

As explained above, whereas soluble ACE2 is expressed at very low levels in healthy subjects (Lew et al., 2008), it is markedly increased in cardiovascular disease. Therefore, although the impact of RAAS blockers on the expression of ACE2 remains highly speculative, the underlying conditions for which patients receive RAAS blockers on ACE2 may actually have a much more pronounced impact on ACE2 than these drugs. The reported increased risk of SARS-CoV-2 infection or severe disease in these conditions may indeed be mediated by modulations of ACE2, but these are more likely to be induced by the disease itself. The role of cardiovascular conditions as risk factors for COVID-19 deserves to be discussed and clarified.

The main reason for the initial concern regarding a potential deleterious role of RAAS blockers in COVID-19 arose from the early observation that more severe infections occurred in patients with cardiovascular comorbidities (Wu C. et al., 2020; Zhou et al., 2020b). An early and widely discussed risk factor was hypertension. In the first fairly large case-series published, describing patients in Wuhan (China), hypertension was highly prevalent in hospitalized-COVID-19 cases, and even more frequent among intensive care unit (ICU)-admitted patients (Wang D. et al., 2020). However, it was quickly noted that the vast majority of these results were not adjusted, even for age, whereas the prevalence of hypertension markedly increases with age (Forouzanfar et al., 2017; Wang et al., 2018). Indeed, although nearly all published studies reported an increased crude risk of mortality, ICU admission or severe disease among patients with hypertension, this association did not remain significant after adjusting for the main covariates, especially age and sex in the majority of the studies (Boulle et al., 2020; Bravi et al., 2020; Chen J. et al., 2020; Fried et al., 2020; Gupta et al., 2020; Iaccarino et al., 2020; Ioannou et al., 2020; Kim et al., 2020; Williamson et al., 2020; Yu et al., 2020).

However, a few large and properly adjusted studies still found a significant association between hypertension and mortality (Albitar et al., 2020; Berenguer et al., 2020; Cunningham et al., 2020; Giorgi Rossi et al., 2020; Hernández-Galdamez et al., 2020; Pan et al., 2020; Parra-Bracamonte et al., 2020; Reilev et al., 2020). A potential explanation for these partially discrepant results is that these studies included rather young patients, with a mean or median age below 50 years (Albitar et al., 2020; Cunningham et al., 2020; Hernández-Galdamez et al., 2020; Parra-Bracamonte et al., 2020). A study among very young hospitalized adults (18–34 years old) across the United States (USA) reported an adjusted odds ratio (OR) for death or mechanical ventilation of 2.36 among hypertensive adults (Cunningham et al., 2020). Accordingly, a large study conducted in the United Kingdom based on the National Health Service surveillance system which accounts for approximately 40% of the patients in the country evaluated the factors associated with COVID-19-related deaths

compared to the general population (Williamson et al., 2020). The authors showed a strong and significant interaction between hypertension and age, hypertension being associated with a higher risk of mortality up to the age of 70 years, and a lower risk in older patients. Another potential explanation for discrepant results regarding hypertension is that obesity is very important confounder (Lighter et al., 2020; Mechanick et al., 2020; Simonnet et al., 2020) which was not always included in the models, especially as body mass index is a frequent missing data in databases.

Besides hypertension, diabetes mellitus, chronic kidney disease, and ischemic heart disease are other conditions frequently treated with RAAS blockers which were reported to be associated with poor outcome early in the pandemic.

Diabetes mellitus was independently associated with death in several studies. Petrilli et al. (2020) reported an adjusted hazard ratio (HR) of 1.24 (95% confidence interval [95% CI] 1.03; 1.5) for mortality among 5279 COVID-19 patients including 35% with diabetes in the United States. Similar results were reported in other occidental cohorts (Bravi et al., 2020; Giorgi Rossi et al., 2020; Iaccarino et al., 2020; Kim et al., 2020; Reilev et al., 2020; Sands et al., 2020; Williamson et al., 2020) and in the two Mexican case-series (Hernández-Galdamez et al., 2020; Parra-Bracamonte et al., 2020). In cohorts from China, results regarding diabetes have been more variable (Cen et al., 2020; Chen J. et al., 2020; Pan et al., 2020).

Chronic kidney disease was reported to be associated with an increased risk for mortality, independently of potential confounder, in multiple large-scaled studies across different regions worldwide (Berenguer et al., 2020; Boulle et al., 2020; Cariou et al., 2020; Chen J. et al., 2020; Fried et al., 2020; Hernández-Galdamez et al., 2020; Iaccarino et al., 2020; Ioannou et al., 2020; Kim et al., 2020; Mikami et al., 2020; Parra-Bracamonte et al., 2020; Perez-Guzman et al., 2020; Reilev et al., 2020; Williamson et al., 2020). Of note, a few other studies reported that the increased crude risk among chronic kidney disease patients was no-longer significant after adjusting for confounders (Cen et al., 2020; Chen L et al., 2020; Petrilli et al., 2020; van Gerwen et al., 2020). The proportion of patients with chronic kidney disease, and etiology of kidney injury varied markedly between all these studies, which may in part explain these differences. Interestingly, in a French multicenter study of 1,317 diabetic patients (CORONADO study), Cariou et al. (2020) reported adjusted odd ratios (ORs) of 2.14 for mortality [95% confidence interval (CI) 1.16; 3.94] for chronic kidney disease and 2.54 [95% CI 1.44; 4.50] for coronary artery disease.

Overall, in large-scale properly adjusted studies, diabetes mellitus and chronic kidney disease appear to be more frequently associated with mortality, and with higher adjusted risk, than hypertension (Chen J. et al., 2020; Williamson et al., 2020; Yu et al., 2020). Results regarding chronic heart disease are more discrepant (Giorgi Rossi et al., 2020; Ioannou et al., 2020; Kim et al., 2020; Parra-Bracamonte et al., 2020; Reilev et al., 2020), in part because most studies did not differentiate properly the underlying baseline cardiac comorbidities.

Results of studies cited in this paragraph which included more than 500 patients are summarized in **Supplementary Table 3** which gives a large, although not exhaustive, overview of studies on the associations between cardio-metabolic comorbidities (in which RAAS blockers are frequently indicated and prescribed) and unadjusted and adjusted risk of adverse outcome in COVID-19.

THE REVERSE HYPOTHESIS: RAAS BLOCKERS MIGHT BE PROTECTIVE IN SARS-COV-2 INFECTION

While some authors were warning against the potential deleterious role of ACE2 and therefore of RAAS blockers, others have been claiming that restoring a disrupted ACE2-Ang1-7/ACE-Ang II homeostasis, for instance by using RAAS blockers, might actually be beneficial in COVID-19.

SARS-CoV and SARS-CoV-2 Share a Common Receptor

SARS-CoV-2, a betacoronavirus belonging to the 2B group, shares 70–80% genetic homology with SARS-CoV(-1) (Lu et al., 2020). When ACE2 was identified as the receptor for SARS-CoV in 2003 (3 years after its discovery), both *in vitro* (Li et al., 2003) and *in vivo* (Kuba et al., 2005), this led to investigate the role of ACE2 signaling in respiratory distress syndromes. In the lung, ACE2 is primarily expressed by type II alveolar epithelial cells, endothelial cells, and vascular smooth muscle cells (Wiener et al., 2007) and appears to play a crucial role in the pathophysiology of lung injury from various origins, even when not induced by SARS-CoV (Gu and Korteweg, 2007).

Early in the COVID-19 pandemic, *in vitro* studies have shown that SARS-CoV-2 and SARS-CoV shared the same receptor (Chen Y et al., 2020; Hoffmann et al., 2020). However, the affinity of SARS-CoV-2 for ACE2 was shown to be much higher than that of SARS-CoV (Chen Y et al., 2020; Wrapp et al., 2020). Shortly after, the pathogenicity of SARS-CoV-2 *via* ACE2 was confirmed *in vivo* in a murine model. Intranasally inoculated SARS-CoV-2 allowed viral replication accompanied by interstitial pneumonia and infiltration of inflammatory cells in the lung of transgenic mice bearing the human ACE2 gene, but not in wild-type mice (Bao et al., 2020).

The many clinical and biological resemblances between SARS-CoV (Lew, 2003; Lam et al., 2004) and SARS-CoV-2-related infections, and most importantly, their common receptor, have renewed interest in previous data generated in the years following the SARS outbreak in 2003.

ACE2-Angiotensin (1-7)/ACE-Angiotensin II Homeostatic Balance and Lung Injury

As summarized in **Table 1**, the protective role of the ACE2-Ang (1-7)-Mas receptor pathway and, conversely, the deleterious role of the ACE-Ang II-AT1 receptor pathway, have been extensively demonstrated in multiple murine models of acute lung injury.

In mouse models of severe lung injury induced by acid aspiration or sepsis, Imai et al., have shown that lung injury

TABLE 1 | Summary of the main experimental studies supporting a role of the ACE-Ang II/ACE2-Ang(1-7) homeostasis in lung injury.

Publication	Model/Strain	Intervention	Main findings/conclusions
Imai – 2005 Nature (Imai et al., 2005)	Mouse	Different models of lung injury: acid aspiration, sepsis (caecal ligation perforation)	ACE2 protein expression was downregulated in lung injury. ACE2 (−/−) knockout mice: worsening of lung injury. Recombinant human ACE2 rescued phenotype. ACE (−/−) knockout mice and Losartan (ARB) decreased lung injury → deleterious ACE-Ang II effects mediated through AT1R; ACE2 is protective
Kuba – 2005 Nat Med (Kuba et al., 2005)	Mouse and cell lines	SARS-CoV infection of wild-type or ACE2−/− knock out mice Recombinant SARS-CoV Spike protein ± acid aspiration	ACE2 is a crucial *in vivo* SARS-CoV receptor required for effective viral replication. SARS-CoV infection of wild-type mice resulted in reduced ACE2 expression in the lungs. Spike-Fc binding to ACE2 induced downregulation of ACE2 in cell lines. Treatment with Spike-Fc in mice enhanced acid-induced lung injury, downregulated ACE2 protein expression in lungs, and further (more than acid aspiration) increased Ang II levels. SARS-CoV spike+acid–mediated lung failure was rescued by an ARB.
Wösten-van Asperen – 2010 Am J Pathol (Asperen et al., 2010)	Rat (Sprague-Dawley)	LPS+mechanical ventilation	LPS+MV increased ACE activity, Ang II levels, and inflammation. Captopril (ACEI) attenuated the lung inflammatory response, and the protective effect of Losartan (ARB) was even greater.
Wösten-van Asperen – 2011 J Pathol (Wösten-van Asperen et al., 2011)	Rat (Sprague-Dawley)	LPS+mechanical ventilation	In bronchoalveolar lavage fluid of ventilated LPS-exposed animals, ACE activity was enhanced, and Ang II levels increased, while ACE2 activity was reduced and Ang (1-7) levels decreased. A cyclic form of Ang-(1-7), and to a lesser extent the ARB losartan, restored the Ang (1-7)/Ang II ratio, attenuated the inflammatory response, markedly decreased lung injury, and improved lung function.
Wong – 2012 Am J Resp Cell Mol Biol (Wong et al., 2012)	Rat (Sprague-Dawley) and primary alveolar T1 cells	LPS-induced lung injury	Alveolar type I cells from LPS-instilled rats, as well as primary TI cells treated with LPS, produced cytokines. ACE2 mRNA was decreased in TI cells of LPS-injured animals. In vitro, ACE2 and losartan (ARB) partially inhibited cytokine production → ACE2 modulates pro-inflammatory cytokines production from pneumocytes I through AT1R.
Zou – 2014 Nat Comm (Zou et al., 2014)	Mouse Human (adults)	Avian influenza A H5N1–induced lung injury	H5N1-infected patients exhibited markedly increased serum levels of Ang II. In mice: protein ACE2 expression in lung was decreased and plasma Ang II levels increased among H5N1 infected mice. ACE2(−/−) knock out mice: aggravated lung injury. Recombinant human ACE2 reduced the severity of lung injury.
Li - 2015 Shock (Li et al., 2015)	Rat (Sprague-Dawley)	LPS-induced lung injury	ACEI captopril pretreatment: - significantly attenuated LPS-induced lung injury - inhibited secretion of tumor necrosis factor α and interleukin 6 (IL6) - reduced the Ang II / Ang (1-7) ratio - reversed the increased ACE/ ACE2 ratio.
Yan – 2015 Sci China Life Sci (Yan et al., 2015)	Mouse (C57/B6)	Avian influenza A H5N1-induced-lung injury	The ARB losartan: - improved the severity of lung injury and survival rate decreased IL6 mRNA expression - increased ACE2 protein expression in lungs.
Yang – 2015 Sci Rep (Yang et al., 2015)	Mouse (C57BL/6) Adult patients	Avian-origin influenza A (H7N9) -induced lung injury	H7N9 infection down-regulated ACE2 protein expression in lungs and increased plasma Ang II levels, Ang II levels were also increased in 6 patients with H7N9 pneumonia. ACE2 deficiency in mice (ACE2-/- knock out mice) aggravated lung injury and reduced survival. Inhibiting AT1 receptor (with losartan, an ARB) alleviated the severity of lung injury in wild-type and ACE2 knock out mice (no effect with AT2R blocker).
Gu – 2016 Sci Rep (Gu et al., 2016)	Mouse (C57/B6) Human (34 children with RSV pneumonia and 20 healthy children)	Respiratory syncytial virus (RSV)-induced lung injury	RSV-infected children and had increased plasma Ang II levels (particularly in the early phase). Mice infected with RSV had decreased ACE2 protein expression in lung and increased plasma Ang II levels. ACE2 deficiency (knock out versus wild-type mice) aggravated, and ARB (losartan) administration markedly attenuated RSV-induced lung injury. Recombinant hACE2 alleviated the severity of RSV-induced lung injury.
Li – 2016 Sci Rep (Li et al., 2016)	Rat (Sprague-Dawley)	LPS-induced lung injury Lentiviral packaged ACE2 cDNA or ACE2 shRNA was intratracheally administrated two weeks before lung injury	ACE2 overexpression prevented (and ACE2 shRNA deteriorated) LPS-induced lung injury and inflammatory response. ACE2 overexpression reversed Ang II/Ang-(1-7) ratio in the bronchoalveolar fluid lavage. ACE2 suppressed the MAPKs (p38-ERK1/2) and NF-kB pathways that mediate LPS-induced lung injury.
Wang – 2016 Am J Transl Res (Wang L. et al., 2016)	Mouse (BALB/c)	Bleomycin-induced acute lung injury	ACE2 diminished lung injury. ACE2 injection antagonized the effects of PLGF (placental growth factor, member of VEGF family) on increase of lung vessel permeability, resulting in improvement of lung function
Zhang – 2018 Am J Physiol Lung Cell Mol Physiol (Zhang et al., 2018)	Rat (Sprague-Dawley)	Seawater aspiration-induced lung injury	Endoplasmic reticulum stress induced by seawater aspiration led to apoptosis in lung tissue This was inhibited by an ARB and by the addition of Ang (1-7).
Fang – 2019 QJM (Fang et al., 2019)	Mouse (BALB/c)	Hyperoxic (95% O_2 for 72 hours) lung injury (HLI)	HLI decreased lung ACE2 expression/activity and increased the Ang II/Ang-(1-7) ratio. Lung injury, inflammation and oxidative stress were attenuated by an ACE2 agonist, and aggravated by an ACE2 inhibitor → Activation of ACE2 can reduce the severity of hyperoxic lung injury by inhibiting inflammatory response and oxidative stress. NK-kB and Nrf2 pathways are involved.

(Continued)

TABLE 1 | Continued

Publication	Model/Strain	Intervention	Main findings/conclusions
Ye – 2020 Exp Mol Pathol (Ye and Liu, 2020)	Mouse (C57BL/6)	Intravenous LPS-induced acute lung injury	LPS administration decreased expression of ACE2, and induced lung injury and inflammation. Lung injury was improved by injection of ACE2. Similar results were found *in vitro*. ACEI and ARB treatments alleviated LPS-induced lung injury.

ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; Ang II, angiotensin II; AT1R, angiotensin II receptor type 1; AT2R, angiotensin II receptor type 2; Ang-(1–7), angiotensin (1-7); ACEI, ACE inhibitor; ARB, angiotensin II receptor blocker; LPS, lipopolysaccharide; ERK, extracellular signal-regulated kinase; NfKB, nuclear factor-kappa B; PLGF, placental growth factor; VEGF, vascular endothelial growth factor; Nrf2, nuclear factor erythroid 2-related factor 2.

was worsened in *ACE2−/− knock out* mice compared to wild-type mice, and that recombinant human ACE2 protein partially rescued the phenotype. Conversely, the severity of acute lung injury was attenuated in *ACE−/− knock out* mice, in mice lacking the AT1 receptor, as well as in wild-type mice treated with the ARB losartan (Imai et al., 2005).

This crucial role of a disrupted ACE2-Ang (1-7)/ ACE-Ang II homeostasis balance has been confirmed by multiple other studies, in many other murine models of lung injury such as those induced by lipopolysaccharide (LPS) administration (Asperen et al., 2010; Wösten-van Asperen et al., 2011; Li et al., 2015; Ye and Liu, 2020), avian influenza A H5N1 (Yan et al., 2015), H7N9 (Yang et al., 2015), or respiratory syncytial virus (Gu et al., 2016) infections, seawater aspiration (Zhang et al., 2018), bleomycine administration (Wang L. et al., 2016), or hyperoxia (Fang et al., 2019). All these experimental models of acute lung injury have consistently shown a decreased lung expression of ACE2, and/or increased levels of plasma Ang II (Imai et al., 2005; Asperen et al., 2010; Wösten-van Asperen et al., 2011; Zou et al., 2014; Li et al., 2015; Yang et al., 2015; Gu et al., 2016; Fang et al., 2019), as well as a protective and deleterious roles of ACE2-Ang(1-7) and ACE-Ang II, respectively.

Regarding the specific pathogenicity of SARS-CoV, a series of experiments on lung injury induced by SARS-CoV or by the spike protein of the virus (Kuba et al., 2005) confirmed the crucial role of ACE2 as a receptor for the virus by showing that *ACE2−/− knock out* mice were markedly protected: viral replication as well as pulmonary lesions were strongly diminished compared with wild type mice. The authors also showed that upon SARS-CoV infection in wild-type mice, ACE2 protein expression in the lung was drastically reduced (Kuba et al., 2006; Gu and Korteweg, 2007). More specifically, binding of the spike protein of SARS-CoV to its receptor downregulated the latter both *in vitro* on cell lines and *in vivo* in the mouse. ACE2 downregulation disrupted the balance between ACE and ACE2 in the lung and increased levels of Ang-II, which then played a key role in lung injury. Downregulation of ACE2 induced by epithelial cell injury during acute respiratory distress syndrome might thereby be amplified by SARS-CoV-2 infection through endocytosis of ACE2 alongside viral particles.

In these models of lung injury, either induced by SARS-CoV or from other origins, the ARB losartan (Imai et al., 2005; Kuba et al., 2005; Asperen et al., 2010; Wösten-van Asperen et al., 2011; Wong et al., 2012; Yan et al., 2015; Gu et al., 2016; Zhang et al., 2018; Ye and Liu, 2020) and to a lesser extent the ACEIs captopril (Li et al., 2015) or enalapril (Ye and Liu, 2020) have been shown to restore the ACE/ACE2 balance and to attenuate lung lesions and inflammation. Likewise, the administration of recombinant ACE2 (Imai et al., 2005; Zou et al., 2014; Gu et al., 2016; Wang L. et al., 2016), of ACE2 agonists (Fang et al., 2019), or of synthetic Ang (1-7) (Wösten-van Asperen et al., 2011; Zhang et al., 2018) have also been shown to attenuate lung injury. Interestingly, the ability of RAAS blockers to restore the ACE2-Ang (1-7)/ ACE-Ang II balance was not only demonstrated in models of lung injury but also in different models of cardiovascular disease (for instance in a pig model of cardiac arrest), as reviewed in **Supplementary Tables 1A,B** (Wang G. et al., 2016).

However, as outlined above for studies on pharmacologically induced modifications of ACE2 expression, it is very important while interpreting these studies to carefully consider the limitations of the biochemical assays to quantify the components of the RAAS (Chappell, 2016; Sparks et al., 2020; Chappell et al., 2021).

Importantly, although detailed mechanisms are beyond the scope of this review, the imbalance between the ACE-Ang II and ACE2-Ang (1-7) pathways also likely contributes to the endothelial dysfunction, to the inflammation and cytokine storm, and to the pro-thrombotic state observed in patients with severe forms of the disease. For instance, animal studies of LPS- or viral-induced lung injury have also shown that pharmacological blockade of the ACE-AngII-AT1 receptor pathway, or recombinant ACE2, two ways of restoring the ACE/ACE2 homeostasis, were associated with a decrease of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) (Wong et al., 2012; Yan et al., 2015), and inhibited pivotal inflammatory mediators such as Toll-like receptor 4 (TLR4) (Ye and Liu, 2020), or NF-κB signaling pathways (Li et al., 2016; Fang et al., 2019). Reviews of these other crucial roles of ACE2 deregulation upon SARS-CoV-2 infection can be found elsewhere (Du et al., 2020; Pons et al., 2020; Zhang J. et al., 2020).

Overall, there are solid experimental data supporting a potential protective role of RAAS-blockers in SARS-CoV-2 pneumonia, through a restored ACE2/ACE balance. This is well-illustrated by the very large numbers of studies implemented early in the pandemic to examine whether ARBs such as losartan may be beneficial in patients with COVID-19, as detailed below.

RAAS and Lung Injury in Human

In line with these solid experimental results, there are some data supporting a similar role for the RAAS, and more specifically protective and deleterious roles for the ACE2-Ang (1-7)-Mas receptor and ACE-Ang II-AT1 receptor pathways, respectively, in human pneumonia and acute respiratory distress syndrome.

Interestingly, a study from China showed increased levels of Ang II in patients with COVID-19 (Liu Y. et al., 2020). In pneumoniae of other origins, such as infections with respiratory syncytial virus in children (Gu et al., 2016), H5N1 (Zou et al., 2014) or H7N9 (Huang et al., 2014; Yang et al., 2015) in adults, small-scaled studies had previously shown increased levels of Ang II in the serum of infected patients, especially in the acute phase of the disease, and that Ang II levels may be associated with disease progression. Of note, in some of these studies (Huang et al., 2014; Zou et al., 2014), the range order of reported Ang II concentrations, including in control subjects (from 1,000 to 10,000 pg/mL, highly variable, and much higher than expected concentrations) questions the methodology used to measure Ang II (Chappell et al., 2021). Importantly, these results obtained in pneumoniae of various origins show that the fact that ACE2 is the viral receptor may amplify disruption of the ACE2/ACE balance, but is not required for this phenomenon.

About half of the variance in plasma ACE activity is explained by the insertion/deletion (I/D) polymorphisms of the enzyme, the D allele being associated with a higher activity. In a population of 96 patients with acute respiratory distress syndrome, the DD genotype frequency was found to be higher than in several control cohorts (Marshall et al., 2002). Similarly, in a cohort of 44 Vietnamese patients with SARS in 2003, the frequency of the D allele was significantly higher in the hypoxemic group than in the non-hypoxemic group (Itoyama et al., 2004).

However, data supporting a protective role of RAAS blockers in human pneumonia are a lot scarcer than in experimental models. In a meta-analysis, Caldeira et al. (2012) have shown that patients treated with ACEIs had a lower incidence of pneumonia (OR 0.66, 95% CI 0.55–0.80 in a total of 19 studies), although a similar effect was not observed for ARBs. In the case of SARS-CoV-2 and COVID-19, numerous observational studies that have examined the link between these treatments and severity of the disease are summarized in the third part of this review.

EVIDENCE FROM CLINICAL STUDIES

Very early in the pandemic, most scientific societies took position (Bavishi et al., 2020), all in favor of a continued use of both ACEIs and ARBs in patients taking these medication as part of their chronic treatment (**Table 2**). These recommendations were based on the theoretical considerations and experimental data detailed above, on the clear cardiovascular and renal benefits associated with these medications (Vaduganathan et al., 2020), and on the well-documented risk associated with the discontinuation of RAAS blockers — at least in some indications such as heart failure (Halliday et al., 2019). However, these recommendations were issued before any clinical evidence was available to directly support them, and since then, many studies, mostly observational, have been published to address this issue and are summarized below and in **Supplementary Tables 4A,B**. However, as recently reviewed (Cohen et al., 2020a), observational studies designed to analyze the effects of RAAS blockers in the COVID-19 pandemic suffer methodological flaws and need to be interpreted with great caution. Many clinical trials have been implemented to provide definite answers regarding the management of these drugs in the context of COVID-19 (**Supplementary Table 5**).

Observational Studies: Association Between Chronic Use of RAAS Blockers and a Positive COVID-19 Test

Several studies have evaluated whether the chronic use of RAAS blockers was associated with an increased risk to contract a SARS-CoV-2 infection (**Supplementary Table 4A**). Mancia et al. (2020) conducted a population-based case-control study in the Lombardy region of Italy including a total of 6,272 patients with a confirmed SARS-CoV-2 infection matched with 30,759 beneficiaries of Regional Health Service as controls. Even though the use of ACEIs and ARBs was more frequent among patients than controls, this was driven by their higher prevalence of cardiovascular diseases. After adjustment, neither ACEIs (adjusted OR 0.96 and 95% CI 0.87–1.07) nor ARBs [0.95 (0.86–1.05)] were associated with COVID-19 or with a severe or fatal course of the disease. Similar conclusions were reported in two studies conducted in the United States. Mehta et al. (2020) analyzed data from 18,742 patients tested for COVID-19 in Ohio and Florida, of whom 2,285 were taking an ACEI or an ARB, and 1,735 had a positive test. No significant association was found between the use of RAAS blockers and test positivity (overlap propensity score–weighted OR, 0.97; 95%CI, 0.81–1.15) (Mehta et al., 2020). Likewise, in 12,594 patients tested for COVID-19 (of whom 5,894 were positive) in the state of New-York, Reynolds et al. (2020) reported that the likelihood of a positive test was not increased in users of ACEIs/ARBs compared to matched patients, in the total population as well as in the subgroup of hypertensive patients. In a case-control analysis of 571 patients with COVID-19 and 5,710 age- and sex-matched controls, all with hypertension, in Denmark, Fosbøl et al. (2020) reported that ACEI/ARB use was not significantly associated with a higher incidence of COVID-19 compared with other antihypertensive drugs [adjusted HR, 1.05 (95%CI, 0.80–1.36)]. In a case-control study conducted in South Korea using data from the Korean National Health Insurance System, 950 COVID-19 cases among 16,281 subjects with hypertension were retrospectively matched with 1,897 not infected controls, and multivariable-adjusted logistic regression demonstrated the absence of a significant association between exposure to RAAS-blockers and risk of COVID-19 (adjusted OR 1.161 [0.958–1.407)] (Son et al., 2020). Finally, in a large Israeli dataset of 14,520 individuals tested for SARS-CoV-2, of whom 1,317 were found positive, although ACEIs/ARBs were more frequent in positive cases than in negative cases, a multivariable logistic regression model found not significant association between the use of these medications and a positive result (adjusted OR = 1.19; 95% CI 0.96–1.47) (Chodick et al., 2020). A study conducted in Spain had a different design as it aimed to analyze the association between chronic use of RAAS blockers (compared to other antihypertensive drugs) and the risk of COVID-19 requiring hospital admission in 1,139 cases admitted with COVID-19 in seven hospitals in Madrid versus 11,390 matched controls admitted in 2018. Compared with

TABLE 2 | Recommendations from scientific societies regarding the use of RAAS blockers in the COVID-19 pandemic.

Society	Recommendation
European Society of Hypertension March 12, 2020	Recommend pursuing ACEIs/ARBs due to lack of evidence to support differential use in COVID-19 patients. In those with severe symptoms or sepsis, antihypertensive decision should be made on a case-by-case basis taking into account current guidelines
British and Irish Hypertension Society March 16, 2020	Recommend pursuing ACEI/ARB
Australian Diabetes Society March 29, 2020	Recommend pursuing anti-hypertensive drugs (including ACEI/ARB)
High Blood Pressure Research Council of Australia March 18, 2020	Recommend pursuing ACEI/ARB in the absence of data
European Renal Association – European Dialysis and Transplant Association March 13, 2020	Based on current evidence, recommend not to stop ARB or ACEI
American Heart Association March 17, 2020	Patients taking ACEI and ARB who contract COVID-19 should continue treatment, unless otherwise advised by their physician
Spanish Society of Hypertension March 16, 2020	Recommend that ACEI/ARB should not be empirically stopped in patients who are already taking them; in seriously ill patients, changes should be made on a case-by-base basis
American College of Physicians March 16, 2020	Recommend pursuing ACEI/ARB (no evidence linking them to COVID-19 severity and potential harm of stopping them)
The Renal Association (UK) March 15, 2020	Based on conflicting evidence from basic science studies about the likely effect that modulation of the renin-angiotensin system would have on infection, advise people taking ACEI/ARB to continue to take them
Canadian Cardiovascular Society March 15, 2020	Patients with confirmed or suspected COVID-19 infection should not stop taking an ACEI/ARB/ARNi unless there is a compelling reason to do so (such as symptomatic hypotension or shock, acute kidney injury, or hyperkalemia)
European Society of Cardiology March 13, 2020	Strongly recommend that physicians and patients should continue treatment with their usual anti-hypertensive therapy because there is no clinical or scientific evidence to suggest that treatment with ACEI or ARBs should be discontinued because of the Covid-19 infection
Hypertension Canada March 13, 2020	Strongly encourage continuing ACEI/ARB and Angiotensin Receptor Neprilysin Inhibitors due to a lack of clinical evidence to support withdrawal of these agents
International Society of Hypertension March 16, 2020	There is no good evidence to change the use of ACE-inhibitors or ARBs for the management of raised blood pressure in the context of avoiding or treating COVID-19 infection

users of other antihypertensive drugs, users of RAAS inhibitors had an adjusted OR for COVID-19 requiring admission to hospital of 0.94 (95% CI 0·77–1·15). Similar results were found for fatal cases and patients admitted to intensive care units (de Abajo et al., 2020).

Overall, the vast majority of observational studies, in different regions of the world, with different designs and adjustment procedures, consistently concluded that long-term treatment with RAAS-blockers was not associated with an increased adjusted risk of SARS-CoV-2 infection (**Supplementary Table 4A**).

Observational Studies: Association Between Chronic Use of RAAS Blockers and Outcome of the Disease in Infected Patients

Multiple observational studies had a different setting as they aimed at evaluating the association between chronic use of RAAS-blockers and outcome of the disease in patients with established COVID-19 infection and are listed in **Supplementary Table 4B**.

Most studies were conducted in hospitalized patients. Some had no restriction on hypertension and analyzed the association between RAAS blockers and outcome in unselected inpatients with COVID-19 (Bean et al., 2020; Holt et al., 2020; Iaccarino et al., 2020; Lahens et al., 2020; Liabeuf et al., 2020; Shah et al., 2020). Except for one study which showed an increased risk of severe disease despite – potentially insufficient – adjustment (Liabeuf et al., 2020), and another study which found a negative association between RAAS blocker exposure and the composite of death or transfer to ICU (Bean et al., 2020), all other studies, and in particular all those which analyzed mortality as an outcome, found no association between exposure and outcome after adjustment, although an increased risk was frequent in crude analyses. For instance, in an Italian cohort of 1,581 patients admitted for COVID-19 in 26 hospitals, Iaccarino et al. (2020) reported that neither ACEIs nor ARBS were associated with mortality after adjustment for confounders, although ACEIs were more frequently used in non-survivors. Similarly, in 531 African American patients hospitalized with COVID-19 in Georgia (United States), of whom 207 were on ACEIs/ARBs at baseline, after adjustment for covariates, there was no difference between users and non-users of these drugs in outcomes including mortality (Shah et al., 2020). Other studies restricted analyses to hypertensive patients (Gao et al., 2020; Pan et al., 2020; Tedeschi et al., 2020), or reported results in the subgroup of hypertensive patients (Andrea et al., 2020; Richardson et al., 2020; Trifirò et al., 2020). Similarly, the vast majority of these studies found no association between RAAS blockers and outcome, and in hypertensive patients this was true in unadjusted as well as adjusted analyses. Trifirò et al. (2020) analyzed the charts from 42,926 COVID-19 hospitalized patients by combining multiple Italian databases accounting for

approximately a quarter of the Italian population. Almost 50% of the patients had at least one antihypertensive drug claim within 3 months prior to admission. Compared to calcium-channel blockers users, adjusted analyses showed no difference in the risk of death among ACEI (Hazard ratio (HR) 1.01, 95% CI [0.92; 1.12)] or ARB (HR 1.03, 95% CI [0.93; 1.14)] users (Trifirò et al., 2020).

A few studies included both inpatients and outpatients. Again, some had no restriction on hypertension (Fosbøl et al., 2020; Mehta et al., 2020) while others reported results in hypertensive patients (Bravi et al., 2020; Felice et al., 2020; Jung S.Y. et al., 2020; Reynolds et al., 2020; Son et al., 2020) or in patients with an indication for RAAS blockers (Giorgi Rossi et al., 2020). Overall, in most studies, findings were similar to those obtained in hospitalized patients, with no significant association between chronic RAAS blocker exposure and outcome of COVID-19 in adjusted analyses. In a nationwide population-based cohort study of 5,179 confirmed COVID-19 cases in South Korea, Jung et al., found that prior use of RAAS blockers was associated with an increased risk of in-hospital mortality in unadjusted analyses [OR 3.88, 95% CI (2.48; 6.05)], but this difference was ironed out when adjusted for age, sex, Charlson comorbidity index, immunosuppression and hospital type [adjusted OR: 0.88, 95% CI ([0.53; 1.44)]. In the subgroup of 1,157 hypertensive patients, there was no association between RAAS blockers and mortality, both in unadjusted [OR 0.74, 95% CI (0.43; 1.28)] and adjusted [0.71, 95% CI (0.40; 1.26)] analyses (Jung S.Y. et al., 2020). In a Danish retrospective cohort of 4,480 patients with COVID-19, of whom 895 were chronic ACEI/ARB users in a 6-month period prior to diagnosis, the unadjusted HR for mortality was 2.65 [95% CI (2.18; 3.23)] while the adjusted HR, after accounting for age and medical history, was 0.83 [95% CI (0.67; 1.03)] (Fosbøl et al., 2020). Reynolds et al. (2020) reported similar results among a cohort of 5,894 patients with COVID-19, of whom 2,573 with hypertension. After careful adjustment, no significant association was found between chronic-exposure to ACEIs/ARBs and severe illness, defined as a composite of intensive care admission, mechanical ventilation, or death, in all patients as well as in hypertensive patients (Reynolds et al., 2020).

Altogether, if some studies conducted in unselected population identified an association of RAAS blocker prescription with mortality in unadjusted analyses, once adjustment for age, sex and comorbidities was performed, chronic use of RAAS-blockers was not associated with worse outcome (in particular mortality) among patients with COVID-19, hospitalized or not. All these studies support the above-mentioned recommendations from scientific societies, not to discontinue these treatments despite the ongoing pandemic.

One randomized trial (CORONACION, NCT 04330300) had been designed to address this specific question in Ireland. The aim was to randomize patients with hypertension taking ACEIs/ARBs to either continue or switch to an alternative blood pressure medication and analyze COVID-19-related events. However, this trial has been interrupted due to a low incidence of COVID-19 in the study site.

Although potentially highly relevant, the specific role of mineralocorticoid receptor antagonists has been less studied, in part because these drugs are much less frequently prescribed than ACEIs and ARBs. In a recent very large observational study from a Swedish national registry, among 1,387,746 patients with a potential indication for RAAS blockers, 5.8% received a mineralocorticoid receptor antagonist. These medications were not associated with the risks of hospitalization for COVID-19 or mortality after adjustment for confounders (Savarese et al., 2020).

Observational Studies: Association Between in-Hospital Use of RAAS Blockers and Outcome of the Disease in Infected Hospitalized Patients

Most studies which analyzed "in-hospital" (and not chronic) exposure to treatment showed a strong protective effect associated with the use of RAAS blockers after adjustment for baseline comorbidities (Cannata et al., 2020; Chaudhri et al., 2020; Meng et al., 2020; Yang et al., 2020; Zhang P et al., 2020; Zhou et al., 2020a). For instance, in a retrospective analysis of 1,128 COVID-19 patients with hypertension admitted in 9 hospitals in the epicenter region of the pandemic in Hubei, China, authors compared patients based on their in-hospital anti-hypertensive regimen. They recorded 188 patients receiving ACEI/ARB during hospitalization and all-cause mortality at 28 days was significantly lower among them. This effect remained significant in a mixed-effect Cox model (using site as a random effect, after adjusting for age, gender, comorbidities, and in-hospital medications), with an adjusted hazard ratio of 0.42 [0.19–0.92] and in a propensity score-matched analysis (adjusted HR, 0.37; 95% CI, 0.15–0.89) (Zhang P et al., 2020).

However, as outlined in a letter by Cohen et al. (2020b) to warn the reader against interpretation of the study by Zhang P et al. (2020), as demonstrated in a dedicated study from our team (Lahens et al., 2020), and as recently discussed in a review on the methodology of observational studies focused on the issue of RAAS blockers and COVID-19 (Cohen et al., 2020a), this protective effect is majorly biased. Indeed, our study showed that cessation of a chronic RAAS blocker exposure upon hospital admission is frequent and occurs in those with the worst outcomes, and conversely for treatment continuation (Lahens et al., 2020). Therefore, treatment discontinuation is related directly or indirectly to disease severity and mortality. This induces a phenomenon of reverse causality whereby severity of the disease causes treatment cessation, and not the reverse. Conversely, the continued treatment arm is prone to immortal-time bias (Suissa, 2008). Overall, this generates a "healthy user-sick stopper bias" explaining that studies based on in-hospital exposure (instead of chronic exposure) find a spurious protective association between RAAS blockers and outcome in COVID-19 (**Supplementary Table 4B**).

Observational pharmaco-epidemiological studies are not suited to analyze the association between in-hospital exposure to RAAS blockers and outcome of COVID-19. The answer to this issue can only be provided by interventional randomized trials.

It is very important to note that most meta-analyses to date meant to analyze the association between RAAS-blockers and outcome have incorporated studies based on in-hospital exposure (Baral et al., 2020; Flacco et al., 2020; Greco et al., 2020; Grover and Oberoi, 2020; Guo X. et al., 2020; Mackey et al., 2020; Pranata et al., 2020; Zhang X. et al., 2020), so that their conclusions should be interpreted with great caution.

Clinical Trials: Randomized Studies on Discontinuation or Continuation of RAAS Blockers in Previously Treated Patients Hospitalized for COVID-19

As of January 2021, optimal management of ACEIs/ARBs in patients with a COVID-19 infection remains uncertain. Several trials randomizing COVID-19 patients previously treated with RAAS blockers and admitted to hospital for treatment continuation or discontinuation (ACORES-2 in France, NCT04329195; ACEI-COVID in Austria, NCT04353596; RASCOVID-19 in Denmark, NCT04351581; RAASCOVID in Canada, NCT04508985; SWITCH-COVID in Brazil, NCT04493359) are currently recruiting (**Supplementary Table 5**), while results have been published for two trials.

The BRACE-CORONA study (NCT04364893) enrolled 659 patients hospitalized with a confirmed diagnosis of COVID-19 from 29 sites in Brazil, with chronic use of ACEIs/ARBs and randomly allocated to continuing or stopping these treatments for 30 days (Lopes et al., 2020). Results were presented at the European Society of Cardiology Congress in September 2020 and published very recently (Lopes et al., 2021). No difference was reported in the number of days alive and out of hospital at 30 days (primary outcome) between the suspending ACEIs/ARBs group and the continuing group. There was no difference in all-cause mortality at 30 days either (HR 0.97 (95% CI [0.38; 2.52)].

An international randomized trial (REPLACE COVID, NCT04338009) was published in January 2021: 152 patients hospitalized for COVID-19 and receiving an ACEI or an ARB before admission were randomly assigned to continuation or discontinuation of this treatment. No difference in the primary endpoint assessing severity of disease course (a global rank

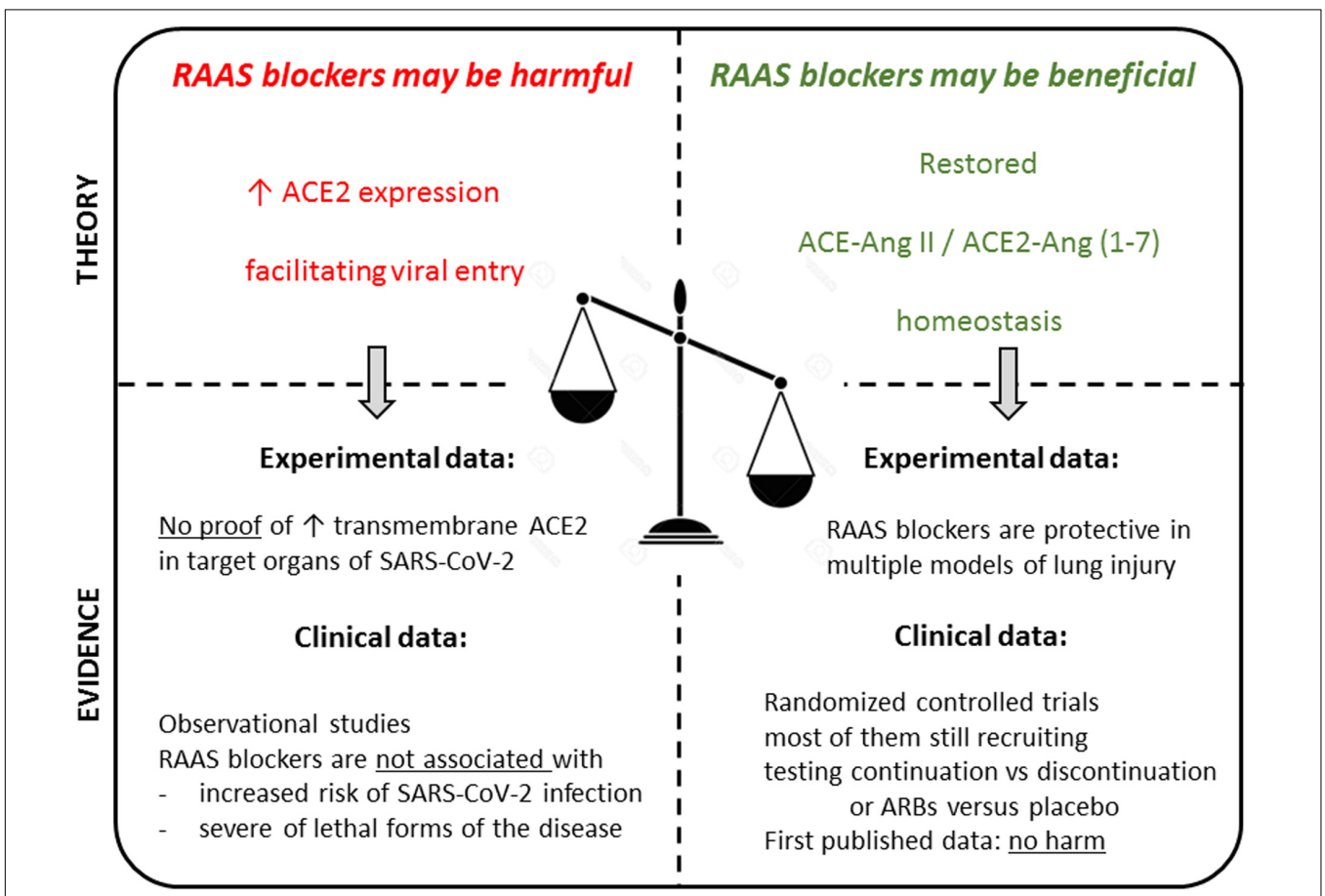


FIGURE 2 | Summarizing illustration. Theoretical pros and cons in the debate on the use of RAAS blockers in the context of the COVID-19 pandemic, and available experimental and clinical evidence to date. Overall, there are no evidence-based data supporting the discontinuation of these medications in the general population, or in patients with COVID-19. In hospitalized patients, these drugs should be managed according to usual clinical practice, taking into account hemodynamics and kidney function.

score across four hierarchical tiers incorporating time to death, duration of mechanical ventilation, time on renal replacement or vasopressor therapy, and multiorgan dysfunction during the hospitalization) was observed between patients who continued or discontinued RAAS blocker therapy (Cohen et al., 2021). The authors concluded that RAAS blockers can be safely continued in patients with COVID-19 requiring hospital admission.

Clinical Trials: Randomized Studies on the Use of ARBs in Patients Infected With COVID-19

An even larger number of trials are testing the hypothesis that RAAS blockers, and in particular ARBs, might be beneficial in patients with SARS-CoV-2 pneumonia. We have identified 21 such trials, listed in **Supplementary Table 5**. For instance, two trials sponsored by the University of Minnesota (United States) are testing the efficiency of losartan in patients with COVID-19 either requiring hospitalization (assessing the respiratory severity at day 7, NCT04312009) or not requiring hospitalization (assessing the rate of hospital admission within 15 days of randomization, NCT04311177). Other trials are focusing on elderly patients: two French trials are assessing the effectiveness of telmisartan in elderly hospitalized patients (COVID-Aging, coordinated in Strasbourg, evaluating the 2-week survival rate, NCT04359953) or in outpatients (COVERAGE, coordinated in Bordeaux, evaluating a composite of hospitalization or death at day 14, NCT04356495). Other ongoing trials worldwide are listed in **Supplementary Table 5**.

CONCLUSION

Whereas an increased tissue expression of ACE2, either due to underlying conditions or to pharmacological treatment, might potentially facilitate SARS-CoV-2 infection and/or more severe forms of the disease on the one hand, on the other hand a higher ACE2 activity could be beneficial in infected patients by attenuating lung injury and inflammation. Because of these opposite potential effects of ACE2 on the disease (Wang K. et al., 2020), the role of RAAS blockers, which may modulate ACE2 expression and activity, is controversial (**Figure 2**).

Several large studies seem to have ruled out that chronic exposure to RAAS blockers may predispose patients to infection, while studies on the association between exposure to RAAS blockers and severity of the disease in patients with COVID-19 have yielded more discrepant results. However, these apparent discrepancies are largely explained by disparities in study design, exposure measurement, adjustment methodologies, and often small sample size. Properly designed studies are also reassuring and consistently found no significant association between chronic exposure to RAAS blockers and outcome in infected patients. Preliminary results from randomized clinical trials did not raise concern regarding the continued use of the treatments in hospitalized patients (**Figure 2**).

Overall, available data corroborate statements from scientific societies against the preventive discontinuation of RAAS blockers in the general population, while management of these medications in infected patients, especially in hospitalized patients, should be clarified by the results of ongoing studies.

Despite extensive worldwide research since the discovery of ACE2 in 2000, the SARS outbreak in 2003, and the COVID-19 pandemic since December 2019, many gaps in knowledge remain regarding the regulation of ACE2 and its implications in the pathogenesis of SARS-CoV-2. The discrepancies in the literature highlight the need for integrated translational research projects, from molecular biology to animal and human pathophysiology, especially as many potential therapeutic targets will be directly impacted by a better knowledge of ACE2, a double-edged sword against the virus.

AUTHOR CONTRIBUTIONS

EV-P conceived the concept of the manuscript, drew the figures, and revised the manuscript and tables. SBG wrote the first draft of the manuscript and tables. All authors contributed to the literature review, wrote iterative drafts of manuscript and tables, and approved the final version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.624052/full#supplementary-material

Supplementary Table 1 | Influence of pharmacological blockade of the renin-angiotensin system on the expression of ACE2: animal models.

Supplementary Table 2 | Influence of the pharmacological blockade of the renin-angiotensin system on the expression of ACE2: human studies.

Supplementary Table 3 | Representative selection of large-scaled studies on the association between cardiovascular or metabolic comorbidities and outcome in COVID-19.

Supplementary Table 4 | Observational studies evaluating the impact of ACEIs/ARBs on the risk of a positive COVID-19 test **(A)** and on the course of the disease in infected patients **(B)**.

Supplementary Table 5 | Ongoing clinical trials (last update: January 25th 2021).

REFERENCES

Agata, J., Ura, N., Yoshida, H., Shinshi, Y., Sasaki, H., Hyakkoku, M., et al. (2006). Olmesartan is an Angiotensin II receptor blocker with an inhibitory effect on angiotensin-converting enzyme. *Hypertens. Res.* 29, 865–874. doi: 10.1291/hypres.29.865

Albitar, O., Ballouze, R., Ooi, J. P., and Sheikh Ghadzi, S. M. (2020). Risk factors for mortality among COVID-19 patients. *Diabetes Res. Clin. Pract.* 166, 108293. doi: 10.1016/j.diabres.2020.108293

Andrea, C., Francesco, M., Antonio, N., Evgeny, F., Marzia, S., Fabio, C., et al. (2020). Renin-angiotensin-aldosterone system inhibitors and outcome in

patients with SARS-CoV-2 pneumonia: a case series study. *Hypertension* 76, e10–e12. doi: 10.1161/HYPERTENSIONAHA.120.15312

Anguiano, L., Riera, M., Pascual, J., Valdivielso, J. M., Barrios, C., Betriu, A., et al. (2015). Circulating angiotensin-converting enzyme 2 activity in patients with chronic kidney disease without previous history of cardiovascular disease. *Nephrol. Dial. Transplant.* 30, 1176–1185. doi: 10.1093/ndt/gfv025

Aronson, J. K., and Ferner, R. E. (2020). Drugs and the renin-angiotensin system in covid-19. *BMJ* 369:m1313. doi: 10.1136/bmj.m1313

Asperen, R. M. W., Lutter, R., Specht, P. A. C., van Woensel, J. B., van der Loos, C. M., Florquin, S., et al. (2010). Ventilator-induced inflammatory response in lipopolysaccharide-exposed rat lung is mediated by angiotensin-converting enzyme. *The Am. J. Pathol.* 176, 2219–2227. doi: 10.2353/ajpath.2010.090565

Bao, L., Deng, W., Huang, B., Gao, H., Liu, J., Ren, L., et al. (2020). The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. *Microbiology* 583, 830–833. doi: 10.1101/2020.02.07.939389

Baral, R., White, M., and Vassiliou, V. S. (2020). Effect of renin-angiotensin-aldosterone system inhibitors in patients with COVID-19: a systematic review and meta-analysis of 28,872 patients. *Curr. Atheroscler. Rep.* 22:61. doi: 10.1007/s11883-020-00880-6

Batlle, D., Wysocki, J., and Satchell, K. (2020). Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy? *Clin. Sci.* 134, 543–545. doi: 10.1042/CS20200163

Bavishi, C., Maddox, T. M., and Messerli, F. H. (2020). Coronavirus Disease 2019 (COVID-19) infection and renin angiotensin system blockers. *JAMA Cardiol.* 5:745. doi: 10.1001/jamacardio.2020.1282

Bean, D. M., Kraljevic, Z., Searle, T., Bendayan, R., Kevin, O., Pickles, A., et al. (2020). Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers are not associated with severe COVID-19 infection in a multi-site UK acute hospital trust. *Eur. J. Heart Fail.* 22, 967–974. doi: 10.1002/ejhf.1924

Berenguer, J., Ryan, P., Rodríguez-Baño, J., Jarrín, I., Carratalà, J., Pachón, J., et al. (2020). Characteristics and predictors of death among 4035 consecutively hospitalized patients with COVID-19 in Spain. *Clin. Microbiol. Infect.* 26, 1525–1536. doi: 10.1016/j.cmi.2020.07.024

Boulle, A., Davies, M.-A., Hussey, H., Ismail, M., Morden, E., Vundle, Z., et al. (2020). Risk factors for COVID-19 death in a population cohort study from the Western Cape Province, South Africa. *Clin. Infect. Dis.* ciaa1198. doi: 10.1093/cid/ciaa1198

Bravi, F., Flacco, M. E., Carradori, T., Volta, C. A., Cosenza, G., De Togni, A., et al. (2020). Predictors of severe or lethal COVID-19, including angiotensin converting enzyme inhibitors and Angiotensin II receptor blockers, in a sample of infected Italian citizens. *PLoS One* 15:e0235248. doi: 10.1371/journal.pone.0235248

Burchill, L. J., Velkoska, E., Dean, R. G., Griggs, K., Patel, S. K., and Burrell, L. M. (2012). Combination renin–angiotensin system blockade and angiotensin-converting enzyme 2 in experimental myocardial infarction: implications for future therapeutic directions. *Clin. Sci.* 123, 649–658. doi: 10.1042/CS20120162

Burrell, L. M., Burchill, L., Dean, R. G., Griggs, K., Patel, S. K., and Velkoska, E. (2012). Chronic kidney disease: cardiac and renal angiotensin-converting enzyme (ACE) 2 expression in rats after subtotal nephrectomy and the effect of ACE inhibition: angiotensin-converting enzyme 2 and chronic kidney disease. *Exp. Physiol.* 97, 477–485. doi: 10.1113/expphysiol.2011.063156

Burrell, L. M., Risvanis, J., Kubota, E., Dean, R. G., MacDonald, P. S., Lu, S., et al. (2005). Myocardial infarction increases ACE2 expression in rat and humans. *Eur. Heart J.* 26, 369–375; discussion 322–324.

Caldeira, D., Alarcao, J., Vaz-Carneiro, A., and Costa, J. (2012). Risk of pneumonia associated with use of angiotensin converting enzyme inhibitors and angiotensin receptor blockers: systematic review and meta-analysis. *BMJ* 345:e4260. doi: 10.1136/bmj.e4260

Cannata, F., Chiarito, M., Reimers, B., Azzolini, E., Ferrante, G., My, I., et al. (2020). Continuation versus discontinuation of ACE inhibitors or angiotensin II receptor blockers in COVID-19: effects on blood pressure control and mortality. *Eur. Heart J. Cardiovasc. Pharmacother.* 6, 412–414. doi: 10.1093/ehjcvp/pvaa056

Cariou, B., Hadjadj, S., Wargny, M., Pichelin, M., Al-Salameh, A., Allix, I., et al. (2020). Phenotypic characteristics and prognosis of inpatients with COVID-19 and diabetes: the CORONADO study. *Diabetologia* 63, 1500–1515. doi: 10.1007/s00125-020-05180-x

Cen, Y., Chen, X., Shen, Y., Zhang, X.-H., Lei, Y., Xu, C., et al. (2020). Risk factors for disease progression in patients with mild to moderate coronavirus disease 2019—a multi-centre observational study. *Clin. Microbiol. Infect.* 26, 1242–1247. doi: 10.1016/j.cmi.2020.05.041

Chappell, M. C. (2016). Biochemical evaluation of the renin-angiotensin system: the good, bad, and absolute? *Am. J. Physiol. Heart Circ. Physiol.* 310, H137–H152. doi: 10.1152/ajpheart.00618.2015

Chappell, M. C., Pirro, N. T., South, A. M., and Gwathmey, T. M. (2021). Concerns on the specificity of commercial ELISAs for the measurement of Angiotensin-(1-7) and Angiotensin II in human plasma. *Hypertension* [Epub ahead of print]. doi: 10.1161/HYPERTENSIONAHA.120.16724

Chaudhri, I., Koraishy, F. M., Bolotova, O., Yoo, J., Marcos, L. A., Taub, E., et al. (2020). Outcomes associated with the use of renin-angiotensin-aldosterone system blockade in hospitalized patients with SARS-CoV-2 infection. *Kidney* 360, 801–809. doi: 10.34067/KID.0003792020

Chen, J., Bai, H., Liu, J., Chen, G., Liao, Q., Yang, J., et al. (2020). Distinct clinical characteristics and risk factors for mortality in female inpatients with Coronavirus Disease 2019 (COVID-19): a sex-stratified, large-scale cohort study in Wuhan, China. *Clinical Infectious Diseases* [Epub ahead of print]. doi: 10.1093/cid/ciaa920

Chen, L., Yu, J., He, W., Chen, L., Yuan, G., Dong, F., et al. (2020). Risk factors for death in 1859 subjects with COVID-19. *Leukemia* 34, 2173–2183. doi: 10.1038/s41375-020-0911-0

Chen, Y., Guo, Y., Pan, Y., and Zhao, Z. J. (2020). Structure analysis of the receptor binding of 2019-nCoV. *Biochem. Biophys. Res. Commun.* 525, 135–140. doi: 10.1016/j.bbrc.2020.02.071

Chirinos, J. A., Cohen, J. B., Zhao, L., Hanff, T., Sweitzer, N., Fang, J., et al. (2020). Clinical and proteomic correlates of Plasma ACE2 (Angiotensin-Converting Enzyme 2) in human heart failure. *Hypertension* 76, 1526–1536. doi: 10.1161/HYPERTENSIONAHA.120.15829

Chodick, G., Nutman, A., Yiekutiel, N., and Shalev, V. (2020). Angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers are not associated with increased risk of SARS-CoV-2 infection. *J. Travel Med.* 27:taaa069. doi: 10.1093/jtm/taaa069

Cohen, J. B., D'Agostino-McGowan, L., Jensen, E. T., Rigdon, J., and South, A. M. (2020a). Evaluating sources of bias in observational studies of angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker use during coronavirus disease 2019: beyond confounding. *J. Hypertens.* [Epub ahead of print]. doi: 10.1097/HJH.0000000000002706

Cohen, J. B., Hanff, T. C., South, A. M., Sparks, M. A., Hiremath, S., Bress, A. P., et al. (2020b). Response by cohen et al to letter regarding article, "association of inpatient use of angiotensin-converting enzyme inhibitors and Angiotensin II receptor blockers with mortality among patients with hypertension hospitalized with COVID-19". *Circ. Res.* 126, e140–e141. doi: 10.1161/CIRCRESAHA.120.317205

Cohen, J. B., Hanff, T. C., William, P., Sweitzer, N., Rosado-Santander, N. R., Medina, C., et al. (2021). Continuation versus discontinuation of renin–angiotensin system inhibitors in patients admitted to hospital with COVID-19: a prospective, randomised, open-label trial. *Lancet Respir. Med.* [Epub ahead of print]. doi: 10.1016/S2213-2600(20)30558-0

Cunningham, J. W., Vaduganathan, M., Claggett, B. L., Jering, K. S., Bhatt, A. S., Rosenthal, N., et al. (2020). Clinical outcomes in young us adults hospitalized with COVID-19. *JAMA Internal Medicine* [Epub ahead of print]. doi: 10.1001/jamainternmed.2020.5313

de Abajo, F. J., Rodríguez-Martín, S., Lerma, V., Mejía-Abril, G., Aguilar, M., García-Luque, A., et al. (2020). Use of renin–angiotensin–aldosterone system inhibitors and risk of COVID-19 requiring admission to hospital: a case-population study. *Lancet* 395, 1705–1714. doi: 10.1016/S0140-6736(20)31030-8

Deshotels, M. R., Xia, H., Sriramula, S., Lazartigues, E., and Filipeanu, C. M. (2014). Angiotensin II mediates angiotensin converting enzyme type 2 internalization and degradation through an Angiotensin II Type I receptor–dependent mechanism. *Hypertension* 64, 1368–1375. doi: 10.1161/HYPERTENSIONAHA.114.03743

Diaz, J. H. (2020). Hypothesis: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the risk of severe COVID-19. *J. Travel Med.* 27:taaa041. doi: 10.1093/jtm/taaa041

Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., et al. (2000). A novel angiotensin-converting enzyme–related carboxypeptidase (ACE2) converts Angiotensin I to Angiotensin 1-9. *Circ. Res.* 87, E1–E9. doi: 10.1161/01.RES.87.5.e1

Du, F., Liu, B., and Zhang, S. (2020). COVID-19: the role of excessive cytokine release and potential ACE2 down-regulation in promoting hypercoagulable state associated with severe illness. *J. Thromb. Thrombol.* [Epub ahead of print]. doi: 10.1007/s11239-020-02224-2

Epelman, S., Shrestha, K., Troughton, R. W., Francis, G. S., Sen, S., Klein, A. L., et al. (2009). Soluble angiotensin-converting enzyme 2 in human heart failure: relation with myocardial function and clinical outcomes. *J. Cardiac. Fail.* 15, 565–571. doi: 10.1016/j.cardfail.2009.01.014

Epelman, S., Tang, W. H. W., Chen, S. Y., Van Lente, F., Francis, G. S., and Sen, S. (2008). Detection of soluble angiotensin-converting enzyme 2 in heart failure. *J. Am. Coll. Cardiol.* 52, 750–754. doi: 10.1016/j.jacc.2008.02.088

Esler, M., and Esler, D. (2020). Can angiotensin receptor-blocking drugs perhaps be harmful in the COVID-19 pandemic? *J. Hypertens.* 38, 781–782. doi: 10.1097/HJH.0000000000002450

Fang, L., Karakiulakis, G., and Roth, M. (2020). Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir. Medicine* 8, e21. doi: 10.1016/S2213-2600(20)30116-8

Fang, Y., Gao, F., and Liu, Z. (2019). Angiotensin-converting enzyme 2 attenuates inflammatory response and oxidative stress in hyperoxic lung injury by regulating NF-κB and Nrf2 pathways. *QJM Int. J. Med.* 112, 914–924. doi: 10.1093/qjmed/hcz206

Felice, C., Nardin, C., Di Tanna, G. L., Grossi, U., Bernardi, E., Scaldaferri, L., et al. (2020). Use of RAAS inhibitors and risk of clinical deterioration in COVID-19: results from an Italian cohort of 133 hypertensives. *Am. J. Hypertens.* 33, 944–948. doi: 10.1093/ajh/hpaa096

Ferrario, C. M., Jessup, J., Chappell, M. C., Averill, D. B., Brosnihan, K. B., Tallant, E. A., et al. (2005a). Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation* 111, 2605–2610. doi: 10.1161/CIRCULATIONAHA.104.510461

Ferrario, C. M., Jessup, J., Gallagher, P. E., Averill, D. B., Brosnihan, K. B., Ann Tallant, E., et al. (2005b). Effects of renin-angiotensin system blockade on renal angiotensin-(1-7) forming enzymes and receptors. *Kid. Int.* 68, 2189–2196. doi: 10.1111/j.1523-1755.2005.00675.x

Flacco, M. E., Acuti Martellucci, C., Bravi, F., Parruti, G., Cappadona, R., Mascitelli, A., et al. (2020). Treatment with ACE inhibitors or ARBs and risk of severe/lethal COVID-19: a meta-analysis. *Heart* 106, 1519–1524. doi: 10.1136/heartjnl-2020-317336

Forouzanfar, M. H., Liu, P., Roth, G. A., Ng, M., Biryukov, S., Marczak, L., et al. (2017). Global burden of hypertension and systolic blood pressure of at least 110 to 115 mm Hg, 1990-2015. *JAMA* 317:165. doi: 10.1001/jama.2016.19043

Fosbøl, E. L., Butt, J. H., Østergaard, L., Andersson, C., Selmer, C., Kragholm, K., et al. (2020). Association of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker use with COVID-19 diagnosis and mortality. *JAMA* 324:168. doi: 10.1001/jama.2020.11301

Fried, M. W., Crawford, J. M., Mospan, A. R., Watkins, S. E., Munoz Hernandez, B., Zink, R. C., et al. (2020). Patient characteristics and outcomes of 11,721 patients with COVID19 hospitalized across the United States. *Clin. Infect. Dis.* ciaa1268. doi: 10.1093/cid/ciaa1268

Furuhashi, M., Moniwa, N., Mita, T., Fuseya, T., Ishimura, S., Ohno, K., et al. (2015). Urinary angiotensin-converting enzyme 2 in hypertensive patients may be increased by olmesartan, an angiotensin II receptor blocker. *Am. J. Hypertens.* 28, 15–21. doi: 10.1093/ajh/hpu086

Gallagher, P. E., Chappell, M. C., Ferrario, C. M., and Tallant, E. A. (2006). Distinct roles for ANG II and ANG-(1–7) in the regulation of angiotensin-converting enzyme 2 in rat astrocytes. *Am. J. Physiol. Cell Physiol.* 290, C420–C426. doi: 10.1152/ajpcell.00409.2004

Gallagher, P. E., Ferrario, C. M., and Tallant, E. A. (2008a). Regulation of ACE2 in cardiac myocytes and fibroblasts. *Am. J. Physiol. Heart Circ. Physiol.* 295, H2373–H2379. doi: 10.1152/ajpheart.00426.2008

Gallagher, P. E., Ferrario, C. M., and Tallant, E. A. (2008b). MAP kinase/phosphatase pathway mediates the regulation of ACE2 by angiotensin peptides. *Am. J. Physiol. Cell Physiol.* 295, C1169–C1174. doi: 10.1152/ajpcell.00145.2008

Gao, C., Cai, Y., Zhang, K., Zhou, L., Zhang, Y., Zhang, X., et al. (2020). Association of hypertension and antihypertensive treatment with COVID-19 mortality: a retrospective observational study. *Eur. Heart J.* 41, 2058–2066. doi: 10.1093/eurheartj/ehaa433

Giorgi Rossi, P., Marino, M., Formisano, D., Venturelli, F., Vicentini, M., and Grilli, R. (2020). Characteristics and outcomes of a cohort of COVID-19 patients in the Province of Reggio Emilia, Italy. *PLoS One* 15:e0238281. doi: 10.1371/journal.pone.0238281

Greco, A., Buccheri, S., D'Arrigo, P., Calderone, D., Agnello, F., Monte, M., et al. (2020). Outcomes of renin–angiotensin–aldosterone system blockers in patients with COVID-19: a systematic review and meta-analysis. *Eur. Heart J. Cardiovasc. Pharmacother.* 6, 335–337. doi: 10.1093/ehjcvp/pvaa074

Grover, A., and Oberoi, M. (2020). A systematic review and meta-analysis to evaluate the clinical outcomes in COVID-19 patients on angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. *Eur. Heart J. Cardiovasc. Pharmacother.* [Epub ahead of print]. doi: 10.1093/ehjcvp/pvaa064

Gu, H., Xie, Z., Li, T., Zhang, S., Lai, C., Zhu, P., et al. (2016). Angiotensin-converting enzyme 2 inhibits lung injury induced by respiratory syncytial virus. *Sci. Rep.* 6:19840. doi: 10.1038/srep19840

Gu, J., and Korteweg, C. (2007). Pathology and pathogenesis of severe acute respiratory syndrome. *Am. J. Pathol.* 170, 1136–1147. doi: 10.2353/ajpath.2007.061088

Guo, X., Zhu, Y., and Hong, Y. (2020). Decreased mortality of COVID-19 with renin-angiotensin-aldosterone system inhibitors therapy in patients with hypertension: a meta-analysis. *Hypertension* 76, e13–e14. doi: 10.1161/HYPERTENSIONAHA.120.15572

Gupta, S., Hayek, S. S., Wang, W., Chan, L., Mathews, K. S., Melamed, M. L., et al. (2020). Factors associated with death in critically ill patients with coronavirus disease 2019 in the US. *JAMA Intern. Med.* 180, 1–12. doi: 10.1001/jamainternmed.2020.3596

Halliday, B. P., Wassall, R., Lota, A. S., Khalique, Z., Gregson, J., Newsome, S., et al. (2019). Withdrawal of pharmacological treatment for heart failure in patients with recovered dilated cardiomyopathy (TRED-HF): an open-label, pilot, randomised trial. *Lancet* 393, 61–73. doi: 10.1016/S0140-6736(18)32484-X

Hamming, I., Timens, W., Bulthuis, M., Lely, A., Navis, G., and van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203, 631–637. doi: 10.1002/path.1570

Hamming, I., Van Goor, H., Turner, A. J., Rushworth, C. A., Michaud, A. A., Corvol, P., et al. (2008). Differential regulation of renal angiotensin-converting enzyme (ACE) and ACE2 during ACE inhibition and dietary sodium restriction in healthy rats: renal ACE and ACE2 during ACE inhibition and low salt. *Exp. Physiol.* 93, 631–638. doi: 10.1113/expphysiol.2007.041855

Han, S.-X., He, G.-M., Wang, T., Chen, L., Ning, Y.-Y., Luo, F., et al. (2010). Losartan attenuates chronic cigarette smoke exposure-induced pulmonary arterial hypertension in rats: possible involvement of angiotensin-converting enzyme-2. *Toxicol. Appl. Pharmacol.* 245, 100–107. doi: 10.1016/j.taap.2010.02.009

Hanff, T. C., Harhay, M. O., Brown, T. S., Cohen, J. B., and Mohareb, A. M. (2020). Is there an association between COVID-19 mortality and the renin-angiotensin system? A call for epidemiologic investigations. *Clin. Infect. Dis.* 71, 870–874. doi: 10.1093/cid/ciaa329

Harmer, D., Gilbert, M., Borman, R., and Clark, K. L. (2002). Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Lett.* 532, 107–110. doi: 10.1016/S0014-5793(02)03640-2

Hernández-Galdamez, D. R., González-Block, M. Á, Romo-Dueñas, D. K., Lima-Morales, R., Hernández-Vicente, I. A., Lumbreras-Guzmán, M., et al. (2020). Increased risk of hospitalization and death in patients with COVID-19 and pre-existing noncommunicable diseases and modifiable risk factors in Mexico. *Arch. Med. Res.* 51, 683–689. doi: 10.1016/j.arcmed.2020.07.003

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271.e8–280.e8. doi: 10.1016/j.cell.2020.02.052

Holt, A., Mizrak, I., Lamberts, M., and Lav Madsen, P. (2020). Influence of inhibitors of the renin–angiotensin system on risk of acute respiratory distress syndrome in Danish hospitalized COVID-19 patients. *J. Hypertens.* [Epub ahead of print]. doi: 10.1097/HJH.0000000000002515

Huang, F., Guo, J., Zou, Z., Liu, J., Cao, B., Zhang, S., et al. (2014). Angiotensin II plasma levels are linked to disease severity and predict fatal outcomes in H7N9-infected patients. *Nat. Commun.* 5:3595. doi: 10.1038/ncomms4595

Iaccarino, G., Grassi, G., Borghi, C., Ferri, C., Salvetti, M., Volpe, M., et al. (2020). Age and multimorbidity predict death among COVID-19 patients: results of the SARS-RAS study of the Italian society of hypertension. *Hypertension* 76, 366–372. doi: 10.1161/HYPERTENSIONAHA.120.15324

Igase, M., Strawn, W. B., Gallagher, P. E., Geary, R. L., and Ferrario, C. M. (2005). Angiotensin II AT 1 receptors regulate ACE2 and angiotensin-(1–7) expression in the aorta of spontaneously hypertensive rats. *Am. J. Physiol. Heart Circ. Physiol.* 289, H1013–H1019. doi: 10.1152/ajpheart.00068.2005

Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., et al. (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 436, 112–116. doi: 10.1038/nature03712

Ioannou, G. N., Locke, E., Green, P., Berry, K., O'Hare, A. M., Shah, J. A., et al. (2020). Risk factors for hospitalization, mechanical ventilation, or death among 10 131 US veterans with SARS-CoV-2 infection. *JAMA Netw. Open* 3:e2022310. doi: 10.1001/jamanetworkopen.2020.22310

Ishiyama, Y., Gallagher, P. E., Averill, D. B., Tallant, E. A., Brosnihan, K. B., and Ferrario, C. M. (2004). Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of Angiotensin II receptors. *Hypertension* 43, 970–976. doi: 10.1161/01.HYP.0000124667.34652.1a

Itoyama, S., Keicho, N., Quy, T., Phi, N. C., Long, H. T., Ha, L. D., et al. (2004). ACE1 polymorphism and progression of SARS. *Biochem. Biophys. Res. Commun.* 323, 1124–1129. doi: 10.1016/j.bbrc.2004.08.208

Jessup, J. A., Gallagher, P. E., Averill, D. B., Brosnihan, K. B., Tallant, E. A., Chappell, M. C., et al. (2006). Effect of angiotensin II blockade on a new congenic model of hypertension derived from transgenic Ren-2 rats. *Am. J. Physiol. Heart Circ. Physiol.* 291, H2166–H2172. doi: 10.1152/ajpheart.00061.2006

Jiang, X., Eales, J. M., Scannali, D., Nazgiewicz, A., Prestes, P., Maier, M., et al. (2020). Hypertension and renin-angiotensin system blockers are not associated with expression of angiotensin converting enzyme 2 (ACE2) in the kidney. *Cardiovasc. Med.* 41, 4580–4588. doi: 10.1101/2020.05.19.20106781

Jung, S.-Y., Choi, J. C., You, S.-H., and Kim, W.-Y. (2020). Association of renin-angiotensin-aldosterone system inhibitors with Coronavirus Disease 2019 (COVID-19)- related outcomes in Korea: a nationwide population-based cohort study. *Clin. Infect. Dis.* 71, 2121–2128. doi: 10.1093/cid/ciaa624

Karram, T., Abbasi, A., Keidar, S., Golomb, E., Hochberg, I., Winaver, J., et al. (2005). Effects of spironolactone and eprosartan on cardiac remodeling and angiotensin-converting enzyme isoforms in rats with experimental heart failure. *Am. J. Physiol. Heart Circ. Physiol.* 289, H1351–H1358. doi: 10.1152/ajpheart.01186.2004

Keidar, S., Gamliel-Lazarovich, A., Kaplan, M., Pavlotzky, E., Hamoud, S., Hayek, T., et al. (2005). Mineralocorticoid receptor blocker increases angiotensin-converting enzyme 2 activity in congestive heart failure patients. *Circ. Res.* 97, 946–953. doi: 10.1161/01.RES.0000187500.24964.7A

Kim, L., Garg, S., O'Halloran, A., Whitaker, M., Pham, H., Anderson, E. J., et al. (2020). Risk factors for intensive care unit admission and in-hospital mortality among hospitalized adults identified through the U.S. Coronavirus Disease 2019 (COVID-19)-associated hospitalization surveillance network (COVID-NET). *Clin. Infect. Dis.* [Epub ahead of print]. doi: 10.1093/cid/ciaa1012

Koka V., Huang, X. R., Chung, A. C., Wang, W., Truong, L. D., and Lan, H. Y. (2008). Angiotensin II up-regulates angiotensin I-converting enzyme (ACE), but down-regulates ACE2 via the AT1-ERK/p38 MAP kinase pathway. *Am. J. Pathol.* 172, 1174–1183. doi: 10.2353/ajpath.2008.070762

Kreutz, R., Algharably, E. A. E.-H., Azizi, M., Dobrowolski, P., Guzik, T., Januszewicz, A., et al. (2020). Hypertension, the renin–angiotensin system, and the risk of lower respiratory tract infections and lung injury: implications for COVID-19. *Cardiovasc. Res.* 116, 1688–1699. doi: 10.1093/cvr/cvaa097

Kuba, K., Imai, Y., and Penninger, J. (2006). Angiotensin-converting enzyme 2 in lung diseases. *Curr. Opin. Pharmacol.* 6, 271–276. doi: 10.1016/j.coph.2006.03.001

Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., et al. (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. *Nat. Med.* 11, 875–879. doi: 10.1038/nm1267

Lahens, A., Mullaert, J., Gressens, S., Gault, N., Flamant, M., Deconinck, L., et al. (2020). Association between renin–angiotensin–aldosterone system blockers and outcome in coronavirus disease 2019: analysing in-hospital exposure generates a biased seemingly protective effect of treatment. *J. Hypertens.* [Epub ahead of print]. doi: 10.1097/HJH.0000000000002658

Lam, C. W. K., Chan, M. H. M., and Wong, C. K. (2004). Severe acute respiratory syndrome: clinical and laboratory manifestations. *Clin. Biochem. Rev.* 25, 121–132.

Larouche-Lebel, É, Loughran, K. A., Oyama, M. A., Solter, P. F., Laughlin, D. S., Sánchez, M. D., et al. (2019). Plasma and tissue angiotensin-converting enzyme 2 activity and plasma equilibrium concentrations of angiotensin peptides in dogs with heart disease. *J. Vet. Intern. Med.* 33, 1571–1584. doi: 10.1111/jvim.15548

Lee, I. T., Nakayama, T., Wu, C.-T., Goltsev, Y., Jiang, S., Gall, P. A., et al. (2020). ACE2 localizes to the respiratory cilia and is not increased by ACE inhibitors or ARBs. *Nat. Commun.* 11:5453. doi: 10.1038/s41467-020-19145-6

Lely, A., Hamming, I., van Goor, H., and Navis, G. (2004). Renal ACE2 expression in human kidney disease. *J. Pathol.* 204, 587–593. doi: 10.1002/path.1670

Lew, R. A., Warner, F. J., Hanchapola, I., Yarski, M. A., Manohar, J., Burrell, L. M., et al. (2008). Angiotensin-converting enzyme 2 catalytic activity in human plasma is masked by an endogenous inhibitor: endogenous inhibitor masks plasma ACE2 activity. *Exp. Physiol.* 93, 685–693. doi: 10.1113/expphysiol.2007.040352

Lew, T. W. K. (2003). Acute respiratory distress syndrome in critically ill patients with severe acute respiratory syndrome. *JAMA* 290:374. doi: 10.1001/jama.290.3.374

Li, W., Moore, M. J., Vasilieva, N., Sui, J., Wong, S. K., Berne, M. A., et al. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 426, 450–454. doi: 10.1038/nature02145

Li, Y., Zeng, Z., Cao, Y., Liu, Y., Ping, F., Liang, M., et al. (2016). Angiotensin-converting enzyme 2 prevents lipopolysaccharide-induced rat acute lung injury via suppressing the ERK1/2 and NF-κB signaling pathways. *Sci. Rep.* 6:27911. doi: 10.1038/srep27911

Li, Y., Zeng, Z., Li, Y., Huang, W., Zhou, M., Zhang, X., et al. (2015). Angiotensin-converting enzyme inhibition attenuates lipopolysaccharide-induced lung injury by regulating the balance between angiotensin-converting enzyme and angiotensin-converting enzyme 2 and inhibiting mitogen-activated protein kinase activation. *Shock* 43, 395–404. doi: 10.1097/SHK.0000000000000302

Liabeuf, S., Moragny, J., Bennis, Y., Batteux, B., Brochot, E., Schmit, J. L., et al. (2020). Association between renin-angiotensin system inhibitors and COVID-19 complications. *Eur. Heart J. Cardiovasc. Pharmacother.* [Epub ahead of print]. doi: 10.1093/ehjcvp/pvaa062

Liang, Y., Deng, H., Bi, S., Cui, Z. A. L., Zheng, D., et al. (2015). Urinary angiotensin converting enzyme 2 increases in patients with Type 2 Diabetic Mellitus. *Kid. Blood Press Res.* 40, 101–110. doi: 10.1159/000368486

Lighter, J., Phillips, M., Hochman, S., Sterling, S., Johnson, D., Francois, F., et al. (2020). Obesity in patients younger than 60 years is a risk factor for COVID-19 Hospital admission. *Clin. Infect. Dis.* 71, 896–897. doi: 10.1093/cid/ciaa415

Liu, Y., Yang, Y., Zhang, C., Huang, F., Wang, F., Yuan, J., et al. (2020). Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci. China Life Sci.* 63, 364–374. doi: 10.1007/s11427-020-1643-8

Liu, Y.-H., Liu, L.-Y., Wu, J.-X., Chen, S.-X., and Sun, Y.-X. (2006). Comparison of captopril and enalapril to study the role of the sulfhydryl-group in improvement of endothelial dysfunction with ACE inhibitors in high dieted methionine mice. *J. Cardiovasc. Pharmacol.* 47, 82–88. doi: 10.1097/01.fjc.0000195306.61719.64

Lopes, R. D., Macedo, A. V. S., de Barros e Silva, P. G. M., Moll-Bernardes, R. J., Feldman, A., D'Andréa Saba Arruda, G., et al. (2020). Continuing versus suspending angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: impact on adverse outcomes in hospitalized patients with severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2)–The BRACE CORONA Trial. *Am. Heart J.* 226, 49–59. doi: 10.1016/j.ahj.2020.05.002

Lopes, R. D., Macedo, A. V. S., de Barros E Silva, P. G. M., Moll-Bernardes, R. J., Dos Santos, T. M., Mazza, L., et al. (2021). Effect of Discontinuing vs Continuing Angiotensin-Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers on Days Alive and Out of the Hospital in Patients Admitted With COVID-19: A Randomized Clinical Trial. *JAMA*. 19, 254–264. doi: 10.1001/jama.2020.25864

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., et al. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 395, 565–574. doi: 10.1016/S0140-6736(20)30251-8

Mackey, K., King, V. J., Gurley, S., Kiefer, M., Liederbauer, E., Vela, K., et al. (2020). Risks and impact of angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers on SARS-CoV-2 infection in adults: a living systematic review. *Ann. Internal Med.* 173, 195–203. doi: 10.7326/M20-1515

Mancia, G., Rea, F., Ludergnani, M., Apolone, G., and Corrao, G. (2020). Renin–angiotensin–aldosterone system blockers and the risk of covid-19. *N. Engl. J. Med.* 382, 2431–2440. doi: 10.1056/NEJMoa2006923

Mariana, C. P., Ramona, P. A., Ioana, B. C., Diana, M., Claudia, R. C., Stefan, V. D., et al. (2016). Urinary angiotensin converting enzyme 2 is strongly related to urinary nephrin in type 2 diabetes patients. *Int. Urol. Nephrol.* 48, 1491–1497. doi: 10.1007/s11255-016-1334-8

Marshall, R. P., Webb, S., Bellingan, G. J., Montgomery, H. E., Chaudhari, B., McAnulty, R. J., et al. (2002). Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 166, 646–650. doi: 10.1164/rccm.2108086

Mechanick, J. I., Rosenson, R. S., Pinney, S. P., Mancini, D. M., Narula, J., and Fuster, V. (2020). Coronavirus and cardiometabolic syndrome. *J. Am. Coll. Cardiol.* 76, 2024–2035. doi: 10.1016/j.jacc.2020.07.069

Mehta, N., Kalra, A., Nowacki, A. S., Anjewierden, S., Han, Z., Bhat, P., et al. (2020). Association of use of angiotensin-converting enzyme inhibitors and Angiotensin II receptor blockers with testing positive for coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 5:1020. doi: 10.1001/jamacardio.2020.1855

Meng, J., Xiao, G., Zhang, J., He, X., Ou, M., Bi, J., et al. (2020). Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension. *Emerg. Microbes Infect.* 9, 757–760. doi: 10.1080/22221751.2020.1746200

Mikami, T., Miyashita, H., Yamada, T., Harrington, M., Steinberg, D., Dunn, A., et al. (2020). Risk factors for mortality in patients with COVID-19 in New York City. *J. Gen. Intern. Med.* [Epub ahead of print]. doi: 10.1007/s11606-020-05983-z

Monteil, V., Kwon, H., Prado, P., Hagelkrüys, A., Wimmer, R. A., Stahl, M., et al. (2020). Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell* 181, 905.e7–913.e7. doi: 10.1016/j.cell.2020.04.004

Narula, S., Yusuf, S., Chong, M., Ramasundarahettige, C., Rangarajan, S., Bangdiwala, S. I., et al. (2020). Plasma ACE2 and risk of death or cardiometabolic diseases: a case-cohort analysis. *Lancet* 396, 968–976. doi: 10.1016/S0140-6736(20)31964-4

Ocaranza, M. P., Godoy, I., Jalil, J. E., Varas, M., Collantes, P., Pinto, M., et al. (2006). Enalapril attenuates downregulation of angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. *Hypertension* 48, 572–578. doi: 10.1161/01.HYP.0000237862.94083.45

Ortiz-Pérez, J. T., Riera, M., Bosch, X., De Caralt, T. M., Perea, R. J., Pascual, J., et al. (2013). Role of circulating angiotensin converting enzyme 2 in left ventricular remodeling following myocardial infarction: a prospective controlled study. *PLoS One* 8:e61695. doi: 10.1371/journal.pone.0061695

Pan, W., Zhang, J., Wang, M., Ye, J., Xu, Y., Shen, B., et al. (2020). Clinical features of COVID-19 in patients with essential hypertension and the impacts of renin-angiotensin-aldosterone system inhibitors on the prognosis of COVID-19 patients. *Hypertension* 76, 732–741. doi: 10.1161/HYPERTENSIONAHA.120.15289

Parra-Bracamonte, G. M., Lopez-Villalobos, N., and Parra-Bracamonte, F. E. (2020). Clinical characteristics and risk factors for mortality of patients with COVID-19 in a large data set from Mexico. *Ann. Epidemiol.* 52, 93.e2–98.e2. doi: 10.1016/j.annepidem.2020.08.005

Perez-Guzman, P. N., Daunt, A., Mukherjee, S., Crook, P., Forlano, R., Kont, M. D., et al. (2020). Clinical characteristics and predictors of outcomes of hospitalized patients with COVID-19 in a multi-ethnic London NHS Trust: a retrospective cohort study. *Clin. Infect. Dis.* [Epub ahead of print]. doi: 10.1093/cid/ciaa1091

Petrilli, C. M., Jones, S. A., Yang, J., Rajagopalan, H., O'Donnell, L., Chernyak, Y., et al. (2020). Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study. *BMJ* 369:m1966. doi: 10.1136/bmj.m1966

Pons, S., Fodil, S., Azoulay, E., and Zafrani, L. (2020). The vascular endothelium: the cornerstone of organ dysfunction in severe SARS-CoV-2 infection. *Crit. Care* 24:353. doi: 10.1186/s13054-020-03062-7

Pranata, R., Permana, H., Huang, I., Lim, M. A., Soetedjo, N. N. M., Supriyadi, R., et al. (2020). The use of renin angiotensin system inhibitor on mortality in patients with coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis. *Diabetes Metab. Syndrome Clin. Res. Rev.* 14, 983–990. doi: 10.1016/j.dsx.2020.06.047

Ramchand, J., and Burrell, L. M. (2020). Circulating ACE2: a novel biomarker of cardiovascular risk. *Lancet* 396, 937–939. doi: 10.1016/S0140-6736(20)32011-0

Ramchand, J., Patel, S. K., Kearney, L. G., Matalanis, G., Farouque, O., Srivastava, P. M., et al. (2020). Plasma ACE2 activity predicts mortality in aortic stenosis and is associated with severe myocardial fibrosis. *JACC Cardiovasc. Imag.* 13, 655–664. doi: 10.1016/j.jcmg.2019.09.005

Ramchand, J., Patel, S. K., Srivastava, P. M., Farouque, O., and Burrell, L. M. (2018). Elevated plasma angiotensin converting enzyme 2 activity is an independent predictor of major adverse cardiac events in patients with obstructive coronary artery disease. *PLoS One* 13:e0198144. doi: 10.1371/journal.pone.0198144

Reich, H. N., Oudit, G. Y., Penninger, J. M., Scholey, J. W., and Herzenberg, A. M. (2008). Decreased glomerular and tubular expression of ACE2 in patients with type 2 diabetes and kidney disease. *Kid. Int.* 74, 1610–1616. doi: 10.1038/ki.2008.497

Reilev, M., Kristensen, K. B., Pottegård, A., Lund, L. C., Hallas, J., Ernst, M. T., et al. (2020). Characteristics and predictors of hospitalization and death in the first 11 122 cases with a positive RT-PCR test for SARS-CoV-2 in Denmark: a nationwide cohort. *Int. J. Epidemiol.* 49, 1468–1481. doi: 10.1093/ije/dyaa140

Reynolds, H. R., Adhikari, S., Pulgarin, C., Troxel, A. B., Iturrate, E., Johnson, S. B., et al. (2020). Renin–angiotensin–aldosterone system inhibitors and risk of Covid-19. *N. Engl. J. Med.* 382, 2441–2448. doi: 10.1056/NEJMoa2008975

Rice, G. I., Jones, A. L., Grant, P. J., Carter, A. M., Turner, A. J., and Hooper, N. M. (2006). Circulating activities of angiotensin-converting enzyme, its homolog, angiotensin-converting enzyme 2, and neprilysin in a family study. *Hypertension* 48, 914–920. doi: 10.1161/01.HYP.0000244543.91937.79

Richardson, S., Hirsch, J. S., Narasimhan, M., Crawford, J. M., McGinn, T., Davidson, K. W., et al. (2020). Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. *JAMA* 323:2052. doi: 10.1001/jama.2020.6775

Sama, I. E., Ravera, A., Santema, B. T., van Goor, H., ter Maaten, J. M., Cleland, J. G. F., et al. (2020). Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin–angiotensin–aldosterone inhibitors. *Eur. Heart J.* 41, 1810–1817. doi: 10.1093/eurheartj/ehaa373

Sands, K. E., Wenzel, R. P., McLean, L. E., Korwek, K. M., Roach, J. D., Miller, K. M., et al. (2020). Patient characteristics and admitting vital signs associated with coronavirus disease 2019 (COVID-19)-related mortality among patients admitted with noncritical illness. *Infect. Control Hosp. Epidemiol.* 1–7. doi: 10.1017/ice.2020.461

Santos, R. A. S., Sampaio, W. O., Alzamora, A. C., Motta-Santos, D., Alenina, N., Bader, M., et al. (2018). The ACE2/Angiotensin-(1–7)/MAS axis of the renin-angiotensin system: focus on angiotensin-(1–7). *Physiol. Rev.* 98, 505–553. doi: 10.1152/physrev.00023.2016

Savarese, G., Benson, L., Sundström, J., and Lund, L. H. (2020). Association between renin–angiotensin–aldosterone system inhibitor use and COVID-19 Hospitalization and death: a 1,4 million patient Nation-Wide registry analysis. *Eur. J. Heart Fail.* [Epub ahead of print]. doi: 10.1002/ejhf.2060

Shah, P., Owens, J., Franklin, J., Jani, Y., Kumar, A., and Doshi, R. (2020). Baseline use of angiotensin-converting enzyme inhibitor/AT1 blocker and outcomes in hospitalized coronavirus disease 2019 African-American patients. *J. Hypertens.* [Epub ahead of print]. doi: 10.1097/HJH.0000000000002584

Simonnet, A., Chetboun, M., Poissy, J., Raverdy, V., Noulette, J., Duhamel, A., et al. (2020). High prevalence of obesity in severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. *Obesity* 28, 1195–1199. doi: 10.1002/oby.22831

Soler, M. J., Ye, M., Wysocki, J., William, J., Lloveras, J., and Batlle, D. (2009). Localization of ACE2 in the renal vasculature: amplification by angiotensin II type 1 receptor blockade using telmisartan. *Am. J. Physiol. Renal Physiol.* 296, F398–F405. doi: 10.1152/ajprenal.90488.2008

Son, M., Seo, J., and Yang, S. (2020). Association between renin-angiotensin-aldosterone system inhibitors and COVID-19 infection in South Korea. *Hypertension* 76, 742–749. doi: 10.1161/HYPERTENSIONAHA.120.15464

Soro-Paavonen, A., Gordin, D., Forsblom, C., Rosengard-Barlund, M., Waden, J., Thorn, L., et al. (2012). Circulating ACE2 activity is increased in patients with type 1 diabetes and vascular complications. *J. Hypertens.* 30, 375–383. doi: 10.1097/HJH.0b013e32834f04b6

South, A. M., Tomlinson, L., Edmonston, D., Hiremath, S., and Sparks, M. A. (2020). Controversies of renin–angiotensin system inhibition during the COVID-19 pandemic. *Nat. Rev. Nephrol.* 16, 305–307. doi: 10.1038/s41581-020-0279-4

Sparks, M. A., South, A. M., Badley, A. D., Baker-Smith, C. M., Batlle, D., Bozkurt, B., et al. (2020). Severe acute respiratory syndrome Coronavirus 2, COVID-19, and the renin-angiotensin system: pressing needs and best research practices. *Hypertension* 76, 1350–1367. doi: 10.1161/HYPERTENSIONAHA.120.15948

Sriram, K., and Insel, P. A. (2020). Risks of ACE inhibitor and ARB usage in COVID-19: evaluating the evidence. *Clin. Pharmacol. Ther.* 108, 236–241. doi: 10.1002/cpt.1863

Stegbauer, J., Kraus, M., Nordmeyer, S., Kirchner, M., Ziehm, M., Dommisch, H., et al. (2020). Proteomic analysis reveals upregulation of ACE2 (Angiotensin-Converting Enzyme 2), the putative SARS-CoV-2 receptor in pressure–but not volume-overloaded human hearts. *Hypertension* 76, e41–e43. doi: 10.1161/HYPERTENSIONAHA.120.16261

Suissa, S. (2008). Immortal time bias in pharmacoepidemiology. *Am. J. Epidemiol.* 167, 492–499. doi: 10.1093/aje/kwm324

Sukumaran, V., Veeraveedu, P. T., Gurusamy, N., Yamaguchi, K., Lakshmanan, A. P., Ma, M., et al. (2011). Cardioprotective effects of telmisartan against heart failure in rats induced by experimental autoimmune myocarditis through the modulation of angiotensin-converting Enzyme-2/Angiotensin 1-7/ *Mas* receptor axis. *Int. J. Biol. Sci.* 7, 1077–1092. doi: 10.7150/ijbs.7.1077

Sukumaran, V., Veeraveedu, P. T., Lakshmanan, A. P., Gurusamy, N., Yamaguchi, K., Ma, M., et al. (2012). Olmesartan medoxomil treatment potently improves cardiac myosin-induced dilated cardiomyopathy via the modulation of ACE-2 and ANG 1–7 mas receptor. *Free Radic. Res.* 46, 850–860. doi: 10.3109/10715762.2012.684878

Takeda, Y., Zhu, A., Yoneda, T., Usukura, M., Takata, H., and Yamagishi, M. (2007). Effects of aldosterone and Angiotensin II receptor blockade on cardiac angiotensinogen and angiotensin-converting enzyme 2 expression in dahl salt-sensitive hypertensive rats. *Am. J. Hypertens.* 20, 1119–1124. doi: 10.1016/j.amjhyper.2007.05.008

Tedeschi, S., Giannella, M., Bartoletti, M., Trapani, F., Tadolini, M., Borghi, C., et al. (2020). Clinical impact of renin-angiotensin system inhibitors on in-hospital mortality of patients with hypertension hospitalized for Coronavirus Disease 2019. *Clin. Infect. Dis.* 71, 899–901. doi: 10.1093/cid/ciaa492

Tipnis, S. R., Hooper, N. M., Hyde, R., Karran, E., Christie, G., and Turner, A. J. (2000). A Human homolog of angiotensin-converting enzyme: cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* 275, 33238–33243. doi: 10.1074/jbc.M002615200

Trifirò, G., Massari, M., Da Cas, R., Menniti Ippolito, F., Sultana, J., Crisafulli, S., et al. (2020). Renin–angiotensin–aldosterone system inhibitors and risk of death in patients hospitalised with COVID-19: a retrospective italian cohort study of 43,000 patients. *Drug Saf.* 43, 1297–1308. doi: 10.1007/s40264-020-00994-5

Uri, K., Fagyas, M., Kertesz, A., Borbely, A., Jenei, C., Bene, O., et al. (2016). Circulating ACE2 activity correlates with cardiovascular disease development. *J. Renin Angiotensin Aldosterone Syst.* 17:1470320316668435. doi: 10.1177/1470320316668435

Vaduganathan, M., Vardeny, O., Michel, T., McMurray, J. J. V., Pfeffer, M. A., and Solomon, S. D. (2020). Renin–angiotensin–aldosterone system inhibitors in patients with Covid-19. *N. Engl. J. Med.* 382, 1653–1659. doi: 10.1056/NEJMsr2005760

van Gerwen, M., Alsen, M., Little, C., Barlow, J., Genden, E., Naymagon, L., et al. (2020). Risk factors and outcomes of COVID-19 in New York City; a retrospective cohort study. *J. Med. Virol.* 93, 907–915. doi: 10.1002/jmv.26337

Velkoska, E., Dean, R. G., Burchill, L., Levidiotis, V., and Burrell, L. M. (2010). Reduction in renal ACE2 expression in subtotal nephrectomy in rats is ameliorated with ACE inhibition. *Clin. Sci.* 118, 269–279. doi: 10.1042/CS20090318

Vuille-dit-Bille, R. N., Camargo, S. M., Emmenegger, L., Sasse, T., Kummer, E., Jando, J., et al. (2015). Human intestine luminal ACE2 and amino acid transporter expression increased by ACE-inhibitors. *Amino Acids* 47, 693–705. doi: 10.1007/s00726-014-1889-6

Walters, T. E., Kalman, J. M., Patel, S. K., Mearns, M., Velkoska, E., and Burrell, L. M. (2016). Angiotensin converting enzyme 2 activity and human atrial fibrillation: increased plasma angiotensin converting enzyme 2 activity is associated with atrial fibrillation and more advanced left atrial structural remodelling. *Europace* 19, 1280–1287. doi: 10.1093/europace/euw246

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA* 323:1061. doi: 10.1001/jama.2020.1585

Wang, K., Gheblawi, M., and Oudit, G. Y. (2020). Angiotensin converting enzyme 2: a double-edged sword. *Circulation* 142, 426–428. doi: 10.1161/CIRCULATIONAHA.120.047049

Wang, G., Zhang, Q., Yuan, W., Wu, J., and Li, C. (2016). Enalapril protects against myocardial ischemia/reperfusion injury in a swine model of cardiac arrest and resuscitation. *Int. J. Mol. Med.* 38, 1463–1473. doi: 10.3892/ijmm.2016.2737

Wang, L., Li, Y., Qin, H., Xing, D., Su, J., and Hu, Z. (2016). Crosstalk between ACE2 and PLGF regulates vascular permeability during acute lung injury. *Am. J. Transl. Res.* 8, 1246–1252.

Wang, Z., Chen, Z., Zhang, L., Wang, X., Hao, G., Zhang, Z., et al. (2018). Status of hypertension in China: results from the China hypertension survey, 2012–2015. *Circulation* 137, 2344–2356. doi: 10.1161/CIRCULATIONAHA.117.032380

Wiener, R. S., Cao, Y. X., Hinds, A., Ramirez, M. I., and Williams, M. C. (2007). Angiotensin converting enzyme 2 is primarily epithelial and is developmentally regulated in the mouse lung. *J. Cell. Biochem.* 101, 1278–1291. doi: 10.1002/jcb.21248

Williamson, E. J., Walker, A. J., Bhaskaran, K., Bacon, S., Bates, C., Morton, C. E., et al. (2020). Factors associated with COVID-19-related death using OpenSAFELY. *Nature* 584, 430–436. doi: 10.1038/s41586-020-2521-4

Wong, M. H., Chapin, O. C., and Johnson, M. D. (2012). LPS-stimulated cytokine production in Type I cells is modulated by the renin–angiotensin system. *Am. J. Respir. Cell Mol. Biol.* 46, 641–650. doi: 10.1165/rcmb.2011-0289OC

Wösten-van Asperen, R. M., Lutter, R., Specht, P. A., Moll, G. N., van Woensel, J. B., van der Loos, C. M., et al. (2011). Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1-7) or an angiotensin II receptor antagonist: ARDS leads to reduced ratio of ACE/ACE2 activities. *J. Pathol.* 225, 618–627. doi: 10.1002/path.2987

Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C.-L., Abiona, O., et al. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Microbiology* 367, 1260–1263. doi: 10.1101/2020.02.11.944462

Wu, C., Ye, D., Mullick, A. E., Li, Z., Danser, A. H. J., Daugherty, A., et al. (2020). Effects of Renin-Angiotensin Inhibition on ACE2 (Angiotensin-Converting Enzyme 2) and TMPRSS2 (Transmembrane Protease Serine 2) Expression: Insights Into COVID-19. *Hypertension* 76, e29–e30. doi: 10.1161/HYPERTENSIONAHA.120.15782

Wu, Y., Guo, W., Liu, H., Qi, B., Liang, K., Xu, H., et al. (2020). Clinical outcomes of 402 patients with COVID-2019 from a single center in Wuhan, China. *J. Med. Virol.* 92, 2751–2757. doi: 10.1002/jmv.26168

Wysocki, J., Lores, E., Ye, M., Soler, M. J., and Batlle, D. (2020). Kidney and lung ACE2 expression after an ACE inhibitor or an Ang II receptor blocker: implications for COVID-19. *JASN* 31, 1941–1943. doi: 10.1681/ASN.2020050667

Wysocki, J., Ye, M., Soler, M. J., Gurley, S. B., Xiao, H. D., Bernstein, K. E., et al. (2006). ACE and ACE2 activity in diabetic mice. *Diabetes* 55, 2132–2139. doi: 10.2337/db06-0033

Yan, Y., Liu, Q., Li, N., Du, J., Li, X., Li, C., et al. (2015). Angiotensin II receptor blocker as a novel therapy in acute lung injury induced by avian influenza A

H5N1 virus infection in mouse. *Sci. China Life Sci.* 58, 208–211. doi: 10.1007/s11427-015-4814-7

Yang, G., Tan, Z., Zhou, L., Yang, M., Peng, L., Liu, J., et al. (2020). Effects of Angiotensin II receptor blockers and ACE (Angiotensin-Converting Enzyme) inhibitors on virus infection, inflammatory status, and clinical outcomes in patients with COVID-19 and hypertension: a single-center retrospective study. *Hypertension* 76, 51–58. doi: 10.1161/HYPERTENSIONAHA.120.15143

Yang, P., Gu, H., Zhao, Z., Wang, W., Cao, B., Lai, C., et al. (2015). Angiotensin-converting enzyme 2 (ACE2) mediates influenza H7N9 virus-induced acute lung injury. *Sci. Rep.* 4:7027. doi: 10.1038/srep07027

Yang, Z., Yu, X., Cheng, L., Miao, L., Li, H., Han, L., et al. (2013). Effects of enalapril on the expression of cardiac angiotensin-converting enzyme and angiotensin-converting enzyme 2 in spontaneously hypertensive rats. *Arch. Cardiovasc. Dis.* 106, 196–201. doi: 10.1016/j.acvd.2013.01.004

Ye, R., and Liu, Z. (2020). ACE2 exhibits protective effects against LPS-induced acute lung injury in mice by inhibiting the LPS-TLR4 pathway. *Exp. Mol. Pathol.* 113:104350. doi: 10.1016/j.yexmp.2019.104350

Yu, C., Lei, Q., Li, W., Wang, X., Liu, W., Fan, X., et al. (2020). Clinical characteristics, associated factors, and predicting COVID-19 mortality risk: a retrospective study in Wuhan, China. *Am. J. Prev. Med.* 59, 168–175. doi: 10.1016/j.amepre.2020.05.002

Zhang, J., Tecson, K. M., and McCullough, P. A. (2020). Endothelial dysfunction contributes to COVID-19-associated vascular inflammation and coagulopathy. *Rev. Cardiovasc. Med.* 21:315. doi: 10.31083/j.rcm.2020.03.126

Zhang, P., Zhu, L., Cai, J., Lei, F., Qin, J.-J., Xie, J., et al. (2020). Association of inpatient use of angiotensin-converting enzyme inhibitors and Angiotensin II receptor blockers with mortality among patients with hypertension hospitalized with COVID-19. *Circ. Res.* 126, 1671–1681. doi: 10.1161/CIRCRESAHA.120.317134

Zhang, X., Yu, J., Pan, L., and Jiang, H. (2020). ACEI/ARB use and risk of infection or severity or mortality of COVID-19: a systematic review and meta-analysis. *Pharmacol. Res.* 158:104927. doi: 10.1016/j.phrs.2020.104927

Zhang, M., Gao, Y., Zhao, W., Yu, G., and Jin, F. (2018). ACE-2/ANG1-7 ameliorates ER stress-induced apoptosis in seawater aspiration-induced acute lung injury. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 315, L1015–L1027. doi: 10.1152/ajplung.00163.2018

Zhang, Y., Li, B., Wang, B., Zhang, J., Wu, J., and Morgan, T. (2014). Alteration of cardiac ACE2/Mas expression and cardiac remodelling in rats with aortic constriction. *Chinese J. Physiol.* 57, 335–342. doi: 10.4077/CJP.2014.BAD268

Zhou, F., Liu, Y.-M., Xie, J., Li, H., Lei, F., Yang, H., et al. (2020a). Comparative impacts of ACE (Angiotensin-Converting Enzyme) inhibitors versus Angiotensin II receptor blockers on the risk of COVID-19 mortality. *Hypertension* 76, e15–e17. doi: 10.1161/HYPERTENSIONAHA.120.15622

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020b). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395, 1054–1062. doi: 10.1016/S0140-6736(20)30566-3

Zou, Z., Yan, Y., Shu, Y., Gao, R., Sun, Y., Li, X., et al. (2014). Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. *Nat. Commun.* 5:3594. doi: 10.1038/ncomms4594

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 23 February 2021
doi: 10.3389/fphys.2021.618929

Correlation of Coagulation Parameters With Clinical Outcomes During the Coronavirus-19 Surge in New York: Observational Cohort

Morayma Reyes Gil[1], Jesus D. Gonzalez-Lugo[2], Shafia Rahman[2], Mohammad Barouqa[1], James Szymanski[1], Kenji Ikemura[1], Yungtai Lo[3] and Henny H. Billett[4]**

[1] Department of Pathology, Albert Einstein College of Medicine, Montefiore Medical Center, New York City, NY, United States, [2] Division of Hematology, Department of Medical Oncology, Albert Einstein College of Medicine, Montefiore Medical Center, New York City, NY, United States, [3] Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Montefiore Medical Center, New York City, NY, United States, [4] Division of Hematology, Departments of Oncology and Medicine, Albert Einstein College of Medicine, Montefiore Medical Center, New York City, NY, United States

OPEN ACCESS

Edited by:
Emmanuelle Vidal-Petiot, Assistance Publique Hopitaux De Paris, France

Reviewed by:
Sulaiman Somani, Icahn School of Medicine at Mount Sinai, United States
Simon Gressens, Hôpital Bichat-Claude-Bernard, France

***Correspondence:**
Morayma Reyes Gil
mreyesgi@montefiore.org
Henny H. Billett
henny.billett@einsteinmed.org

Specialty section:
This article was submitted to Clinical and Translational Physiology, a section of the journal Frontiers in Physiology

Received: *19 October 2020*
Accepted: *22 January 2021*
Published: *23 February 2021*

Citation:
Reyes Gil M, Gonzalez-Lugo JD, Rahman S, Barouqa M, Szymanski J, Ikemura K, Lo Y and Billett HH (2021) Correlation of Coagulation Parameters With Clinical Outcomes During the Coronavirus-19 Surge in New York: Observational Cohort. Front. Physiol. 12:618929. doi: 10.3389/fphys.2021.618929

Importance: COVID-19 has caused a worldwide illness and New York became the epicenter of COVID-19 in the United States from Mid-March to May 2020.

Objective: To investigate the coagulopathic presentation of COVID and its natural course during the early stages of the COVID-19 surge in New York. To investigate whether hematologic and coagulation parameters can be used to assess illness severity and death.

Design: Retrospective case study of positive COVID inpatients between March 20, 2020-March 31, 2020.

Setting: Montefiore Health System main hospital, Moses, a large tertiary care center in the Bronx.

Participants: Adult inpatients with positive COVID tests hospitalized at MHS.

Exposure (for observational studies): Datasets of participants were queried for demographic (age, sex, socioeconomic status, and self-reported race and/or ethnicity), clinical and laboratory data.

Main Outcome and Measures: Relationship and predictive value of measured parameters to mortality and illness severity.

Results: Of the 225 in this case review, 75 died during hospitalization while 150 were discharged home. Only the admission PT, absolute neutrophil count (ANC) and first D-Dimer could significantly differentiate those who were discharged alive and those who died. Logistic regression analysis shows increased odds ratio for mortality by first D-Dimer within 48 hrs. of admission. The optimal cut-point for the initial D-Dimer to predict mortality was found to be 2.1 μg/mL. 15% of discharged patients required

readmission and more than a third of readmitted patients died (5% of all initially discharged).

Conclusion: We describe here a comprehensive assessment of hematologic and coagulation parameters in COVID-19 and examine the relationship of these to mortality. We demonstrate that both initial and maximum D-Dimer values are biomarkers that can be used for survival assessments. Furthermore, D-Dimer may be useful to follow up discharged patients.

Keywords: coagulopathy, D-Dimer, COVID-19, New York City, prothrombin time

BACKGROUND

COVID-19 is a heterogenous disease caused by 2019-nCoV/SARS-CoV-2 virus, a new member of the coronavirus family. Clinical manifestations vary from an asymptomatic illness in some to rapid death in others (Chen et al., 2020; Huang C. et al., 2020; Wu et al., 2020; Zhou et al., 2020). COVID-19 has caused a worldwide illness, causing the World Health Organization to declare this a pandemic on January 30th 2020. (Cucinotta and Vanelli, 2020) The United States is currently one of the global hot spots with a fatality rate ranging from 10% to 27% among adults aged more than 85 years, 3 to 11% among adults of age group 65-84 years and 1% to 3% among age group 55-64 years (Cucinotta and Vanelli, 2020). New York became the epicenter of COVID-19 in the United States and the Bronx had the highest prevalence, hospitalizations and deaths per capita in New York during mid-March to May 2020 (Goyal et al., 2020; NYC, 2020; Wadhera et al., 2020).

Our hospital is a large tertiary care center and is the primary medical system for the Bronx. Faced with a human crisis of enormous proportions, we decided to try and understand the potential correlations with illness severity and death and the natural course of both complicated and less complicated disease during the first surge in New York. Coagulopathy and D-Dimer elevations are reported in 3.75-68.0% of the COVID-19 patients (Berger et al., 2020; Gungor et al., 2020; Huang Y. et al., 2020; Valerio et al., 2020; Yao et al., 2020; Yu H. H. et al., 2020; Yu B. et al., 2020; Zhang L. et al., 2020). Initial studies from patients in Wuhan showed an association of high D-Dimer, a marker for thrombosis, with mortality (Tang et al., 2020). A recent study from Manhattan, New York, showed that 76% of COVID + patients that required hospitalization had elevated D-Dimer on admission and those patients were more likely to developed critical illness and complications including thrombotic events, kidney injury and death (Berger et al., 2020). The primary objective of this study is to examine baseline, and dynamic changes in D-Dimer and other laboratory data and to determine the relation between these laboratory parameters, in particular tests of coagulation, to illness severity.

Herein we studied if D-Dimer correlated with other laboratory parameters and clinical complications, including thrombosis and mortality in the Bronx population.

METHODS

Study Design

Data Gathering and Variables

In our retrospective, single-center study, we included confirmed COVID-19 cases in Montefiore Medical Center/University Hospital for Albert Einstein College of Medicine, Moses Campus, who were hospitalized and had routine coagulation tests done between March 20th to March 31st 2020. For those samples without an ordered D-Dimer, D-Dimer was performed alongside with prothrombin time (PT) as part of this study. All cases were established with reverse-transcriptase–polymerase-chain-reaction real-time (RT PCR) assay of the nasal and the pharyngeal swabs. We excluded patients younger than 18 years of age, those admitted for "COVID-19-like" illnesses but negative initial test results, and patients for whom data were missing. The study was approved by the Albert Einstein College of Medicine Institutional Review Board. Electronic medical records of the patients were reviewed to obtain epidemiological, demographic, clinical and laboratory data.

These variables included demographic attributes (age, sex, and self-reported race and/or ethnicity) and baseline comorbidities (body mass index, previous history of hypertension, diabetes, kidney, pulmonary, liver, autoimmune, cancer, or sickle cell disease on presentation). Initial vital signs, including oxygen saturation, and laboratory values were documented. Obesity was defined as BMI more than 30. Cancer was defined as malignancy with active treatment or diagnosed within the last 5 years. Laboratory values consisted of a complete blood count, a metabolic profile, assessments of liver and renal function, procalcitonin and coagulation testing [prothrombin time (PT), partial thromboplastin time (PTT), D-Dimer values, fibrinogen]. Management and clinical outcomes were followed up to June 10th 2020. We assessed for interventions and time to interventions including ICU admission, intubation, thrombosis and anticoagulation, mortality, hospital discharge and post-discharge readmission. Thrombosis was document only if a thrombus was identified on radiological imaging. *Ex vivo* clotting while on hemodialysis (HD) or continuous renal replacement therapy (CRRT) was based on the need for kit/filter change and/or visual clots as documented in the clinical progress notes. We documented anticoagulation medications given to each patient within 48 h preceding the thrombus or clotting event. Doses for prophylactic anticoagulation were: apixaban 2.5 mg

twice a day, enoxaparin 40 mg subcutaneously once a day (BMI < 40, GFR = 30) or enoxaparin 30 mg subcutaneously twice a day (BMI = 40). Therapeutic anticoagulation doses were apixaban 5 mg twice a day, enoxaparin 1-5 mg/kg/day (1 mg/kg/day if GFR = 30) or 1 mg/kg twice a day. All intravenous unfractionated heparin (UFH) administrations were deemed therapeutic, typically 80 units/kg IV bolus followed by continuous infusion of 18 units/kg/h or 5000 units IV bolus followed by continuous infusion of 1300 units/h. All intravenous bivalirudin administrations were deemed therapeutic. Patients on warfarin prior to admission that continued warfarin while hospitalized were considered on a therapeutic regimen.

The earliest symptoms were categorically defined: New onset cough, dyspnea, and diarrhea, intubation and dialysis requirements. Maximum and, when appropriate, minimum values were noted and the day of these values from admission date were noted. Levels of parameters of particular interest were recorded daily whenever possible. Chest imaging done on presentation to the emergency department was documented.

TABLE 1 | Demographics and clinical features of COVID positive patients at admission.

Demographics/Clinical Features	Died *n* = 75	Discharged *n* = 150	*P*-value
Age, yrs. (median (IQR))	71.0 (62.0, 77.5)	59.0 (48.3, 67.0)	<0.001
Gender			
Female, n (%)	26 (34.7)	69 (46.0)	0.139
Male, n (%)	49 (65.3)	81 (54.0)	
Comorbidity			
Hypertension, n (%)	56 (74.7)	90 (60.0)	0.03
Diabetes, n (%)	36 (48.0)	51 (34.0)	0.04
Chronic Kidney Disease, n (%)	17 (22.6)	26 (17.3)	0.92
Pulmonary Disease, n (%)	17 (22.6)	38(25.3)	0.19
Liver Disease, n (%)	4 (5.3)	6 (4.0)	0.21
Autoimmune Disease, n (%)	5 (6.7)	8 (5.5)	0.16
Cancer, n (%)	9 (12.0)	7 (4.6)	0.04
Sickle Cell Disease, n (%)	1 (1.3)	1 (0.7)	0.25
Race			
Black, n (%)	35 (46.7)	54 (36.0)	0.361
White, n (%)	11 (14.7)	29 (19.3)	
Asian, n (%)	2 (2.7)	2 (1.3)	
Other/Declined, n (%)	27 (36.0)	65 (43.3)	
Ethnicity			
Hispanic, n (%)	20 (26.7)	57 (38.0)	0.1
Non- Hispanic, n (%)	51 (68.0)	75 (50.0)	
Other/Declined, n (%)	4 (5.3)	18 (12.0)	
BMI (mean)	30.5 (8.28)	29.7 (6.08)	0.46
Presentation			
Fever, n (%)	55 (73.3)	104 (69.3)	0.641
Cough, n (%)	54 (72.0)	100 (66.7)	0.51
SOB, n (%)	56 (74.7)	98 (65.3)	0.205
Diarrhea, n (%)	9 (12.0)	33 (22.0)	0.102
Infiltrate on initial X-ray, n (%)	69 (92)	136 (90.6)	0.11
O2 Sat (median (IQR))	90.0 (80.0, 95.0)	96.0 (92.3, 97.0)	<0.001

Laboratory Testing

All cases were established with reverse-transcriptase–polymerase-chain-reaction real-time (RT PCR) assay of the nasal and the pharyngeal swabs. Coagulation tests (prothrombin time, D-Dimer, partial thromboplastin time and fibrinogen) were performed by STA-R Max instruments. STA Liatest LIA D-Dimer assay was performed as per manufacturer recommendations and reported as FEU μg/mL with a cut-off of <0.5 ug/ml to rule out PE. Complete blood counts were performed by Sysmex XN9000. Chemistry assays were performed by Roche instrumentation and reagents as per manufacturer recommendations.

Statistical Methods

Data analysis was performed using R software, version 3.6.2. Differences in demographic, clinical variables and laboratory assessments between patients who died in the hospital and patients discharged alive were compared using chi-square tests, or Fisher's exact tests for categorical variables and two-sample Student *t*-tests, or the Mann-Whitney *U*-test for continuous variables. Logistic regression was carried out to examine the relationship between the factors and lab parameters under examination and in-hospital mortality. Parameters for the logistic regression analysis were selected based on a *p*-value = 0.1 (Bursac et al., 2008).

The receiver operating characteristic curves (ROC), Youden's J statistics and Kaplan-Meier were used to assess performance of D-Dimer in the first 48 h on predicting in-hospital mortality adjusted for age, O2 saturation and sex.

RESULTS

Study Population: Admission and Mortality Data

Of the 225 patients who tested positive for COVID, 75 (33%) patients died during hospitalization while 150 (67%) were discharged alive. Analysis of demographics, comorbidities, and clinical parameters are included in **Table 1**. Similar to other reports, patients that succumbed to death were significantly older than survivors (median [interquartile range (IQR)]; 71.00 [62.0, 77.5] vs. 59.00 [48.3, 67.0] years, $p < 0.001$). Among pre-existing comorbidities, hypertension (74.7% vs. 60.0%, $p = 0.03$), diabetes (48.0% vs. 34.0%, $p = 0.04$) and cancer (12.0% vs. 4.6%, $p = 0.04$) were more prevalent in non-survivors. The only clinical parameter on admission that showed statistical significance between non-survivors vs. survivors were the oxygen saturation on RA (median [interquartile range (IQR)]; 90.00 [80.00, 95.00] vs. 96.00 [92.00, 97.00], $p < 0.001$). We could not detect a statistical significance for gender or race between survivors vs. non-survivors in this population.

TABLE 2 | Laboratory and clinical course of COVID positive patients during hospitalization.

	Died *n* = 75	Discharged *n* = 150	*P*
Lab Test			
Absolute Neutrophilic Count, $\times 10^9$/L (median (IQR))			
- Initial (median (IQR))	5.40 (3.45, 7.70)	4.45 (3.23, 6.60)	0.038
- Minimum (median (IQR))	4.70 (3.0, 6.75)	3.00 (2.20, 4.00)	<0.001
- Hospital days to minimum	1.00 (0.00, 3.00)	3.00 (1.00, 5.00)	<0.001
- Maximum (median (IQR))	12.60 (9.60, 16.50)	5.95 (4.20, 9.03)	<0.001
- Hospital days to maximum	7.00 (3.00, 11.00)	3.00 (0.00, 5.00)	<0.001
Absolute Lymphocytic Count, $\times 10^9$/L (median (IQR))			
- Initial (median (IQR))	0.90 (0.60, 1.20)	0.90 (0.70, 1.28)	0.457
- Minimum (median (IQR))	0.50 (0.40, 0.80)	0.80 (0.60, 1.10)	<0.001
- Hospital days to minimum	3.00 (1.50, 5.00)	1.00 (0.00, 4.00)	<0.001
Hemoglobin, g/dl (median (IQR))			
- First	12.70 (10.45, 14.25)	13.10 (11.72, 14.20)	0.48
- Minimum	10.00 (7.50, 11,60)	11.30 (9.30, 12.80)	0.001
- Hospital days to minimum	6.00 (3.00, 10.00)	4.00 (2.00, 7.00)	0.003
Platelets, $\times 10^9$/L (median (IQR))			
- Initial	193.00 (153.75, 236.25)	198.00 (145.50, 245.00)	0.793
- Minimum	150.00 (120.50, 193.50)	178.00 (136.00, 218.00)	0.004
- Hospital days to minimum	2.00 (0.00, 4.00)	1.00 (0.00, 2.00)	0.003
Procalcitonin, ng/ml (median (IQR))			
- Initial (median (IQR))	0.10 (0.10, 0.50)	0.30 (0.10, 1.40)	0.17
- Maximum (median (IQR))	2.00 (0.00, 8.25)	0.00 (0.00, 1.00)	0.0011
Initial AST (median (IQR))	51.00 (29.50, 78.50)	38.00 (27.00, 60.00)	0.29
Initial ALT (median (IQR))	29.00 (18.00, 41.50)	28.00 (19.00, 43.75)	1.0
Initial Creatinine	1.40 (1.10, 2.00)	1.00 (0.80, 1.37)	0.41
Clinical Course			
Cardiac arrest (%)	36 (48.0)	0 (0.0)	<0.001
Dialysis required (%)	39 (52.0)	15 (10.0)	<0.001
LFT = 2.5x ULN at any time	15 (20.0)	16 (10.7)	0.034
Intubation required (%)	47 (62.7)	17 (11.3)	<0.00001
Days to intubation (mean)	1.00 (0.00, 3.00)	1.00 (0.00, 4.00)	0.68
Intubated days (mean)	6.00 (3.00, 11.00)	9.00 (5.00, 11.75)	0.292
LOS, days (median (IQR))	10.00 (6.00, 14.50)	7.00 (4.00, 12.00)	0.012

TABLE 3 | Coagulation parameters and anticoagulation treatments during hospitalization.

	Died *n* = 75	Discharged *N* = 150	*p*
Coagulation Profile			
PT sec (median [IQR])			
- Initial	14.50 (13.70, 16.10)	13.80 (13.30, 14.65)	0.002
- Maximum (mean)	16.40 (15.20, 20.20)	14.70 (13.80, 16.30)	<0.001
- Hospital days to maximum (mean (SD))	4.00 (0.00, 9.00)	2.00 (0.00, 6.00)	0.273
PTT sec (Median (IQR))			
- Initial	34.00 (30.67, 38.15)	32.60 (29.65, 36.50)	0.109
- Maximum	41.20 (33.40, 50.30)	34.70 (31.20, 40.08)	<0.001
- Hospital days to maximum (mean (SD))	3.00 (0.00, 8.00)	1.00 (0.00, 2.00)	0.003
D-Dimer ug/ml FEU			
- Within first 48 h (Median (IQR))	2.85 (1.36, 10.36)	1.05 (0.64, 2.23)	<0.001
- Maximum (Median (IQR))	4.66 (2.16, 12.41)	1.19 (0.68, 3.01)	<0.0001
- Hospital days to maximum	5.00 (2.00, 10.00)	3.00 (1.00, 5.00)	0.006
Fibrinogen mg/dl (mean (SD))			
- Initial	641.00 (449.00, 736.00)	656.50 (505.25, 743.75)	0.64
- Maximum	690.91 (255.21)	715.00 (274.60)	0.75
- Hospital days to maximum (mean (SD))	5.00 (2.00, 11.50)	4.50 (2.00, 11.00)	0.839

TABLE 4 | Characteristics of Discharged patients with initial D-Dimer < 48 h vs. > 48 h since admission.

Characteristics of Discharged Patients	1^{st} D-Dimer < 48 h	1^{st} D-Dimer > 48 h	*p*
n	96	54	
1st D-Dimer (median [IQR])	1.02 [0.64, 2.04]	1.18 [0.66, 3.18]	0.26
Age (mean (SD))	55.50 [46.75, 67.50]	60.00 [52.25, 66.00]	0.33
Sex = Male (%)	55 (57.3)	26 (48.1)	0.36
Comorbidity			
Hypertension, n (%)	53 (55.2)	37 (68.5)	0.16
Diabetes, n (%)	30 (31.2)	21 (38.9)	0.44
Chronic Kidney disease (%)	15 (14.9)	13 (22.8)	0.30
Pulmonary disease, n (%)	27 (26.7)	12 (21.1)	0.55
Liver Disease, n (%)	4 (4.2)	2 (3.7)	1.00
Autoimmune Disease, n (%)	4 (4.2)	4 (7.4)	0.64
Cancer, n (%)	5 (5.2)	2 (3.7)	1.00
Sickle Cell Disease, n (%)	0 (0.0)	1 (1.9)	0.77
Race (%)			
Black	32 (33.3)	22 (40.7)	0.27
White	16 (16.7)	13 (24.1)	
Asian	2 (2.1)	0 (0.0)	
Other	46 (47.9)	19 (35.2)	
Ethnicity (%)			
Declined	12 (12.5)	6 (11.1)	0.59
Hispanic	39 (40.6)	18 (33.3)	
Non-Hispanic	45 (46.9)	30 (55.6)	
Presentation			
Fever, n (%)	63 (65.6)	41 (75.9)	0.26
Cough, n (%)	67 (69.8)	33 (61.1)	0.37
SOB, n (%)	65 (67.7)	33 (61.1)	0.53
Diarrhea, n (%)	21 (21.9)	12 (22.2)	1
O2 Sat (median [IQR])	96.00 [91.75, 97.00]	96.00 [93.00, 97.00]	0.43
Clinical laboratory data on admission			
ANC$\times 10^9$/L (median [IQR])	4.90 [3.60, 7.00]	4.05 [2.83, 5.56]	0.03
ALC$\times 10^9$/L (median [IQR])	0.90 [0.68, 1.23]	0.90 [0.80, 1.28]	0.59
Hb g/dl (median [IQR])	13.10 [11.80, 14.20]	12.80 [11.55, 14.17]	0.58
PLT$\times 10^9$/L (median [IQR])	202.50 [161.00, 240.25]	180.50 [145.00, 228.50]	0.12
PT (median [IQR])	13.80 [13.20, 14.40]	13.95 [13.40, 16.02]	0.16
PTT (median [IQR])	32.55 [30.03, 36.75]	32.60 [28.90, 35.25]	0.79
AST (median [IQR])	40.00 [28.00, 61.00]	33.00 [25.00, 54.00]	0.17
ALT (median [IQR])	28.00 [19.00, 48.00]	28.00 [18.00, 38.00]	0.32
CRT (median [IQR])	1.00 [0.80, 1.32]	1.00 [0.80, 1.37]	0.73
Clinical Course			
Cardiac arrest (%)	0 (0.0)	0 (0.0)	NA
Dialysis required (%)	8 (8.3)	7 (13.0)	0.54
LFTs = 2.5X ULN (%)	8 (8.3)	8 (14.8)	0.34

Clinical Characteristics

As shown in **Table 2**, intubation ($p < 0.00001$), cardiac arrest ($p < 0.001$), dialysis requirement ($p < 0.001$) and significant liver disease) (liver enzyme elevation greater than 2.5 fold the upper limit of normal range, $p < 0.034$) during hospitalization were all associated with decreased survival ($p < 0.001$). The overall length of stay for survivors discharged home was significantly shorter than for patients that died during hospitalization.

Laboratory Data

Table 2 contains non-coagulation labs and **Table 3** contains coagulation labs. The only significantly different admission lab tests between deceased vs. survivors were absolute neutrophil count PT and D-Dimer within first 48 h. (**Tables 2**, **3**). D-Dimer results within first 48 h. of admission were missing in a significant number of patients. The lack of D-Dimer data however did not translate into any statistical differences in other values, such as demographics, clinical and other laboratory characteristics.

TABLE 5 | Characteristics of patients that died during hospitalization with initial D-Dimer < 48 h vs. > 48 h since admission.

Characteristics of Deceased Patients	1^{st} D-Dimer < 48 h	1^{st} D-Dimer > 48 h	*p*
n	38	37	
1st D-Dimer (median [IQR])	1.85 [0.88, 3.16]	6.26 [2.25, 13.72]	0.003
Age (mean (SD))	73.00 [65.00, 78.00]	68.00 [56.00, 75.00]	0.13
Sex = Male (%)	25 (65.8)	24 (64.9)	1
Comorbidity			
Hypertension, n (%)	30 (78.9)	26 (70.3)	0.55
Diabetes, n (%)	20 (52.6)	16 (43.2)	0.56
Chronic Kidney disease (%)	5 (13.2)	12 (32.4)	0.09
Pulmonary disease, n (%)	7 (18.4)	10 (27.0)	0.54
Liver Disease, n (%)	2 (5.3)	2 (5.6)	1
Autoimmune Disease, n (%)	4 (10.5)	1 (2.7)	0.37
Cancer, n (%)	4 (10.5)	5 (13.5)	0.97
Sickle Cell Disease, n (%)	0 (0.0)	1 (2.7)	0.99
Race (%)			
Black	19 (50.0)	16 (43.2)	0.38
White	4 (10.5)	7 (18.9)	
Asian	2 (5.3)	0 (0.0)	
Other	13 (34.2)	14 (37.8)	
Ethnicity (%)			
Declined	0 (0.0)	4 (10.8)	0.09
Hispanic	12 (31.6)	8 (21.6)	
Non-Hispanic	26 (68.4)	25 (67.6)	
Presentation			
Fever, n (%)	27 (71.1)	28 (75.7)	0.85
Cough, n (%)	28 (73.7)	25 (69.4)	0.88
SOB, n (%)	28 (73.7)	28 (75.7)	1
Diarrhea, n (%)	6 (15.8)	3 (8.1)	0.50
O2 Sat (median [IQR])	86.00 [80.00, 94.75]	90.00 [80.00, 95.00]	0.67
Clinical laboratory data on admission			
ANC $\times 10^9$/L (median [IQR])	6.55 [4.23, 8.78]	5.10 [3.30, 6.00]	0.06
ALC $\times 10^9$/L (median [IQR])	0.85 [0.60, 1.20]	0.90 [0.80, 1.10]	0.49
Hb g/dl (median [IQR])	12.60 [10.70, 14.30]	12.70 [10.40, 14.20]	0.71
PLT $\times 10^9$/L (median [IQR])	194.50 [148.25, 241.50]	199.00 [144.00, 244.00]	0.90
PT (median [IQR])	14.60 [13.70, 16.10]	14.30 [13.60, 15.80]	0.60
PTT (median [IQR])	34.70 [30.52, 37.85]	33.30 [30.67, 38.23]	0.74
AST (median [IQR])	51.00 [32.00, 73.50]	51.00 [28.00, 85.00]	0.84
ALT (median [IQR])	25.00 [18.00, 40.75]	31.00 [20.00, 42.00]	0.34
CRT (median [IQR])	1.44 [1.12, 2.63]	1.30 [0.90, 1.80]	0.56
Clinical Course			
Cardiac arrest (%)	22 (57.9)	14 (37.8)	0.13
Dialysis required (%)	22 (57.9)	17 (45.9)	0.42
LFTs = 2.5X ULN (%)	6 (15.8)	9 (24.3)	0.53

The same differences were observed among survivors and non-survivors in patients who were discharged who had D-Dimer values within 48 hrs. as were observed in those who did not (**Tables 4**, **5**).

The maximum PT, PTT, D-Dimer, procalcitonin, absolute neutrophil count (ANC) were statistically significantly higher in non-survivors vs. survivors. Likewise, the minimum absolute lymphocyte count (ALC), hemoglobin and platelet count were statistically significantly lower in non-survivors compared to survivors. Inflammation can mildly increase D-Dimer. Likewise, renal and hepatic disease can increase D-Dimer levels due to decreased clearance. However, no obvious positive correlation was seen in regression plots of D-Dimer vs. CRP (inflammation marker), ALT and AST (liver function markers), PT and CRT (renal function marker) (**Figures 1A-E**).

On univariable logistic regression analysis, older age (OR = 1.06, 95%CI: 1.03–1.09; $p < 0.001$), hypertension (OR 3.04, 95%CI: 1.31-7.74, $p = 0.01$), diabetes (OR 2.44, 95%CI: 1.14-5.33, $p = 0.02$), lower oxygen saturation (OR 0.90, 95%CI: 0.85-0.94, $p < 0.001$), PT [OR = 1.17 (1.02-1.37, $p = 0.028$)]

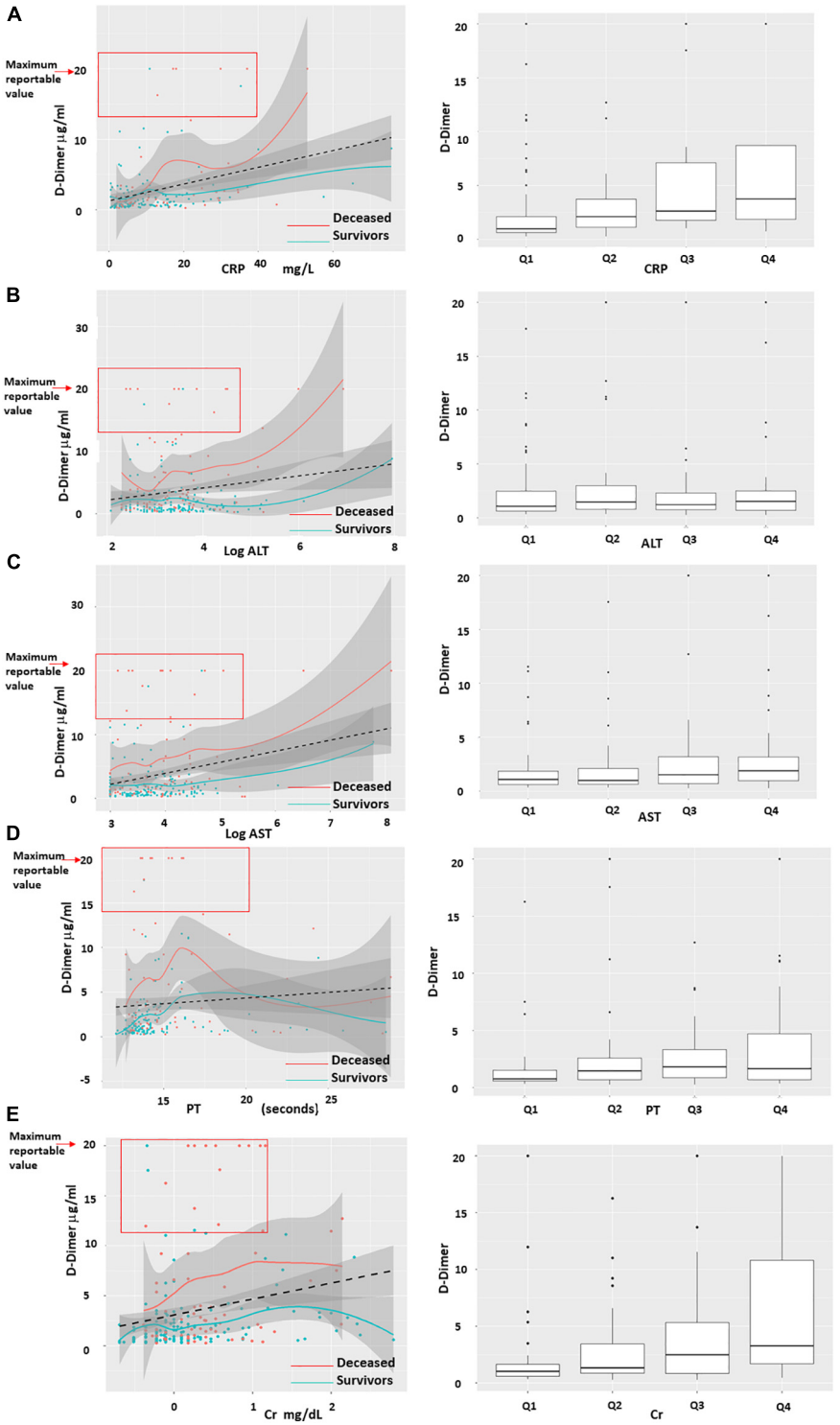


FIGURE 1 | Regression plots of D-Dimer correlation with C-Reactive Protein, AST, ALT, PT and Creatinine. D-Dimer levels of patients that died (red) vs. patients that survived (blue) did not significantly correlate with C-Reactive Protein (CRP), AST, ALT, Prothrombin Time (PT) and Creatinine (Cr). **(A–E)** Left column shows density plots with 95% intervals (gray solid zones). Black dotted line = linear regression. Blue and red line = smooth regression, also known as "Distribution free" which assumes no correlation and finds the best fit to the trajectory of the points. Red square highlights that the majority of cases with maximum D-Dimer of >20 µg/mL were non-survivors and had corresponding levels of CRP, AST, ALT, PT, and Cr in the lowest quartiles. **(A–E)** Right columns represents the quartiles of CRP, AST, ALT, PT, and Cr distribution based on D-Dimer.

TABLE 6 | Logistic regression analysis of risk factors for mortality in hospitalized COVID patients.

Dependent: expired		no	yes	Odds ratio (univariable)	Odds ratio (multivariable)
D-Dimer < 48 h	Mean (SD)	1.7 (1.8)	4.4 (6.1)	1.23 (1.09-1.43, $p = 0.003$)	1.24 (1.04-1.49, $p = 0.018$)
Age	Mean (SD)	56.9 (16.9)	70.4 (12.6)	1.06 (1.03-1.09, $p < 0.001$)	1.06 (1.01-1.11, $p = 0.011$)
Sex	Female	41 (42.7)	13 (34.2)	-	-
	Male	55 (57.3)	25 (65.8)	1.43 (0.66-3.20, $p = 0.367$)	1.18 (0.32-4.35, $p = 0.806$)
Ethnicity	hispanic	39 (40.6)	12 (31.6)		
	not hispanic or declined	57 (59.4)	26 (68.4)	1.48 (0.68-3.37, $p = 0.332$)	1.18 (0.33-4.18, $p = 0.801$)
Diarrhea	no	75 (78.1)	32 (84.2)	-	-
	yes	21 (21.9)	6 (15.8)	0.67 (0.23-1.73, $p = 0.431$)	1.63 (0.35-7.62, $p = 0.533$)
HT	no	43 (44.8)	8 (21.1)	-	-
	yes	53 (55.2)	30 (78.9)	3.04 (1.31-7.74, $p = 0.013$)	1.93 (0.46-8.04, $p = 0.365$)
DM	no	66 (68.8)	18 (47.4)	-	-
	yes	30 (31.2)	20 (52.6)	2.44 (1.14-5.33, $p = 0.023$)	1.21 (0.34-4.32, $p = 0.766$)
Cancer	no	91 (94.8)	34 (89.5)	-	-
	yes	5 (5.2)	4 (10.5)	2.14 (0.50-8.56, $p = 0.277$)	1.05 (0.15-7.12 $p = 0.961$)
Oxygen saturation	Mean (SD)	93.6 (5.9)	83.7 (15.1)	0.90 (0.85-0.94, $p < 0.001$)	0.89 (0.83-0.95, $p = 0.001$)
ANC	Mean (SD)	6094.8 (4603.2)	7171.1 (3993.4)	1.00 (1.00-1.00, $p = 0.214$)	1.00 (1.00-1.00, $p = 0.173$)
PT	Mean (SD)	14.4 (2.4)	15.6 (3.2)	1.17 (1.02-1.37, $p = 0.028$)	1.10 (0.90-1.34, $p = 0.366$)
PTT	Mean (SD)	33.6 (5.6)	35.3 (6.8)	1.05 (0.98-1.13, $p = 0.177$)	1.04 (0.94-1.16, $p = 0.437$)
Chest infiltrates	no	9 (9.4)	3 (7.9)	-	-
	yes	87 (90.6)	35 (92.1)	1.21 (0.34-5.68, $p = 0.787$)	0.96 (0.13-7.32, $p = 0.968$)

and increased first 48 h D-Dimer level (OR = 1.23, 95%CI 1.09–1.43; $p = 0.003$), were associated with increased odds for mortality. Using multivariable logistic analysis only D-Dimer, age and oxygen saturation remain statistically significant, and the odds ratio for D-Dimer increased slightly (OR = 1.24, 95%CI 1.04–1.49; $p = 0.018$), highlighting the strength of D-Dimer as an independent risk factor for mortality (**Table 6**).

The receiver operating characteristic curve (ROC) of first 48 h. D-Dimer adjusted for age and oxygen saturation, showed an area under the curve (AUC) of 0.86 and a very similar AUC 0.81 with only D-Dimer and age, underscoring D-Dimer as an important admission lab test as predictor of mortality (**Figure 2**). Using Youde's J statistic, the optimal cut-point for the initial D-Dimer to predict mortality was found to be 2.1 μg/mL (**Figure 3**). The cumulative survival by Kaplan Meier using a cutoff of initial D-Dimer of 2 μg/mL shows a clear separation between the two groups: 78% (71/91) of patients with D-Dimer < 2 μg/mL survived whereas only 57% (24/42) of the patients with D-Dimer = 2 μg/mL survived (**Figure 4**).

Thrombosis and Anticoagulation

A total of 10 patients (4.4%) had documented *in vivo* thrombosis, mainly venous thromboembolism (DVT). Although *in vivo* thrombosis was not significantly different between survivors and non-survivors, *ex vivo* clotting, mainly in hemodialysis lines, was significantly higher non-survivors compared to survivors. 187 of the 225 patients (83%) were on some anticoagulation. Of those who were on anticoagulation, 14.7% of the survivors were given therapeutic doses as compared to 21.3% of those who died. These data, and the medications involved, are detailed in **Table 7**. Maximum D-Dimer was not statistically significant between patients not anticoagulated compared to anticoagulated patients (**Table 7**). However, when comparing discharged vs. deceased patients, maximum D-Dimer was significantly higher in non-survivor patients not anticoagulated and prophylactically anticoagulated but not among patients that received therapeutic anticoagulation (**Table 7**). Among discharged patients maximum D-Dimer, but not initial D-Dimer, was significantly higher in patients that develop clots (including both *in vivo* and *ex vivo* clots). Whereas within patients that eventually died during hospitalization both initial D-Dimer and maximum D-Dimer levels were significantly higher in those that developed clots (**Table 8**).

Sequela Post-discharge

In **Table 9**, 22 of the 150 (14.7%) patients that were discharged alive had complications that required readmission. Complications included respiratory (5/22), cardiovascular (4/22), renal (4/22), and infectious (9/22) etiologies. 8 patients (5% of the discharged patients) died due to post-discharge complications and all happened within 1 month since discharge.

DISCUSSION

This study of COVID19 patients in the Bronx, NY, United States confirms the original observations made by Wuhan studies regarding the association of D-Dimer with mortality in COVID19 patients (Huang C. et al., 2020; Vidali et al., 2020). Goyal et al. (2020) described a population in New York City (NYC) that is majority White (37%), minority Black (12.5%) and unknown percentage of Hispanics, as no classification for Hispanics was provided. Richardson et al. (2020) also described the demographics and comorbidities of a NYC that is majority

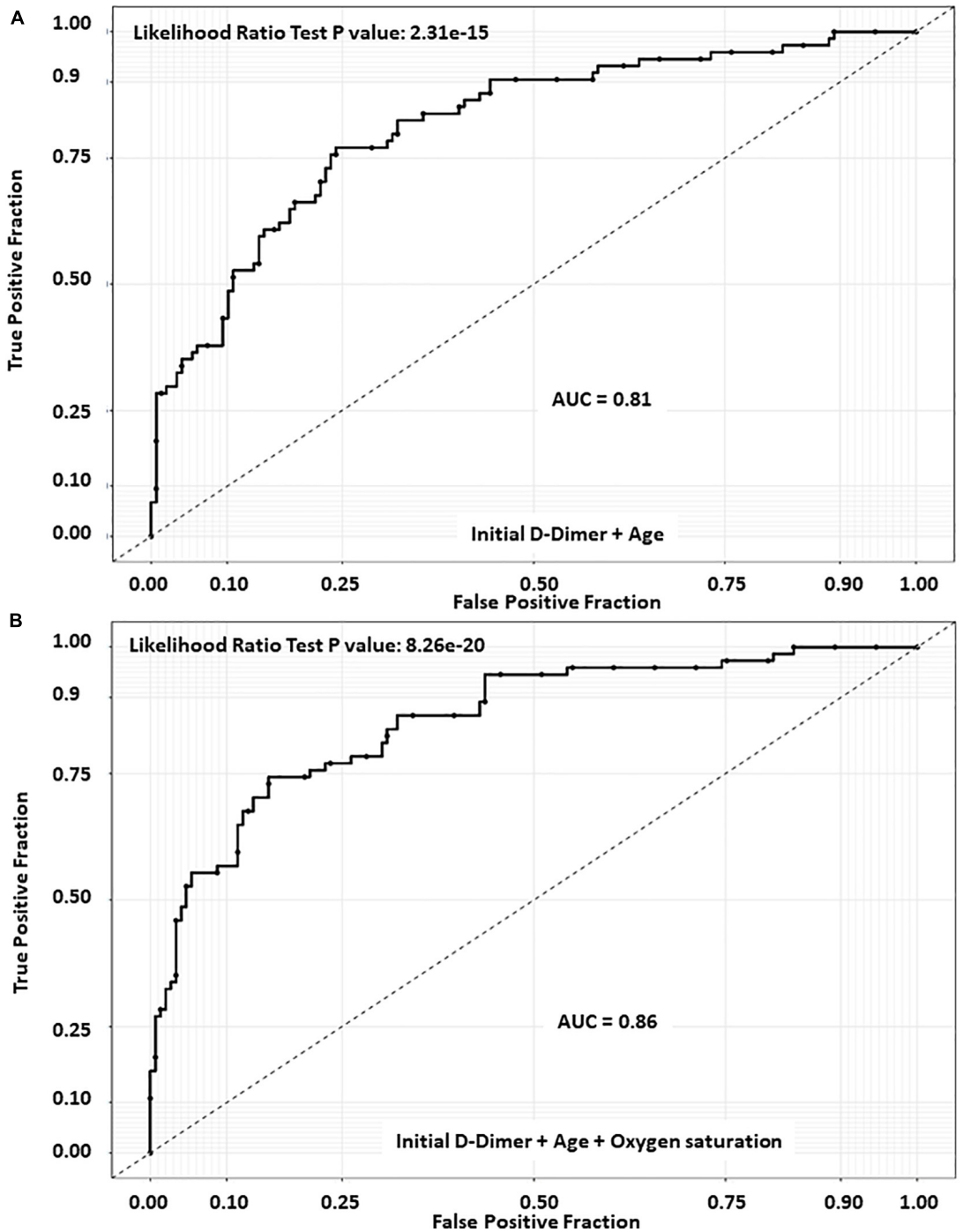


FIGURE 2 | Receiver operating characteristic curve (ROC) of D-Dimer. **(A)** D-Dimer combined with age only displayed an AUC of 0.81. **(B)** D-Dimer combined with age and Oxygen saturation showed an area under the curve (AUC) of 0.86.

White (39.8%), followed by Hispanics (23%) and Blacks (22.6%). Although Richardson et al. (2020) showed laboratory data, no comparison or statistical analysis was shown between discharged patients vs. survivors. In contrast we studied a population of Blacks (40%), Hispanics (33%) and a minority Whites (18%), representative of the Bronx and overall NYC demographics and we were able to analyze physiological and laboratory parameters as predictors of mortality in our cohort of US COVID19 infected patients.

Our cohort consists of 225 patients seen at the main Montefiore Medical Center hospital at the beginning of the pandemic peak in NYC. Montefiore comprises a population of

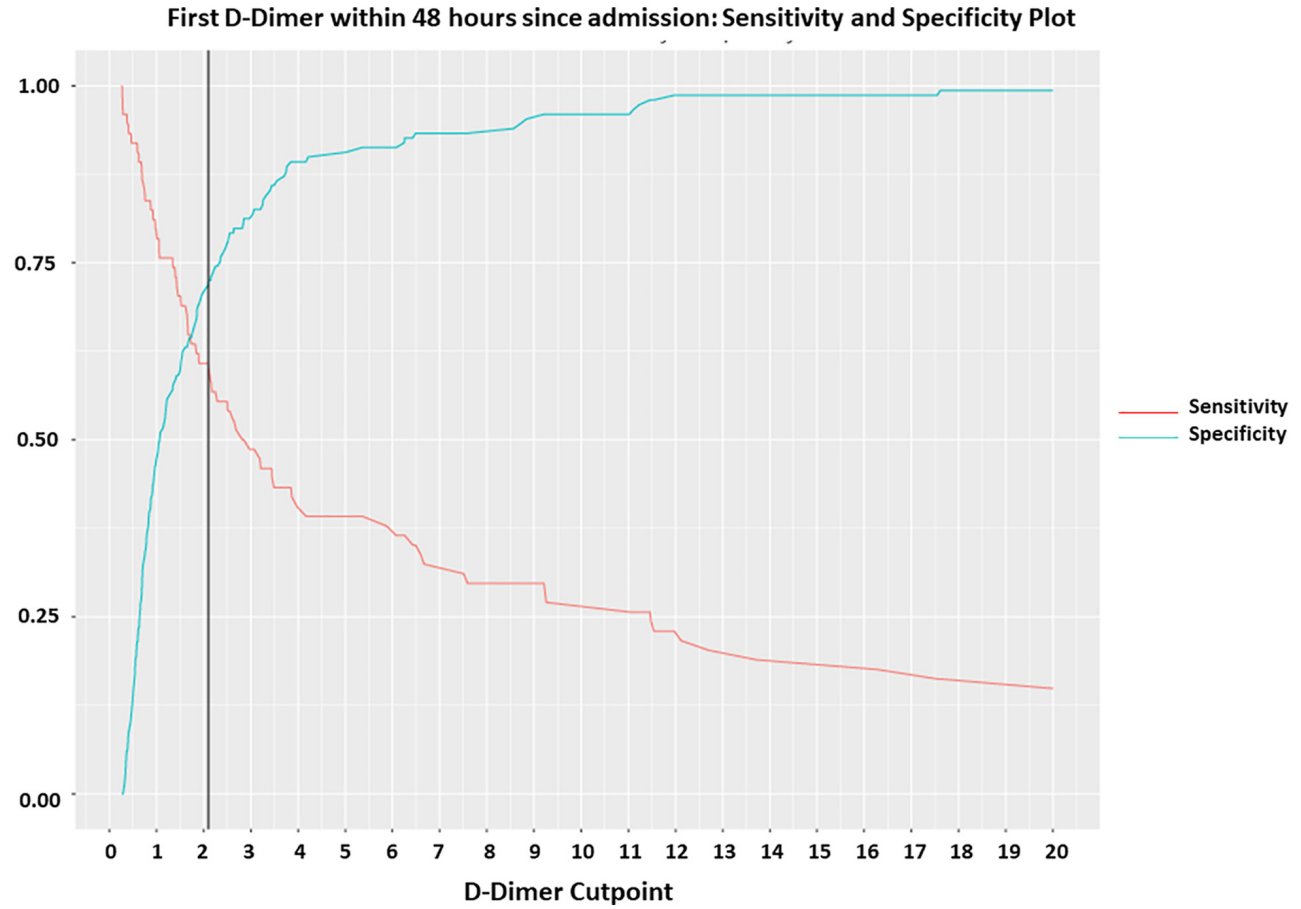


FIGURE 3 | Optimal cut off of initial D-Dimer by Youden's *J* statistics. Youden index measuring the optimal cut point for initial D-Dimer as a differentiating marker when equal weight is given to sensitivity and specificity for the values in the cohort. The optimal cut-point for the initial D-Dimer within 48 h since admission to predict mortality was found to be at 2.1 g/ml at a sensitivity of 0.61, specificity 0.73 and AUC 0.71.

minorities that is largely underserved and understudied (Blacks and Hispanics with a minor population of Whites and Asians). Given the huge patient load, only the very sick were being admitted in the Bronx. Survival in our hospitalized patients during this period was poor, with 33% of the hospitalized patients dying. This is comparable to the death rate of 28.3% for the rest of New York City during the COVID-19 peak (Filardo et al., 2020; Kalyanaraman Marcello et al., 2020; Lamb et al., 2020; Thompson et al., 2020).

Although both male gender and Black race were increased in those who died vs. those who were discharged, we did not get a significant association. This is probably due to the smaller sample size in our cohort which limited comprehensive study of the influences of comorbidities, race and socioeconomic status. Indeed, larger studies have shown a relationship of Black race with increased mortality (Laurencin and McClinton, 2020), including a recent study from our same institution (Neugarten et al., 2020) that demonstrated that Blacks have a higher mortality even after adjusting by age and comorbidities.

Several published cohorts have examined laboratory characteristics of patient admissions (Huang C. et al., 2020; Richardson et al., 2020; Zhang L. et al., 2020). Concordantly with those, we show that PT and D-Dimer within first 48hrs of admission were associated with mortality, underscoring the association of COVID-19 coagulopathy with mortality (Long et al., 2020). There is evidence of both an increase in venous and arterial disease in COVID-19 and many patients have been demonstrated to have antiphospholipid antibodies (Harzallah et al., 2020; Mathian et al., 2020; Reyes Gil et al., 2020; Zhang Y. et al., 2020). 81% of the non-survivors and 76% of survivors received anticoagulation. Our data do not show any differences in outcome based on anticoagulation likely due to size limitations of our small cohort. Indeed, we and others, have demonstrated in a larger cohort that prophylactic anticoagulation reduces mortality (Billett et al., 2020). Despite proper anticoagulation the rate thrombosis is high in COVID inpatients (Al-Ani et al., 2020; Alonso-Fernandez et al., 2020; Demelo-Rodriguez et al., 2020; Helms et al., 2020; Le Jeune et al., 2020; Llitjos et al., 2020). We observed a rate of *in vivo* thrombosis 4.4% (10/225), which is likely an underestimate due to lack of surveillance and limited imaging studies during the COVID peak. Although maximum D-Dimer levels were not significantly different

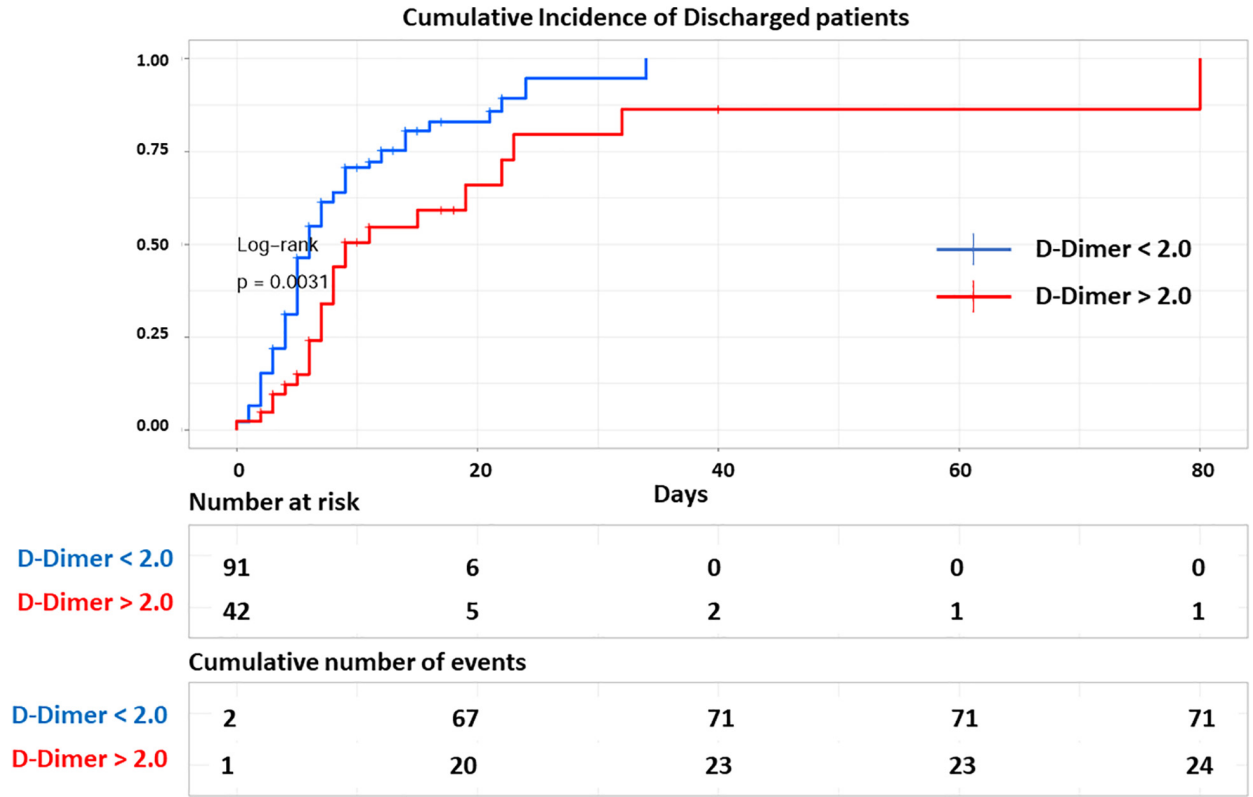


FIGURE 4 | Cumulative discharged curve. Kaplan Meier curve shows cumulative number of discharged COVID-19 positive patients over time (n = 133) based on initial D-Dimer. Patients with initial D-Dimer < 2 ug/ml (blue) showed higher rate of discharge alive compared to patients with initial D-Dimer > 2 ug/ml (red) (log rank, p = 0.0031). Bottom table shows the number of COVID-19 positive patients admitted and at risk of mortality over time and the cumulative number of discharged patients in each group in increments of every 10 days. Each line represents a discharged patient.

between anticoagulation groups vs. non-anticoagulated patients, maximum D-Dimer levels were significantly higher in patients that developed clots (including *in vivo* and *ex vivo* clots) in both categories discharged and deceased patients. Whereas initial D-Dimer was significantly higher among patients that developed clots and eventually died but not in patients that survived. Interestingly, we observed a higher rate of *ex vivo* thrombosis (mainly in hemodialysis lines) 7% (16/225), significantly higher in non-survivors vs. survivors (16% vs. 2.7%, p = 0.0002). This difference is likely a reflection of the higher hemodialysis needs in non-survivors compared to survivors (90% vs. 48%). Nonetheless, the observation of *ex vivo* clots in hemodialysis lines despite anticoagulation suggests that this disease may present with more of a thrombotic microangiopathy (TMA) picture and may be more amenable to TMA therapeutics (Henry et al., 2020; Sweeney et al., 2020; Wang et al., 2020; Cugno et al., 2021). Studies are ongoing looking at therapies with anticoagulation, anti-complement, fibrinolysis (Bikdeli et al., 2020; Laurence et al., 2020).

Modeling showed that an initial D-Dimer value of about 2 μg/ml could distinguish between those that would survive and those that would not. However, sensitivity and specificity of Youden's cut off were <0.8, indicating that a single initial D-Dimer provides limited information and may need to be coupled with other parameters and/or followed by serial trending. Indeed, analysis of the maximum and minimum levels of important lab parameters indicated that, in addition to the importance of the initial D-Dimer to screen patients that present with coagulopathy and are at higher risk of mortality, the maximum D-Dimer during hospitalization was also associated with mortality. Creel-Bulos et al. (2020) demonstrated that the D-Dimer maximum, magnitude and rate of rise in the first 10 days of admission correlated with VTE but not mortality in a cohort of 115 COVID-19 + inpatients. Similarly, we showed that initial D-Dimer and maximum D-Dimer correlated with clot development but also mortality. The lack of D-Dimer association with mortality in the Creel-Bulos et al. (2020) study may be due to a sample size limitation. Huang et al., showed that an initial D-Dimer = 1 ug/mL correlates with increased risk of mortality. In another cohort from Zhang L. et al. (2020) showed that a D-Dimer cut off = 2 ug/mL better predicted mortality. Similarly, our study showed that a cut off close to 2 ug/mL on initial D-Dimer best stratified our patients at higher risk of mortality. Blacks are known to have higher mean baseline D-Dimers than Europeans and Asians (Naik et al., 2016; Raffield et al., 2017) and the majority of the Hispanics in the Bronx are Afro-Caribbean descendants. Thus, we believe that racial, ethnical, demographic and socioeconomic characteristic

are important factors to consider when establishing guidelines utilizing D-Dimer for patient stratification and patient care. Given these data, we would suggest that D-Dimer be factored in the decision-making algorithm of whom to dismiss from the ED. In the Richardson study 2.2% were readmitted within 3 days, although follow-up time was short at 4.5 days (Richardson et al., 2020). Examining the D-Dimer of these patients before discharge may enable us to make more informed decisions. There is evidence that thrombotic and bleeding events may occur in COVID patients post-discharge (Patell et al., 2020); following D-Dimers might be a way to distinguish who should get prolonged thromboprophylaxis. Indeed, the majority of the patients who were readmitted had elevated D-Dimer on readmission (**Table 9**). 4 of these patients (18% (4/22) among readmitted and 2.7% (4/150) among all discharged patients) had documented thrombosis.

TABLE 7 | Thrombosis and anticoagulation in patients that died during hospitalization vs. patient discharged.

	Died (*n* = 75)	Discharged (*n* = 150)	*p*
Thrombosis	2 (2.7)	8 (5.3)	0.837
• Deep Venous Thrombosis	1 (1.3)	6 (4.0)	0.277
• Pulmonary Embolism	0 (0.0)	1 (0.7)	1
• Arterial Thrombosis	1 (1.3)	1 (0.7)	1
• Stroke	0 (0.0)	0 (0.0)	1
Ex Vivo Clotting	12 (16)	4 (2.7)	0.0002
Anticoagulation, n (%)			
None	14 (18.7)	24 (16.0)	0.902
Prophylactic	45 (60)	104 (69.3)	0.720
• Heparin	6 (8.0)	14 (9.3)	1.000
• Enoxaparin	38 (50.7)	89 (59.3)	0.899
• Apixaban	1 (1.3)	1 (0.7)	0.660
Therapeutic	16 (21.3)	22 (14.7)	0.576
• Heparin	5 (6.7)	5 (3.3)	1.000
• Enoxaparin	1 (1.3)	3 (2.0)	0.146
• Apixaban	5 (6.7)	12 (8.0)	0.718
• Bivalirudin	2 (2.7)	0 (0.0)	0.530
• Warfarin	3 (4.0)	2 (1.3)	1.000
Maximum D-Dimer by anticoagulation			
None, D-Dimer μg/ml FEU (Median (IQR))	7.58 (2.81, 11.02)	1.20 (0.70, 3.13)	0.03
Prophylactic, D-Dimer μg/ml FEU (Median (IQR))	3.45 (1.82, 17.07)	1.35 (0.68, 2.63)	<0.0001
Therapeutic, D-Dimer μg/ml FEU (Median (IQR))	6.18 (3.03, 11.16)	0.94 (0.71, 3.76)	0.11
One-Way ANOVA *p*-Value	0.29	0.99	

TABLE 8 | Initial D-Dimer and maximum D-Dimer levels in discharged and expired patients that developed clots during hospitalization.

	Clot	No Clot	*P*-value
Discharged patients			
1st D-Dimer ug/ml FEU (Median (IQR))	1.64 (0.97, 5.50)	1.02 (0.63, 2.17)	0.20
Max D-Dimer ug/ml FEU (Median (IQR))	9.83 (4.33, 15.98)	1.13 (0.66, 2.61)	0.01
Expired patients			
1st D-Dimer ug/ml FEU (Median (IQR))	12.08 (5.49, 20)	2.16 (1.05, 6.6)	0.006
Max D-Dimer ug/ml FEU (Median (IQR))	14.48 (7.78, 20)	3.21 (1.7, 9.26)	0.002

TABLE 9 | Description of complications in discharged patients that required readmission.

cases	Respiratory	Cardiovascular	Renal	Thrombosis	Infection	Readmission Days since discharge	D-Dimer ug/ml readmission	Death
66 M	Acute hypoxia RF				Septic shock	11	6.25	yes
38 F			AKI	Sickle cell VOC DVT, splenic infarct		26	ND	yes
72 M	Acute respiratory failure/ventilation				Aspiration PNA	27	1.20	
74 M		CAD				124	ND	
81 M					E Coli bacteremia	94	1.58	
50 M		Hypertensive urgency				71	ND	
75 M	Hypoxic RF					19	9.90	yes
62 F				PE/DVT		32	ND	
61 M		CHF				9	ND	yes
44 M					Acute cholecystitis	84	0.91	
80 M					epididymitis/ochitis	95	ND	
75 M	Hypoxic RF		AKI			6	2.44	yes
68 M					MRSA PNA	20	0.40	
51 M				RLE thrombosis		58	ND	
56 F			CKD HD			51	ND	
58 F			Volume overload/dialysis			6	ND	
35 M					Fever, viral URI	161	ND	
77 F				DVT	Extended spectrum beta-lactamase sepsis	7	2.31	yes
52 M		myocarditis				3	1.20	
81 F				R foot ischemia arterial occlusion		66	ND	
87 M	Hypoxic RF					14	13.5	yes
85 F					Septic shock	28	ND	yes

Among the patients that required readmission (22/150, 15%) more than a third died (8/22, 5% of all discharged patients) mainly due to respiratory failure or septic shock. D-Dimer on readmission was available in 4 out of 16 patients that survived readmission and 5 out of 8 patients that died during readmission. In those patients with available D-Dimer that died during readmission, the D-Dimer levels were > 2 ug/ml (**Table 6**).

Given the strength of D-Dimer as a predictor of mortality, future studies should focus on establishing guidelines on how to use D-Dimer trending in different settings to better predict mortality, monitor disease progression and response to treatment (Hardy et al., 2021).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Albert Einstein College of Medicine IRB committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MR and HB conceptualized the study design, performed the methodology, and wrote IRB protocol. JG-L, SR, MB, KI, and JS collected the data and analyzed the data. MR, MB, and JS performed the formal analysis and validation. YL advised on statistical analysis. JS and MB provided visual graphics. MR and HB wrote the manuscript. All authors reviewed, edited, and approved final manuscript submission.

ACKNOWLEDGMENTS

We greatly appreciate the efforts of our fellow health care workers and support staff at the Montefiore Medical Center for providing outstanding patient care at considerable personal risk on the front lines of this pandemic. We express our support for our patients with Covid-19 and their families. We also thank Dr. Eran Bellin, MD (Vice President Clinical IT Research and Development Montefiore Information Technology Professor of Clinical Epidemiology and Population Health Albert Einstein College of Medicine) and Lindsay Stahl for providing guidance about study design and interpretation of results. This manuscript has been released as a pre-print at MedRXiv (Lamb et al., 2020).

REFERENCES

Al-Ani, F., Chehade, S., and Lazo-Langner, A. (2020). Thrombosis risk associated with COVID-19 infection. A scoping review. *Thromb. Res.* 192, 152–160. doi: 10.1016/j.thromres.2020.05.039

Alonso-Fernandez, A., Toledo-Pons, N., Cosio, B. G., Millan, A., Calvo, N., Ramon, L., et al. (2020). Prevalence of pulmonary embolism in patients with COVID-19 pneumonia and high D-dimer values: A prospective study. *PLoS One* 15:e0238216. doi: 10.1371/journal.pone.0238216

Berger, J. S., Kunichoff, D., Adhikari, S., Ahuja, T., Amoroso, N., Aphinyanaphongs, Y., et al. (2020). Prevalence and Outcomes of D-Dimer Elevation in Hospitalized Patients With COVID-19. *Arterioscler. Thromb. Vasc. Biol.* 40, 2539–2547. doi: 10.1161/atvbaha.120.314872

Bikdeli, B., Madhavan, M. V., Jimenez, D., Chuich, T., Dreyfus, I., Driggin, E., et al. (2020). COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-up. *J. Am. Coll. Cardiol.* 75, 2950–2973.

Billett, H. H., Reyes-Gil, M., Szymanski, J., Ikemura, K., Stahl, L. R., Lo, Y., et al. (2020). Anticoagulation in COVID-19: Effect of Enoxaparin, Heparin, and Apixaban on Mortality. *Thromb. Haemost.* 120, 1691–1699. doi: 10.1055/s-0040-1720978

Bursac, Z., Gauss, C. H., Williams, D. K., and Hosmer, D. W. (2008). Purposeful selection of variables in logistic regression. *Source Code Biol. Med.* 3:17.

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395, 507–513. doi: 10.1016/s0140-6736(20)30211-7

Creel-Bulos, C., Liu, M., Auld, S. C., Gaddh, M., Kempton, C. L., Sharifpour, M., et al. (2020). Trends and diagnostic value of D-dimer levels in patients hospitalized with coronavirus disease 2019. *Med.* 99:e23186. doi: 10.1097/md.0000000000023186

Cucinotta, D., and Vanelli, M. W. H. O. (2020). Declares COVID-19 a Pandemic. *Acta Biomed.* 91, 157–160.

Cugno, M., Meroni, P. L., Gualtierotti, R., Griffini, S., Grovetti, E., Torri, A., et al. (2021). Complement activation and endothelial perturbation parallel COVID-19 severity and activity. *J. Autoimmun.* 116:102560. doi: 10.1016/j.jaut.2020.102560

Demelo-Rodriguez, P., Cervilla-Munoz, E., Ordieres-Ortega, L., Parra-Virto, A., Toledano-Macias, M., Toledo-Samaniego, N., et al. (2020). Incidence of asymptomatic deep vein thrombosis in patients with COVID-19 pneumonia and elevated D-dimer levels. *Thromb. Res.* 192, 23–26. doi: 10.1016/j.thromres.2020.05.018

Filardo, T. D., Khan, M. R., Krawczyk, N., Galitzer, H., Karmen-Tuohy, S., Coffee, M., et al. (2020). Comorbidity and clinical factors associated with COVID-19 critical illness and mortality at a large public hospital in New York City in the early phase of the pandemic (March-April 2020). *PLoS One* 15:e0242760. doi: 10.1371/journal.pone.0242760

Goyal, P., Choi, J. J., Pinheiro, L. C., Schenck, E. J., Chen, R., Jabri, A., et al. (2020). Clinical Characteristics of Covid-19 in New York City. *N. Engl. J. Med.* 382, 2372–2374.

Gungor, B., Atici, A., Baycan, O. F., Alici, G., Ozturk, F., Tugrul, S., et al. (2020). Elevated D-dimer levels on admission are associated with severity and increased risk of mortality in COVID-19: A systematic review and meta-analysis. *Am. J. Emerg. Med.* 39, 173–179. doi: 10.1016/j.ajem.2020.09.018

Hardy, M., Michaux, I., Lessire, S., Douxfils, J., Dogne, J. M., Bareille, M., et al. (2021). Prothrombotic disturbances of hemostasis of patients with severe COVID-19: A prospective longitudinal observational study. *Thromb. Res.* 197, 20–23. doi: 10.1016/j.thromres.2020.10.025

Harzallah, I., Debliquis, A., and Drenou, B. (2020). Lupus anticoagulant is frequent in patients with Covid-19. *J. Thromb. Haemost.* 18, 2064–2065. doi: 10.1111/jth.14867

Helms, J., Tacquard, C., Severac, F., Leonard-Lorant, I., Ohana, M., Delabranche, X., et al. (2020). High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med.* 46, 1089–1098. doi: 10.1007/s00134-020-06062-x

Henry, B. M., Benoit, S. W., de Oliveira, M. H. S., Lippi, G., Favaloro, E. J., and Benoit, J. L. (2020). ADAMTS13 activity to von Willebrand factor antigen ratio

predicts acute kidney injury in patients with COVID-19: Evidence of SARS-CoV-2 induced secondary thrombotic microangiopathy. *Int. J. Lab. Hematol.* 2020:3415.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan. China. *Lancet* 395, 497–506.

Huang, Y., Lyu, X., Li, D., Wang, L., Wang, Y., Zou, W., et al. (2020). A cohort study of 676 patients indicates D-dimer is a critical risk factor for the mortality of COVID-19. *PLoS One* 15:e0242045. doi: 10.1371/journal.pone.0242045

Kalyanaraman Marcello, R., Dolle, J., Grami, S., Adule, R., Li, Z., Tatem, K., et al. (2020). Characteristics and outcomes of COVID-19 patients in New York City's public hospital system. *PLoS One* 15:e0243027. doi: 10.1371/journal.pone.0243027

Lamb, M. R., Kandula, S., and Shaman, J. (2020). Differential COVID-19 case positivity in New York City neighborhoods: Socioeconomic factors and mobility. *Influenza Other Respir. Viruses*. Preprint.

Laurence, J., Mulvey, J. J., Seshadri, M., Racanelli, A., Harp, J., Schenck, E. J., et al. (2020). Anti-complement C5 therapy with eculizumab in three cases of critical COVID-19. *Clin. Immunol.* 219:108555. doi: 10.1016/j.clim.2020.108555

Laurencin, C. T., and McClinton, A. (2020). The COVID-19 Pandemic: a Call to Action to Identify and Address Racial and Ethnic Disparities. *J. Racial Ethn. Health Disparities* 7, 398–402. doi: 10.1007/s40615-020-00756-0

Le Jeune, S., Suhl, J., Benainous, R., Minvielle, F., Purser, C., Foudi, F., et al. (2020). High prevalence of early asymptomatic venous thromboembolism in anticoagulated COVID-19 patients hospitalized in general wards. *J. Thromb. Thrombol.* 2020, 1–5.

Llitjos, J. F., Leclerc, M., Chochois, C., Monsallier, J. M., Ramakers, M., Auvray, M., et al. (2020). High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. *J. Thromb. Haemost.* 18, 1743–1746. doi: 10.1111/jth.14869

Long, H., Nie, L., Xiang, X., Li, H., Zhang, X., Fu, X., et al. (2020). D-Dimer and Prothrombin Time Are the Significant Indicators of Severe COVID-19 and Poor Prognosis. *Biomed. Res. Int.* 2020:6159720.

Mathian, A., Mahevas, M., Rohmer, J., Roumier, M., Cohen-Aubart, F., Amador-Borrero, B., et al. (2020). Clinical course of coronavirus disease 2019 (COVID-19) in a series of 17 patients with systemic lupus erythematosus under long-term treatment with hydroxychloroquine. *Ann. Rheum. Dis.* 79, 837–839.

Naik, R. P., Wilson, J. G., Ekunwe, L., Mwasongwe, S., Duan, Q., Li, Y., et al. (2016). Elevated D-dimer levels in African Americans with sickle cell trait. *Blood* 127, 2261–2263. doi: 10.1182/blood-2016-01-694422

Neugarten, J., Fisher, M., Billett, H. H., Gil, M. R., Johns, T., et al. (2020). The association of race and COVID-19 mortality. *EClinicalMedicine* 25: 100455.

NYC, (2020). Available online at: https://www1.nyc.gov/site/doh/covid/covid-19-data.page#download [nyc.gov]. https://www1.nyc.gov/site/doh/covid/covid-19-data.

Patell, R., Bogue, T., Koshy, A., Bindal, P., Merrill, M., Aird, W. C., et al. (2020). Postdischarge thrombosis and hemorrhage in patients with COVID-19. *Blood* 136, 1342–1346.

Raffield, L. M., Zakai, N. A., Duan, Q., Laurie, C., Smith, J. D., Irvin, M. R., et al. (2017). D-Dimer in African Americans: Whole Genome Sequence Analysis and Relationship to Cardiovascular Disease Risk in the Jackson Heart Study. *Arterioscler. Thromb. Vasc. Biol.* 37, 2220–2227. doi: 10.1161/atvbaha.117.310073

Reyes Gil, M., Barouqa, M., Szymanski, J., Gonzalez-Lugo, J. D., Rahman, S., and Billett, H. H. (2020). Assessment of Lupus Anticoagulant Positivity in Patients With Coronavirus Disease 2019 (COVID-19). *JAMA Netw. Open* 3:e2017539. doi: 10.1001/jamanetworkopen.2020.17539

Richardson, S., Hirsch, J. S., Narasimhan, M., Crawford, J. M., McGinn, T., Davidson, K. W., et al. (2020). Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* 323, 2052–2059. doi: 10.1001/jama.2020.6775

Sweeney, J. M., Barouqa, M., Krause, G. J., Gonzalez-Lugo, J. D., Rahman, S., and Gil, M. R. (2020). Evidence for secondary thrombotic microangiopathy in COVID-19. *medRxiv*. Preprint.

Tang, N., Li, D., Wang, X., and Sun, Z. (2020). Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J. Thromb. Haemost.* 18, 844–847. doi: 10.1111/jth.14768

Thompson, C. N., Baumgartner, J., Pichardo, C., Toro, B., Li, L., Arciuolo, R., et al. (2020). COVID-19 Outbreak - New York City, February 29-June 1, 2020. *MMWR Morb. Mortal Wkly Rep.* 69, 1725–1729.

Valerio, L., Ferrazzi, P., Sacco, C., Ruf, W., Kucher, N., Konstantinides, S. V., et al. (2020). Course of D-Dimer and C-Reactive Protein Levels in Survivors and Nonsurvivors with COVID-19 Pneumonia: A Retrospective Analysis of 577 Patients. *Thromb. Haemost.* 121, 98–101.

Vidali, S., Morosetti, D., Cossu, E., Luisi, M. L. E., Pancani, S., Semeraro, V., et al. (2020). D-dimer as an indicator of prognosis in SARS-CoV-2 infection: a systematic review. *ERJ Open Res.* 6, 00260–2020. doi: 10.1183/23120541.00260-2020

Wadhera, R. K., Wadhera, P., Gaba, P., Figueroa, J. F., Joynt Maddox, K. E., Yeh, R. W., et al. (2020). Variation in COVID-19 Hospitalizations and Deaths Across New York City Boroughs. *JAMA* 323, 2192–2195. doi: 10.1001/jama.2020.7197

Wang, X., Sahu, K. K., and Cerny, J. (2020). Coagulopathy, endothelial dysfunction, thrombotic microangiopathy and complement activation: potential role of complement system inhibition in COVID-19. *J. Thromb. Thrombol.* 2020, 1–6.

Wu, J., Liu, J., Zhao, X., Liu, C., Wang, W., Wang, D., et al. (2020). Clinical Characteristics of Imported Cases of COVID-19 in Jiangsu Province: A Multicenter Descriptive Study. *Clin. Infect. Dis.* 71, 706–712. doi: 10.1093/cid/ciaa199

Yao, Y., Cao, J., Wang, Q., Shi, Q., Liu, K., Luo, Z., et al. (2020). D-dimer as a biomarker for disease severity and mortality in COVID-19 patients: a case control study. *J. Intensive Care* 8:49.

Yu, B., Li, X., Chen, J., Ouyang, M., Zhang, H., Zhao, X., et al. (2020). Evaluation of variation in D-dimer levels among COVID-19 and bacterial pneumonia: a retrospective analysis. *J. Thromb. Thrombol.* 50, 548–557. doi: 10.1007/s11239-020-02171-y

Yu, H. H., Qin, C., Chen, M., Wang, W., and Tian, D. S. D. - (2020). dimer level is associated with the severity of COVID-19. *Thromb. Res.* 195, 219–225. doi: 10.1016/j.thromres.2020.07.047

Zhang, L., Yan, X., Fan, Q., Liu, H., Liu, X., Liu, Z., et al. (2020). D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. *J. Thromb. Haemost.* 18, 1324–1329. doi: 10.1111/jth.14859

Zhang, Y., Xiao, M., Zhang, S., Xia, P., Cao, W., Jiang, W., et al. (2020). Coagulopathy and Antiphospholipid Antibodies in Patients with Covid-19. *N. Engl. J. Med.* 382:e38.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395, 1054–1062. doi: 10.1016/s0140-6736(20)30566-3

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

REVIEW
published: 25 February 2021
doi: 10.3389/fphys.2021.624698

Metabolic Alterations in SARS-CoV-2 Infection and Its Implication in Kidney Dysfunction

Magaiver Andrade Silva[1,2], Ana Ruth Paolinetti Alves da Silva[1], Mariana Abrantes do Amaral[2], Matheus Garcia Fragas[2] and Niels Olsen Saraiva Câmara[1,2]*

[1]Laboratory of Experimental and Clinical Immunology, Department of Clinical Medicine, Faculty of Medicine, Federal University of São Paulo, São Paulo, Brazil, [2]Laboratory of Transplantation Immunobiology, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Clinical strategies focusing on pathogen elimination are expected in an infectious-disease outbreak, such as the severe coronavirus disease 2019 (COVID-19), to avoid organ dysfunction. However, understanding the host response to viral infection is crucial to develop an effective treatment to optimize the patient's conditions. The pathogenic viruses can promote metabolic changes during viral infection, favoring its survival, altering cell phenotype and function, and causing sustained inflammation and tissue injury. Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19, provokes systemic and cell metabolic changes and possibly altering lipid and glucose metabolism. Besides severe acute respiratory syndrome (SARS), SARS-CoV-2 can cause acute kidney injury, which has been associated with the severity of the disease. Although it is not clear the mechanisms whereby SARS-CoV-2 induces kidney dysfunction, it is known that the virus presents kidney tropism, namely, podocytes and proximal tubular epithelial cells. Changes in renal cell metabolism and systemic metabolic disorders are important events in kidney injury progression. Here, we explored the metabolism and its interface with SARS-CoV-2 infection and raised the perspective on metabolism disturbances as a critical event to kidney dysfunction in COVID-19.

Keywords: metabolism, tubular epithelial cells, glycolysis, fat acid oxidation, COVID-19

OPEN ACCESS

Edited by:
Ravi Nistala, University of Missouri, United States

Reviewed by:
Yanlin Wang, University of Connecticut School of Medicine, United States
Sophie De Seigneux, Geneva University Hospitals (HUG), Switzerland

****Correspondence:***
Magaiver Andrade Silva
magaiver.andrade@unifesp.br

Specialty section:
This article was submitted to Renal and Epithelial Physiology, a section of the journal Frontiers in Physiology

Received: *01 November 2020*
Accepted: *27 January 2021*
Published: *25 February 2021*

Citation:
Andrade-Silva M, da Silva ARPA, do Amaral MA, Fragas MG and Câmara NOS (2021) Metabolic Alterations in SARS-CoV-2 Infection and Its Implication in Kidney Dysfunction. Front. Physiol. 12:624698. doi: 10.3389/fphys.2021.624698

INTRODUCTION

The role of metabolic pathways has been little explored in the pathogenesis of several diseases. More recently, a substantial number of studies have reported that abnormal systemic or cellular metabolism is a central point in several disorders (Hotamisligil, 2006; DeBerardinis and Thompson, 2012). The metabolic functions involve different pathways, such as glycolysis, the tricarboxylic acid cycle, the pentose phosphate pathway, oxidative phosphorylation, and fatty acid oxidation, among many others, which act in an integrated manner to maintain the balance and organism homeostasis. Thereof, perturbations in these pathways are associated with the development and progression of infection and non-infection disorders (Heaton and Randall, 2010; Vastag et al., 2011; DeBerardinis and Thompson, 2012; Thai et al., 2014; Ayres, 2020).

In the last decade, an increasing number of studies aimed at investigating the crosstalk between cell metabolism and viral infection (Heaton and Randall, 2010; Vastag et al., 2011; Thai et al., 2014; Moreno-Altamirano et al., 2019; Thaker et al., 2019). These studies demonstrated that several viruses cause cell metabolic reprogramming in immune cells, including alterations on the glycolic pathway, tricarboxylic acid cycle, amino acids, and lipid synthesis (Heaton and Randall, 2010; Vastag et al., 2011; Thai et al., 2014; Moreno-Altamirano et al., 2019; Thaker et al., 2019). The fate of this metabolic perturbation is the development of viral strategies to escape from immune response and to induce severe tissue inflammation, as reported in patients infected with severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2; Huang et al., 2020; Lucas et al., 2020), the etiological agent of the coronavirus disease 2019 (COVID-19). Initial studies reported that SARS-CoV-2 causes alterations in systemic and cellular homeostasis, affecting energy metabolism (Bruzzone et al., 2020; Wu et al., 2020), and it may influence the normal function of several organs, contributing to the severity of COVID-19.

The entry of SARS-CoV-2 in host cells depends on the interaction between the Spike protein and angiotensin-converting enzyme 2 (ACE2). The Spike protein needs to be priming by specific proteases (Sungnak et al., 2020), such as TMPRSS2 (Hoffmann et al., 2020) and furin protease (Vankadari, 2020). Initially, SARS-CoV-2 infects the epithelial cells in the lungs (Zou et al., 2020). However, it can target other organs, which can considerably aggravate the clinical condition of hospitalized patients (Gupta et al., 2020), becoming COVID-19 a multiorgan disease. Kidneys are one of the main organs affected in COVID-19, resulting in elevated proteinuria, hematuria, and even acute kidney injury (AKI; Cummings et al., 2020; Hirsch et al., 2020), a severe complication in the intensive care unit associated with high mortality and morbidity (Braun et al., 2020). Studies performed in China, the United States, and the United Kingdom reported an AKI incidence of 17–43% in hospitalized patients with COVID-19, but these numbers, which are higher in patients in critical condition, range from 61 to 76% (Vijayan and Humphreys, 2020).

Histological analysis of post-mortem tissue demonstrated that viral RNA in kidneys correlates with the renal tropism of SARS-CoV-2 with early death and AKI development (Braun et al., 2020). SARS-CoV-2 preferentially infects tubular epithelial cells (Puelles et al., 2020), considered the epicenter of renal damage in the kidneys. A recent study has shown a frequency of symptoms related to kidney damage in confirmed COVID-19 patients hospitalized in Wuhan (China), where 43.9% of patients had proteinuria, and 26.7% had hematuria, increased serum creatinine levels and blood urea nitrogen, and a glomerular filtration less than 60 ml/min/1.73 m2 was observed in around ~13% of patients (Cheng et al., 2020). Other studies have also suggested that kidney function is a marker for mortality in COVID-19 patients (Brienza et al., 2020; Naicker et al., 2020; Trabulus et al., 2020).

Currently, the exact mechanisms involved in renal damage during COVID-19 have not been clear, and probably are multifactorial. Changes in systemic and cell metabolism in COVID-19 may exert an essential contribution to kidney dysfunction. In this review, we first explore the interface of metabolism and SARS-CoV-2 infection (especially at the cellular level), then raising a perspective that systemic and cellular metabolism disorders should be considered an important mechanism of renal dysfunction in COVID-19.

EMERGING PERSPECTIVES OF METABOLISM IN COVID-19 PATHOGENESIS

Previous reports about virus infections demonstrated the importance of metabolism on the disease outcome. In 2003, the most critical cases of SARS happened in patients with metabolic disorders (Booth et al., 2003; Ayres, 2020), which demonstrate the importance to understand how metabolic changes could affect the course of infectious diseases as a warning of what to expect on future viral infections. Obesity, type 2 diabetes (T2D), and hypertension are related to the worst prognosis of COVID-19 (Guan et al., 2020).

It is well established that one of the critical phases of COVID-19 is the cytokine storm generated by the host response due to infection, causing an extreme inflammation process (Tay et al., 2020). Patients with previous state of chronic inflammation, as observed in most metabolic disorders (Luft et al., 2013), have more chances of presenting the cytokine storm, causing a physiological unbalance and increased health aggravation. In obesity cases, the poor condition could be due to the difficulty of ventilation associated with diaphragm excursion hampered (Burns et al., 1994); or T2D that causes decreased respiratory function, pulmonary fibrosis, and chronic obstructive pulmonary disorder (Ehrlich et al., 2010). However, the significant risk for patients with an impairment in metabolic health could be beyond respiration problems. It can also be associated with the modification of metabolism in different organs. This section described the emerging studies focusing on SARS-CoV-2 infection and its interface with energy metabolism.

Lipid Metabolism and COVID-19

Recent studies have demonstrated the multifaceted roles of lipids in viral infection, involving lipid signaling, synthesis, and host cell metabolism modulation to subvert the protective immune response (Heaton and Randall, 2011; Murillo et al., 2015). Some studies have demonstrated that interruption in lipid synthesis impairs virus replication, suggesting that lipid pathways can represent a relevant target in the investigation of viral disorders (Merino-Ramos et al., 2016). Patients infected with SARS-CoV-2 presented altered levels of lipids. Diglycerides, free fatty acids, and triglycerides were identified in higher amounts in the fatality group (Wu et al., 2020). Furthermore, *ex vivo* and *in vitro* studies reported increased viral replication in cells with excessive intracellular lipid accumulation (Dias et al., 2020). These initial findings suggest that in SARS-CoV-2 infection, systemic and cell lipid metabolism disturbances can be critical event in COVID-19 progression.

In a recent study conducted by Thomas et al. (2020), the authors investigated the metabolic effects of SARS-CoV-2 infection by analyzing serum metabolites from patients with COVID-19 in comparison with COVID-19-negative controls (Thomas et al., 2020). The results demonstrated an increase of free fatty acids in circulation, especially in patients with high inflammatory cytokine levels (Thomas et al., 2020). Accordingly, another finding revealed alterations in a diversity of metabolites in serum of the COVID-19 patients, highlighting the expressive reduction of malic acid and glycerol 3-phosphate in fatality, severe and mild COVID-19 groups (Wu et al., 2020). Both metabolites, malic acid and glycerol 3-phosphate, are involved in energy metabolism, the first enters in tricarboxylic acid cycle in mitochondria, and the latter is a chemical intermediate in the glycolysis pathway, evidencing the alteration in metabolites that participate in human energy metabolism (Wu et al., 2020). Similar altered lipid profile was observed in SARS-CoV infection, even 12 years after recovery from the disease, patients infected with SARS-CoV revealed dysregulated levels of free fatty acids in the serum (Wu et al., 2017).

Also, SARS-CoV-2-infected human bronchial epithelial cells presented 59–65% of the differentially expressed genes related to metabolism, including 8–18% of the genes associated with lipid metabolic pathways (Ehrlich et al., 2020). However, cellular and molecular mechanisms that orchestrate lipid metabolism during SARS-CoV-2 infection are poorly described so far. Recently, it was observed the lipid bodies formation in monocytes from infected patients and *in vitro* assay of SARS-CoV-2 infection (Dias et al., 2020). The lipid bodies have been described as a source of inflammatory mediators and contribute to pathogen escape from immune system elimination (D'Avila et al., 2006, 2008; Mattos et al., 2011; Almeida et al., 2014). Dias et al. (2020) observed the colocalization of lipid bodies and SARS-CoV-2, suggesting them as a fuel for viral replication. The inhibition of lipid bodies formation reduced the viral load, cell death, and levels of inflammatory mediators. Mechanistically, the authors reported an increase in expression of transcription factor sterol regulatory element-binding protein 1 (SREBP-1) and the nuclear receptor peroxisome proliferator-activated receptor (PPARγ) after SARS-CoV-2 infection, which could be an indicative of cell reprogramming toward a lipogenic phenotype. The inhibition of the SREBP in isolated lung epithelial cells and mice infected with the Mers-CoV virus suppresses viral replication (Yuan et al., 2019), since SREBP is considered a master regulator of lipogenesis (Eberlé et al., 2004).

SARS-CoV-2 changes lipid profile in the lung epithelial cells by interfering in PPARα and PPARγ expression or activity (Ehrlich et al., 2020), culminating in lipotoxicity, which became these molecules an attractive potential therapeutic target in COVID-19 patients (Heffernan et al., 2020). PPARγ acts as a transcription factor important to CD36 expression, involved in lipid uptake (Lim et al., 2006). While PPARα is associated with control of nuclear genes encoding fatty acid oxidation enzymes (Song et al., 2010). Clinical trials using fenofibrate, a PPARα agonist, are in course in the United States as a metabolic intervention in COVID-19,[1] which evidence the importance of lipid metabolism dysfunction in COVID-19 pathogenesis and progression.

Glucose Metabolism and COVID-19

Besides lipid homeostasis disruption, several studies observed an increase of glycolysis activity in immune and epithelial cells from patients with COVID-19 (Codo et al., 2020; Moolamalla et al., 2020). An unmanageable blood glucose level is associated with poor diagnoses and risk of mortality, according to a study with 7,000 patients infected with coronavirus (Zhu et al., 2020).

Codo et al. (2020) demonstrated that monocytes infected with SARS-CoV-2 presented increase of ACE2 expression and viral load depending on glucose concentration. SARS-CoV-2-infected human monocytes presented a greater glycolytic capacity and reserve. The same was not observed in human monocytes infected with influenza A virus and respiratory syncytial virus (RSV). Besides, the expression of inflammatory genes (such as TNF-α, IL-6, IL-1β, INF-α, and INF-β) was glucose dose-dependent and viral replication and enhanced ACE2 expression and cytokines are decreased once the flux of glucose was blocked by 2-deoxy-D-glucose (2-DG). However, when the ATP synthase was blocked (by oligomycin), the viral load was even higher (Codo et al., 2020). Therefore, glycolysis was essential for viral replication in monocytes, being a good source of carbon, similarly observed in epithelial cells (Caco-2 cells) infected with SARS-CoV-2 (Bojkova et al., 2020; Codo et al., 2020). One factor that may explain glycolysis metabolic changes in the infected cells is the increased expression of HIF-1α, which has been implicated in the increase of glycolytic genes expression and IL-1β release (Tannahill et al., 2013). HIF-1α can regulate the activity of genes related to glucose transport and processing (LDH-A, PFKFB3, GLUT-1, PKM2), which seems to be overexpressed in monocytes from COVID-19 patients, but not at the same intensity as influenza virus and RSV-infected monocytes (Codo et al., 2020).

Monocytes and macrophages are the most common immune cell types found in the lungs of patients infected with COVID-19 recruited in response to infection and injured lung cells (Bost et al., 2020). These cells respond to infection with the exacerbated release of several inflammatory cytokines and subsidy COVID-19 outcome (Blanco-Melo et al., 2020; Tay et al., 2020). The previous study demonstrated that during SARS-CoV infection, a delay in type I interferon (IFN) expression (which is involved in the antiviral response) was associated with an inappropriate inflammatory response and lung pathology (Channappanavar et al., 2016), providing a favorable environment for viral replication and tissue injury. Similarly, in SARS-CoV-2 infection, Blanco-Melo et al. (2020) revealed that occurs a reduction of antiviral response, concomitantly with an exacerbated inflammatory response evidenced by chemokines and IL-6 production (Blanco-Melo et al., 2020). However, it was not clear whether type I IFN response was delayed, which could drive COVID-19 progression. Based on previous studies, it is plausible to suggest

[1]clinicaltrials.gov/ct2/show/NCT04517396

that modulation of glycolysis in early type I IFN response could be a strategy to increase the host defense against the virus at the beginning of infection (Zhang et al., 2019; Ayres, 2020).

Recently, it was described how glycolysis can interfere with antiviral signaling. The hexokinase-2 is the initial enzyme of glycolysis, and its activity is suggested to be dependent on physical interaction with mitochondrial antiviral-signaling protein (MAVS) and dampened IFN-I production (Zhang et al., 2019). The IFN-I production in viral infection is dependent on the virus RNA recognition in the cytosol by retinoic-acid-inducible gene I (RIG-I)-like receptor (RLR), which leads to the formation of the RIG-I-MAVS-type I IFN axis (Zhang et al., 2019). The disruption of the MAVS interaction with hexokinase-2 increases type I IFN production. Corroborating this, cells incubated with a hexokinase inhibitor increased type I IFN production, supporting the idea that the glycolysis activity interferes with the protective response in viral infection. The excessive glycolysis affects interferon production due to lactate production (one of the metabolites produced in glycolysis), which is internalized and binds to MAVS, impairing its interaction with RIG-I (Zhang et al., 2019). In SARS-CoV-2, the role of the nucleic acid sensor in the inflammation and metabolism of the different organs target by SARS-CoV-2, such as kidneys, still needs to be investigated to better understand the mechanisms in COVID-19 progression.

The kidneys are one of main organs in the regulation of systemic glucose metabolism. Because renal cells express ACE2, the kidneys become one of the main targets for SARS-CoV-2, and changes in renal metabolism may underlie the mechanisms by which SARS-CoV-2 induces AKI and aggravates clinical conditions of COVID-19 patients. Changes in systemic metabolism (as occurs in metabolic diseases) and in renal cell metabolism are reported as crucial events on decline of renal function.

KIDNEY DYSFUNCTION AND METABOLISM

Kidney dysfunction has long been known as an important consequence of metabolic disorders (Cohen, 1962; Joven et al., 1993). In metabolic syndrome, a clinical condition characterized by cardiovascular problems, disturbances in the metabolism of lipid and glucose have high impact on renal function (Locatelli et al., 2006; Ikee et al., 2008). Conversely, the progressive decline of the kidney function, dependent or independent of metabolic etiology, causes changes in the systemic metabolism (de Boer and Utzschneider, 2017). In physiological conditions, kidneys are responsible for up to 40% of the glucose production by gluconeogenesis, and perturbation in the metabolism of the renal cells, such as proximal tubular epithelial cells (PTECS), profoundly impacts on glucose metabolism, affecting glycolytic and gluconeogenic pathways (Legouis et al., 2020). Besides, other metabolic routes can be affected in renal injury, such as lipid and mitochondrial metabolism, starting in renal cortex, followed by medulla and plasma (Wei et al., 2014), demonstrating that altered renal energy metabolism, specifically in renal cell, is correlated with kidney injury development and it can affect systemic metabolism.

The metabolic changes occur at the cellular level and perturbations in cell energy hemostasis can lead to acute and chronic disorders. The source of energy for each renal cell type is specific, for instance, glucose is the primary energy source of podocytes, mesangial, and endothelial cells (Forbes, 2016). While PTECS supply their energy demand from fatty acid oxidation (Kang et al., 2015; Han et al., 2017). PTECS are the ones that need the most significant production of ATP because of the intense transport and reabsorption of solutes in the kidney (Bhargava and Schnellmann, 2017), and are among the renal cell types the most sensitive to renal damage.

Fatty acids act as mitochondrial substrates for oxidative metabolism in proximal tubules, and transportation of fatty acids into mitochondria is controlled by carnitine palmitoyltransferase (CPT) 1 and 2. To produce ATP from β-oxidation, fatty acids receive a coenzyme A (CoA) group through enzyme fatty acyl action synthase, resulting in a fatty acyl CoA. The fatty acyl CoA is converted to acylcarnitine by the action of CPT1 and transported to the mitochondrial inner space. In the mitochondria, acylcarnitine returns to a fatty acid acyl CoA form by the CPT2, located in mitochondrial inner membrane (O'Neill et al., 2016). In a recent study, CPT1a overexpression in renal tubule decreases renal injury by restoring mitochondrial homeostasis (Miguel et al., 2020), evidencing that mitochondria dysfunction is crucial in kidney disease development, and enzymes involved in fatty acids oxidation have a fundamental role in maintaining the mitochondria homeostasis. Kang et al. (2015) demonstrated that CPT1 inhibition reduced ATP production, causing cell death, dedifferentiation, and intracellular lipid accumulation in PTECS, which are common renal injury features. These enzymes expression is regulated by transcription factors named PPAR-α. Reduction in PPARα leads to a decreased expression of CPT1 and the peroxisomal acyl-coenzyme A oxidase 1, reflecting in the fatty acid oxidation (Kang et al., 2015). PTECS are susceptible to lipid accumulation, and a large number of studies demonstrated that excess of renal lipids causes tissue damage (Bobulescu et al., 2008; Bobulescu, 2010; Falkevall et al., 2017; Yan et al., 2018).

Mitochondrial damage and inflammatory response are classical events in AKI. The increase of mitochondria number is a protective event during experimental AKI (Tran et al., 2016). In another context, *in vitro* experiments using human PTECS stimulated with cisplatin (anticancer drug that causes nephrotoxicity and AKI development), it was observed a reduction in the mitochondrial fatty acid oxidation (Maekawa et al., 2019), leading to lipid accumulation. The lipid excess induces reactive oxygen species production, apoptosis, inflammation, profibrotic factors release, and organelle damage (Weinberg, 2006; Bobulescu, 2010). In addition, lipotoxicity can occur due to the impact of hypoxia on them (Ruidera et al., 1988; Bobulescu et al., 2008), which is one of the mechanisms that potentially causes tubular damage. Based on these findings, renal lipotoxicity may be contribute to kidney damage in COVID-19 patients, since individuals with COVID-19 present respiratory insufficiency

that leads them to hypoxemia, worsening peripheral tissue ischemia (Del Vecchio and Locatelli, 2020).

Besides the alterations in lipid metabolism observed in renal dysfunction, the metabolism of glucose can also be altered leading to deleterious events. During AKI, the PTECs present an increased glycolytic profile, and this change is exceptionally critical in their physiology during recovery after AKI. In ischemia-reperfusion injury, the metabolic switch occurs early during regeneration after insult and tubules become atrophic. However, even regenerating tubules present increased glycolytic enzyme expression, and this irreversibility of metabolic profile led the cell to hypoxia and induced the profibrotic signaling (Lan et al., 2016), which can contribute to the progression from AKI to chronic pathology. In line with this, it was observed an increase in glycolytic profile in experimental and clinical AKI, in contrast with the reduction of gluconeogenesis (Legouis et al., 2020). It was observed that rate-limiting gluconeogenesis enzymes were decreased during the early phase following ischemia-reperfusion injury, but the expression of glycolytic enzymes was increased. The reduction of renal gluconeogenesis can contribute to hypoglycemia in stress conditions, compromising the systemic metabolism and contributing to worsening patient condition. Metabolic reprogramming of glucose metabolism during AKI was associated with mortality, as reported by Legouis et al. (2020). COVID-19 patients with metabolic disorders have a worsening of the clinical condition associated with acute kidney disease, which suggests that dysfunction in systemic metabolism may contribute to renal injury in COVID-19.

METABOLISM AND SARS-CoV-2: POSSIBLE IMPLICATIONS ON RENAL INJURY DEVELOPMENT

Currently, it is already known that SARS-CoV-2 can change host metabolism. The consequences of the metabolic alteration in COVID-19 for organ functions, especially the kidneys, are poorly described. An investigation with 33 diagnosed patients with COVID-19 in comparison with COVID-19-negative individuals demonstrated that altered metabolite levels of the fatty acid and tryptophan metabolism in infected patients were correlated with clinical markers of inflammation (IL-6 and C-reactive protein) and renal function (BUN and creatinine; Thomas et al., 2020). Besides, *in vitro* studies demonstrated the SARS-CoV-2 potential of modulating the lipid metabolism in monocytes and lung epithelial cells (Dias et al., 2020). The abnormal metabolism functioning is critical for renal injury development, which makes systemic and cellular metabolism in COVID-19 an exciting issue of investigation for further studies in the context of renal injury.

A retrospective analysis found that patients with COVID-19 presented altered blood glucose levels (hypoglycemia and hyperglycemia) in the course of disease accompanied by poor outcomes, including AKI. In the patient's group that achieves a mean glycemia of 140 mg/ml, 24% of them experienced at least one episode of hypoglycemia (blood glucose levels below 70 mg/dl) and presented an increased risk of AKI and mortality. However, the exact cause of hypoglycemia in these patients is unknown (Klonoff et al., 2020). Legouis et al. (2020) observed that gluconeogenesis is impaired in renal PTECs in clinical and experimental AKI. In this study, the author verified the increase of glycolytic enzymes and reduction of gluconeogenesis, demonstrating that the glucose metabolism reprogramming in renal PTECs had an effect on systemic levels of glucose and was correlated with patient mortality. The high death rates in COVID-19 associated to AKI may be due to alterations in the metabolism of PTECs caused by systemic or direct infection of renal cells by SARS-CoV-2.

The molecular mechanisms involved in metabolic dysfunction in COVID-19 are still sparsely described. An *in silico* study demonstrated the interaction of the spike protein (S protein) from SARS-CoV-2 with human innate immune receptor, named Toll-like receptors (TLRs), which are a type of pattern recognition receptors. Molecular docking revealed the potential binding of the S protein of SARS-CoV-2 to TLR-1, -4, and -6, presenting binding energy value of −57.3, −120.2, and −68.4, indicating that TLR4 has a high affinity to S protein following TLR6 and TLR1 (Choudhury and Mukherjee, 2020). TLR4 has been associated with inflammatory conditions and its activation induces metabolic changes in macrophages and dendritic cells, altering mitochondrial, lipid, and glycolytic homeostasis (Everts et al., 2014; Perrin-Cocon et al., 2018; Lauterbach et al., 2019). In renal context, TLR4 activation induces severe inflammation and AKI (Cenedeze et al., 2007; Andrade-Silva et al., 2018). However, whether the metabolic dysfunction of SARS-CoV-2-infected patients can be associated with TLRs signaling in the kidneys remains unclear.

Therefore, further studies aiming at cellular and molecular mechanisms in SARS-CoV-2 infection and kidney pathology are urgent topics of investigation.

A proposal mechanism for the acute renal dysfunction development in COVID-19 and its interface with metabolism is shown in **Figure 1**.

FINAL REMARKS AND PERSPECTIVES

The interface between cell metabolism and inflammation is an emerging topic in immune and non-immune disorders. Disturbances in metabolism are associated with inflammation and targeting host cellular metabolism in severe disease is undoubted point to be considered in clinical management of the affected patients. Urgently, the world hopes for solutions for COVID-19 complications. Undoubtedly, the kidney represents a critical organ that, when affected, can be determinative in morbidity and mortality of COVID-19 patients. The focus on COVID-19 should be directed not only on pathogen elimination but also on the physiological alterations during infectious processes, such as systemic and cellular metabolism changes and more studies to clear how

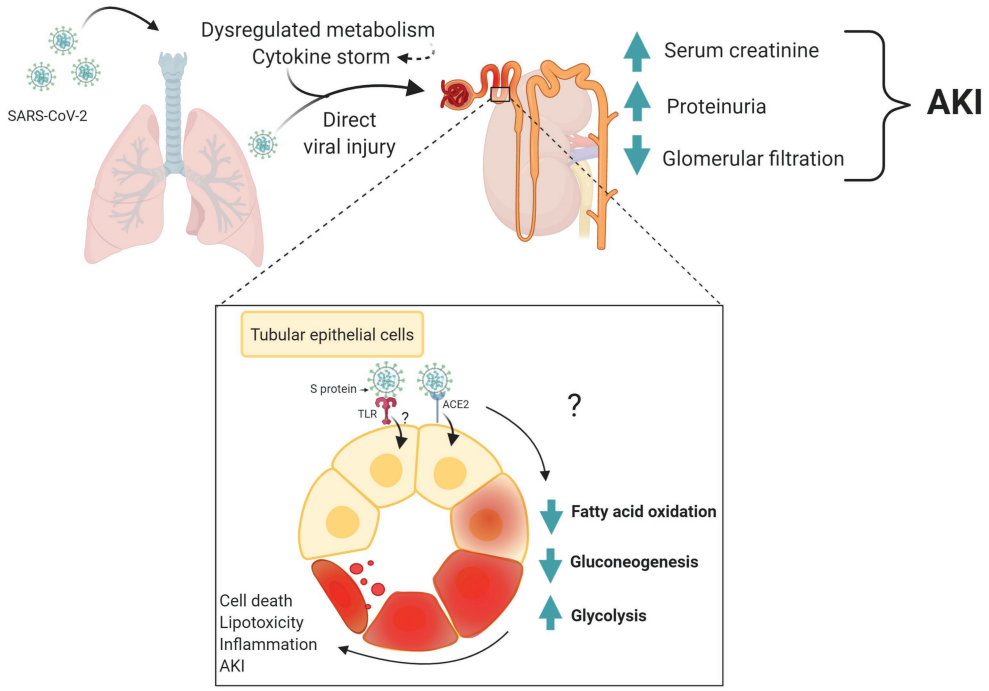


FIGURE 1 | Metabolic changes as a potential mechanism of acute kidney dysfunction in coronavirus disease 2019 (COVID-19). Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) infects lung epithelial and lead to extrapulmonary systemic hyperinflammation. Products of inflammation and metabolites may cause renal metabolic dysregulation. This affects could be triggered by direct infection of kidney cells by SARS-CoV-2. Patter recognition receptor, such Toll-like receptor (TLR) may be important molecular mechanism activate by virus proteins and triggering changes in tubular epithelial cell metabolism and inflammation. Both scenarios, systemic and/or direct changes in metabolism may be associated to acute kidney injury in COVID-19. Created with www.BioRender.com.

metabolism can be determinative in tissue injury progression. Currently, little is known about the long-term effects of SARS-CoV-2, but another species of coronavirus already demonstrated the potential to cause metabolic disorders even many years after the patient recovery of infection. Understanding the systemic and intracellular metabolic alterations and its consequences in COVID-19 will help to design better pharmacological therapy, repurposing drugs used in metabolic disorders aiming at improvement of hospitalized patient clinical conditions, and reduction of death rates or sequelae.

AUTHOR CONTRIBUTIONS

MA-S and NC conceived the concept of the manuscript. All authors contributed to the literature review and writing of the manuscript and approved for its publication.

ACKNOWLEDGMENTS

We thank Ingrid K. M. Watanabe for valuable comments on the manuscript.

REFERENCES

Almeida, P. E., Roque, N. R., Magalhães, K. G., Mattos, K. A., Teixeira, L., Maya-Monteiro, C., et al. (2014). Differential TLR2 downstream signaling regulates lipid metabolism and cytokine production triggered by Mycobacterium bovis BCG infection. *Biochim. Biophys. Acta* 1841, 97–107. doi: 10.1016/j.bbalip.2013.10.008

Andrade-Silva, M., Cenedeze, M. A., Perandini, L. A., Felizardo, R. J. F., Watanabe, I. K. M., Agudelo, J. S. H., et al. (2018). TLR2 and TLR4 play opposite role in autophagy associated with cisplatin-induced acute kidney injury. *Clin. Sci.* 132, 1725–1739. doi: 10.1042/CS20170262

Ayres, J. S. (2020). A metabolic handbook for the COVID-19 pandemic. *Nat. Metab.* 2, 572–585. doi: 10.1038/s42255-020-0237-2

Bhargava, P., and Schnellmann, R. G. (2017). Mitochondrial energetics in the kidney. *Nat. Rev. Nephrol.* 13, 629–646. doi: 10.1038/nrneph.2017.107

Blanco-Melo, D., Nilsson-Payant, B. E., Liu, W. C., Uhl, S., Hoagland, D., Møller, R., et al. (2020). Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell* 181, 1036.e1039–1045.e1039. doi: 10.1016/j.cell.2020.04.026

Bobulescu, I. A. (2010). Renal lipid metabolism and lipotoxicity. *Curr. Opin. Nephrol. Hypertens.* 19, 393–402. doi: 10.1097/MNH.0b013e32833aa4ac

Bobulescu, I. A., Dubree, M., Zhang, J., McLeroy, P., and Moe, O. W. (2008). Effect of renal lipid accumulation on proximal tubule Na+/H+ exchange and ammonium secretion. *Am. J. Physiol. Ren. Physiol.* 294, F1315–F1322. doi: 10.1152/ajprenal.00550.2007

Bojkova, D., Klann, K., Koch, B., Widera, M., Krause, D., Ciesek, S., et al. (2020). Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. *Nat.* 583, 469–472. doi: 10.1038/s41586-020-2332-7

Booth, C. M., Matukas, L. M., Tomlinson, G. A., Rachlis, A. R., Rose, D. B., Dwosh, H. A., et al. (2003). Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. *JAMA* 289, 2801–2809. doi: 10.1001/jama.289.21.JOC30885

Bost, P., Giladi, A., Liu, Y., Bendjelal, Y., Xu, G., David, E., et al. (2020). Host-viral infection maps reveal signatures of severe COVID-19 patients. *Cell* 181, 1475.e1412–1488.e1412. doi: 10.1016/j.cell.2020.05.006

Braun, F., Lütgehetmann, M., Pfefferle, S., Wong, M. N., Carsten, A., Lindenmeyer, M. T., et al. (2020). SARS-CoV-2 renal tropism associates with acute kidney injury. *Lancet* 396, 597–598. doi: 10.1016/S0140-6736(20)31759-1

Brienza, N., Puntillo, F., Romagnoli, S., and Tritapepe, L. (2020). Acute kidney injury in coronavirus disease 2019 infected patients: a meta-analytic study. *Blood Purif.* 50, 35–41. doi: 10.1159/000509274

Bruzzone, C., Bizkarguenaga, M., Gil-Redondo, R., Diercks, T., Arana, E., García de Vicuña, A., et al. (2020). SARS-CoV-2 infection dysregulates the metabolomic and lipidomic profiles of serum. *iScience* 23:101645. doi: 10.1016/j.isci.2020.101645

Burns, S. M., Egloff, M. B., Ryan, B., Carpenter, R., and Burns, J. E. (1994). Effect of body position on spontaneous respiratory rate and tidal volume in patients with obesity, abdominal distension and ascites. *Am. J. Crit. Care* 3, 102–106. doi: 10.4037/ajcc1994.3.2.102

Cenedeze, M. A., Gonçalves, G. M., Feitoza, C. Q., Wang, P. M., Damião, M. J., Bertocchi, A. P., et al. (2007). The role of toll-like receptor 4 in cisplatin-induced renal injury. *Transplant. Proc.* 39, 409–411. doi: 10.1016/j.transproceed.2007.01.032

Channappanavar, R., Fehr, A. R., Vijay, R., Mack, M., Zhao, J., Meyerholz, D. K., et al. (2016). Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. *Cell Host Microbe* 19, 181–193. doi: 10.1016/j.chom.2016.01.007

Cheng, Y., Luo, R., Wang, K., Zhang, M., Wang, Z., Dong, L., et al. (2020). Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int.* 97, 829–838. doi: 10.1016/j.kint.2020.03.005

Choudhury, A., and Mukherjee, S. (2020). In silico studies on the comparative characterization of the interactions of SARS-CoV-2 spike glycoprotein with ACE-2 receptor homologs and human TLRs. *J. Med. Virol.* 92, 2105–2113. doi: 10.1002/jmv.25987

Codo, A. C., Davanzo, G. G., Monteiro, L. B., de Souza, G. F., Muraro, S. P., Virgilio-da-Silva, J. V., et al. (2020). Elevated glucose levels favor SARS-CoV-2 infection and monocyte response through a HIF-1α/glycolysis-dependent axis. *Cell Metab.* 32, 437.e435–446.e435. doi: 10.1016/j.cmet.2020.07.007

Cohen, B. D. (1962). Abnormal carbohydrate metabolism in renal disease. Blood glucose unresponsiveness to hypoglycemia, epinephrine, and glucagon. *Ann. Intern. Med.* 57, 204–213. doi: 10.7326/0003-4819-57-2-204

Cummings, M. J., Baldwin, M. R., Abrams, D., Jacobson, S. D., Meyer, B. J., Balough, E. M., et al. (2020). Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. *Lancet* 395, 1763–1770. doi: 10.1016/S0140-6736(20)31189-2

D'Avila, H., Maya-Monteiro, C. M., and Bozza, P. T. (2008). Lipid bodies in innate immune response to bacterial and parasite infections. *Int. Immunopharmacol.* 8, 1308–1315. doi: 10.1016/j.intimp.2008.01.035

D'Avila, H., Melo, R. C., Parreira, G. G., Werneck-Barroso, E., Castro-Faria-Neto, H. C., and Bozza, P. T. (2006). Mycobacterium bovis bacillus Calmette-Guérin induces TLR2-mediated formation of lipid bodies: intracellular domains for eicosanoid synthesis in vivo. *J. Immunol.* 176, 3087–3097. doi: 10.4049/jimmunol.176.5.3087

de Boer, I. H., and Utzschneider, K. M. (2017). The kidney's role in systemic metabolism-still much to learn. *Nephrol. Dial. Transplant.* 32, 588–590. doi: 10.1093/ndt/gfx027

DeBerardinis, R. J., and Thompson, C. B. (2012). Cellular metabolism and disease: what do metabolic outliers teach us? *Cell* 148, 1132–1144. doi: 10.1016/j.cell.2012.02.032

Del Vecchio, L., and Locatelli, F. (2020). Hypoxia response and acute lung and kidney injury: possible implications for therapy of COVID-19. *Clin. Kidney J.* 13, 494–499. doi: 10.1093/ckj/sfaa149

Dias, S. S. G., Soares, V. C., Ferreira, A. C., Sacramento, C. Q., Fintelman-Rodrigues, N., Temerozo, J. R., et al. (2020). Lipid droplets fuel SARS-CoV-2 replication and production of inflammatory mediators. *PLoS Pathog.* 16:e1009127. doi: 10.1371/journal.ppat.1009127

Eberlé, D., Hegarty, B., Bossard, P., Ferré, P., and Foufelle, F. (2004). SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie* 86, 839–848. doi: 10.1016/j.biochi.2004.09.018

Ehrlich, S. F., Quesenberry, C. P., Van Den Eeden, S. K., Shan, J., and Ferrara, A. (2010). Patients diagnosed with diabetes are at increased risk for asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, and pneumonia but not lung cancer. *Diabetes Care* 33, 55–60. doi: 10.2337/dc09-0880

Ehrlich, A., Uhi, S., Ioannidis, K., Hofree, M., tenOeve, B. R., and Nahmias, Y. (2020). The SARS-CoV-2 transcriptional metabolic signature in lung epithelium. *Cell Metab.* doi: 10.2139/ssrn.3650499

Everts, B., Amiel, E., Huang, S. C., Smith, A. M., Chang, C. H., Lam, W. Y., et al. (2014). TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nat. Immunol.* 15, 323–332. doi: 10.1038/ni.2833

Falkevall, A., Mehlem, A., Palombo, I., Heller Sahlgren, B., Ebarasi, L., and He, L. (2017). Reducing VEGF-B signaling ameliorates renal lipotoxicity and protects against diabetic kidney disease. *Cell Metab.* 25, 713–726. doi: 10.1016/j.cmet.2017.01.004

Forbes, J. M. (2016). Mitochondria-power players in kidney function? *Trends Endocrinol. Metab.* 27, 441–442. doi: 10.1016/j.tem.2016.05.002

Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W. H., Ou, C. Q., He, J. X., et al. (2020). Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* 382, 1708–1720. doi: 10.1056/NEJMoa2002032

Gupta, A., Madhavan, M. V., Sehgal, K., Nair, N., Mahajan, S., Sehrawat, T. S., et al. (2020). Extrapulmonary manifestations of COVID-19. *Nat. Med.* 26, 1017–1032. doi: 10.1038/s41591-020-0968-3

Han, S. H., Wu, M. Y., Nam, B. Y., Park, J. T., Yoo, T. H., Kang, S. W., et al. (2017). PGC-1α protects from notch-induced kidney fibrosis development. *J. Am. Soc. Nephrol.* 28, 3312–3322. doi: 10.1681/ASN.2017020130

Heaton, N. S., and Randall, G. (2010). Dengue virus-induced autophagy regulates lipid metabolism. *Cell Host Microbe* 8, 422–432. doi: 10.1016/j.chom.2010.10.006

Heaton, N. S., and Randall, G. (2011). Multifaceted roles for lipids in viral infection. *Trends Microbiol.* 19, 368–375. doi: 10.1016/j.tim.2011.03.007

Heffernan, K. S., Ranadive, S. M., and Jae, S. Y. (2020). Exercise as medicine for COVID-19: on PPAR with emerging pharmacotherapy. *Med. Hypotheses* 143:110197. doi: 10.1016/j.mehy.2020.110197

Hirsch, J. S., Ng, J. H., Ross, D. W., Sharma, P., Shah, H. H., Barnett, R. L., et al. (2020). Acute kidney injury in patients hospitalized with COVID-19. *Kidney Int.* 98, 209–218. doi: 10.1016/j.kint.2020.05.006

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271.e278–280.e278. doi: 10.1016/j.cell.2020.02.052

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature* 444, 860–867. doi: 10.1038/nature05485

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Ikee, R., Hamasaki, Y., Oka, M., Maesato, K., Mano, T., Moriya, H., et al. (2008). Glucose metabolism, insulin resistance, and renal pathology in non-diabetic chronic kidney disease. *Nephron Clin. Pract.* 108, c163–c168. doi: 10.1159/000115329

Joven, J., Vilella, E., Ahmad, S., Cheung, M. C., and Brunzell, J. D. (1993). Lipoprotein heterogeneity in end-stage renal disease. *Kidney Int.* 43, 410–418. doi: 10.1038/ki.1993.60

Kang, H. M., Ahn, S. H., Choi, P., Ko, Y. A., Han, S. H., Chinga, F., et al. (2015). Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. *Nat. Med.* 21, 37–46. doi: 10.1038/nm.3762

Klonoff, D. C., Messler, J. C., Umpierrez, G. E., Peng, L., Booth, R., Crowe, J., et al. (2020). Association between achieving inpatient glycemic control and clinical outcomes in hospitalized patients with COVID-19: a multicenter, retrospective hospital-based analysis. *Diabetes Care* 44, 578–585. doi: 10.2337/dc20-1857

Lan, R., Geng, H., Singha, P. K., Saikumar, P., Bottinger, E. P., Weinberg, J. M., et al. (2016). Mitochondrial pathology and glycolytic shift during proximal tubule atrophy after ischemic AKI. *J. Am. Soc. Nephrol.* 27, 3356–3367. doi: 10.1681/ASN.2015020177

Lauterbach, M. A., Hanke, J. E., Serefidou, M., Mangan, M. S. J., Kolbe, C. C., Hess, T., et al. (2019). Toll-like receptor signaling rewires macrophage metabolism and promotes histone acetylation via ATP-citrate lyase. *Immunity* 51, 997.e1017–1011.e1017. doi: 10.1016/j.immuni.2019.11.009

Legouis, D., Ricksten, S.-E., Faivre, A., Verissimo, T., Gariani, K., Verney, C., et al. (2020). Altered proximal tubular cell glucose metabolism during acute kidney injury is associated with mortality. *Nat. Metab.* 2, 732–743. doi: 10.1038/s42255-020-0238-1

Lim, H. J., Lee, S., Lee, K. S., Park, J. H., Jang, Y., Lee, E. J., et al. (2006). PPARgamma activation induces CD36 expression and stimulates foam cell like changes in rVSMCs. *Prostaglandins Other Lipid Mediat.* 80, 165–174. doi: 10.1016/j.prostaglandins.2006.06.006

Locatelli, F., Pozzoni, P., and Del Vecchio, L. (2006). Renal manifestations in the metabolic syndrome. *J. Am. Soc. Nephrol.* 17, S81–S85. doi: 10.1681/ASN.2005121332

Lucas, C., Wong, P., Klein, J., Castro, T. B. R., Silva, J., Sundaram, M., et al. (2020). Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature* 584, 463–469. doi: 10.1038/s41586-020-2588-y

Luft, V. C., Schmidt, M. I., Pankow, J. S., Couper, D., Ballantyne, C. M., Young, J. H., et al. (2013). Chronic inflammation role in the obesity-diabetes association: a case-cohort study. *Diabetol. Metab. Syndr.* 5:31. doi: 10.1186/1758-5996-5-31

Maekawa, H., Inoue, T., Ouchi, H., Jao, T. M., Inoue, R., Nishi, H., et al. (2019). Mitochondrial damage causes inflammation via cGAS-STING signaling in acute kidney injury. *Cell Rep.* 29, 1261.e1266–1273.e1266. doi: 10.1016/j.celrep.2019.09.050

Mattos, K. A., Lara, F. A., Oliveira, V. G., Rodrigues, L. S., D'Avila, H., Melo, R. C., et al. (2011). Modulation of lipid droplets by Mycobacterium leprae in Schwann cells: a putative mechanism for host lipid acquisition and bacterial survival in phagosomes. *Cell. Microbiol.* 13, 259–273. doi: 10.1111/j.1462-5822.2010.01533.x

Merino-Ramos, T., Vázquez-Calvo, Á., Casas, J., Sobrino, F., Saiz, J. C., and Martín-Acebes, M. A. (2016). Modification of the host cell lipid metabolism induced by hypolipidemic drugs targeting the acetyl coenzyme A carboxylase impairs west nile virus replication. *Antimicrob. Agents Chemother.* 60, 307–315. doi: 10.1128/AAC.01578-15

Miguel, V., Tituaña, J., Herrero, J. I., Herrero, L., Serra, D., Cuevas, P., et al. (2020). Renal tubule Cpt1a overexpression mitigates kidney fibrosis by restoring mitochondrial homeostasis. bioRxiv [Preprint]. doi:10.1101/2020.02.18.952440

Moolamalla, S. T. R., Chauhan, R., Deva Priyakumar, U., and Vinod, P. K. (2020). Host metabolic reprogramming in response to SARS-Cov-2 infection. bioRxiv [Preprint]. doi:10.1101/2020.08.02.232645

Moreno-Altamirano, M. M. B., Kolstoe, S. E., and Sánchez-García, F. J. (2019). Virus control of cell metabolism for replication and evasion of host immune responses. *Front. Cell. Infect. Microbiol.* 9:95. doi: 10.3389/fcimb.2019.00095

Murillo, A., Vera-Estrella, R., Barkla, B. J., Méndez, E., and Arias, C. F. (2015). Identification of host cell factors associated with astrovirus replication in Caco-2 cells. *J. Virol.* 89, 10359–10370. doi: 10.1128/JVI.01225-15

Naicker, S., Yang, C. W., Hwang, S. J., Liu, B. C., Chen, J. H., and Jha, V. (2020). The novel coronavirus 2019 epidemic and kidneys. *Kidney Int.* 97, 824–828. doi: 10.1016/j.kint.2020.03.001

O'Neill, L. A., Kishton, R. J., and Rathmell, J. (2016). A guide to immunometabolism for immunologists. *Nat. Rev. Immunol.* 16, 553–565. doi: 10.1038/nri.2016.70

Perrin-Cocon, L., Aublin-Gex, A., Diaz, O., Ramière, C., Peri, F., André, P., et al. (2018). Toll-like receptor 4-induced glycolytic burst in human monocyte-derived dendritic cells results from p38-dependent stabilization of HIF-1α and increased hexokinase ii expression. *J. Immunol.* 201, 1510–1521. doi: 10.4049/jimmunol.1701522

Puelles, V. G., Lütgehetmann, M., Lindenmeyer, M. T., Sperhake, J. P., Wong, M. N., Allweiss, L., et al. (2020). Multiorgan and renal tropism of SARS-CoV-2. *N. Engl. J. Med.* 383, 590–592. doi: 10.1056/NEJMc2011400

Ruidera, E., Irazu, C. E., Rajagopalan, P. R., Orak, J. K., Fitts, C. T., and Singh, I. (1988). Fatty acid metabolism in renal ischemia. *Lipids* 23, 882–884. doi: 10.1007/BF02536209

Song, S., Attia, R. R., Connaughton, S., Niesen, M. I., Ness, G. C., Elam, M. B., et al. (2010). Peroxisome proliferator activated receptor alpha (PPARalpha) and PPAR gamma coactivator (PGC-1alpha) induce carnitine palmitoyltransferase IA (CPT-1A) via independent gene elements. *Mol. Cell. Endocrinol.* 325, 54–63. doi: 10.1016/j.mce.2010.05.019

Sungnak, W., Huang, N., Bécavin, C., Berg, M., Queen, R., Litvinukova, M., et al. (2020). SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat. Med.* 26, 681–687. doi: 10.1038/s41591-020-0868-6

Tannahill, G. M., Curtis, A. M., Adamik, J., Palsson-McDermott, E. M., McGettrick, A. F., Goel, G., et al. (2013). Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* 496, 238–242. doi: 10.1038/nature11986

Tay, M. Z., Poh, C. M., Rénia, L., MacAry, P. A., and Ng, L. F. P. (2020). The trinity of COVID-19: immunity, inflammation and intervention. *Nat. Rev. Immunol.* 20, 363–374. doi: 10.1038/s41577-020-0311-8

Terry, L. J., Vastag, L., Rabinowitz, J. D., and Shenk, T. (2012). Human kinome profiling identifies a requirement for AMP-activated protein kinase during human cytomegalovirus infection. *Proc. Natl. Acad. Sci. USA.* 109, 3071–3076. doi: 10.1073/pnas.1200494109

Thai, M., Graham, N. A., Braas, D., Nehil, M., Komisopoulou, E., Kurdistani, S. K., et al. (2014). Adenovirus E4ORF1-induced MYC activation promotes host cell anabolic glucose metabolism and virus replication. *Cell Metab.* 19, 694–701. doi: 10.1016/j.cmet.2014.03.009

Thaker, S. K., Ch'ng, J., and Christofk, H. R. (2019). Viral hijacking of cellular metabolism. *BMC Biol.* 17:59. doi: 10.1186/s12915-019-0678-9

Thomas, T., Stefanoni, D., Reisz, J. A., Nemkov, T., Bertolone, L., Francis, R. O., et al. (2020). COVID-19 infection alters kynurenine and fatty acid metabolism, correlating with IL-6 levels and renal status. *JCI Insight* 5:e140327. doi: 10.1172/jci.insight.140327

Trabulus, S., Karaca, C., Balkan, I. I., Dincer, M. T., Murt, A., Ozcan, S. G., et al. (2020). Kidney function on admission predicts in-hospital mortality in COVID-19. *PLoS One* 15:e0238680. doi: 10.1371/journal.pone.0238680

Tran, M. T., Zsengeller, Z. K., Berg, A. H., Khankin, E. V., Bhasin, M. K., Kim, W., et al. (2016). PGC1α drives NAD biosynthesis linking oxidative metabolism to renal protection. *Nature* 531, 528–532. doi: 10.1038/nature17184

Vankadari, N. (2020). Structure of furin protease binding to SARS-CoV-2 spike glycoprotein and implications for potential targets and virulence. *J. Phys. Chem. Lett.* 11, 6655–6663. doi: 10.1021/acs.jpclett.0c01698

Vastag, L., Koyuncu, E., Grady, S. L., Shenk, T. E., and Rabinowitz, J. D. (2011). Divergent effects of human cytomegalovirus and herpes simplex virus-1 on cellular metabolism. *PLoS Pathog.* 7:e1002124. doi: 10.1371/journal.ppat.1002124

Vijayan, A., and Humphreys, B. D. (2020). SARS-CoV-2 in the kidney: bystander or culprit? *Nat. Rev. Nephrol.* 16, 703–704. doi: 10.1038/s41581-020-00354-7

Wei, Q., Xiao, X., Fogle, P., and Dong, Z. (2014). Changes in metabolic profiles during acute kidney injury and recovery following ischemia/reperfusion. *PLoS One* 9:e0116239. doi: 10.1371/journal.pone.0116239

Weinberg, J. M. (2006). Lipotoxicity. *Kidney Int.* 70, 1560–1566. doi: 10.1038/sj.ki.5001834

Wu, D., Shu, T., Yang, X., Song, J. X., Zhang, M., Yao, C., et al. (2020). Plasma metabolomic and lipidomic alterations associated with COVID-19. *Natl. Sci. Rev.* 7, 1157–1168. doi: 10.1093/nsr/nwaa086

Wu, Q., Zhou, L., Sun, X., Yan, Z., Hu, C., Wu, J., et al. (2017). Altered lipid metabolism in recovered SARS patients twelve years after infection. *Sci. Rep.* 7:9110. doi: 10.1038/s41598-017-09536-z

Yan, Q., Song, Y., Zhang, L., Chen, Z., Yang, C., Liu, S., et al. (2018). Autophagy activation contributes to lipid accumulation in tubular epithelial cells during kidney fibrosis. *Cell Death Dis.* 4:2. doi: 10.1038/s41420-018-0065-2

Yuan, S., Chu, H., Chan, J. F., Ye, Z. W., Wen, L., Yan, B., et al. (2019). SREBP-dependent lipidomic reprogramming as a broad-spectrum antiviral target. *Nat. Commun.* 10:120. doi: 10.1038/s41467-018-08015-x

Zhang, W., Wang, G., Xu, Z. G., Tu, H., Hu, F., Dai, J., et al. (2019). Lactate is a natural suppressor of RLR signaling by targeting MAVS. *Cell* 178, 176.e115–189.e115. doi: 10.1016/j.cell.2019.05.003

Zhu, L., She, Z. G., Cheng, X., Qin, J. J., Zhang, X. J., Cai, J., et al. (2020). Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes. *Cell Metab.* 31, 1068.e1063–1077.e1063. doi: 10.1016/j.cmet.2020.04.021

Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., et al. (2020). SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N. Engl. J. Med.* 382, 1177–1179. doi: 10.1056/NEJMc2001737

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 09 March 2021
doi: 10.3389/fphys.2021.611772

Proteinuria as a Biomarker for COVID-19 Severity

Hajar Ouahmi[1,2], Johan Courjon[3,4], Lucas Morand[1], Juliette François[5], Vincent Bruckert[6], Romain Lombardi[1], Vincent Esnault[2], Barbara Seitz-Polski[2,7,8,9], Elisa Demonchy[3], Jean Dellamonica[1,9†] and Sonia Boyer-Suavet[8,9†]*

[1] Service de Médecine Intensive Réanimation, CHU de Nice, Université Côte d'Azur, Nice, France, [2] Service de Néphrologie-Dialyse-Transplantation, CHU de Nice, Université Côte d'Azur, Nice, France, [3] Service d'Infectiologie, CHU de Nice, Université Côte d'Azur, Nice, France, [4] Université Côte d'Azur, Centre Méditerranéen de Médecine Moléculaire (C3M), CHU de Nice, INSERM U1065, Nice, France, [5] Service de Réanimation CH Antibes-Juan les Pins, Antibes, France, [6] Département de réanimation médico-chirugicale et transplantation d'organe, hôpital L'Archet 2, CHU de Nice, Université Côte d'Azur, Nice, France, [7] Laboratoire d'Immunologie, CHU de Nice, Université Côte d'Azur, Nice, France, [8] Centre de référence Maladies rares Syndrome néphrotique idiopathique, CHU de Nice, Université Côte d'Azur, Nice, France, [9] Unité de Recherche Clinique de la Côte d'Azur (UR2CA), Université Côte d'Azur, Nice, France

OPEN ACCESS

Edited by:
Ravi Nistala,
University of Missouri, United States

Reviewed by:
Theodoros Eleftheriadis,
University of Thessaly, Greece
Carlos A. Flores,
Centro de Estudios Científicos, Chile

***Correspondence:**
Sonia Boyer-Suavet
boyer-suavet.s@chu-nice.fr

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Renal and Epithelial Physiology, a section of the journal Frontiers in Physiology

Received: *29 September 2020*
Accepted: *08 February 2021*
Published: *09 March 2021*

Citation:
Ouahmi H, Courjon J, Morand L, François J, Bruckert V, Lombardi R, Esnault V, Seitz-Polski B, Demonchy E, Dellamonica J and Boyer-Suavet S (2021) Proteinuria as a Biomarker for COVID-19 Severity. Front. Physiol. 12:611772. doi: 10.3389/fphys.2021.611772

Background: Renal involvement in syndrome coronavirus 2 (SARS-CoV-2) infection has been retrospectively described, especially acute kidney injury (AKI). However, quantitative proteinuria assessment and its implication in coronavirus disease 2019 (COVID-19) remain unknown.

Methods: In this prospective, multicenter study in France, we collected clinical and biological data including urinary protein to creatine ratio (UPCR) in patients presenting with moderate to severe COVID-19. Clinical outcome was analyzed according to the level of UPCR.

Results: 42/45 patients (93.3%) had renal involvement (abnormal urinary sediment and/or AKI). Significant proteinuria occurred in 60% of patients. Urine protein electrophoresis showed tubular protein excretion in 83.8% of patients with proteinuria. Inflammatory parametersand D-dimer concentrations correlated with proteinuria level. Patients who required intensive care unit (ICU) admission had higher proteinuria (p = 0.008). On multivariate analysis, proteinuria greater than 0.3 g/g was related to a higher prevalence of ICU admission [OR = 4.72, IC95 (1.16–23.21), p = 0.03], acute respiratory distress syndrome (ARDS) [OR = 6.89, IC95 (1.41–53.01, p = 0.02)], nosocomial infections [OR = 3.75, IC95 (1.11–13.55), p = 0.03], longer inpatient hospital stay (p = 0.003).

Conclusion: Renal involvement is common in moderate to severe SARS-CoV-2 infection. Proteinuria at baseline is an independent risk factor for increased hospitalization duration and ICU admission in patients with COVID-19.

Keywords: acute kidney injury, biomarker, COVID-19, proteinuria, SARS-CoV-2, kidney involvement, pronostic and predictive factors

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a transmitted disease caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It primarily manifests itself as an acute respiratory illness but can affect multiple organs such as kidneys, heart, digestive tract, and nervous system (Wang et al., 2020). In previous reports of SARS and Middle East Respiratory Syndrome coronavirus, acute kidney injury (AKI) was described in 5 – 15% of patients and was associated with high mortality rates (60–90%) (Chu et al., 2005; Cha et al., 2015). Recent reports showed renal abnormalities in COVID-19 patients (Naicker et al., 2020), but kidney involvement has not been yet well characterized (only retrospective cohorts using urine dip-stick tests) (Pei et al., 2020; Wang et al., 2020). A recent Chinese study also reported that AKI was an independent risk factor for mortality (Cheng et al., 2020). However, the exact mechanism of kidney involvement remains unclear: sepsis-related cytokine storm (Mehta et al., 2020) or direct cellular injury induced by the virus (Sun et al., 2020). Human angiotensin-converting enzyme 2 (ACE2) receptor has been identified as the functional receptor for SARS-CoV-2 and is highly expressed in kidneys (Hoffmann et al., 2020; Walls et al., 2020). These data suggest that the kidney might be a target of this SARS-CoV-2 as highlighted in pathological examinations (Su et al., 2020). Reports about the natural course of renal complications during SARS-CoV-2 are scarce, most of them concern only AKI or specific populations (e.g., Chinese) (Cheng et al., 2020; Pei et al., 2020), or kidney transplant recipients (Akalin et al., 2020; Alberici et al., 2020; Banerjee et al., 2020). We aimed to prospectively identify renal involvement, more especially proteinuria (quantitative) at baseline and its prognosis in a French cohort with moderate to severe SARS-CoV-2 infection.

MATERIALS AND METHODS

Study Protocol

Multicenter prospective observational study.

Study Approval

Use of routinely collected health data as a current care practice study (NCT04355624).

Patient Population

Inclusion criteria: all patients aged ≥18 years-old with symptomatic proven moderate to severe COVID-19 according to the WHO classification, admitted in one infectious diseases department or in three different intensive care unit (ICU) of Nice University Hospital and Antibes Juan-les-Pins hospital general hospital, between March 15th to April 19th, 2020.

Patients with a history chronic kidney disease and pregnant women were non-included.

Patients with documented urinary tract infection at inclusion were excluded to avoid confusing results.

Data Sources

The demographic characteristics (medical history, clinical symptoms, laboratory data, and medications) were extracted from electronic medical records. Urinary data were collected on urine sample: microalbuminuria, urine protein/creatinine ratio (UPCR), urine protein electrophoresis, red blood, and white blood cells counts.

Definitions

Proven COVID-19 was defined as a positive SARS-CoV-2 real-time polymerase chain reaction (RT-PCR) assay in nasopharyngeal swabs.

Severe COVID-19 was defined as respiratory failure or need for mechanical ventilation, shock or organ failure or need for ICU admission.

Acute kidney injury was defined according to KDIGO guidelines.

The date of disease onset was defined as the day of first symptom.

The last day of follow up was the in-hospital death or hospital discharge.

Statistical Analysis

For descriptive statistics, data are presented as median (ranges) or mean ± standard deviation. Shapiro-Wilk test was used to test for normal distribution of variable. Comparison of qualitative criteria was performed using Chi-square test or Fisher's exact test. Comparison of quantitative variables was performed using the Student *t*-test or Wilcoxon-Mann–Whitney test. Spearman rank-order test was used to find correlations between continuous variables. Multivariate analysis was performed using logistic regression. The multivariate model was built by including variables that met the 20% significance threshold in univariate analysis. Choice among colinear variables was performed using Akaike Iteration Criteria. Receiver Operating Characteristic (ROC) curve was used to evaluate the performance of the test. Comparisons for survival curves were performed using Kaplan-Meier analysis. A p-value < 0.05 indicated statistical significance. Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, United States) and RStudio [RStudio Team (2019). RStudio. Inc., Boston].

RESULTS

Baseline Characteristics

A total of 45 patients were enrolled and followed for a median of 11 days (5–23 days). Twenty-two patients (49%) developed severe COVID-19 and required ICU admission. **Table 1** shows the characteristics of the cohort. Prevalence of hypertension and diabetes mellitus was 37.8 and 26.7%, respectively. Less than 25% of patients were on renin-angiotensin system (RAS) blockers. Two patients died from SARS-CoV-2 infection: one in the ICU from refractory respiratory failure, one patient died from respiratory failure in conventional unit with DNR order.

TABLE 1 | Clinical and biological characteristics of the cohort.

Variables	All patients (*n* = 45)	Severe COVID-19 (*n* = 22)	Non-severe COVID-19 (*n* = 23)	*P*-value
Clinical characteristics				
Age, years	64.0 (48.5–71.5)	66.0 (58.8–72.0)	58 (47.0–68.0)	0.11
Male patients, *n* (%)	31.0 (68.9)	18.0 (81.8)	13.0 (56.5)	0.07
Respiratory disease, *n* (%)	9.0 (20.0)	5.0 (22.7)	4.0 (17.4)	0.65
Hypertension, *n* (%)	17.0 (37.8)	9.0 (40.9)	8.0 (34.8)	0.67
Diabetes mellitus, *n* (%)	12.0 (26.7)	6.0 (27.3)	6.0 (26.1)	0.93
Cardiac disease, *n* (%)	5.0 (11.1)	3.0 (13.6)	2.0 (8.7)	0.60
Vascular disease, *n* (%)	3.0 (6.7)	1.0 (45.5)	2.0 (8.7)	0.58
Active and former smokers, *n* (%)	13.0 (28.9)	9.0 (39.1)	4.0 (17.4)	0.08
Immunosuppression, *n* (%)	6.0 (13.3)	3.0 (13.6)	3.0 (13)	0.95
ACE inhibitor, *n* (%)	1.0 (22.2)	1.0 (4.5)	0.0	0.30
ARB, *n* (%)	8.0 (17.8)	2.0 (9.0)	6.0 (26.1)	0.14
BMI (kg/m^2)	27.8 (24.3–32.5)	28.9 (25.0–32.9)	25.8 (23.4–32.6)	0.47
Systolic blood pressure, mmHg	125.0 (120.0–137.5)	128.5 (120.0–144.0)	120.0 (115.0–130.0)	0.03*
Diastolic blood pressure, mmHg	75.0 (67.0–80.0)	80.0 (71.5–80.0)	70.0 (60.0–80.0)	0.02*
Days from illness onset to admission	8.0 (5.0–12.0)	11.0 (6.0–14.0)	7.0 (4.0–10.0)	0.03*
Biological data				
Leukocytes, G/L	5.7 (4.4–8.3)	7.7 (5–9.3)	4.9 (4–6.9)	0.03*
Lymphocytes, G/L	0.9 (0.6–1.2)	0.7 (0.5–1)	1 (0.7–1.3)	0.11
Neutrophils, G/L	4.7 (3.2–6.8)	5.9 (4.2–7.9)	3.4 (2.5–4.9)	0.008*
Hemoglobin, G/L	13.0 (12.2–14.2)	13.0 (12.1–14.2)	13.2 (12.2–14.2)	0.93
Platelets, G/L	205.0 (165.5–270.0)	255.5 (174.8–288.5)	203.0 (157.0–256.0)	0.11
D-dimer, ng/ml	1764.0 (779.0–5689.0)	4944.0 (1814.0–9374.0)	913.0 (476.0–1422.0)	<0.001*
Fibrinogen, g/l	7.1 (5.8–8.7)	7.8 (6.8–9.7)	5.5 (4.8–7.4)	0.001*
Procalcitonin, ng/ml	0.2 (0.1–0.5)	0.4 (0.2–0.8)	0.1 (0.1–0.3)	0.002*
C-reactive protein, mg/l	87.8 (57.3–150.9)	123.3 (80.3–219.2)	83.6 (41.1–112.8)	0.02*
Lactose deshydrogenase, U/L	666.0 (488.0–790.5)	677.0 (490.0–992.3)	649.0 (470.0–694.0)	0.15
Sodium, mmol/L	137.0 (134.0–139.0)	135.5 (133.0–141.0)	138.0 (135.0–139.0)	0.24
Potassium, mmol/l	4.0 (3.7–4.3)	4.2 (3.8–4.5)	3.8 (3.6–4.1)	0.04*
Bicarbonate, mmol/l	23.0 (22.0–25.0)	22.0 (20.0–23.3)	24.0 (23.0–26.0)	0.003*
Albumin, g/l	28.9 (25.4–32.8)	27.0 (24.7–30.0)	30.8 (27.3–34.3)	0.02*
BUN, mmol/l	5.2 (3.9–7.3)	4.7 (2.8–6.3)	3.7 (1.7–4.6)	0.06
Creatinine, μmol/l	72.0 (57.0–87.5)	75.5 (59.3–94.0)	72.0 (55.0–82.0)	0.44
eGFR, ml/min per 1.73 m^2	87 (82–101)	89 (80–97)	87 (82–106)	0.45
Renal involvement				
AKI, *n* (%)	12.0 (26.7)	8.0 (36.4)	4.0 (17.4)	0.15
KDIGO 1	6/12 (50.0)	4/8 (50)	2/4 (50)	
KDIGO 2	4/12 (33.3)	2/8 (25)	2/4 (50)	
KDIGO 3	2/12 (16.7)	2/8 (25)	0	
RRT	2/12 (16.7)	2/8 (25)	0	
Proteinuria, g/g	0.50 ± 0.47	0.59 ± 0.40	0.42 ± 0.53	0.008*
Proteinuria >0.3 g/g, *n* (%)	27 (60.0)	17 (77.3)	10 (43.5)	0.02*
Non-albumin proteinuria, *n* (%)	37 (82.2)	18 (81.8)	19 (82.6)	0.94
Microalbuminuria, *n* (%)	5 (11.1)	3 (13.6)	2 (8.7)	0.6
Hematuria, *n* (%)	21 (46.7)	12 (54.5)	9 (39.1)	0.3
Leukocyturia, *n* (%)	21 (46.7)	13 (59.1)	8 (34.8)	0.10
Normoglycemic glycosuria, *n* (%)	0	0	0	
Treatment				
Steroids, *n* (%)	22 (48.9)	17 (77.3)	5 (21.7)	<0.001*
Antibiotics, *n* (%)	21 (46.7)	17 (77.3)	4 (17.4)	0.001*
Lopinavir/Ritonavir, *n* (%)	3 (6.7)	3 (13.6)	0	0.07
Hydroxychloroquine, *n* (%)	17 (37.8)	14 (63.6)	3 (13.0)	<0.001*
Tocilizumab, *n* (%)	1 (2.2)	1 (4.5)	0	0.97

(Continued)

TABLE 1 | Continued

Variables	All patients (*n* = 45)	Severe COVID-19 (*n* = 22)	Non-severe COVID-19 (*n* = 23)	*P*-value
Vasopressor, *n* (%)	12 (26.7)	12 (54.5)	12 (52.2)	0.001*
Invasive ventilation, *n* (%)	15 (33.3)	15 (68.1)	0	0.001*
Outcome				
Nosocomial infection, *n* (%)	15 (33.3)	13 (59.1)	2 (8.7)	<0.001*
In-hospital death, *n* (%)	2 (4.4)	1 (4.5)	1 (4.3)	0.97
Length of stay, days	11 (5–23)	23 (14–32)	7 (5–11)	0.001*

*ACE, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blockers; BMI, body mass index; BUN, blood urea nitrogen; AKI, acute kidney injury; RRT, renal replacement therapy. *Significant, P < 0.05.*

Renal Involvement

On admission, 93.3% of patients (42 of 45) presented with renal involvement.

Urine Sediment Abnormalities

Significant proteinuria (>0.3 g/g) occurred in 27 patients (60%) with a mean of 0.50 ± 0.47 g/g (77.3% patients in the severe disease group versus 43% in the non-severe group, $p = 0.02$). Higher UPCR was observed in patients with severe COVID-19 (0.59 ± 0.40 versus 0.42 ± 0.53 g/g, $p = 0.008$). Urine protein electrophoresis showed non-albumin proteinuria excretion in 77.7% of patients with significant proteinuria. Inflammatory variables, D-dimers and length of hospital stay were correlated with the level of proteinuria (**Supplementary Table 1**).

Acute Kidney Injury

The incidence of AKI in the overall cohort was 26.7% (12 of 45 patients) according to KDIGO definition (**Table 1**). Stage 1 AKI accounted for 50% (6/12 patients with AKI), stage 2 comprised 33.3% (4/12), and 16.7% (2/12) reached stage 3 and required renal replacement therapy (RRT). Among patients with AKI: proteinuria, hematuria and leukocyturia were not different compared with non-AKI patients (**Supplementary Table 2**). Mortality was not different between AKI and non-AKI patients. Among patients in whom AKI developed, 50% recovered.

Impact of Proteinuria in SARS-CoV-2 Infection

Patients who required ICU admission had significantly higher level of proteinuria (0.59 ± 0.40 versus 0.42 ± 0.53 g/g, $p = 0.008$).

Patients with significant proteinuria defined as proteinuria above 0.3 g/g had a longer hospital stay [19 days (9–31) versus 7 days (5–11), $p = 0.001$], a higher prevalence of nosocomial infection (48.1 versus 11.1%, $p = 0.01$) and acute respiratory distress syndrome (ARDS) (48.1 versus 11.1%, $p = 0.01$) but mortality was not significantly higher ($p = 0.24$) (**Table 2**). Using univariate and multivariate analyses, proteinuria was related to a higher prevalence of ICU admission, ARDS and nosocomial infection (**Table 3**). Kaplan-Meier analysis revealed a significantly longer hospitalization duration for patients with a significant proteinuria (**Figure 1**). For in-hospital mortality and AKI, proteinuria was not an independent predictor (**Table 3**).

DISCUSSION

Among the 45 patients prospectively analyzed in the present study, a high proportion presented renal involvement. Proteinuria at baseline was associated with poor outcome in SARS-CoV-2 infection. Our data are consistent with a recent retrospective study among 333 patients in China (Pei et al., 2020). Our study confirmed that kidney involvement is common in hospitalized COVID-19 patients, but not only in severe cases. AKI has been previously described as associated with in-hospital mortality in COVID-19 (Cheng et al., 2020) and renal complications in COVID-19 remains associated with poor outcome (Pei et al., 2020). To our knowledge, this is the first study using quantitative assessment of urinary protein excretion and identifying proteinuria as a factor associated with poor outcome in COVID-19 (higher prevalence of ARDS, nosocomial infection, ICU admission). Furthermore, we could show that a significant proteinuria (i.e., above 0.3 g/g) was associated with a higher risk of ICU admission, developing ARDS, requiring vasopressor, developing nosocomial infections. Proteinuria higher than 0.3 g/g was a predictor for length of stay. Similar results have been described in a retrospective cohort with bacterial community-acquired pneumonia (Spoorenberg et al., 2012) but also in non-infectious diseases such as cirrhosis (Lin et al., 2014) or lung cancer (Hsu et al., 2018).

The tubular profile of kidney involvement we describe is consistent with recent pathologic reports (Su et al., 2020). The analysis of 26 autopsies from COVID-19 patients showed diffuse proximal tubule injury with the loss of brush border, non-isometric vacuolar degeneration, necrosis; hyaline casts and microthrombi were also observed. Interestingly, in this study, some patients did not present AKI according to KDIGO definition suggesting subclinical kidney injury. Proteinuria appears as a sensitive tool, comparing to creatinine and/or BUN, for renal assessment in COVID-19, even in subclinical kidney injury.

In our study, hematuria and leukocyturia were present in almost half of the patients but we found no difference between severe and non-severe COVID-19. Our results suggest an interstitial participation in kidney. Histopathologic reports did not show interstitial inflammation but 61.5% of patients had steroids before kidney biopsy was performed (and data are not available for 23% of patients) (Su et al., 2020). Even if a case

TABLE 2 | Characteristics of patients with and without proteinuria >0.3 g/g.

Variable	Proteinuria (*n* = 27)	No proteinuria (*n* = 18)	*P*-value
Age, years	66.0 (49.0–72.0)	61.0 (43.5–68.3)	0.18
Male patients, *n* (%)	22 (81.5)	9 (50.0)	0.03*
Day from illness onset to admission, days	9.0 (5.0–11.0)	8.0 (5.5–12.5)	0.83
Systolic blood pressure, mmgh	127.0 (120.0–140.0)	120.0 (120.0–130.0)	0.40
Diastolic blood pressure, mmgh	80.0 (70.0–80.0)	70.0 (60.0–80.0)	0.54
Respiratory disease, *n* (%)	7 (25.9)	2 (11.1)	0.22
Hypertension, *n* (%)	11 (40.7)	6 (33.3)	0.62
Diabetes mellitus, *n* (%)	10 (37.0)	2 (11.1)	0.05
Cardiac disease, *n* (%)	3 (11.1)	2 (11.1)	1
Vascular disease, *n* (%)	3 (11.1)	0	0.14
Active and former smokers, *n* (%)	9 (33.3)	4 (22.2)	0.42
ACEI, *n* (%)	1 (3.7)	0	0.41
ARB, *n* (%)	3 (0.11)	5 (27.8)	0.15
BMI (Kg/m^2)	26.5 (24.3–32.9)	28.3 (24.1–32.6)	0.74
C Reactive protein, mg/l	112.8 (75.1–168.1)	51.6 (81.2–104.0)	0.04*
Procaciltonin, ng/ml	0.3 (0.1–0.7)	0.2 (0.1–0.4)	0.27
D–Dimer ng/ml	4349.0 (1701.0–6828.0)	779.0 (472.0–1501.0)	<0.001*
AKI, *n* (%)	9 (33.3)	3 (16.7)	0.22
ICU admission, *n* (%)	17 (62.9)	5 (27.8)	0.02*
ARDS, *n* (%)	13 (48.1)	2 (11.1)	0.01*
Length of stay, days	19 (9.0–31.0)	6.5 (4.8–11.3)	0.001*
Nosocomial infection, *n* (%)	13 (48.1)	2 (11.1)	0.01*
In-hospital death, *n* (%)	2 (7.4)	0	0.24

*ACE, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blockers; ARDS, acute respiratory distress syndrom; BMI, body mass index; AKI, acute kidney injury; ICU, intensive care unit. *Significant, P < 0.05.*

TABLE 3 | Association between significant proteinuria and outcomes.

Variable	Univariate analysis			Multivariate analysis		
	Odds ratio	95% Confidence interval	*P* value	Odds ratio	95% Confidence interval	*P* value
AKI	2.33	0.57–12.02	0.26			
RRT	5.33	0.48–119.84	0.18			
ARDS	6.96	1.55–49.87	0.02*	6.89	1.41–53.01	0.02*
ICU admission	4.08	1.15–16.19	0.03*	4.72	1.16–23.21	0.03*
Vasopressor	11.0	1.82–213.24	0.03*	12.32	1.83–254.97	0.03*
Nosocomial infection	6.96	1.56–49.87	0.02*	3.75	1.11–13.55	0.03*
In-hospital death	4.17	0.63–34.32	0.13			

*AKI, acute kidney injury; RRT, renal replacement therapy; ARDS, acute respiratory distress syndrome; ICU, intensive care unit. *Significant, P < 0.05.*

of collapsing glomerulopathy has been reported in COVID-19 (Gaillard et al., 2020), acute tubular necrosis (ATN) with interstitial component probably remains the main cause of kidney injury. Other factors may contribute to kidney involvement: systemic inflammation and cytokine storm (Burton and Harris, 1996; Coletta et al., 2000; Gohda et al., 2001; D'Amico and Bazzi, 2003; Imai, 2003; Thorevska et al., 2003; Mäkelä et al., 2004; Wassmann et al., 2004; Kielar et al., 2005; Abbate et al., 2006; Liu et al., 2007; Delvaeye and Conway, 2009; Murugan et al., 2010; Ranganathan et al., 2013; Darmon et al., 2014, 2017; Su et al., 2017; Sarhan et al., 2018; Meissner et al., 2019; Cremoni et al., 2020; Mehta et al., 2020; Pan et al., 2020; Rossi et al., 2020; Siddiqi and Mehra, 2020; Tang et al., 2020; Vaninov, 2020; Zhang et al., 2020; Ruetsch et al., 2021), release of pathogen-associated molecular patterns (PAMPs), high levels of damage-associated molecular proteins (DAMPS) from lung injury and severe hypoxemic respiratory failure (Burton and Harris, 1996; Coletta et al., 2000; Gohda et al., 2001; D'Amico and Bazzi, 2003; Thorevska et al., 2003; Mäkelä et al., 2004; Wassmann et al., 2004; Kielar et al., 2005; Abbate et al., 2006; Delvaeye and Conway, 2009; Ranganathan et al., 2013; Darmon et al., 2014, 2017; Su et al., 2017; Sarhan et al., 2018; Meissner et al., 2019; Cremoni et al., 2020; Pan et al., 2020; Rossi et al., 2020; Siddiqi and Mehra, 2020; Tang et al., 2020; Vaninov, 2020; Zhang et al., 2020; Ruetsch et al., 2021). All these factors could also lead to tissue factor release and the hypercoagulate state

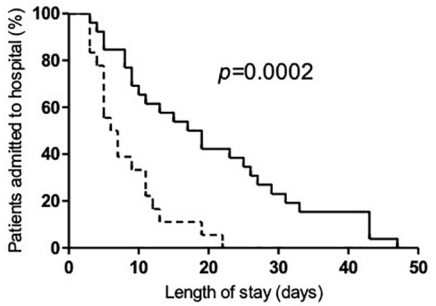


FIGURE 1 | Significant proteinuria is associated with an increased risk to develop severe disease and longer hospitalization in patients with COVID-19 disease. Patients with higher proteinuria presented an increased risk to develop severe disease and to require admission to the intensive care unit. Kaplan-Meier analysis of the effect of proteinuria >0.3 g/g on length of stay in patients hospitalized with SARS-CoV-2 infection showed that patients with proteinuria greater than 0.3 g/g presented a longer hospitalization (log-rank $p = 0.0002$).

described in SARS-COV-2 infection (Delvaeye and Conway, 2009; Tang et al., 2020). In our cohort, D-dimer correlated with higher proteinuria and severe disease. Moreover, reports suggested that patients with COVID-19 presented an increased risk for thrombosis or microangiopathy in autopsy reports (Tang et al., 2020). This hypercoagubility might participate in kidney involvement in COVID-19.

Proximal tubules injury has been described (Su et al., 2020), but our results don't support Fanconi's syndrome. Microalbuminuria was present in 11.1% of the cohort. High prevalence of microalbuminuria has been described in critically ill patients and is associated with mortality (Thorevska et al., 2003) but here, no difference was found in microalbuminuria according to disease severity. This is the reason why we focused on significant proteinuria. Immunostaining with SARS-CoV-2 nucleoprotein antibody was found positive in tubules and podocytes (Su et al., 2020). One mechanism of kidney impairment may be direct viral infection of renal epithelium, but not all biopsy specimens found viral genetic material and cytopathic effects (Rossi et al., 2020). Mechanisms for proteinuria may result from a defect in proximal tubular resorption and also from impairment of glomerular permeability due to pathophysiologic changes due to pro-inflammatory cytokines (Chu et al., 2005). Proteinuria in COVID 19 could not be differentiate from febrile proteinuria. As in septic state, proteinuria could be predictive of ICU survival. We found higher C-reactive protein levels with higher proteinuria, SARS-CoV-2 infection is known to induce cytokines storm and this pro-inflammatory disorder has been associated with severity and poor outcome (Mehta et al., 2020; Vaninov, 2020; Zhang et al., 2020). Pro-inflammatory cytokines might play a role as previously explored in AKI (Liu et al., 2007; Murugan et al., 2010) but not in proteinuria. Interestingly, the entire cohort was studied during the hyperinflammatory phase of the disease (Siddiqi and Mehra, 2020). These facts can support the relation with proteinuria and cytokine storm in SARS-CoV-2 infection. As described previously, inflammatory cytokines IL1β, IL6, IL8, and TNFα increased in the plasma of moderate and severe COVID-19 patients (Ruetsch et al., 2021). Cytokine storm may contribute to COVID-19 AKI by cooperating with renal resident cells and promoting tubular and endothelial dysfunction. Previous studies described a relevant role for IL6 (Ruetsch et al., 2021). Interleukin-6 can lead renal endothelial cells to produce pro-inflammatory cytokines and chemokines, and can induce kidney vascular permeability, acting in microcirculatory dysfunction. Pro-inflammatory cytokines can also induce capillary leak syndrome and the production of thrombosis. Interestingly, IL6 could be produced by renal resident cells, including podocytes (Burton and Harris, 1996; Coletta et al., 2000; Gohda et al., 2001; Mäkelä et al., 2004; Wassmann et al., 2004; Kielar et al., 2005; Abbate et al., 2006; Ranganathan et al., 2013; Su et al., 2017; Cremoni et al., 2020; Pan et al., 2020; Ruetsch et al., 2021), mesangial cells (Coletta et al., 2000; Gohda et al., 2001), endothelial cells (Wassmann et al., 2004) and tubular epithelial cells (Kielar et al., 2005; Ranganathan et al., 2013). All these cells would actively respond to IL6 (Su et al., 2017). Moreover, plasma and urinary IL6 concentrations correlated with proteinuria in acute Hantavirus-induced nephritis (Mäkelä et al., 2004) and increased IL6 plasma levels and a TH17 profile have already been described in glomerular diseases such as membranous nephropathy (Cremoni et al., 2020).

This study has several limitations. One is the number of patients studied, nevertheless all consecutive patients were prospectively screened in different wards and hospitals strengthening the results obtained. This small sample size could explain our non-significative results on correlating proteinuria to AKI and mortality. Secondly, we could not detect SARS-CoV-2 in urine samples but this is not performed routinely and kidney involvement in SARS-CoV-2 infection seems multifactorial and not only due to viral infection. Last, we did not have renal histopathological data to correlate to biological data since there were no formal indication for kidney biopsy.

Nevertheless, our study is prospective and multicentric. This is the first conducted in an European population. Differences have been described between Caucasian and Asian populations in kidney disease and also in ACE2 expression (Hoffmann et al., 2020). We used quantitative urinary protein excretion that is more accurate but still an easy and inexpensive test in contrast with other urinary markers.

In conclusion, kidney involvement in SARS-CoV-2 infection is common and not only in severe forms. Renal impairment in SARS-CoV-2 infection and more precisely proteinuria is an independent predictor for length of stay and admission to the ICU. Proteinuria is an easily measurable marker to predict outcome and may be used to assess the severity of SARS-CoV-2 infection. Evidence suggest that proteinuria is a marker of chronic disease progression (Burton and Harris, 1996; Abbate et al., 2006). In SARS-CoV-2 infection, quantitative proteinuria should be monitored at admission and during follow-up, even in patients without AKI or severe disease to assess long-term implication of SARS-CoV-2 infection.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

COLLABORATORS

Mathieu Buscot: Service de Médecine Intensive Réanimation, CHU de Nice, Université Côte d'Azur, Nice, France; Clément Saccheri: Service de Médecine Intensive Réanimation, CHU de Nice, Université Côte d'Azur, Nice, France; Hervé Hyvernat: Service de Médecine Intensive Réanimation, CHU de Nice, Université Côte d'Azur, Nice, France; Denis Doyen: Service de Médecine Intensive Réanimation, CHU de Nice, Université Côte d'Azur, Nice, France; Nihal Martis: Service de Médecine Intensive Réanimation, CHU de Nice, Université Côte d'Azur, Nice, France; Yanis Kouchit: Service de Médecine Intensive Réanimation, CHU de Nice, Université Côte d'Azur, Nice, France; Thomas Citti: Service de Médecine Intensive Réanimation, CHU de Nice, Université Côte d'Azur, Nice, France; Éric Cua: Service d'Infectiologie, CHU de Nice, Université Côte d'Azur, Nice, France; Véronique Mondain: Service d'Infectiologie, CHU de Nice, Université Côte d'Azur, Nice, France; David Chirio: Service d'Infectiologie, CHU de Nice, Université Côte d'Azur, Nice, France; Karine Risso: Service d'Infectiologie, CHU de Nice, Université Côte d'Azur, Nice, France; Philippe Deswardt: Service de Réanimation CH Antibes-Juan les Pins, Antibes, France; Cécilia Benard: Service de Réanimation CH Antibes-Juan les Pins, Antibes, France; Marine Clavaud: Département de réanimation médico-chirugicale et anesthésie, CHU de Nice, Université Côte d'Azur, Nice, France; Abdlazize Sahraoui: Département de réanimation médico-chirugicale et anesthésie, CHU de Nice, Université Côte d'Azur, Nice, France; Thibaud Chapelle: Département de réanimation médico-chirugicale et anesthésie, CHU de Nice, Université Côte d'Azur, Nice, France.

AUTHOR CONTRIBUTIONS

HO, SB-S, and JD designed the study and drafted and revised the manuscript. HO, LM, JC, JF, VB, RL, and ED collected clinical and biological data. HO, LM, and SB-S analyzed and interpreted the data. HO, JC, LM, JF, VB, RL, VE, BS-P, ED, JD, and SB-S provided medical oversight. All authors approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors greatly appreciate all the hospital staff from Nice, Antibes, Cannes, and Grasse for their efforts in recruiting and treating patients and thank all patients involved in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.611772/full#supplementary-material

REFERENCES

Abbate, M., Zoja, C., and Remuzzi, G. (2006). How does proteinuria cause progressive renal damage? *J. Am. Soc. Nephrol.* 17, 2974–2984. doi: 10.1681/asn.2006040377

Akalin, E., Azzi, Y., Bartash, R., Seethamraju, H., Parides, M., Hemmige, V., et al. (2020). Covid-19 and kidney transplantation. *N. Engl. J. Med.* 382, 2475–2477. doi: 10.1056/NEJMc2011117

Alberici, F., Delbarba, E., Manenti, C., Econimo, L., Valerio, F., Pola, A., et al. (2020). A single center observational study of the clinical characteristics and short-term outcome of 20 kidney transplant patients admitted for SARS-CoV2 pneumonia. *Kidney Int.* 97, 1083–1088. doi: 10.1016/j.kint.2020.04.002

Banerjee, D., Popoola, J., Shah, S., Ster, I. C., Quan, V., and Phanish, M. (2020). COVID-19 infection in kidney transplant recipients. *Kidney Int.* 97, 1076–1082. doi: 10.1016/j.kint.2020.03.018

Burton, C., and Harris, K. P. (1996). The role of proteinuria in the progression of chronic renal failure. *Am. J. Kidney Dis.* 27, 765–775. doi: 10.1016/s0272-6386(96)90512-0

Cha, R.-H., Joh, J.-S., Jeong, I., Lee, J. Y., Shin, H.-S., Kim, G., et al. (2015). Renal complications and their prognosis in korean patients with middle east respiratory syndrome-coronavirus from the central MERS-CoV designated hospital. *J. Korean Med. Sci.* 30, 1807–1814. doi: 10.3346/jkms.2015.30.12.1807

Cheng, Y., Luo, R., Wang, K., Zhang, M., Wang, Z., Dong, L., et al. (2020). Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int.* 97, 829–838. doi: 10.1016/j.kint.2020.03.005

Chu, K. H., Tsang, W. K., Tang, C. S., Lam, M. F., Lai, F. M., To, K. F., et al. (2005). Acute renal impairment in coronavirus-associated severe acute respiratory syndrome. *Kidney Int.* 67, 698–705.

Coletta, I., Soldo, L., Polentarutti, N., Mancini, F., Guglielmotti, A., Pinza, M., et al. (2000). Selective induction of MCP-1 in human mesangial cells by the IL-6/sIL-6R complex. *Nephron Exp. Nephrol.* 14, 37–43. doi: 10.1159/000059327

Cremoni, M., Brglez, V., Perez, S., Decoupigny, F., Zorzi, K., Andreani, M., et al. (2020). Th17-Immune response in patients with membranous nephropathy is associated with thrombosis and relapses. *Front. Immunol.* 11:574997. doi: 10.3389/fimmu.2020.574997

D'Amico, G., and Bazzi, C. (2003). Pathophysiology of proteinuria. *Kidney Int.* 63, 809–825. doi: 10.1046/j.1523-1755.2003.00840.x

Darmon, M., Clec'h, C., Adrie, C., Argaud, L., Allaouchiche, B., Azoulay, E., et al. (2014). Acute respiratory distress syndrome and risk of AKI among critically Ill patients. *Clin. J. Am. Soc. Nephrol.* 9, 1347–1353.

Darmon, M., Legrand, M., and Terzi, N. (2017). Understanding the kidney during acute respiratory failure. *Intens. Care Med.* 43, 1144–1147. doi: 10.1007/s00134-016-4532-z

Delvaeye, M., and Conway, E. M. (2009). Coagulation and innate immune responses: can we view them separately? *Blood* 17, 2367–2374. doi: 10.1182/blood-2009-05-199208

Gaillard, F., Ismael, S., Sannier, A., Tarhini, H., Volpe, T., Greze, C., et al. (2020). Tubuloreticular inclusions in COVID-19-related collapsing glomerulopathy. *Kidney Int.* 98:241. doi: 10.1016/j.kint.2020.04.022

Gohda, T., Makita, Y., Shike, T., Funabiki, K., Shirato, I., and Tomino, Y. (2001). Dilazep hydrochloride, an antiplatelet drug, inhibits lipopolysaccharide-induced mouse mesangial cell IL-6 secretion and proliferation. *Kidney Blood Press. Res.* 24, 33–38. doi: 10.1159/000054203

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271–280.e8.

Hsu, S.-N., Hsu, Y.-J., Lin, C., Su, S.-L., and Lin, S.-H. (2018). Proteinuria: associated with poor outcome in patients with small cell lung cancer. *J. Cancer Res. Ther.* 14, S688–S693.

Imai, Y. (2003). Injurious mechanical ventilation and end-organ epithelial cell apoptosis and organ dysfunction in an experimental model of acute respiratory distress syndrome. *JAMA* 289:2104. doi: 10.1001/jama.289.16.2104

Kielar, M. L., John, R., Bennett, M., Richardson, J. A., Shelton, J. M., Chen, L., et al. (2005). Maladaptive role of IL-6 in ischemic acute renal failure. *J. Am. Soc. Nephrol.* 16, 3315–3325. doi: 10.1681/asn.2003090757

Lin, L.-Y., Jenq, C.-C., Liu, C.-S., Huang, C.-S., Fan, P.-C., Chang, C.-H., et al. (2014). Proteinuria can predict short-term prognosis in critically ill cirrhotic patients. *J. Clin. Gastroenterol.* 48, 377–382. doi: 10.1097/mcg.0000000000000060

Liu, K. D., Glidden, D. V., Eisner, M. D., Parsons, P. E., Ware, L. B., Wheeler, A., et al. (2007). Predictive and pathogenetic value of plasma biomarkers for acute kidney injury in patients with acute lung injury. *Crit. Care Med.* 35, 2755–2761. doi: 10.1097/01.ccm.0000291649.72238.6d

Mäkelä, S., Mustonen, J., Ala-Houhala, I., Hurme, M., Koivisto, A.-M., Vaheri, A., et al. (2004). Urinary excretion of interleukin-6 correlates with proteinuria in acute Puumala hantavirus-induced nephritis. *Am. J. Kidney Dis.* 43, 809–816. doi: 10.1053/j.ajkd.2003.12.044

Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., and Manson, J. J. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 395, 1033–1034. doi: 10.1016/s0140-6736(20)30628-0

Meissner, M., Viehmann, S. F., and Kurts, C. (2019). DAMPening sterile inflammation of the kidney. *Kidney Int.* 95, 489–491. doi: 10.1016/j.kint.2018.12.007

Murugan, R., Karajala-Subramanyam, V., Lee, M., Yende, S., Kong, L., Carter, M., et al. (2010). Acute kidney injury in non-severe pneumonia is associated with an increased immune response and lower survival. *Kidney Int.* 77, 527–535. doi: 10.1038/ki.2009.502

Naicker, S., Yang, C.-W., Hwang, S.-J., Liu, B.-C., Chen, J.-H., and Jha, V. (2020). The novel coronavirus 2019 epidemic and kidneys. *Kidney Int.* 97, 824–828. doi: 10.1016/j.kint.2020.03.001

Pan, X., Xu, D., Zhang, H., Zhou, W., Wang, L., and Cui, X. (2020). Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. *Intensive Care Med.* 46, 1114–1116. doi: 10.1007/s00134-020-06026-1

Pei, G., Zhang, Z., Peng, J., Liu, L., Zhang, C., Yu, C., et al. (2020). Renal involvement and early prognosis in patients with COVID-19 pneumonia. *J. Am. Soc. Nephrol.* 31, 1157–1165. doi: 10.1681/asn.2020030276

Ranganathan, P., Jayakumar, C., and Ramesh, G. (2013). Proximal tubule-specific overexpression of netrin-1 suppresses acute kidney injury-induced interstitial fibrosis and glomerulosclerosis through suppression of IL-6/STAT3 signaling. *Am. J. Physiol. Renal Physiol.* 304, F1054–F1065.

Rossi, G. M., Delsante, M., Pilato, F. P., Gnetti, L., Gabrielli, L., Rossini, G., et al. (2020). Kidney biopsy findings in a critically ill COVID-19 patient with dialysis-dependent acute kidney injury: a case against "SARS-CoV-2 nephropathy.". *Kidney Int. Rep.* 5, 1100–1105. doi: 10.1016/j.ekir.2020.05.005

Ruetsch, C., Brglez, V., Crémoni, M., Zorzi, K., Fernandez, C., Boyer-Suavet, S., et al. (2021). Functional exhaustion of Type I and II interferons production in severe COVID-19 patients. *Front. Med.* 7:603961. doi: 10.3389/fmed.2020.603961

Sarhan, M., von Mässenhausen, A., Hugo, C., Oberbauer, R., and Linkermann, A. (2018). Immunological consequences of kidney cell death. *Cell Death Dis.* 9:114.

Siddiqi, H. K., and Mehra, M. R. (2020). COVID-19 illness in native and immunosuppressed states: a clinical–therapeutic staging proposal. *J. Heart Lung. Transplant.* 39, 405–407. doi: 10.1016/j.healun.2020.03.012

Spoorenberg, S. M. C., Meijvis, S. C. A., Navis, G., Ruven, H. J., Biesma, D. H., Grutters, J. C., et al. (2012). Incidence and predictive value of proteinuria in community-acquired pneumonia. *Nephron Clin. Pract.* 122, 67–74. doi: 10.1159/000348833

Su, H., Lei, C.-T., and Zhang, C. (2017). Interleukin-6 signaling pathway and its role in kidney disease: an update. *Front. Immunol.* 8:405. doi: 10.3389/fimmu.2017.00405

Su, H., Yang, M., Wan, C., Yi, L.-X., Tang, F., Zhu, H.-Y., et al. (2020). Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int.* 98, 219–227. doi: 10.1016/j.kint.2020.04.003

Sun, J., Zhu, A., Li, H., Zheng, K., Zhuang, Z., Chen, Z., et al. (2020). Isolation of infectious SARS-CoV-2 from urine of a COVID-19 patient. *Emerg. Microbes Infect.* 28, 1–8.

Tang, N., Li, D., Wang, X., and Sun, Z. (2020). Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J. Thromb Haemost JTH.* 18, 844–847. doi: 10.1111/jth.14768

Thorevska, N., Sabahi, R., Upadya, A., Manthous, C., and Amoateng-Adjepong, Y. (2003). Microalbuminuria in critically ill medical patients: prevalence, predictors, and prognostic significance. *Crit. Care Med.* 31, 1075–1081. doi: 10.1097/01.ccm.0000059316.90804.0b

Vaninov, N. (2020). In the eye of the COVID-19 cytokine storm. *Nat. Rev. Immunol.* 20:277. doi: 10.1038/s41577-020-0305-6

Walls, A. C., Park, Y.-J., Tortorici, M. A., Wall, A., McGuire, A. T., and Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 181, 281–292.e6.

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. (2020). Clinical characteristics of 138 hospitalized patients With 2019 novel coronavirus–infected pneumonia in wuhan. China. *JAMA* 17 323:1061. doi: 10.1001/jama.2020.1585

Wassmann, S., Stumpf, M., Strehlow, K., Schmid, A., Schieffer, B., Böhm, M., et al. (2004). Interleukin-6 induces oxidative stress and endothelial dysfunction by overexpression of the angiotensin II Type 1 receptor. *Circ. Res.* 5, 534–541. doi: 10.1161/01.res.0000115557.25127.8d

Zhang, C., Wu, Z., Li, J.-W., Zhao, H., and Wang, G.-Q. (2020). The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist tocilizumab may be the key to reduce the mortality. *Int. J. Antimicrob. Agents* 29:105954. doi: 10.1016/j.ijantimicag.2020.105954

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 09 March 2021
doi: 10.3389/fphys.2021.622987

Olfactory Dysfunction in Frontline Health Care Professionals During COVID-19 Pandemic in Brazil

Mariana Ferreira Sbrana[1], Marco Aurélio Fornazieri[2], Alexandre Bruni-Cardoso[3], Vivian I. Avelino-Silva[4], Deborah Schechtman[3], Richard Louis Voegels[1], Bettina Malnic[3], Isaias Glezer[5]* and Fabio de Rezende Pinna[1]*

[1] Department of Otorhinolaryngology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [2] Department of Clinical Surgery, Universidade Estadual de Londrina and Pontifical Catholic University of Paraná, Londrina, Brazil, [3] Department of Biochemistry, Institute of Chemistry, Universidade de São Paulo, São Paulo, Brazil, [4] Department of Infectious and Parasitic Diseases, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [5] Department of Biochemistry, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil

Upper respiratory viral infections can decrease the sense of smell either by inflammatory restriction of nasal airflow that carries the odorant molecules or through interference in olfactory sensory neuron function. During the coronavirus disease 2019 (COVID-19) pandemic, triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), worldwide reports of severe smell loss (anosmia/hyposmia) revealed a different type of olfactory dysfunction associated with respiratory virus infection. Since self-reported perception of smell is subjective and SARS-CoV-2 exposure is variable in the general population, we aimed to study a population that would be more homogeneously exposed to the virus. Here, we investigated the prevalence of olfactory loss in frontline health professionals diagnosed with COVID-19 in Brazil, one of the major epicenters of the disease. We also analyzed the rate of olfactory function recovery and the particular characteristics of olfactory deficit in this population. A widely disclosed cross-sectional online survey directed to health care workers was developed by a group of researchers to collect data concerning demographic information, general symptoms, otolaryngological symptoms, comorbidities, and COVID-19 test results. Of the 1,376 health professionals who completed the questionnaire, 795 (57.8%) were working directly with COVID-19 patients, either in intensive care units, emergency rooms, wards, outpatient clinics, or other areas. Five-hundred forty-one (39.3%) participants tested positive for SARS-CoV-2, and 509 (37%) were not tested. Prevalence of olfactory dysfunction in COVID-19-positive subjects was 83.9% (454 of 541) compared to 12.9% (42 of 326) of those who tested negative and to 14.9% (76 of 509) of those not tested. Olfactory dysfunction incidence was higher in those working in wards, emergency rooms, and intensive care units compared to professionals in outpatient clinics. In general, remission from olfactory symptoms was frequent by the time of responses. Taste disturbances were present in 74.1% of infected participants and were significantly associated with hyposmia. In conclusion, olfactory dysfunction is highly correlated with exposure to SARS-CoV-2 in health care professionals, and remission rates up to 2 weeks are high.

Keywords: coronavirus, COVID-19, olfaction disorders, respiratory tract infection, health care, sense of smell, SARS-CoV-2, anosmia

OPEN ACCESS

Edited by:
Emmanuelle Vidal-Petiot, Assistance Publique Hopitaux De Paris, France

Reviewed by:
Jerry Polesel, Aviano Oncology Reference Center (IRCCS), Italy
Siddharth Sridhar, The University of Hong Kong, Hong Kong

****Correspondence:***
Mariana Ferreira Sbrana
mariana.sbrana@yahoo.com.br
Isaias Glezer
iglezer@unifesp.br

Specialty section:
This article was submitted to Respiratory Physiology, a section of the journal Frontiers in Physiology

Received: *29 October 2020*
Accepted: *20 January 2021*
Published: *09 March 2021*

Citation:
Sbrana MF, Fornazieri MA, Bruni-Cardoso A, Avelino-Silva VI, Schechtman D, Voegels RL, Malnic B, Glezer I and de Rezende Pinna F (2021) Olfactory Dysfunction in Frontline Health Care Professionals During COVID-19 Pandemic in Brazil. Front. Physiol. 12:622987. doi: 10.3389/fphys.2021.622987

INTRODUCTION

Clinical presentation of patients infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) varies from asymptomatic infection to mild and severe systemic symptoms (Chan et al., 2020). Common symptoms include fever, myalgia, cough, and fatigue (Guan et al., 2020). Sore throat, nasal congestion, rhinorrhea, headache, and diarrhea have also been described (Chen et al., 2020; Huang et al., 2020). Since the beginning of the pandemic, an increasing number of patients have sought medical assistance reporting loss of smell (Hopkins and Kumar, 2020; Iran News, 2020) and, thereby, a number of studies have been conducted to analyze the prevalence and determinants of olfactory dysfunction in coronavirus disease 2019 (COVID-19) patients.

The studies published so far have shown a prevalence of loss of smell in SARS-CoV-2-infected patients ranging from 5.1 to 85.6% (Lechien et al., 2020; Mao et al., 2020; Menni et al., 2020; Parma et al., 2020; Spinato et al., 2020) and suggested that SARS-CoV-2-related anosmia/hyposmia may differ from that associated with other respiratory virus infections, affecting patients with no other upper respiratory tract symptoms (Gane et al., 2020).

The primary objective of this study was to investigate the prevalence of olfactory loss among frontline health professionals according to exposure to COVID-19 in Brazil, one of the major epicenters of the disease. As secondary objectives, we aimed to analyze the frequency of olfactory function recovery during the period of study and the particular characteristics of hyposmia/anosmia (relation to other nasal symptoms, duration, and recovery time) in this population.

MATERIALS AND METHODS

The current study was approved by the ethics committee of *Hospital das Clinicas of University of São Paulo, Brazil* (approval number: 4.047.527). All participants provided consent for participation through the electronic questionnaire platform.

Subject Population

Health care workers were invited to answer an online questionnaire widely disclosed through social media, institutional mailing lists, regional professional councils, and radio. We excluded health professionals who did not inform a valid professional registration number, those who did not live in Brazil, those working in administrative jobs, those not in direct contact with patients, and those who reported contradictory answers related to olfactory/taste symptoms, for instance, answered "loss or reduction of smell" for one question and "I did not lose sense of smell" for a subsequent question (see **Supplementary File** for detailed survey questions).

COVID-19 epidemiological dataset (Brazilian cities) is publicly available at https://brasil.io.

Clinical Outcomes

A cross-sectional study was carried out, and clinical data were collected from May 29 to July 8. By the time we started this work, we did not have standard questionnaires to evaluate olfactory and taste functions available in Portuguese. Therefore, participants answered an electronic nonstandard questionnaire[1] developed by a group of researchers and based on previous studies that assessed olfactory complaints in COVID-19 patients. It consisted of 17 questions concerning demographic information (age, sex, e-mail, professional council number, state, occupation, area of professional practice, care for COVID-19 patients), general symptoms, ear/nose/throat (ENT) symptoms (including olfactory and gustatory symptoms), comorbidities that are known to impair smell function (such as chronic rhinosinusitis, neurodegenerative diseases, smoking, traumatic brain injury, stroke, epilepsy, brain tumor, the use of psychiatric or heart disease medications), and COVID-19 test results (the questionnaire is available as **Supplementary Material**).

Statistical Analysis

Statistical analysis was performed using statistical software STATA 13.0 (StataCorp LP, College Station, TX, United States) and R software environment (v3.6 Linux version; using standard packages like ggplot, tidyverse, ggmap, sf, etc., in addition to easyalluvial and ComplexUpset) (Wickham, 2016; Koneswarakantha, 2020; Krassowski, 2020; R Core Team, 2020). Continuous data were described as mean and standard deviation or as median and interquartile range, and categorical data were described as percentages. Potential associations between categorical variables have been assessed through chi-square test. The risk of olfactory disturbances between different groups of health care professionals has been assessed through logistic regression analysis. The independent variables used in logistic regression analysis were age, sex, SARS-CoV-2 positivity, area of practice [ward, emergency room, intensive care unit (ICU), clinic], comorbidities, occupation, and nasal symptoms (obstruction, rhinorrhea, burning sensation). The Bonferroni approach was used to adjust the level of statistical significance in multiple hypothesis testing. A level of $p < 0.05$ was set to determine statistical significance.

Correlations between some survey answers were displayed through alluvial plots, which employ the style of Sankey diagram to visualize categorical data over multiple dimensions as flows (Rosvall and Bergstrom, 2010). Due to figure complexity of Venn and Euler diagrams when depicting group intersections, we employed UpSet as a visualization technique (Lex et al., 2014). Figures were assembled with Inkscape[2] and converted to TIFF files with GIMP[3].

RESULTS

Subject Population

Of the 1,499 individuals who answered the questionnaire, 123 were excluded because the access to the online survey was tracked to foreign regions (not Brazil) or because the respondent was

[1] https://pt.surveymonkey.com/r/ANOSMIACOVIDSAUDE

[2] inkscape.org

[3] gimp.org

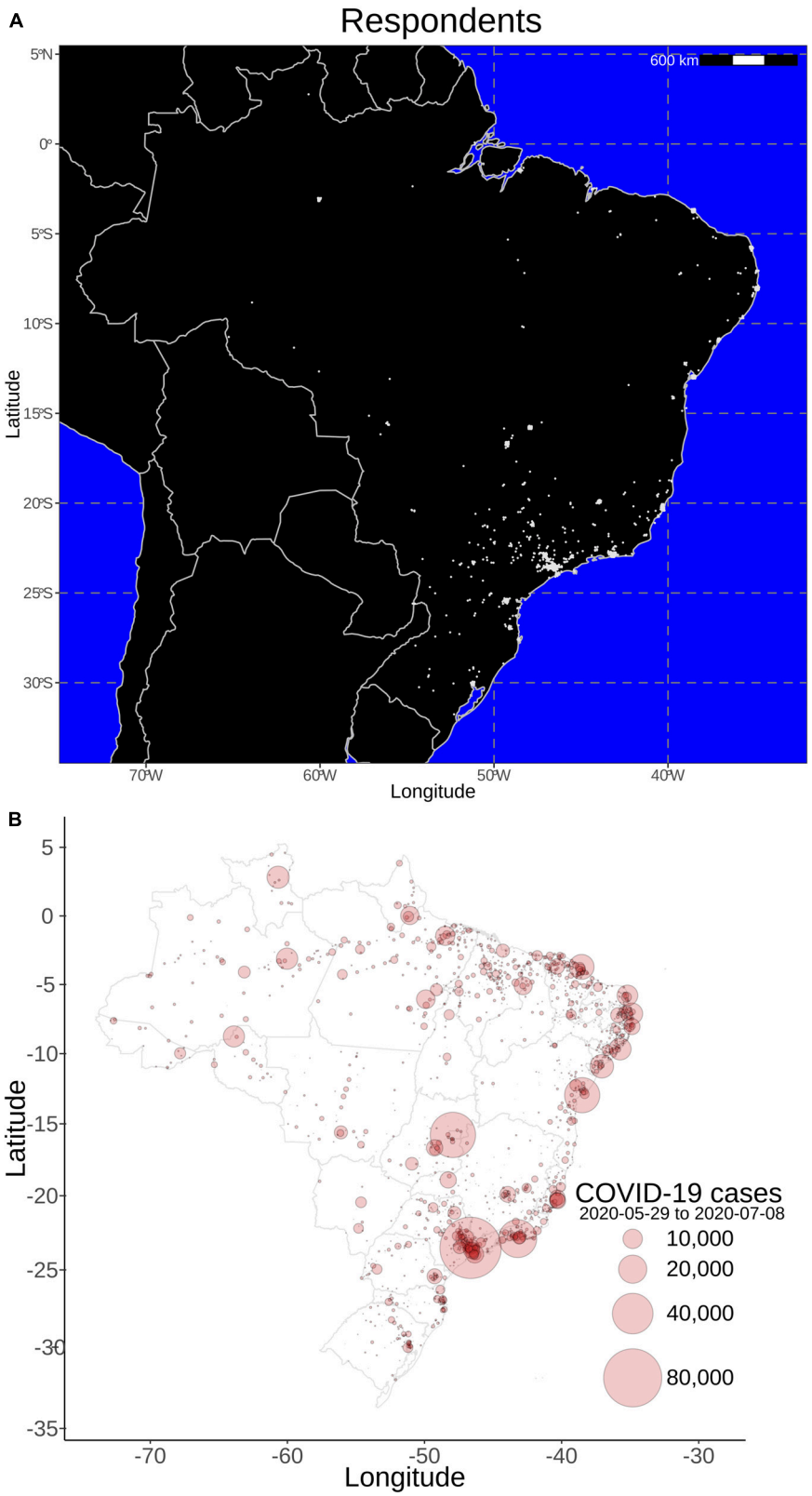


FIGURE 1 | Geo-localization of online survey participants and epidemiological data. Approximate location of respondents who enrolled in the online survey **(A)**, indicating that the major coronavirus disease 2019 (COVID-19) hot spots in Brazil **(B)** are represented in the collected data (May 29, 2020 to July 8, 2020). Most of participants were from São Paulo, which has been the Brazilian epicenter.

identified as a non-health care professional, did not provide a valid professional council number, or provided contradictory answers. Therefore, 1,376 health professionals who completed the questionnaire were included in the analysis. Our survey collected nationwide answers concentrated in endemic Brazilian regions that registered a high number of COVID-19 cases in the period of data collection (from May 29, 2020, to July 8, 2020; **Figure 1**). Mean age of respondents was 38.9 ± 10.4 years old (range 20–80), 1,021 were females (74.2%). Seventy-eight professionals (5.6%) reported previous olfactory or taste disturbances. A total of 795 (57.8%) were working directly with COVID-19 patients, 356 (25.8%) participants worked in outpatient clinics, 228 (16.5%) in emergency rooms, 183 (13.3%) in ICUs, 109 (8%) in wards, and 495 (36%) in other areas. **Table 1** summarizes clinical and demographic data of the health care professionals. As some questions were not mandatory to answer, there were participants who did not provide all information. These participants were not excluded because the missing questions would not compromise the analysis of our main objective.

TABLE 1 | Health professionals' clinical and demographic data.

	Total (*N* = 1,376)
Age, mean (SD), years	38.9 (10.4)
Female sex, No. (%)	1,021 (74.2)
Comorbidities, No. (%)	
Yes	299 (21.7)
No	1,020 (74.1)
No answer	57 (4.2)
Professional practice local, No. (%)	
Clinic	356 (25.8)
Emergency room	228 (16.5)
Intensive care unit	183 (13.3)
Ward	109 (8)
Other	495 (36)
No answer	5 (0.4)
Working with COVID-19 patients? No. (%)	
Yes	795 (57.8)
No	572 (41.5)
No answer	9 (0.7)
COVID-19 tests, No. (%)	
Positive	541 (39.3)
Negative	326 (23.7)
Non-tested	509 (37)
Type of test, No. (% total) [% tested]	
Only positive RT-PCR	427 (31) [49.2]
Only positive anti-COVID-19 IgG/IgM rapid test	82 (6) [9.5]
Positive RT-PCR and positive anti-COVID-19 IgG/IgM rapid test	24 (1.7) [2.8]
Positive RT-PCR and negative anti-COVID-19 IgG/IgM rapid test	3 (0.2) [0.3]
Negative RT-PCR and positive anti-COVID-19 IgG/IgM rapid test	5 (0.4) [0.6]
Only negative RT-PCR	106 (7.7) [12.2]
Only negative anti-COVID-19 IgG/IgM rapid test	188 (13.6) [21.7]
Negative RT-PCR and negative anti-COVID-19 IgG/IgMrapid test	32 (2.3) [3.7]
Previous olfactory deficit? No. (%)	
Yes	78 (5.7)
No	1,249 (90.7)
No answer	49 (3.6)

Clinical Outcomes

Five-hundred forty-one (39.3%) health care professionals tested positive for SARS-CoV-2 infection. Prevalence of olfactory dysfunction in COVID-19-positive subjects was 83.9% (454 of 541) compared to 12.9% (42 of 326) of those who tested negative and to 14.3% (73 of 509) of those not tested (Pearson chi-square 407.2, $p < 0.001$). **Supplementary Table 1** summarizes overall symptoms reported by survey respondents, and **Supplementary Tables 2**, **3** present general variables and other symptoms associated with hyposmia.

Direct care provision to COVID-19 patients tended to be associated with a positive test result: 64.8% of health care professionals working with SARS-COV-2-infected patients tested positive comparing to 58.4% of those who did not (Pearson chi-square 3.42, $p = 0.064$; proportions are depicted in **Figure 2**). While 38.7% of professionals providing health care to COVID-19 patients (795; 58% of respondents) tested positive for SARS-CoV-2 and reported loss of smell or taste (red color flow in **Figure 2**), only 25.7% of the group that did not provide health care to COVID-19 patients (572, 42% of respondents) tested positive for SARS-CoV-2 and showed these same symptoms. Only 2.9% of respondents providing health care to COVID-19 patients tested negative for coronavirus (blue color flows) and reported loss of smell or taste. Similarly, only 5.9% of the professionals who provided care to COVID-19 patients, but were not tested for SARS-CoV-2, reported hyposmia or hypogeusia. Professionals who tested negative or did not test for SARS-CoV-2 predominate in the subset that did not report olfactory or taste impairment (blocks identified as "No symptoms" or "No olfactory or taste symptoms"; **Figure 2**), independent of treating COVID-19 patients or not.

Prevalence of olfactory dysfunction was higher in professionals working in wards (61.5%), ICUs (60.1%), and emergency rooms (44.3%) compared to those working in outpatient clinics (22.3%), either positive for SARS-CoV-2 infection or not (Pearson chi-square 93.4, $p < 0.001$; **Supplementary Figure 1**). Physiotherapists were the professional category with the highest prevalence of olfactory dysfunction (59.6%), followed by nurses (54.1%), doctors (36.5%), and speech therapists (25.7%; Pearson chi-square 45.3, $p < 0.001$). Physiotherapists were also the category represented by a higher proportion of positive tests (Pearson chi-square 24.9, $p < 0.001$).

We performed a logistic regression analysis to evaluate the factors related to the risk of presenting smell loss, and we found an odds two times higher in subjects working in wards compared to professionals working in outpatient clinics [odds ratio (OR): 2.4; CI 95% 1.1–5.4, $p = 0.03$]. It was also higher for those working in the ICU (OR: 1.8; CI 95% 1–3.4, $p = 0.049$). We found no statistically significant association between olfactory loss and comorbidities, occupation, or providing direct care to SARS-CoV-2-infected patients ($p > 0.05$). **Table 2** illustrates

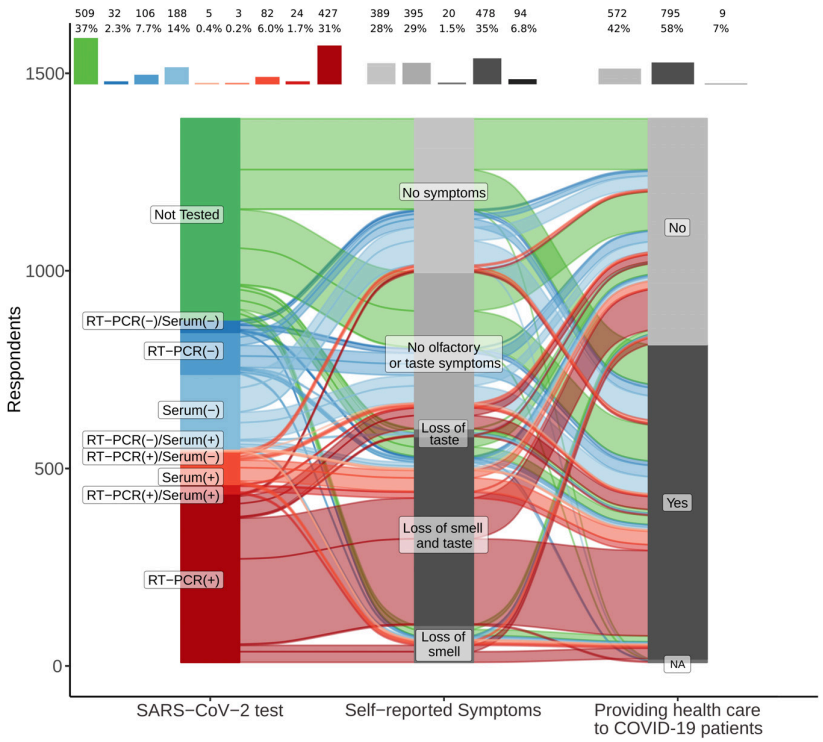


FIGURE 2 | Loss of smell is associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive testing in professionals providing health care to coronavirus disease 2019 (COVID-19) patients. Alluvial diagram depicting SARS-CoV-2-positive respondents (shades of red) as the dominant proportion of those who self-reported loss of smell, combined or not with loss of taste. A higher proportion of participants tested positive in the group providing health care to COVID-19 patients. In contrast, SARS-CoV-2-negative (shades of blue) and not-tested (green) participants reported other symptoms or no symptoms more often than SARS-CoV-2-positive respondents. Frequency and percentages of each block are reported at the top of the diagram. Correlations between individual answers are represented as flows between one block to another (each block in the same column represents a different answer for the same question). The larger the width of a flow, the larger is the number/proportion of correlated answers from different questions. Questions (Q)/answer (A) pairs were (from left to right): (1) **Q** – Have you been tested for COVID-19? **A** – Yes or no (specify test method); (2) **Q** – Are you feeling or have you felt any of these symptoms in the last days? / **A** – Answers to loss or reduction of smell or taste were aggregated as "loss of smell," "loss of smell and taste," "loss of taste," "no olfactory or taste symptoms," "no symptoms"; (3) **Q** – Are you providing direct care to patients with COVID-19 diagnosis? / **A** – Yes or no. NA: not answered.

the risk of diminished smell perception according to the variables studied.

We also analyzed the association between nasal symptoms (rhinorrhea, obstruction, and burning sensation) and olfactory loss in all participants complaining of hyposmia (**Figure 3** and **Supplementary Figure 2**). Thirty-one percent of subjects without nasal obstruction had olfactory loss, while 67.8% with nasal obstruction complained of olfactory deficit. When considering only patients with COVID-19, 80.8% of patients who did not report nasal obstruction had olfactory dysfunction. The proportion of subjects with nasal burning sensation and nasal obstruction were higher in those with olfactory loss (Pearson chi-squares 146.8 and 219.1, respectively; $p < 0.001$). Participants who presented nasal burning sensation and nasal obstruction had a higher risk of complaining of olfactory loss (OR: 4.2, CI 95% 2.7–6.6, $p < 0.001$ and OR: 2.1, CI 95% 1.4–3.2, $p < 0.001$, respectively) independent of the SARS-CoV-2 test result. While loss of smell and taste were the most frequent symptoms in the infected respondents, other symptoms were variably concurrent (**Figure 3A** and **Supplementary Table 3**). In SARS-CoV-2-negative or not-tested respondents, the absence of symptoms and non-nasal symptoms were the most frequent reports, followed by rhinorrhea and nasal obstruction without concurrent loss of smell (**Figure 3B**).

Loss of smell usually developed simultaneously with other COVID-19 symptoms, but in 7.5% of cases, olfactory dysfunction was the first disease indicator (**Table 3**). Altogether, remission from olfactory symptoms was frequent in positive subjects by the time of responses (57.2%), and in 48.4%, it happened in the first 2 weeks. When considering participants who tested negative or who did not test for SARS-CoV-2 infection and who presented loss of smell, 66.9% had recovered the olfactory function by the time they answered the questionnaire, a percentage of 9.7% higher than that of infected participants (**Figure 4**).

Taste dysfunction was present in 74.1% of infected subjects (401 of 541) compared to 11% (36 of 326) of noninfected individuals and was significantly associated with olfactory disturbances (Pearson chi-square 951.4, $p < 0.001$). By the time they answered the questionnaire, gustatory function had recovered in 65.3% of COVID-19-positive participants (**Figure 4**).

TABLE 2 | Logistic regression for the variables related to diminished smell perception.

Presence of olfactory dysfunction	Coef.	St. err.	*t* value	*p* value	[95% Conf. Interval]		Sig
Positivity for COVID-19	23.28	4.164	17.60	0.000	16.398	33.059	***
Sex (male as ref.)	1.163	0.239	0.74	0.462	0.778	1.739	
Age	0.985	0.009	−1.72	0.086	0.968	1.002	*
Area of practice							
Clinic (ref.)	1	.	.	.	.	.	
Intensive Care Unit	1.841	0.572	1.97	0.049	1.002	3.384	**
Ward	2.424	1.001	2.14	0.032	1.079	5.446	**
Emergency room	1.552	0.418	1.63	0.103	0.915	2.633	
Other places	1.71	0.506	1.82	0.07	0.958	3.053	*
Working with COVID-19 patients	0.915	0.174	−0.47	0.641	0.63	1.329	
Profession							
Speech therapist (ref.)	1	.	.	.	.	.	
Nurse	1.423	0.512	0.98	0.328	0.702	2.881	
Physician	1.091	0.427	0.22	0.823	0.507	2.348	
Physiotherapist	1.41	0.794	0.61	0.542	0.467	4.253	
Nasal symptoms							
Coryza	1.393	0.28	1.65	0.099	0.94	2.065	*
Nasal obstruction	2.148	0.444	3.70	0.000	1.433	3.222	***
Nasal burning	4.212	0.981	6.18	0.000	2.669	6.648	***
Comorbidities	1.099	0.254	0.41	0.681	0.699	1.728	
Constant	0.095	0.057	−3.94	0.000	0.03	0.307	***
Mean dependent var		0.406	SD dependent var			0.491	
Pseudo r-squared		0.454	Number of obs			1,256	
Chi-square		770.696	Prob > chi^2			0.000	
Akaike crit. (AIC)		957.882	Bayesian crit. (BIC)			1, 040.053	

****p < 0.01, **p < 0.05, *p < 0.1, ref. = reference.*

DISCUSSION

This study analyzed smell and taste disorders in a large sample of frontline health care workers, a population widely and homogeneously exposed to SARS-CoV-2 infection. We found a high prevalence of chemosensory disorders among professionals with confirmed COVID-19 in Brazil, in accordance with previous studies (Bagheri et al., 2020; Lechien et al., 2020; Menni et al., 2020; Neto et al., 2020; Parma et al., 2020; Spinato et al., 2020), which was significantly higher than those who tested negative. Interestingly, these symptoms were not associated with previous comorbidities known to impair olfactory function, such as chronic rhinosinusitis, neurodegenerative diseases, smoking, traumatic brain injury, stroke, or the use of psychiatric or heart disease medications. Chemosensory dysfunction occurred mostly concomitant to other COVID-19 symptoms and was the first sign of disease in 7.5% of participants. Olfactory disturbances were not more common in those providing direct care to infected patients. It suggests that to be exposed to a higher viral load does not increase the risk of olfactory impairment in health care professionals.

Almost half of all COVID-19-positive participants had recovered their olfactory function within 2 weeks of onset of symptoms. This early recovery was also observed by other authors (Hopkins et al., 2020; Lechien et al., 2020; Neto et al., 2020) and supports the hypothesis that the olfactory impairment is a transient symptom of COVID-19 patients. In fact, it has been demonstrated in animal studies that olfactory epithelium appeared intact at 7 days after SARS-CoV-2 intranasal inoculation (Zhang et al., 2020).

It is noteworthy that participants with olfactory complaints who were not tested or the ones who tested negative presented a recovery rate 9.7% higher than COVID-19-positive subjects by the time they answered the questionnaire (66.9 vs. 57.2%). This is an indication that, even though the mechanism is probably reversible, the degree of damage to the olfactory neuroepithelium caused by SARS-CoV-2 might be worse than that caused by other upper respiratory viruses; alternatively, the recovery of neuroepithelium damage following COVID-19 may take longer, increasing the risk of prolonged olfactory dysfunction. This long-lasting smell dysfunction was also documented by other authors, through objective measurement of olfactory function, even after clinical recovery and nasopharyngeal virologic clearance (Chung et al., 2020). A possible explanation might be SARS-CoV-2 infection of neuron progenitor cells, as suggested by Zhang et al. (2020), could impair olfactory sensory neuron regeneration.

In this study, we did not assess overall symptom severity because we tried to simplify the survey to completion in 3 min on average as a way to enlarge the study sample. We also did not ask participants about the need for hospitalization during the course

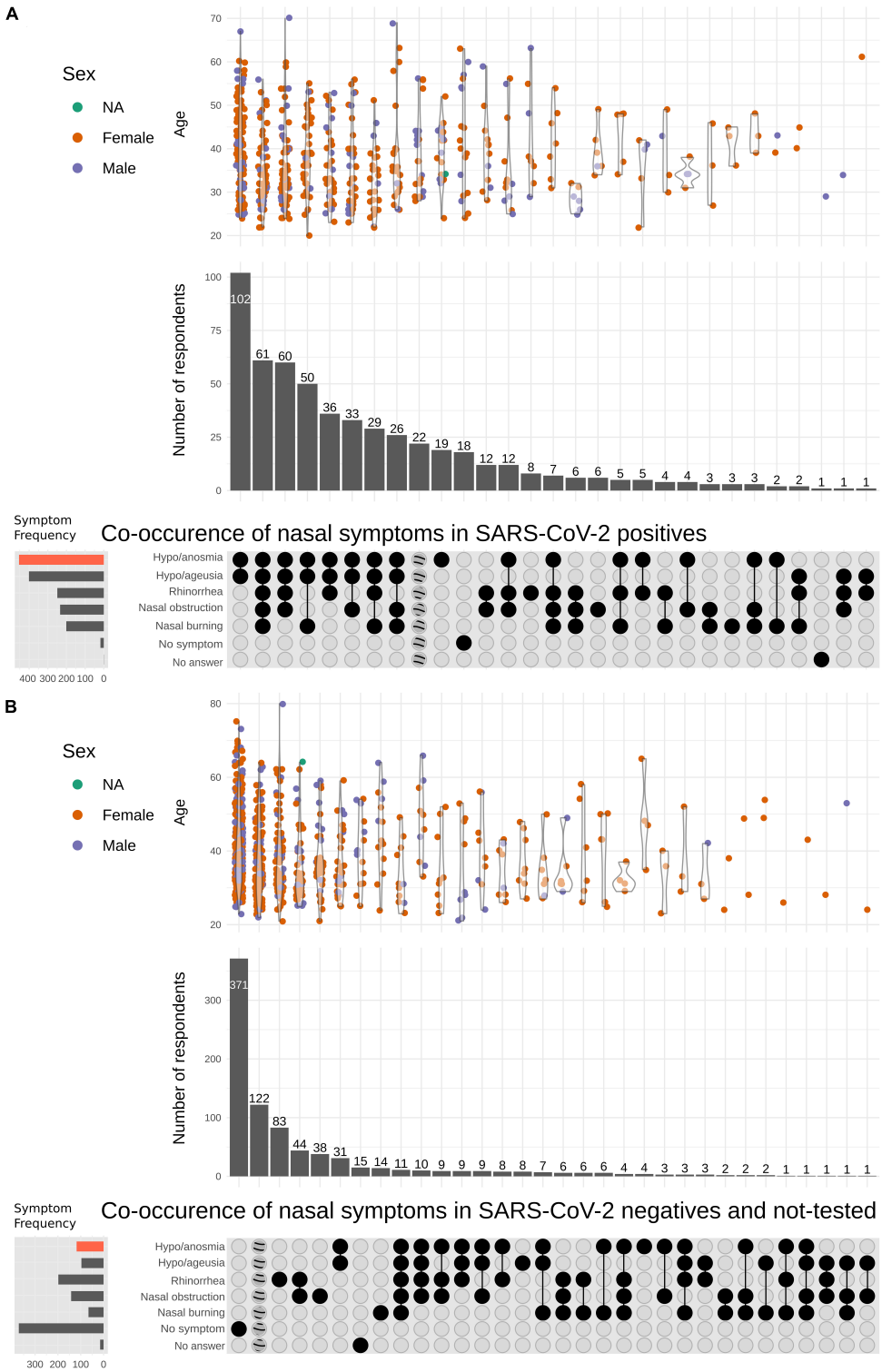


FIGURE 3 | Nasal symptoms in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected participants. Co-occurrence of different nasal symptoms (rhinorrhea, nasal obstruction, and nasal burning sensation) in SARS-CoV-2-positive **(A)** and SARS-CoV-2-negative or not-tested respondents **(B)**. Age and sex are represented as violin plots for each symptom co-occurrence (top). UpSet plots depict the relationships between the symptom sets (bottom). The vertical bars represent the number of respondents who reported each one of the symptom co-occurrences. The horizontal bars shown to the left indicate the total frequencies of each individual symptom, with the red shade-filled bar denoting the sharp contrast between the frequency of hyposmia/anosmia symptoms in SARS-CoV-2-infected participants and in those who tested negative/not tested. Subjects who did not report any of the symptoms are identified as "No symptom." Those who only reported symptoms unrelated to the nasal symptoms are identified by the dashed circles.

of COVID-19, and since it was a self-reported questionnaire and we did not have access to participants' medical records, we could not evaluate overall disease severity. However, if we observe the reported symptoms, only 91 of the infected respondents (16.8%) complained of dyspnea, chest pain, or difficulty to breath, which could suggest severe disease and may indicate that our sample is composed mostly of mild cases. Some authors suggested that, in mild-to-moderate cases, chemosensory dysfunction is more prevalent than in severe cases for which hospitalization is needed (Koneswarakantha, 2020). In fact, surveys including hospitalized patients show a lower prevalence of olfactory dysfunction, varying from 5 to 49% (Beltran-Corbelini et al., 2020; Giacomelli et al., 2020; Mao et al., 2020; Patel et al., 2020). Liu Y. et al. (2020) demonstrated that the mean viral load of patients with severe disease is higher than that of mild cases. However, when patients were stratified according to the day of disease onset, severe cases had lower nasopharyngeal viral loads in the first 12 days after disease onset than corresponding mildly symptomatic patients (Liu Y. et al., 2020). It is possible that, by the time nasopharyngeal viral loads increased in these patients, enhancing inflammatory response in upper airways, the respiratory distress overlaps with the olfactory symptoms so that they end up being neglected. Alternatively, nasopharyngeal and pulmonary/systemic viral replication and epithelial damage may occur independently.

It is of interest that smell loss was found in more than 80% of COVID-19 health professionals without nasal obstruction. This fact supports the hypothesis that olfactory impairment might be associated with an inflammatory response in olfactory neuroepithelium triggered by the virus or by cellular death secondary to SARS-CoV-2 infection. Both the respiratory epithelium and the olfactory neuroepithelium express angiotensin-converting enzyme 2 (ACE2) receptors, which are used by SARS-CoV-2 to enter cells (Brann et al., 2020; Hoffman et al., 2020; Liu M. et al., 2020; Sungnak et al., 2020). It is possible that the infection of supporting cells, which present the highest levels of ACE2 expression in the olfactory neuroepithelium (Fodoulian et al., 2020), triggers an inflammatory response that could either impair cellular signaling or cause death of the supporting cells, leading to loss of olfactory function (Glezer et al., 2020).

Another possible mechanism that could explain the presence of olfactory dysfunction in the absence of nasal obstruction is a cerebral involvement through virus dissemination from systemic circulation or from the cribriform plate (Baig et al., 2020; Zhou et al., 2020). Although olfactory neurons do not express ACE2 receptors, olfactory bulb vascular cells, glial cells, and brain neurons do, and these cells could be implicated in the pathogenesis of the disease (Alenina and Bader, 2019; Zhou et al., 2020). It has already been demonstrated that SARS-CoV, which is structurally similar to SARS-CoV-2 and uses the same ACE2 receptor, leads to neuronal death and invades

TABLE 3 | Moment of occurrence of olfactory loss in COVID-19-positive participants and COVID-19-negative and non-tested participants.

Moment of smell lost compared to others symptoms	COVID-19 positive *N* (%)	COVID-19 negative or non-tested *N* (%)
Before others symptoms	34 (7.5)	21 (17.8)
Concurrent to others symptoms	239 (52.6)	60 (50.8)
After other symptoms	177 (39)	34 (28.8)

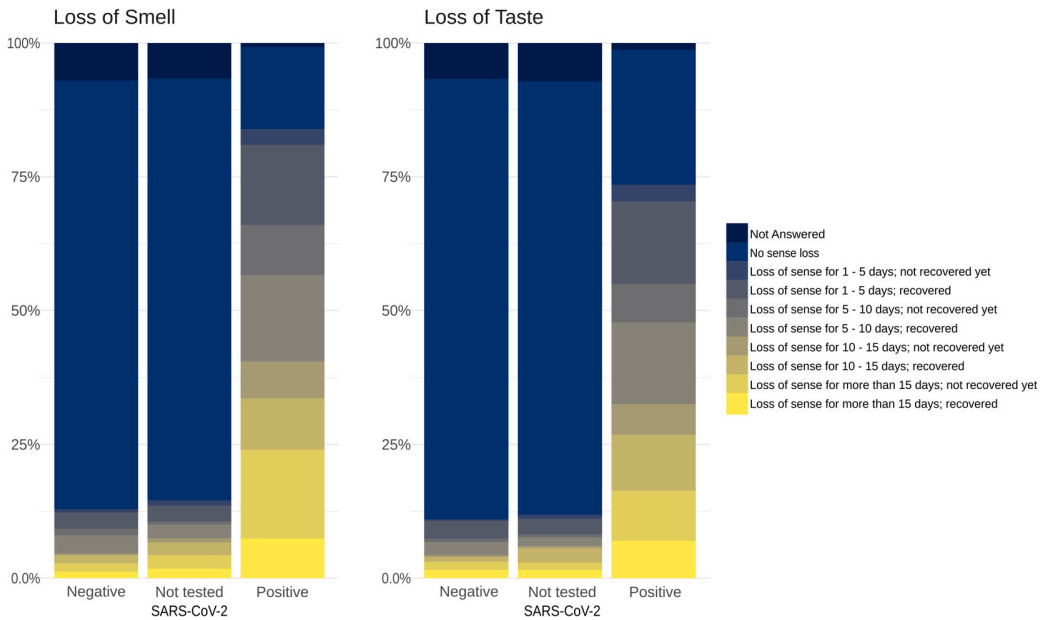


FIGURE 4 | Duration and recovery of loss of smell and taste. Proportions based on the period of olfactory/taste function recovery are represented as stacked bar charts that depict the longer duration of loss of smell and loss of taste in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive participants. Color scale shows the duration of loss of sense and the remission status.

the central nervous system after intranasal inoculation (Netland et al., 2008). Moreover, animal studies demonstrated that rodent coronaviruses invade the olfactory bulb even though olfactory neurons do not express their main receptor (Youngentob et al., 2001). It was also demonstrated that SARS-CoV-2 infects both mature and immature olfactory sensory neurons in hamsters after intranasal inoculation (Zhang et al., 2020). Kirschbaum et al. (2020) described autopsy findings of two patients with SARS-CoV-2 infection with olfactory neuropathy suggestive of axonal damage. However, studies with larger samples are necessary to confirm these theories in humans.

We found a statistically significant association between olfactory impairment and nose burning sensation in both positive and negative participants complaining of hyposmia. The sense of smell in humans is mostly mediated by cranial nerve I (CN I), responsible for odor sensation, through activation of olfactory neurons present in the olfactory epithelium (Doty and Mishra, 2001). The intranasal trigeminal nerve endings can also detect chemical stimuli and are typically activated by irritant stimuli, leading to varied sensations such as burning, cooling, and pungent sensations (Silver and Finger, 2009; Viana, 2011). There is evidence that the olfactory and trigeminal systems can interact and regulate each other, and it has been proposed that olfactory loss would lead to increased trigeminal sensitivity (Frasnelli and Hummel, 2007). This could explain the association between burning sensation and olfactory loss in the studied subjects, which appears to be independent of SARS-CoV-2 infection.

Our study has some limitations such as the fact that it was a self-reported electronic questionnaire, therefore subjects diagnosed with COVID-19 and who presented olfactory and gustatory disturbances are more inclined to voluntarily participate than those who did not. This could explain the high percentage of infected subjects (39.3%), the female predominance, and it could be partially responsible of the high prevalence of olfactory and taste disturbances. It has been demonstrated that women outperform men in olfactory tests (Doty et al., 1984), and therefore, they could be more sensitive to identify olfactory function deficits and, thus, more inclined to participate in surveys. This preponderance was also found in other online surveys (Bagheri et al., 2020; Hopkins et al., 2020; Lechien et al., 2020), despite the fact that COVID-19 has been suggested to be more prevalent in males (Hu et al., 2020; Remuzzi and Remuzzi, 2020). Giacomelli et al. (2020) performed a cross-sectional study in hospitalized patients with oral interviews and also found a female predominance with chemosensory disturbances even in a sample with a male majority. Besides that, it is possible that, once SARS-CoV-2 infection has a higher morbidity and mortality in males (Guo et al., 2020), and chemosensory disturbances have been associated with mild-to-moderate cases, this group would be less likely to report olfactory or taste dysfunctions.

As it was self-reported, there was no objective measurement of olfactory or gustatory functions. Research shows that self-report measures of smell are specific but not sensitive, and a considerable proportion of people do not recognize the loss of olfactory function (Adams et al., 2017). However, despite the possible lack of sensitivity, we found a large prevalence of chemosensory disturbances.

Another possible limitation was the use of a nonstandard questionnaire. However, by the time we started this work, we did not have standard questionnaires to evaluate olfactory and taste functions available in Portuguese.

Alongside the abovementioned conclusions, it is important to note the high rate of non-tested subjects (37%), particularly between participants who did not provide direct care to COVID-19 patients (46.6%). When considering the non-tested group, 14.9% of respondents presented olfactory loss—what could indicate the first sign of disease. However, since there were no other symptoms, these individuals were not tested. Therefore, it is possible that the prevalence of chemosensory disturbances would be even higher in the study sample.

CONCLUSION

COVID-19 is associated with olfactory and gustatory disturbances, and this dysfunction occurs even in the absence of nose obstruction. It usually develops concurrent to other symptoms, but 7.5% of infected people can present it as the first sign of disease. Chemosensory impairment appears to have a good prognosis, and almost half of individuals presenting loss of smell recover olfactory function in the first 2 weeks of symptom onset, albeit a small proportion may maintain it for a longer period of time. However, when compared to non-tested and to SARS-CoV-2-negative subjects, COVID-19 participants had a lower rate of recovery of smell function during the period of study.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of Hospital das Clinicas of University of São Paulo, Brazil (approval number: 4.047.527). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MS, AB-C, DS, BM, IG, and FR conceptualized and designed the study and developed the questionnaire. MS, IG, VA-S, and FR conducted the data collection. MF and IG conducted the analyses. MS drafted the initial manuscript. MS, MF, VA-S, DS, BM, IG, RV, and FR revised the manuscript and approved the final manuscript as submitted. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the following grants from FAPESP 2016/24471-0 (BM); 2013/07937-8 and 2018/18633-3 (IG); 2019/26767-2 (AB-C) and 2019/06982-6 (DS). IG is a member of the CEPID Redoxoma (FAPESP 2013/07937-8).

ACKNOWLEDGMENTS

We would like to thank the efforts of the Regional Medical Councils of the states of São Paulo, Goiás, and Tocantins, of the Regional Nursing Councils of the states of Paraná, Rio Grande do Sul, and Espírito Santo, and the University of São Paulo Medical School in helping us with the disclosure of this research. We would also like to thank Prof. Roger Chammas for the great suggestion of studying the characteristics of SARS-CoV-2 infection in frontline health care professionals.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.622987/full#supplementary-material

REFERENCES

Adams, D. R., Wroblewski, K. E., Kern, D. W., Kozloski, M. J., Dale, W., McClintock, M. K., et al. (2017). Factors associated with inaccurate self-reporting of olfactory dysfunction in older US adults. *Chem. Senses.* 42, 223–231.

Alenina, N., and Bader, M. (2019). ACE2 in brain physiology and pathophysiology: evidence from transgenic animal models. *Neurochem* 44, 1323–1329.

Bagheri, S. H., Asghari, A., Farhadi, M., Shamshiri, A. R., Kabir, A., Kamrava, S. K., et al. (2020). Coincidence of COVID-19 epidemic and olfactory dysfunction outbreak. *medRvix* [Preprint]. doi: 10.1101/2020.03.23.20041889

Baig, A. M., Khaleeq, A., Ali, U., and Syeda, H. (2020). Evidence of COVID-19 virus targeting the CNS: tissue distribution, host-virus interaction, and proposed neurotropic mechanism. *ACS Chem. Neurosci.* 11, 995–998.

Beltran-Corbelini, A., Chico-Garcia, J. L., Martinez-Poles, J., Rodriguez-Jorge, F., Natera-Villalba, E., Gomez-Corral, J., et al. (2020). Acute-onset smell and taste disorders in the context of COVID-19: a pilot multicenter polymerase chain reaction based case-control study. *Eur. J. Neurol.* 27, 1738–1741. doi: 10.1111/ene.14273

Brann, D., Tsukahara, T., Weinreb, C., Logan, D., and Datta, S. (2020). Non-neural expression of SARS-CoV-2 entry genes in the olfactory epithelium suggests mechanisms underlying anosmia in COVID-19 patients. *Sci. Adv.* 6:eabc5801.

Chan, J. F., Yuan, S., Kok, K., To, K. K., Chu, H., Yang, J., et al. (2020). A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 395, 514–523. doi: 10.1016/S0140-6736(20)30154-9

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 395, 507–513.

Chung, T. W., Sridhar, S., Zhang, A. J., Chan, K. H., Li, H. L., Wong, F. K. C., et al. (2020). Olfactory dysfunction in coronavirus disease 2019 patients: observational cohort study and systematic review. *Open Forum Infect. Dis.* 7:ofaa199.

Doty, R. L., and Mishra, A. (2001). Olfaction and its alteration by nasal obstruction, rhinitis and rhinosinusitis. *Laryngoscope* 111, 409–423.

Doty, R. L., Shaman, P., Applebaum, S. L., Giberson, R., Siksorski, L., and Rosenberg, L. (1984). Smell identification ability: changes with age. *Science* 226, 1441–1443. doi: 10.1126/science.6505700

Fodoulian, L., Tuberosa, J., Rossier, D., Landis, B. N., Carleton, A., Rodriguez, I., et al. (2020). SARS-CoV-2 receptor and entry genes are expressed by sustentacular cells in the human olfactory neuroepithelium. *bioRxiv* [Preprint]. doi: 10.1101/2020.03.31.013268

Frasnelli, J., and Hummel, T. (2007). Interaction between the chemical senses: trigeminal function in patients with olfactory loss. *Int. J. Psychophysiol.* 65, 177–181.

Gane, S. B., Kelly, C., and Hopkins, C. (2020). Isolated sudden onset anosmia in COVID-19 infection. A novel syndrome? *Rhinology* 58, 299–301. doi: 10.4193/Rhin20.114

Giacomelli, A., Pezzati, L., Conti, F., Bernacchia, D., Siano, M., Oreni, L., et al. (2020). Self-reported olfactory and taste disorders in patients with severe acute respiratory coronavirus 2 infection: a cross-sectional study. *Clin. Infect. Dis.* 71, 889–890.

Glezer, I., Bruni-Cardoso, A., Schechtman, D., and Malnic, B. (2020). Viral infection and smell loss: the case of COVID-19. *J. Neurochem.* doi: 10.1111/jnc.15197. [Epub ahead of print].

Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W., Ou, C., He, J., et al. (2020). Clinical characteristics of 2019 novel coronavirus infection in China. *medRxiv* [Preprint]. doi: 10.1101/2020.02.06.20020974

Guo, G., Ye, L., Pan, K., Chen, Y., Xing, D., Yan, K., et al. (2020). New insights of emerging SARS-CoV-2: epidemiology, etiology, clinical features, clinical treatment, and prevention. *Front. Cell Dev. Biol.* 8:410. doi: 10.3389/fcell.2020.00410

Hoffman, M., Kleine-Webber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271–280.

Hopkins, C., and Kumar, N. (2020). *Loss of Sense of Smell as Marker of COVID-19 Infection [Internet]*. London: ENT UK.

Hopkins, C., Surda, P., Whitehead, E., and Kumar, B. N. (2020). Early recovery following new onset anosmia during COVID-19 pandemic – an observational cohort study. *J. Otolaryngol. Head Neck Surg.* 49:26.

Hu, T., Liu, Y., Zhao, M., Zhuang, Q., Xu, L., and He, Q. (2020). A comparison of COVID-19. SARS and MERS. *PeerJ* 8:e9725. doi: 10.7717/peerj.9725

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506.

Iran News (2020). *Iran News*. Avaliable at: https://en.radiofarda.com/a/loss-of-sense-of-smell-among-iranians-coinciding-with-coronavirus-epidemic/30478044.html. (Accessed at August 14, 2020).

Kirschbaum, D., Imbach, L. L., Ulrich, S., Rushing, E. J., Keller, E., Reimann, R. R., et al. (2020). Inflammatory olfactory neuropathy in two patients with COVID-19. *Lancet* 396:166.

Koneswarakantha, B. (2020). *easyalluvial: Generate Alluvial Plotswith a Single Line of Code. R Package Version 0.2.3*. Avalibale at: https://CRAN.R-project.org/package=easyalluvial (accessed January 13, 2021).

Krassowski, M. (2020). *ComplexUpset: Create Complex UpSet PlotsUsing ggplot2 Components. R Package Version 0.5.18*. Zenodo. doi: 10.5281/zenodo.3700590

Lechien, J. R., Chiesa-Estomba, C. M., De Siati, D. R., Horoi, M., Le Bon, S. B., Rodriguez, A., et al. (2020). Olfactory and gustatory dysfunction as a clinical presentation of mild to moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur. Arch. Otorhinolaryngol.* 277, 2251–2261. doi: 10.1007/s00405-020-05965-1

Lex, A., Gehlenborg, N., Strobelt, H., Vuillemot, R., and Pfister, H. (2014). UpSet: visualization of Intersecting Sets. *IEEE Trans. Vis. Comput. Graph.* 20, 1983–1992. doi: 10.1109/TVCG.2014.2346248

Liu, M., Wang, T., Zhou, Y., Zhao, Y., Zhang, Y., and Li, J. (2020). Potential role of ACE2 in coronavirus disease 2019 (COVID-19) prevention and management. *J. Transl. Int. Med.* 8, 9–19.

Liu, Y., Yan, L. M., Wan, L., Xiang, T. X., Le, A., Liu, J. M., et al. (2020). Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect. Dis.* 20, 656–657.

Mao, L., Wang, M., Chen, S., He, Q., Chang, J., Hong, C., et al. (2020). Neurological manifestations of hospitalized patients with COVID-19 in Wuhan, China: a retrospective case series study. *medRxiv* [Preprint]. doi: 10.1101/2020.02.22.20026500

Menni, C., Valdes, A. M., Freidin, M. B., Sudre, C. H., Nguyen, L. H., Drew, D. A., et al. (2020). Real-time tracking of self-reported symptoms to predict potential COVID-19. *Nat. Med.* 26, 1037–1040. doi: 10.1038/s41591-020-0916-2

Netland, J., Meyerholz, D. K., Moore, S., Cassell, M., and Perlman, S. (2008). Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. *J. Virol.* 82, 7264–7275.

Neto, D. B., Fornazieri, M. A., Dib, C., Di Francesco, R. C., Doty, R. L., Voegels, R. L., et al. (2020). Chemosensory dysfunction in COVID-19: prevalences, recovery rates, and clinical associations on a large Brazilian Sample. *Otolaryngol. Head Neck Surg.* 0194599820954825. doi: 10.1177/0194599820954825

Parma, V., Ohla, K., Veldhuizen, M. G., Niv, M. Y., Kelly, C. E., Bakke, A. J., et al. (2020). More than smell – COVID-19 is associated with severe impairment of smell, taste and chemesthesis. *Chem. Senses.* 45, 609–622.

Patel, A., Charani, E., Ariyanayagam, D., Abdulaal, A., Denny, S. J., Mughal, N., et al. (2020). New-onset anosmia and ageusia in adult patients diagnosed with SARS-CoV-2 infection. *Clin. Microbiol. Infect.* 26, 1236–1241.

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Remuzzi, A., and Remuzzi, G. (2020). COVID-19 and Italy: what next? *Lancet.* 395, 1225–1228. doi: 10.1016/S0140-6736(20)30627-90

Rosvall, M., and Bergstrom, C. T. (2010). Mapping change in large networks. *PLoS One* 5:e8694. doi: 10.1371/journal.pone.0008694

Silver, W. L., and Finger, T. E. (2009). The anatomical and electrophysiological basis of peripheral nasal trigeminal chemoreception. *Ann. N. Y. Acad. Sci.* 1170, 202–205. doi: 10.1111/j.1749-6632.2009.03894.x

Spinato, G., Fabbris, C., Polesel, J., Cazzador, D., Borsetto, D., Hopkins, C., et al. (2020). Alterations in smell or taste in mildly symptomatic outpatients with SARS-CoV2 infection. *JAMA* 323, 2089–2090. doi: 10.1001/jama.2020.6771

Sungnak, W., Huang, N., Bécavin, C., Berg, M., Queen, R., Litvinukova, M., et al. (2020). HCA lung biological network. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med.* 26, 681–687. doi: 10.1038/s41591-020-0868-6

Viana, F. (2011). Chemosensory properties of the trigeminal system. *ACS Chem. Neurosci.* 2, 38–50. doi: 10.1021/cn100102c

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysi*. New York, NY: Springer-Verlag New York.

Youngentob, S. L., Schwob, J. E., Saha, S., Manglapus, G., and Jubelt, B. (2001). Functional consequences following infection of olfactory system by intranasal infusion of olfactory bulb line variant (OBLV) of mouse hepatitis strain JHM. *Chem. Senses* 26, 953–963.

Zhang, A. J., Lee, A. C. Y., Chu, H., Chan, J. F. W., Fan, Z., Li, C., et al. (2020). Severe acute respiratory syndrome coronavirus 2 infects and damages the mature and immature olfactory sensory neurons of hamsters. *Clin. Infect. Dis.* ciaa995. doi: 10.1093/cid/ciaa995

Zhou, Z., Kang, H., Li, S., and Zhao, X. (2020). Understanding the neurotropic characteristics of SARS-CoV-2: from neurological manifestations of COVID-19 to potential neurotropic mechanisms. *J. Neurol.* 267, 2179–2184. doi: 10.1007/s00415-020-09929-7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

MINI REVIEW
published: 11 March 2021
doi: 10.3389/fphys.2021.613019

The Good Treatment, the Bad Virus, and the Ugly Inflammation: Pathophysiology of Kidney Involvement During COVID-19

*Marie-Bénédicte Le Stang[1], Jordan Desenclos[2,3], Martin Flamant[1,3], Benjamin G. Chousterman[4,5] and Nahid Tabibzadeh[1,3]**

[1] Université de Paris, U1149 INSERM, Paris, France, [2] Nephrology, Dialysis and Transplantation Department, CHU Clermont Ferrand, University Clermont Auvergne, Clermont Ferrand, France, [3] Department of Physiology, Hôpital Bichat, FHU APOLLO, DMU Dream, APHP.Nord, Paris, France, [4] INSERM U942 MASCOT, Université de Paris, Paris, France, [5] Department of Anesthesia and Critical Care, Hôpital Lariboisière, FHU PROMICE, DMU Parabol, APHP.Nord, Paris, France

OPEN ACCESS

Edited by:
Ravi Nistala,
University of Missouri, United States

Reviewed by:
Francois Verrey,
University of Zurich, Switzerland
Matthieu Jamme,
Centre Hospitalier Intercommunal de Poissy, France
Muner Mohamed,
Ochsner Medical Center, United States

***Correspondence:**
Nahid Tabibzadeh
nahid.tabibzadeh@inserm.fr

Specialty section:
This article was submitted to Renal and Epithelial Physiology, a section of the journal Frontiers in Physiology

Received: 01 October 2020
Accepted: 08 February 2021
Published: 11 March 2021

Citation:
Le Stang M-B, Desenclos J, Flamant M, Chousterman BG and Tabibzadeh N (2021) The Good Treatment, the Bad Virus, and the Ugly Inflammation: Pathophysiology of Kidney Involvement During COVID-19. Front. Physiol. 12:613019. doi: 10.3389/fphys.2021.613019

Kidney involvement is a common complication during SARS-CoV-2 infection. Its association with poor outcomes, especially in critically ill patients, raises issues whether kidney involvement reflects multi-organ damage or if it is a specific feature of the infection. Based on observational studies, autopsy series, and on current understanding of the route of entry of the virus, this review will highlight the different types of kidney involvement during COVID-19 and put them in the perspective of the different pathophysiological hypotheses. Virus entry route through ACE2 ligation and TMPRSS2 coligation allows identifying potential viral targets in the kidney, including tubules, endothelial cells, and glomerulus. While reports have described damages of all these structures and virus kidney tropism has been identified in renal extracts in autopsy series, no direct viral infection has been found in the latter structures thus far on kidney biopsies. Notwithstanding the technical challenge of disclosing viral invasion within tissues and cells, viral direct cytopathogenic effect generally does not appear as the cause of the observed renal damage. Inflammation and altered hemodynamics, described as "viral sepsis," might rather be responsible for organ dysfunction, including kidneys. We shall place these various mechanisms into an integrated vision where the synergy between direct viral pathogenicity and systemic inflammation enhances renal damage. As SARS-CoV-2 inexorably continues its rampant spread, understanding the sequence of events in the kidneys might thus help inform improved therapeutic strategies, including antiviral drugs and immunomodulators.

Keywords: COVID, viral sepsis, inflammation, AKI (acute kidney injury), kidney, SARS – CoV – 2, COVID–19

INTRODUCTION

Since the COVID-19 outbreak in January 2020, SARS-CoV-2 infection has affected so far more than 80 million people around the world with nearly 2 million reported deaths according to the WHO (World Health Organization [WHO], 2021). Although the most populous countries have gone through their first wave, the pandemic is still ongoing worldwide with some countries, including

France, worryingly experiencing a recent outbreak in new cases and new admissions in hospital, notably in intensive care units (ICUs). Finding therapeutic strategies is thus of major importance to mitigate the impact of the pandemics, as measures of social distancing seem insufficient to contain the spread of the virus.

The main organ involvement during COVID-19 infection is the lung, but extrapulmonary manifestations are emerging not only as more frequent than initially hypothesized but also as of major impact during the clinical course of the infection. In particular, kidney impairment has been extensively reported and is associated with poor outcomes (Robbins-Juarez et al., 2020). However, pathophysiology of kidney involvement during SARS-CoV-2 infection remains to be elucidated. Indeed, mechanistic and experimental studies are still lacking and most of the hypotheses rely on observational retrospective clinical data, with frequently missing information, and some pathological findings that might give insights on potential mechanisms.

Kidneys are richly vascularized organs, as renal blood flow (RBF) accounts for 25% of cardiac output. While this high RBF allows efficient homeostasis of electrolytes and acid–base balance, the inevitable backlash is a high susceptibility to hemodynamic changes and systemic diseases, infectious or immune related (Maher, 1981). Even though the regulating system of tubuloglomerular feedback allows preserving glomerular filtration rate (GFR) in physiology, this refined adapting system cannot suffice in major pathophysiological states, such as that encountered during severe COVID-19.

Efficient as kidneys may be, they are consequently prone to be the target of various diseases and, as such, reflect these diseases and their severity. This paradigm should thus apply to SARS-CoV-2–related acute kidney injury (AKI), which reflects the systemic phase of the infection. However, whether the severity of the disease is due to viral dissemination and/or systemic inflammation is still a matter of debate.

Consequently, understanding kidney involvement might be the bridge to a better understanding of the disease itself. This might thus lead to optimal therapeutic targets, including antiviral and/or anti-inflammatory drugs.

In this view, we will focus on emerging data regarding kidney involvement during COVID-19 and infer pathophysiological hypotheses that might finally shed light on potential therapeutic interventions.

ACUTE KIDNEY INJURY AND COVID-19

Epidemiology

Acute kidney injury (AKI), defined by a rapid increase in serum creatinine and/or a sudden decrease in urinary output (KDIGO Guidelines, 2012), has been reported in several studies on COVID-19. The prevalence vary widely depending not only on the severity of the disease but also on geographical factors, and consequently the population studied (Robbins-Juarez et al., 2020): from 0.5% in the report from Guan et al. (2020) gathering findings from 1,099 outpatients and hospitalized patients throughout China, to 80% of patients while in ICU in the study from Rubin et al. (2020). Consistently, severe AKI requiring renal replacement therapy (RRT) also occurs more frequently in critically ill patients, in 40 to 55% of cases (Chand et al., 2020; Mohamed et al., 2020) compared with a general prevalence of 0.8 to 14.7% in the meta-analysis gathering 20 reported cohorts from Robbins-Juarez et al. (2020).

Acute kidney injury also seems to be close-related to the temporal evolution of pulmonary signs (Hirsch et al., 2020). Specifically, AKI and mechanical ventilation seem intricately linked in several reports. For example, Hirsch et al. (2020) reported that AKI occurred in 89.7% of patients on mechanical ventilation, compared with only 21.7% of the non-ventilated patients, and that almost all (96.8%) the patients of their cohort requiring RRT were on ventilator support. These results suffer, however, from an immortality bias as patients on mechanical ventilation must survive enough to be integrated in the analysis (Jamme and Geri, 2020). Besides mechanical ventilation, other commonly identified risk factors for AKI are age, male sex, and pre-existing comorbidities [cardiovascular disease, diabetes mellitus, hypertension, and chronic kidney disease (CKD)] (Fu et al., 2020), as well as black race and obesity (Bowe et al., 2020). In several studies, kidney involvement has been reported as an independent risk factor of mortality, with a meta-analysis from Fu et al. (2020) showing a pooled risk ratio of mortality from 142 studies of 4.6 (95% CI 3.3–6.5) compared with patients with no AKI. Other events undeniably contribute both to general deterioration and to AKI and are not taken into account in these analyses. We thus cannot exclude residual confounding factors such as hemodynamic instability during the course of the disease, degree of hypoxemia, or septic events.

Features of Kidney Involvement During SARS-CoV-2 Infection: Are They Specific to COVID-19?

Kidney involvement in COVID-19 usually presents with non-specific features of AKI: rise in serum creatinine and/or decrease in urine output. The presence of AKI does not prejudge the cause of kidney damage and might exist regardless of the underlying etiology. Authors have reported general features of kidney impairment during COVID-19, as well as more specific descriptions and histopathological data in case series or case reports. However, the precise rate of specific tubular, glomerular, and vascular involvement is still unknown, and whether they represent key features of SARS-CoV-2–related kidney injury remains to be determined.

Prerenal Azotemia

The effective decrease of extracellular volume, induced by poor intake of water and food, high fever, diarrhea, and ultimately hypovolemic, cardiogenic, or septic shock observed in the course of COVID-19, might induce a decrease in renal blood flow resulting in GFR decrease. Moreover, rapid recovering of AKI after volume supplementation has been described in 12.2% of critically ill patients (Xia et al., 2020), which might conduct retrospectively to the diagnosis of prerenal acute kidney failure. When measured, a fractional excretion of urinary sodium

<1% was observed in 38% of cases upon admission in ICU (Mohamed et al., 2020).

It is interesting to note that among patients with severe COVID-19 admitted to ICUs, AKI is frequently associated with invasive ventilation (Hirsch et al., 2020). As a matter of fact, patients with invasive ventilation display altered abdominal pressure, especially when they are exposed to high expiratory pressure during mechanical ventilation. In this setting, the mechanical ventilation–induced high abdominal pressure compromises abdominal venous drainage, resulting in renal venous congestion, which is a prominent cause of ischemic kidney injury. Mechanical ventilation is also correlated with more profound and longer hypoxemia, the latter potentially resulting in sustained renal ischemia. Other factors have been suggested to contribute to kidney injury during invasive ventilation, including neurohormonal changes and inflammatory mediators (Koyner and Murray, 2008).

Acute Tubular Injury: Is Tubular Injury A Specific Feature of COVID-19 and Does it Reflect Viral Invasion?

Kidney involvement during COVID-19 usually presents with features of tubular injury: mild proteinuria, in 15.5 (Xia et al., 2020) to 69% of patients (Cheng et al., 2020; Mohamed et al., 2020; Pei et al., 2020), of low molecular weight when assessed (Werion et al., 2020), tubular casts, and renal tubular epithelial cell casts on urine sediment microscopic analysis (Hernandez-Arroyo et al., 2020). Based on these findings and others, several authors suggest that a direct and specific tubular viral invasion occurs during SARS-CoV-2 infection. There are some limitations in this interpretation that we will summarize in this section.

Is There a Specific Fanconi Syndrome During SARS-CoV-2-Related AKI?

Fanconi syndrome is a specific proximal tubular dysfunction characterized by abnormal handling of solutes that are secreted and/or reabsorbed by proximal tubule. Fanconi syndrome features are the following:

Tubular proteinuria

Low molecular weight proteinuria and/or urinary albumin/protein ratio <50% are key features of acute tubular injury, irrespective of the cause of tubular damage. Consistently, tubular injury markers such as NGAL and KIM-1 have been extensively studied to better detect acute tubular injury before the increase in serum creatinine and the decrease in estimated GFR (Parikh et al., 2010). Consequently, low molecular weight proteinuria cannot be considered a specific feature of Fanconi syndrome.

Aminoaciduria, uric acid and phosphate renal wasting, normoglycemic glycosuria

Two reports have studied these parameters in COVID-19 patients. Werion et al. (2020) found unquantified aminoaciduria in 6 out of 13 tested SARS-CoV-2–infected patients, 18/39 with hypouricemia and fractional excretion of uric acid (FeUA) > 10%, and 6/32 with hypophosphatemia and fractional excretion of phosphate >20%. Kormann et al. (2020) found hypouricemia and FeUA > 10% in 14/35 patients, a calculated maximal threshold for phosphate reabsorption (TmPi/GFR) <0.77 mmol/L in 19/48 patients, and, conversely to Werion et al., normoglycemic glycosuria in 11/28 patients.

Specific cautions in proximal tubular functions interpretation during AKI

First, in the same line with tubular proteinuria, proximal tubular transports, especially sodium co-transporters (including sodium-phosphate co-transporters), are disturbed during AKI in experimental models as well as in humans (Basile et al., 2012; Vallon, 2016). Second, FeUA might be increased in patients with volume overload as frequently seen in critically ill patients (Kazory et al., 2020); this situation has even been described during the first SARS outbreak, where it was associated with inflammatory cytokines (Wu et al., 2005). Third, phosphatemia is often decreased in critically ill patients, mostly due to an intracellular transfer mechanism, falsely decreasing the TmPi/GFR calculation while phosphate tubular transport is not affected (Suzuki et al., 2013). Finally, proximal tubules are the mainstay of injury during AKI (Takaori et al., 2016); hence, it should not be surprising to find these features during any type of acute tubular injury. However, as these markers are not usually assessed in acute tubular injury, it remains uncertain whether they are more frequent during SARS-CoV-2–related AKI.

Specific cautions in urinary biochemistry interpretation during AKI

Another limitation of these findings is the possible flaws in urine biochemistry during acute illness. First, during AKI, urine creatinine concentration rapidly decreases. Ratios based on its level might thus be inaccurate. In the same line, fractional excretion of all the solutes will appear elevated even with no modification of tubular handling. Finally, proteinuria can increase in conditions such as fever, oliguria, or hematuria in patients with urine catheter (Nguyen et al., 2009; Gabarre et al., 2020).

Could SARS-CoV-2–Related AKI Be a Toxic Acute Tubular Injury?

Rhabdomyolysis

Some authors have reported results suggestive of a contribution of myoglobin to kidney damage. Mohamed et al. (2020) found high levels of plasmatic creatine phosphokinase (CPK), above 1000 U/L in more than 30% of their patients. In most cases, values were not as high as in typical rhabdomyolysis-induced AKI (above 15,000 IU/ml) (Bosch et al., 2009). Pigmented casts were also found in 3 out of 26 autopsy analysis of kidney tubules from deceased patients with high CPK levels (Su et al., 2020). The association of myoglobinuria with dehydration, sepsis, and acidosis might thus trigger AKI in a subpopulation of SARS-CoV-2–infected patients.

Toxic acute tubular injury

A high incidence of AKI, especially in critically ill patients, along with a lack of efficacy on primary endpoints during preliminary data analysis has led the Discovery trial investigators to an early termination of inclusions in the lopinavir/ritonavir

arm (Inserm, 2020). While it was not confirmed by the lopinavir/ritonavir versus standard of care randomized trial by Cao et al. (2020), Binois et al. (2020) found an association between AKI and this treatment regimen in patients admitted in their ICU unit, notwithstanding potential confounding factors, as this study involved critically ill patients with features of viral sepsis. Interestingly, Arrestier et al. (2020) explored three critically ill patients with AKI while on lopinavir/ritonavir treatment and found neither urinary crystals nor evidence of drugs by infrared spectroscopy analysis of urinary sediment. Conversely, they mostly found cellular debris and granular casts. Overall, these results suggest that during SARS-CoV-2 infection, potentially nephrotoxic treatments might contribute to AKI on underlying subclinically damaged kidneys.

SARS-CoV-2–Related Acute Tubular Injury: Other Pathophysiological Hypotheses

Ischemic ATI

Prerenal azotemia often overlaps with ischemic tubular injury or might rapidly evolve toward organic tubular damage when hemodynamic changes are severe. Several other factors, in particular systemic inflammation, microvascular damage, and reduction in kidney medullary perfusion, contribute to parenchymal injury during ischemic ATI (Basile et al., 2012).

Viral renal invasion

As far as we currently know, establishing that SARS-CoV-2–related AKI is related to viral invasion needs simultaneous ACE2 (type 2 angiotensin-converting enzyme) and TMPRSS2 (transmembrane protease, serine 2) expressions in the same site, and detection of viral RNA in those tissues during infection. Indeed, host cell entry of SARS-CoV-2 involves two major steps: binding of the Spike (S) protein to ACE2 and cleavage in two subunits (S1 and S2) by the host TMPRSS2, thus initiating fusion and endocytosis of the virus (Batlle et al., 2020). In both rodent and human kidneys, ACE2 protein and transcript are highly expressed in the proximal tubule, in parietal and visceral epithelial cells of the glomerulus, in vascular smooth muscle cells, and in the endothelium of interlobular arteries (Lely et al., 2004; Ye et al., 2006; Batlle et al., 2020). TMPRSS2 is expressed at lower levels in the proximal tubule and the glomerulus compared with distal nephron, questioning the kidney infectivity of SARS-CoV-2, and raising the possibility of SARS-CoV-2 priming by other TMPRSS subtypes. Of note, Pan et al. (2020) have found differential expressions of ACE2 and TMPRSS2 in Asian and European populations, potentially explaining different susceptibility to SARS-CoV-2–related AKI.

Viral RNA and proteins have been extensively reported in upper respiratory tract and pulmonary cells, by various direct techniques including spatial identification (Best Rocha et al., 2020; Bussani et al., 2020; Ehre, 2020; Hou et al., 2020; Schaefer et al., 2020). In contrast, findings are far more conflicting in the kidneys. Viral RNA has been found in 40 to 78% of studied kidney extracts in autopsy series (Bradley et al., 2020; Braun et al., 2020; Edler et al., 2020; Puelles et al., 2020; Remmelink et al., 2020; Wichmann et al., 2020), with similar viral loads in the liver and the heart, which appear to be substantially lower than in respiratory samples. It correlates with viremia when assessed (Wichmann et al., 2020), but not always with clinical and histopathological findings. Kidney histology of these autopsy series found either normal tissue or aspecific shock lesions, tubular injury, and autolysis (Bradley et al., 2020; Edler et al., 2020; Remmelink et al., 2020; Santoriello et al., 2020; Wichmann et al., 2020). Interestingly, a high proportion of chronic vascular and glomerular lesions has also been reported. Braun et al. (2020) reported the presence of pre-mortem AKI in 23 out of 32 patients with SARS-CoV-2–positive kidney samples. A report in May 2020 by Puelles et al. (2020) suggested the presence of SARS-CoV-2 RNA and protein, respectively, by *in situ* hybridization (ISH) and immunofluorescent staining within podocytes, glomerular endothelial cells, and tubular cells, whereas none of the more recent kidney biopsy series found evidence of viral RNA with validated techniques including ISH (Couturier et al., 2020; Kudose et al., 2020; Sharma et al., 2020). Consistently, the main finding in these kidney biopsy series is acute tubular injury. Besides these conflicting results, the presence of virus in cells and tissues does not imply that there is a cytopathogenic infection, as demonstrated by *in vitro* studies by Eckerle et al. (2013) showing the absence of SARS-CoV infectivity in kidney epithelial cells.

Specific cautions in interpreting indirect ultrastructural evidence of virus. Upon electron microscopic analysis of kidney structures, some authors have interpreted the presence of intracellular inclusions as direct evidence of the presence of SARS-CoV-2. Since then, several authors have disclosed these non-specific microvesicular bodies in biopsies from non-infected patients with various disorders (Calomeni et al., 2020; Cassol et al., 2020; Goldsmith and Miller, 2020). Consequently, intracellular inclusions should not be considered as viral inclusions if not associated with specific virus identification techniques.

Viral septic AKI

On the whole, even though viral RNA is found in kidneys of autopsies, current evidence does not support a major role of direct viral pathogenicity on the kidneys. Yet, severe viral infections, in particular with respiratory viruses, can induce multi-organ damage, including acute respiratory distress syndrome (ARDS) and AKI (Gu et al., 2020). Before the recent outbreak of COVID-19, public health concerns about mortality during influenza viruses, SARS-CoV, and MERS-CoV infections have yielded increasing interest in on "viral sepsis," defined as a virus-related "life-threatening organ dysfunction resulting from dysregulated host responses to infection" (Singer et al., 2016). This implies that organ damage does not directly depend on viral invasion and SARS-CoV-2–related local inflammation, but is rather considered as a remote inflammation, due to pulmonary involvement resulting in a massive systemic response that is deleterious in itself. This crosstalk between distant organs is likely mediated by several factors including cytokine and DAMP (damage-associated molecular pattern) release by injured tissues. Consistently with this hypothesis, patients with severe COVID-19 often present with multi-organ and hemodynamic failure, which appears late in the time course of the infection. This presentation is often associated with a

pro-inflammatory phenotype (Azoulay et al., 2020), including fever, high levels of C-reactive protein, and high levels of pro-inflammatory cytokines, particularly IL-6 (Chen et al., 2020), which is an established mediator of AKI in experimental models (Nechemia-Arbely et al., 2008). Besides, renal blood flow decrease has also been demonstrated in COVID-19 patients and was comparable with patients with bacterial sepsis in a case–control study (Watchorn et al., 2020). Consequently, sepsis-associated hemodynamic changes and inflammation might be of major importance in the pathophysiology of SARS-CoV-2–related AKI.

Specific cautions in extrapolating from pulmonary endothelial dysfunction and hypercoagulability to the kidney injury

Pulmonary hypercoagulability is indeed a major feature during COVID-19, raising the issue of endothelial dysfunction (Ackermann et al., 2020) as part of the viral sepsis, or as a distinct and specific mechanism of multi-organ damage (Kaur et al., 2020; Pons et al., 2020). Indeed, hypercoagulability and endothelial dysfunction are interrelated especially in the setting of thrombo-inflammation (Abou-Ismail et al., 2020). However, endotheliitis and thrombosis have been mostly described in lungs, and systemic hypercoagulability is rather occasional based on published data (Klok et al., 2020). Regarding kidneys, two cases of multiple renal infarctions have been reported (Post et al., 2020), occurring in both cases simultaneously with general clinical worsening; renal outcome eventually happened to be favorable along with respiratory and general improvement.

Renal microvascular or endothelial involvement seems rare as to date few cases of thrombotic microangiopathy (TMA) have been reported to be unequivocally related to COVID-19 (Akilesh et al., 2020; Jhaveri et al., 2020; Sharma et al., 2020). In autopsy series, Santoriello et al. (2020) found focal fibrin thrombi in only 6 out of 42 autopsies, and Varga et al. (2020) found lymphocytic endotheliitis in the kidney of one out of three deceased patients, with no precision as to which renal vascular structure was involved. These results suggest that if present, endothelial lesions are mild and do not account for the majority of SARS-CoV-2–related AKI. In the same line, while complement activation has been suggested as a major contributor to endothelial dysfunction and hypercoagulability, there is currently no evidence of such mechanism in the kidney, as no specific complement mediated renal lesions have been reported so far (such as membranoproliferative glomerulonephritis or C3 glomerulopathy). In the aforementioned published case of TMA, however, a comprehensive complement testing showed a slightly decreased level of circulating factor H, and increased circulating CBb and SC5b-9 levels, suggesting an activation of the alternative pathway of the complement in this specific case, in which genetic testing was not performed (Jhaveri et al., 2020).

The Particular Case of COVID-19–Associated Collapsing Glomerulopathy

Although probably rare, glomerular involvement seems a characteristic feature during SARS-CoV-2 infection (**Table 1**). It presents with severe AKI and heavy proteinuria, with a nephrotic syndrome (i.e., with hypoalbuminemia) in a majority of cases. Hematuria is inconstant. Histopathological findings are those of a collapsing glomerulopathy, which is a variant form of focal segmental glomerulosclerosis associated with poor renal prognosis. This lesion can be observed in other viral-associated nephropathies (HIV, CMV, EBV, and Parvovirus B19) (Velez et al., 2020). In particular, it has been described in HIV patients from African origin expressing risk variants for APOL1 gene. When tested, all the cases of collapsing glomerulopathy during

TABLE 1 | Summary of the type of reported kidney involvement during SARS-CoV-2 infection according to the underlying site of kidney damage.

Renal involvement	Features	Underlying condition	References	Commentaries
Prerenal azotemia	AKI Signs of ECV decrease FeNa <1% RBF decrease Favorable outcome after volume repletion	***Hemodynamic changes*** Hypovolemia Venous congestion Mechanical ventilation	Chand et al., 2020 Xia et al., 2020 Watchorn et al., 2020 Mohamed et al., 2020	
Tubular	AKI Low-range proteinuria Low molecular weight proteinuria ±Hypouricemia ±Hypophosphatemia ±Aminoaciduria	***Ischemic ATI Sepsis-associated ATI*** *Rhabdomyolysis*	Werion et al., 2020 Kormann et al., 2020 Mohamed et al., 2020 Kudose et al., 2020 Santoriello et al., 2020 Sharma et al., 2020	No direct identification of SARS-CoV-2 (ISH, IHC, and PCR) (Unspecific microvesicular bodies on electron microscopy)
Glomerular	AKI Nephrotic-range proteinuria Albuminuria ±Hematuria	***Collapsing glomerulopathy*** *Membranous nephropathy Minimal change disease Anti-GBM GN Pauci-immune crescentic GN* ***Chronic glomerulosclerosis***	Kudose et al., 2020 Santoriello et al., 2020 Sharma et al., 2020 Wu et al., 2020 Gaillard et al., 2020	*APOL-1* variant–associated collapsing glomerulopathy Role of interferon?
Vascular	AKI Hematuria ±Low-range proteinuria Severe COVID-19	*Microvascular* 6 cases of TMA Focal fibrin thrombi in 6/42 *Macrovascular* 2 cases of renal infarction ***Chronic vascular lesions***	Jhaveri et al., 2020 Akilesh et al., 2020 Santoriello et al., 2020 Post et al., 2020	Evidence of complement activation in one case of TMA Evidence of multiple thrombosis in one case of renal infarction

In bold the most frequently reported lesions. AKI, acute kidney injury; ECV, extracellular volume; ATI, acute tubular injury; ISH, in situ hybridization; IHC, immunohistochemistry; GBM, glomerular basal membrane; GN, glomerulonephritis; TMA, thrombotic microangiopathy.

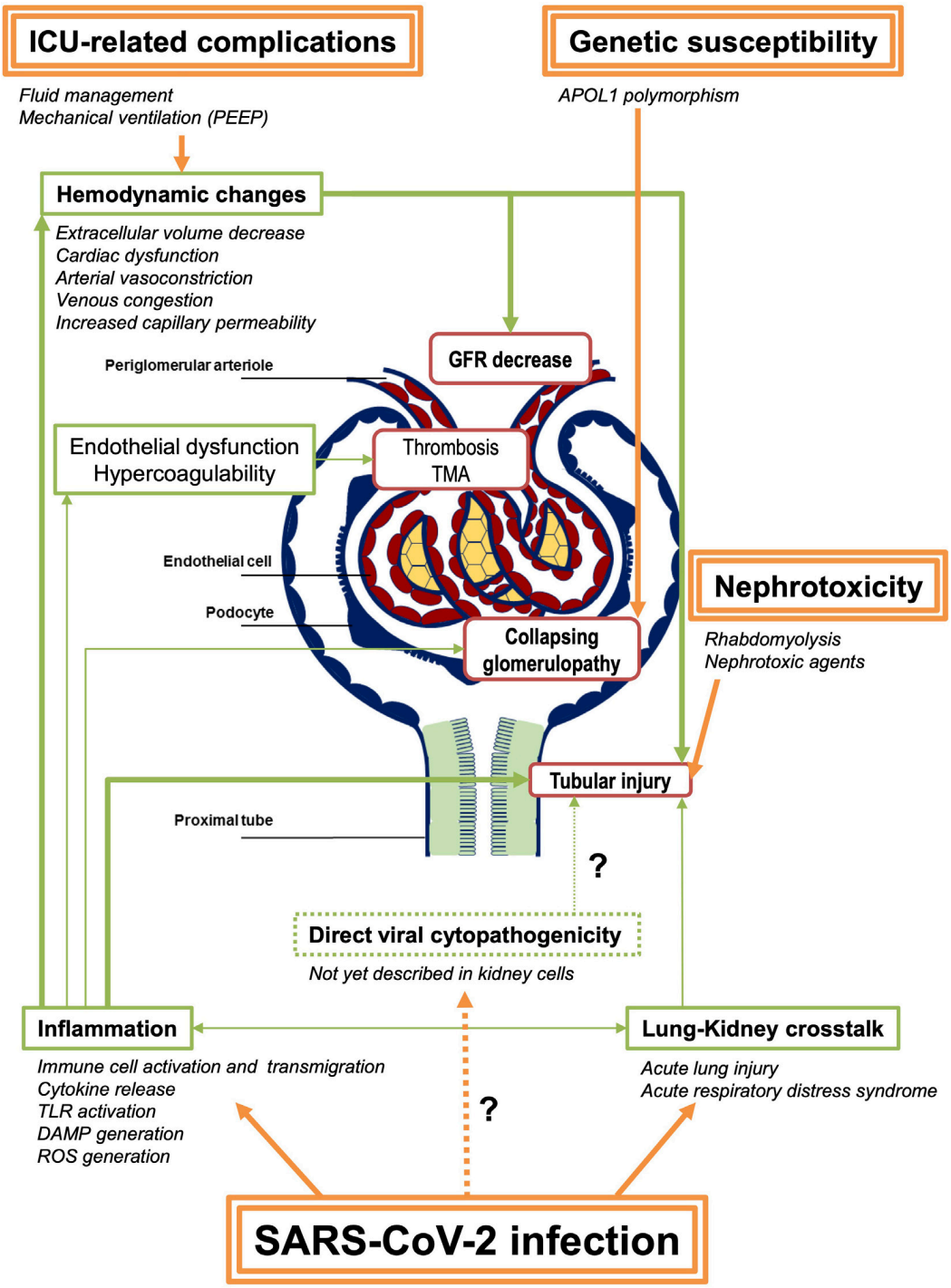


FIGURE 1 | Summary of the potential pathophysiological mechanisms of SARS-CoV-2–associated AKI. SARS-CoV-2 infection induces direct pulmonary injury that might lead to systemic inflammation. Hemodynamic changes are also frequent in patients admitted in ICU, due to the infection and its complication, as well as to medical interventions. These modifications result in GFR decrease and thus prerenal azotemia, potentially leading to acute ischemic tubular injury. Other factors including inflammation itself and tubular toxicity due to nephrotoxic agents (antibiotics, antiviral drugs, etc.) contribute to acute tubular injury in these patients. Few cases of TMA and renal vascular thrombosis have also been reported, raising the hypothesis of endothelial dysfunction and systemic hypercoagulability in the most severe patients. Collapsing glomerulopathy is a specific feature of SARS-CoV-2–related AKI, also called COVAN (COVID-associated nephropathy) in reference to HIVAN (HIV-associated nephropathy), as they probably share common mechanisms, including the strong association with APOL1 genetic variants. Finally, following the report of the autopsy series from Puelles et al. (2020), a direct tubular or glomerular viral invasion has not yet been confirmed in other reports. Consequently, this mechanism remains controversial. Arrows in bold represent the proposed major mechanisms. PEEP, positive end-expiratory pressure; GFR, glomerular filtration rate; TMA, thrombotic microangiopathy; TLR, Toll-like receptors; DAMP, damage-associated molecular patterns; ROS, reactive oxygen species.

COVID-19 occurred in patients with the same APOL1 risk variants, suggesting a common underlying susceptibility and a common second-hit mechanism (Wu et al., 2020). Noteworthy, none of the published cases found SARS-CoV-2 viral RNA in the injured glomeruli using validated direct identification techniques (PCR, ISH, and IHC). Conversely, Wu et al., found increased *in situ* chemokine gene expression consistent with electron microscopy findings of endothelial reticular aggregates often associated with conditions presenting with elevated α-interferon (Gaillard et al., 2020).

CONCLUSION: PATHOPHYSIOLOGIC HYPOTHESES AND THERAPEUTIC PERSPECTIVES

On the whole, current evidence does mostly not support the direct role of SARS-CoV-2 viral invasion in the pathophysiology of SARS-CoV-2–related AKI, even in the severe cases with systemic symptoms. Systemic complement activation is not corroborated either thus far. Rather, emerging knowledge of renal involvement during COVID-19 suggests that a state of viral sepsis results in acute tubular injury with concurrent hemodynamic changes and systemic inflammation, potentially aggravated by nephrotoxic treatments and myoglobinuria (**Figure 1**). Based on these published data on kidney involvement, in the setting of severe or late-stage SARS-CoV-2 infection, antiviral drugs and complement inhibitors might not be effective, at least if administered alone. Strict fluid management, eviction when possible of nephrotoxic agents, and hemodynamic control as well as anti-inflammatory or immunomodulatory drugs, contrariwise, might be promising in these situations.

Future experimental studies and interventional trials should unravel the natural history of SARS-CoV-2 infection and the best therapeutic options.

AUTHOR CONTRIBUTIONS

M-BL, NT, and BC wrote the outline of the review. M-BL and NT wrote the first draft of the manuscript and drafted the table. JD wrote the first draft of the table and edited the manuscript. MF contributed to the revised draft of the manuscript and new figure. BC edited the manuscript, table, and figure. All authors edited and approved the final version of the manuscript.

REFERENCES

Abou-Ismail, M. Y., Diamond, A., Kapoor, S., Arafah, Y., and Nayak, L. (2020). The hypercoagulable state in COVID-19: incidence, pathophysiology, and management. *Thromb. Res.* 194, 101–115. doi: 10.1016/j.thromres.2020.06.029

Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al. (2020). Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N. Engl. J. Med.* 383, 120–128. doi: 10.1056/NEJMoa2015432

Akilesh, S., Nast, C. C., Yamashita, M., Henriksen, K., Charu, V., Troxell, M. L., et al. (2020). Multicenter clinicopathologic correlation of kidney biopsies performed in COVID-19 patients presenting with acute kidney injury or proteinuria. *Am. J. Kidney Dis.* 77, 82.e–93.e. doi: 10.1053/j.ajkd.2020.10.001

Arrestier, R., Stehle, T., Letavernier, E., and Mekontso-Dessap, A. (2020). Lopinavir-ritonavir associated acute kidney injury is not related to crystalluria in critically-ill COVID-19 patients. Lopinavir-ritonavir associated acute kidney injury is not related to crystalluria in critically-ill COVID-19 patients. *Kidney Int. Rep.* 5:2119. doi: 10.1016/j.ekir.2020.08.021

Azoulay, E., Zafrani, L., Mirouse, A., Lengliné, E., Darmon, M., and Chevret, S. (2020). Clinical phenotypes of critically ill COVID-19 patients. *Intens. Care Med.* 46, 1651–1652. doi: 10.1007/s00134-020-06120-4

Basile, D. P., Anderson, M. D., and Sutton, T. A. (2012). Pathophysiology of Acute Kidney Injury. *Comp. Physiol. Am. Cancer Soc.* 2, 1303–1353. doi: 10.1002/cphy.c110041

Batlle, D., Soler, M. J., Sparks, M. A., Hiremath, S., South, A. M., Welling, P. A., et al. (2020). Acute kidney injury in COVID-19: emerging evidence of a distinct pathophysiology. *J. Am. Soc. Nephrol.* 31, 1380–1383. doi: 10.1681/ASN.2020040419

Best Rocha, A., Stroberg, E., Barton, L. M., Duval, E. J., Mukhopadhyay, S., Yarid, N., et al. (2020). Detection of SARS-CoV-2 in formalin-fixed paraffin-embedded tissue sections using commercially available reagents. *Lab Investig. J. Tech. Methods Pathol.* 100, 1485–1489. doi: 10.1038/s41374-020-0464-x

Binois, Y., Hachad, H., Salem, J.-E., Charpentier, J., Lebrun-Vignes, B., Pène, F., et al. (2020). Acute kidney injury associated with lopinavir/ritonavir combined therapy in patients with COVID-19. *Kidney Int. Rep.* 5, 1787–1790. doi: 10.1016/j.ekir.2020.07.035

Bosch, X., Poch, E., and Grau, J. M. (2009). Rhabdomyolysis and acute kidney injury. *N. Engl. J. Med.* 361, 62–72. doi: 10.1056/NEJMra0801327

Bowe, B., Cai, M., Xie, Y., Gibson, A. K., Maddukuri, G., and Al-Aly, Z. (2020). Acute kidney injury in a national cohort of hospitalized US veterans with COVID-19. *Clin. J. Am. Soc. Nephrol.* 16, 14–25. doi: 10.2215/CJN.09610620

Bradley, B. T., Maioli, H., Johnston, R., Chaudhry, I., Fink, S. L., Xu, H., et al. (2020). Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington State: a case series. *Lancet Lond. Engl.* 396, 320–332. doi: 10.1016/S0140-6736(20)31305-2

Braun, F., Lütgehetmann, M., Pfefferle, S., Wong, M. N., Carsten, A., Lindenmeyer, M. T., et al. (2020). SARS-CoV-2 renal tropism associates with acute kidney injury. *Lancet Lond. Engl.* 396, 597–598. doi: 10.1016/S0140-6736(20)31759-1

Bussani, R., Schneider, E., Zentilin, L., Collesi, C., Ali, H., Braga, L., et al. (2020). Persistence of viral RNA, pneumocyte syncytia and thrombosis are hallmarks of advanced COVID-19 pathology. *EBioMedicine* 61:103104. doi: 10.1016/j.ebiom.2020.103104

Calomeni, E., Satoskar, A., Ayoub, I., Brodsky, S., Rovin, B. H., and Nadasdy, T. (2020). Multivesicular bodies mimicking SARS-CoV-2 in patients without COVID-19. *Kidney Int.* 98, 233–234. doi: 10.1016/j.kint.2020.05.003

Cao, B., Wang, Y., Wen, D., Liu, W., Wang, J., Fan, G., et al. (2020). A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. *N. Engl. J. Med.* 382, 1787–1799. doi: 10.1056/NEJMoa2001282

Cassol, C. A., Gokden, N., Larsen, C. P., and Bourne, T. D. (2020). Appearances can be deceiving - viral-like inclusions in COVID-19 negative renal biopsies by electron microscopy. *Kidney360* 1, 824–828. doi: 10.34067/KID.0002692020

Chand, S., Kapoor, S., Orsi, D., Fazzari, M. J., Tanner, T. G., Umeh, G. C., et al. (2020). COVID-19-associated critical illness—report of the first 300 patients admitted to intensive care units at a New York city medical center. *J. Intensive Care Med.* 35, 963–970. doi: 10.1177/0885066620946692

Chen, G., Wu, D., Guo, W., Cao, Y., Huang, D., Wang, H., et al. (2020). Clinical and immunological features of severe and moderate coronavirus disease 2019. *J. Clin. Invest.* 130, 2620–2629. doi: 10.1172/JCI137244

Cheng, Y., Luo, R., Wang, K., Zhang, M., Wang, Z., Dong, L., et al. (2020). Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int.* 97, 829–838. doi: 10.1016/j.kint.2020.03.005

Couturier, A., Ferlicot, S., Chevalier, K., Guillet, M., Essig, M., Jauréguiberry, S., et al. (2020). Indirect effects of severe acute respiratory syndrome coronavirus

2 on the kidney in coronavirus disease patients. *Clin. Kidney J.* 13, 347–353. doi: 10.1093/ckj/sfaa088

Eckerle, I., Müller, M. A., Kallies, S., Gotthardt, D. N., and Drosten, C. (2013). In-vitro renal epithelial cell infection reveals a viral kidney tropism as a potential mechanism for acute renal failure during Middle East Respiratory Syndrome (MERS) Coronavirus infection. *Virol. J.* 10:359. doi: 10.1186/1743-422X-10-359

Edler, C., Schröder, A. S., Aepfelbacher, M., Fitzek, A., Heinemann, A., Heinrich, F., et al. (2020). Dying with SARS-CoV-2 infection-an autopsy study of the first consecutive 80 cases in Hamburg. *Germany Int. J. Legal Med.* 134, 1275–1284. doi: 10.1007/s00414-020-02317-w

Ehre, C. (2020). SARS-CoV-2 infection of airway cells. *N. Engl. J. Med.* 383:969. doi: 10.1056/NEJMicm2023328

Fu, E. L., Janse, R. J., de Jong, Y., van der Endt, V. H. W., Milders, J., van der Willik, E. M., et al. (2020). Acute kidney injury and kidney replacement therapy in COVID-19: a systematic review and meta-analysis. *Clin. Kidney J.* 13, 550–563. doi: 10.1093/ckj/sfaa160

Gabarre, P., Dumas, G., Dupont, T., Darmon, M., Azoulay, E., and Zafrani, L. (2020). Acute kidney injury in critically ill patients with COVID-19. *Intens. Care Med.* 46, 1339–1348. doi: 10.1007/s00134-020-06153-9

Gaillard, F., Ismael, S., Sannier, A., Tarhini, H., Volpe, T., Greze, C., et al. (2020). Tubuloreticular inclusions in COVID-19–related collapsing glomerulopathy. *Kidney Int.* 98:241. doi: 10.1016/j.kint.2020.04.022

Goldsmith, C. S., and Miller, S. E. (2020). Caution in identifying coronaviruses by electron microscopy. *J. Am. Soc. Nephrol.* 31, 2223–2224. doi: 10.1681/ASN.2020050755

Gu, X., Zhou, F., Wang, Y., Fan, G., and Cao, B. (2020). Respiratory viral sepsis: epidemiology, pathophysiology, diagnosis and treatment. *Eur. Respir. Rev.* 29:200038. doi: 10.1183/16000617.0038-2020

Guan, W.-J., Ni, Z.-Y., Hu, Y., Liang, W.-H., Ou, C.-Q., He, J.-X., et al. (2020). Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* 382, 1708–1720. doi: 10.1056/NEJMoa2002032

Hernandez-Arroyo, C. F., Varghese, V., Mohamed, M. M. B., and Velez, J. C. Q. (2020). Urinary sediment microscopy in acute kidney injury associated with COVID-19. *Kidney360* 1, 819–823. doi: 10.34067/KID.0003352020

Hirsch, J. S., Ng, J. H., Ross, D. W., Sharma, P., Shah, H. H., Barnett, R. L., et al. (2020). Acute kidney injury in patients hospitalized with COVID-19. *Kidney Int.* 98, 209–218. doi: 10.1016/j.kint.2020.05.006

Hou, Y. J., Okuda, K., Edwards, C. E., Martinez, D. R., Asakura, T., Dinnon, K. H., et al. (2020). SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. *Cell* 182, 429.e–446.e. doi: 10.1016/j.cell.2020.05.042

Inserm (2020). *Discovery: Stopping Inclusions in Two Treatment Groups. Newsroom | Inserm*. https://presse.inserm.fr/en/discovery-stopping-inclusions-in-two-treatment-groups/40087/ (accessed January, 2021).

Jamme, M., and Geri, G. (2020). Time-dependent effect, immortal bias, and competing risk: 3 components that should be handled to assess the impact of covariates on occurrence of acute kidney injury. *Kidney Int.* 98:1348. doi: 10.1016/j.kint.2020.07.049

Jhaveri, K. D., Meir, L. R., Flores Chang, B. S., Parikh, R., Wanchoo, R., Barilla-LaBarca, M. L., et al. (2020). Thrombotic microangiopathy in a patient with COVID-19. *Kidney Int.* 98, 509–512. doi: 10.1016/j.kint.2020.05.025

Kaur, S., Tripathi, D. M., and Yadav, A. (2020). The enigma of endothelium in COVID-19. *Front. Physiol.* 11:989. doi: 10.3389/fphys.2020.00989

Kazory, A., Ronco, C., and McCullough, P. A. (2020). SARS-CoV-2 (COVID-19) and intravascular volume management strategies in the critically ill. *Proc. Bayl. Univ. Med. Cent.* 33, 370–375. doi: 10.1080/08998280.2020.1754700

KDIGO Guidelines (2012). *Acute Kidney Injury (AKI) – KDIGO*. Available online at: https://kdigo.org/guidelines/acute-kidney-injury/ (accessed January, 2021).

Klok, F. A., Kruip, M. J. H. A., van der Meer, N. J. M., Arbous, M. S., and Gommers, D. (2020). Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb. Res.* 191, 145–147. doi: 10.1016/j.thromres.2020.04.013

Kormann, R., Jacquot, A., Alla, A., Corbel, A., Koszutski, M., Voirin, P., et al. (2020). Coronavirus disease 2019: acute Fanconi syndrome precedes acute kidney injury. *Clin. Kidney J.* 13, 362–370. doi: 10.1093/ckj/sfaa109

Koyner, J. L., and Murray, P. T. (2008). Mechanical ventilation and lung–kidney interactions. *Clin. J. Am. Soc. Nephrol.* 3, 562–570. doi: 10.2215/CJN.03090707

Kudose, S., Batal, I., Santoriello, D., Xu, K., Barasch, J., Peleg, Y., et al. (2020). Kidney biopsy findings in patients with COVID-19. *J. Am. Soc. Nephrol.* 31, 1959–1968. doi: 10.1681/ASN.2020060802

Lely, A. T., Hamming, I., van Goor, H., and Navis, G. J. (2004). Renal ACE2 expression in human kidney disease. *J. Pathol.* 204, 587–593. doi: 10.1002/path.1670

Maher, J. F. (1981). Pathophysiology of renal hemodynamics. *Nephron* 27, 215–221. doi: 10.1159/000182057

Mohamed, M. M., Lukitsch, I., Torres-Ortiz, A. E., Walker, J. B., Varghese, V., Hernandez-Arroyo, C. F., et al. (2020). Acute kidney injury associated with coronavirus disease 2019 in Urban New Orleans. *Kidney360* 1, 614–622. doi: 10.34067/KID.0002652020

Nechemia-Arbely, Y., Barkan, D., Pizov, G., Shriki, A., Rose-John, S., Galun, E., et al. (2008). IL-6/IL-6R axis plays a critical role in acute kidney injury. *J. Am. Soc. Nephrol.* 19, 1106–1115. doi: 10.1681/ASN.2007070744

Nguyen, M. T., Maynard, S. E., and Kimmel, P. L. (2009). Misapplications of commonly used kidney equations: renal physiology in practice. *Clin. J. Am. Soc. Nephrol.* 4, 528–534. doi: 10.2215/CJN.05731108

Pan, X. W., Xu, D., Zhang, H., Zhou, W., Wang, L. H., and Cui, X. G. (2020). Identification of a potential mechanism of acute kidney injury during the COVID-19 outbreak: a study based on single-cell transcriptome analysis. *Intens. Care Med.* 46, 1114–1116. doi: 10.1007/s00134-020-06026-1 doi: 10.1007/s00134-020-06026-196

Parikh, C. R., Lu, J. C., Coca, S. G., and Devarajan, P. (2010). Tubular proteinuria in acute kidney injury: a critical evaluation of current status and future promise. *Ann. Clin. Biochem.* 47, 301–312. doi: 10.1258/acb.2010.010076

Pei, G., Zhang, Z., Peng, J., Liu, L., Zhang, C., Yu, C., et al. (2020). Renal involvement and early prognosis in patients with COVID-19 Pneumonia. *J. Am. Soc. Nephrol.* 31, 1157–1165. doi: 10.1681/ASN.2020030276

Pons, S., Fodil, S., Azoulay, E., and Zafrani, L. (2020). The vascular endothelium: the cornerstone of organ dysfunction in severe SARS-CoV-2 infection. *Crit. Care Lond. Engl.* 24:353. doi: 10.1186/s13054-020-03062-7

Post, A., den Deurwaarder, E. S. G., Bakker, S. J. L., de Haas, R. J., van Meurs, M., Gansevoort, R. T., et al. (2020). Kidney infarction in patients with COVID-19. *Am. J. Kidney Dis. Off J. Natl. Kidney Found.* 76, 431–435. doi: 10.1053/j.ajkd.2020.05.004

Puelles, V. G., Lütgehetmann, M., Lindenmeyer, M. T., Sperhake, J. P., Wong, M. N., Allweiss, L., et al. (2020). Multiorgan and renal tropism of SARS-CoV-2. *N. Engl. J. Med.* 383, 590–592. doi: 10.1056/NEJMc2011400

Remmelink, M., De Mendonça, R., D'Haene, N., De Clercq, S., Verocq, C., Lebrun, L., et al. (2020). Unspecific post-mortem findings despite multiorgan viral spread in COVID-19 patients. *Crit. Care Lond. Engl.* 24:495. doi: 10.1186/s13054-020-03218-5

Robbins-Juarez, S. Y., Qian, L., King, K. L., Stevens, J. S., Husain, S. A., Radhakrishnan, J., et al. (2020). A systematic review and meta-analysis of outcomes for patients with COVID-19 and acute kidney injury. *Kidney Int. Rep.* 5, 1149–1160. doi: 10.1016/j.ekir.2020.06.013

Rubin, S., Orieux, A., Prevel, R., Garric, A., Bats, M.-L., Dabernat, S., et al. (2020). Characterization of acute kidney injury in critically ill patients with severe coronavirus disease 2019. *Clin. Kidney J.* 13, 354–361. doi: 10.1093/ckj/sfaa099

Santoriello, D., Khairallah, P., Bomback, A. S., Xu, K., Kudose, S., Batal, I., et al. (2020). Postmortem kidney pathology findings in patients with COVID-19. *J. Am. Soc. Nephrol.* 31, 2158–2167. doi: 10.1681/ASN.2020050744

Schaefer, I.-M., Padera, R. F., Solomon, I. H., Kanjilal, S., Hammer, M. M., Hornick, J. L., et al. (2020). In situ detection of SARS-CoV-2 in lungs and airways of patients with COVID-19. *Mod. Pathol.* 33, 2104–2114.

Sharma, P., Uppal, N. N., Wanchoo, R., Shah, H. H., Yang, Y., Parikh, R., et al. (2020). COVID-19-associated kidney injury: a case series of kidney biopsy findings. *J. Am. Soc. Nephrol.* 31, 1948–1958.

Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., et al. (2016). The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA.* 315, 801–810.

Su, H., Yang, M., Wan, C., Yi, L.-X., Tang, F., Zhu, H.-Y., et al. (2020). Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int.* 98, 219–227.

Suzuki, S., Egi, M., Schneider, A. G., Bellomo, R., Hart, G. K., and Hegarty, C. (2013). Hypophosphatemia in critically ill patients. *J. Crit. Care* 28, 536.e9–536.e19.

Takaori, K., Nakamura, J., Yamamoto, S., Nakata, H., Sato, Y., Takase, M., et al. (2016). Severity and frequency of proximal tubule injury determines renal prognosis. *J Am. Soc. Nephrol.* 27, 2393–2406.

Vallon, V. (2016). Tubular transport in acute kidney injury: relevance for diagnosis, prognosis and intervention. *Nephron* 134, 160.

Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., et al. (2020). Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 395, 1417–1418.

Velez, J. C. Q., Caza, T., and Larsen, C. P. (2020). COVAN is the new HIVAN: the re-emergence of collapsing glomerulopathy with COVID-19. *Nat. Rev. Nephrol.* 16, 565–567.

Watchorn, J., Huang, D. Y., Joslin, J., Bramham, K., and Hutchings, S. D. (2020). Critically ill COVID-19 patients with acute kidney injury have reduced renal blood flow and perfusion despite preserved cardiac function; a case-control study using contrast enhanced ultrasound. *Shock Augusta Ga [Online ahead of print]* doi: 10.1097/SHK.0000000000001659

Werion, A., Belkhir, L., Perrot, M., Schmit, G., Aydin, S., Chen, Z., et al. (2020). SARS-CoV-2 causes a specific dysfunction of the kidney proximal tubule. *Kidney Int.* 98, 1296–1307. doi: 10.1016/j.kint.2020.07.019

Wichmann, D., Sperhake, J.-P., Lütgehetmann, M., Steurer, S., Edler, C., Heinemann, A., et al. (2020). Autopsy findings and venous thromboembolism in patients With COVID-19: a prospective cohort study. *Ann. Intern. Med.* doi: 10.7326/M20-2003 [Epub ahead of print].

World Health Organization [WHO] (2021). *WHO Coronavirus Disease (COVID-19) Dashboard*. Available online at: https://covid19.who.int (accessed January, 2021).

Wu, H., Larsen, C. P., Hernandez-Arroyo, C. F., Mohamed, M. M. B., Caza, T., Sharshir, M., et al. (2020). AKI and collapsing glomerulopathy associated with COVID-19 and APOL1 high-risk genotype. *J. Am. Soc. Nephrol.* 31, 1688–1695.

Wu, V.-C., Huang, J.-W., Hsueh, P.-R., Yang, Y.-F., Tsai, H.-B., Kan, W.-C., et al. (2005). Renal hypouricemia is an ominous sign in patients with severe acute respiratory syndrome. *Am. J. Kidney Dis.* 45, 88–95.

Xia, P., Wen, Y., Duan, Y., Su, H., Cao, W., Xiao, M., et al. (2020). Clinicopathological features and outcomes of acute kidney injury in critically ill COVID-19 with prolonged disease course: a retrospective cohort. *J. Am. Soc. Nephrol.* 31, 2205–2221.

Ye, M., Wysocki, J., William, J., Soler, M. J., Cokic, I., and Batlle, D. (2006). Glomerular localization and expression of angiotensin-converting enzyme 2 and angiotensin-converting enzyme: implications for albuminuria in diabetes. *J. Am. Soc. Nephrol.* 17, 3067–3075.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

PERSPECTIVE
published: 25 March 2021
doi: 10.3389/fphys.2021.624062

Alteration of Diffusion Capacity After SARS-CoV-2 Infection: A Pathophysiological Approach

Justine Frija-Masson[1,2], Catherine Bancal[2], Laurent Plantier[3,4], Hélène Benzaquen[2], Laurence Mangin[2,5], Dominique Penaud[2], Florence Arnoult[2], Martin Flamant[2,6] and Marie-Pia d'Ortho[1,2]*

[1] Neurodiderot, INSERM, Université de Paris, Paris, France, [2] Physiologie-Explorations Fonctionnelles, FHU APOLLO, Assistance Publique Hôpitaux de Paris, Hôpital Bichat-Claude Bernard, Paris, France, [3] Centre de Ressources et de Compétences de la Mucoviscidose, Service de Pneumologie et Explorations Fonctionnelles Respiratoires, CHRU de Tours, Tours, France, [4] CEPR/INSERM, UMR 1100, Université de Tours, Tours, France, [5] Laboratoire Matière et Systèmes Complexes, UMR 7505, CNRS, Paris, France, [6] INSERM U 1149, Center for Research in Inflammation, Université de Paris, Paris, France

OPEN ACCESS

Edited by:
Ravi Nistala, University of Missouri, United States

Reviewed by:
Jordan A. Guenette, University of British Columbia, Canada
Hariharan Regunath, University of Missouri, United States

***Correspondence:**
Justine Frija-Masson
justine.frija@aphp.fr
orcid.org/0000-0001-5575-3913

Specialty section:
This article was submitted to Respiratory Physiology, a section of the journal Frontiers in Physiology

Received: *30 October 2020*
Accepted: *27 January 2021*
Published: *25 March 2021*

Citation:
Frija-Masson J, Bancal C, Plantier L, Benzaquen H, Mangin L, Penaud D, Arnoult F, Flamant M and d'Ortho M-P (2021) Alteration of Diffusion Capacity After SARS-CoV-2 Infection: A Pathophysiological Approach. Front. Physiol. 12:624062. doi: 10.3389/fphys.2021.624062

Severe acute respiratory syndrome coronavirus 2 (*SARS-CoV-2*) infection has affected millions of people worldwide, and pneumonia affects 90% of patients. This raises the possibility of millions of people with altered lung function. Few data exist to date on pulmonary function after SARS-CoV-2 infection, but alteration of diffusion capacity of CO (D_{LCO}) is the most frequently described abnormality. First, we present original data on lung function at 3 months after SARS-CoV-2 infection and discuss the effect of using European Coal and Steel Community (ECSC) or Global Lung Function Initiative (GLI) reference equations to diagnose diffusion capacity. Second, we review existing data on D_{LCO} alteration after SARS-CoV-2 infection and discuss the implication of restrictive disorder in D_{LCO} alteration. Last, we discuss the pathophysiology of D_{LCO} alteration and try to disentangle vascular damage and fibrosis.

Keywords: SARS-CoV-2, D_{LCO}, pneumonia, pulmonary function test, COVID-19

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has affected more than 100 million of people worldwide and more than 60 million have recovered (Jonhs Hopkins University of Medicine, 2018). Pneumonia affects more than 90% of patients and can range clinically from asymptomatic to acute respiratory distress syndrome. The radiological extent of pneumonia can be classified as absent, mild (<10% of parenchyma involved), moderate (10–24%), wide (25–49%), severe (50–74%), or very severe (>75%), according to European guidelines (Revel et al., 2020). The high number of affected people raises concern about the possibility of having millions of people with altered pulmonary function tests (PFTs). To date, few data exist on the frequency of clinically relevant PFT abnormalities after coronavirus disease 2019 (COVID-19), but alteration of diffusion capacity (D_{LCO}) is the most frequently described feature (Frija-Masson et al., 2020; Mo et al., 2020; Zhao et al., 2020a). However, PFT are not accessible worldwide, and a better knowledge of the pathophysiology underlying D_{LCO} alteration could help prioritize patients for PFT access. In this article, we review existing data on PFT results after SARS-CoV-2 infection, discuss the

possible pathophysiology of D_{LCO} alteration, and present original data on the importance of using appropriate reference equations for assessing normality of PFT.

DIFFUSION CAPACITY ALTERATION AFTER COVID-19 IS FREQUENT AND INDEPENDENT OF INITIAL CLINICAL SEVERITY

The transfer (or diffusion) conductance of the lung for carbon monoxide (D_{LCO}) is measured using the single apnea method, by multiplying two values that can be considered independent, namely (1) the alveolar volume (VA), which is the lung volume where inhaled helium diffuses in gas state following inspiration from residual volume to total lung capacity and (2) the carbon monoxide transfer coefficient (kCO), which is the rate constant for carbon monoxide uptake in the lung. Thus, D_{LCO} can be reduced by reduction in lung volumes (restriction) or reduction in carbon monoxide uptake. Since carbon monoxide uptake depends on the integrity of the alveolar–capillary membrane and the presence of hemoglobin across the alveolar–capillary membrane, any alteration in either alveolar lung regions or the pulmonary vasculature results in reduced D_{LCO}. In addition, although kCO increases with reductions in lung inflation, this relationship is not proportional (Johnson, 2000); thus, restriction due to reduced chest wall compliance or respiratory muscle weakness may result in reduced D_{LCO}. Since D_{LCO} depends on the integrity of almost all structures of the respiratory system (conducting airways excluded), it is highly sensitive to detect lung disease. Because kCO depends on lung inflation, it is difficult to interpret in restrictive lung disease where low lung volumes may result from reductions in chest wall compliance, inspiratory muscle weakness, or reduced lung compliance.

The Importance of Choosing the Right Predicted Values

We conducted a retrospective study on patients with confirmed SARS-CoV-2 infection (PCR) referred at 3 months after symptom onset for pulmonary PFT. Patients were referred to the PFT laboratory if they had presented with severe COVID-19 (i.e., had required at least 6 L/min oxygen or mechanical ventilation during acute infection) or if they still had respiratory symptoms at 3 months. Comparisons between groups used Mann–Whitney and Kruskal–Wallis (with Dunns' multiple comparisons tests) tests for continuous variables and chi-2 or Fisher's exact tests for categorical variables (Prims 8, Graphpad, San Diego, United States). Non-opposition was obtained for all patients, according to French law. The study was approved by the Institutional Review Board of the French Learned Society for Respiratory Medicine—Société de Pneumologie de Langue Française (ref 2020-056).

We included 146 patients [median age, 58 (Q1 = 49; Q3 = 67); median body mass index (BMI), 27.15 kg/m (24.72;30.35), 89 (61%) men]. Complete characteristics of patients are presented in **Table 1**.

TABLE 1 | Patients' characteristics.

	All (*n* = 151)
Age, years	57 (49; 67)
Male sex	91 (60)
BMI, kg/m^2	27.29 (24.72; 30.35)
Respiratory comorbidities	
Asthma	10 (7)
COPD	9 (6)
Bronchiectasis	2 (1)
Sarcoidosis	3 (2)
Idiopathic pulmonary fibrosis	1 (0.01)
Lymphangioleiomyomatosis	1 (0.01)
Lung transplant	1 (0.01)
Hypertension	25 (17)
Smoking status	
Active	11 (7)
Former	9 (6)
Respiratory support	
None	37 (25)
Oxygen 0–6 L/min	61 (40)
Oxygen > 6 L/min	13 (8)
High flow nasal canula/CPAP	14 (9)
Invasive ventilation	26 (17)
Initial pneumonia on chest CT	
No chest CT	22 (15)
Absent	3 (2)
Mild	13 (8)
Moderate	37 (25)
Wide	42 (28)
Severe/extremely severe	34 (23)
Pulmonary function tests	
FEV_1 (% pred)	109 (91; 119)
FVC (% pred)	109 (93; 119)
FEV_1/FVC	0.72 (0.71; 0.73)
TLC (% pred)	103 (90; 115)
D_{LCO} (CECA,% pred)	71 (69; 72)
D_{LCO} (GLI,% pred)	74 (63; 84)

Results are presented as median (Q1;Q3) for continuous variables or n (%) for categorical variables. Extent of pneumonia on initial CT was defined as absent, mild (<10%), moderate (10–24%), wide (25–49%), or severe/extremely severe (≥50%) (Revel et al., 2020).

To assess the effect of reference equations on altered diffusion capacity prevalence, we determined percent of predicted value for European Coal and Steel Community (ECSC) 93 (Pellegrino, 2005) and Global Lung Function Initiative (GLI) (Stanojevic et al., 2017) reference equations; altered diffusion capacity was defined as D_{LCO} < LLN. Median (% pred) D_{LCO} were 70 (69;72) for ECSC 93 and 72 (63;84) for GLI, $p < 0.0001$. Interestingly, when using ECSC 93 reference equations, 71 (49%) patients had altered diffusion capacity (among which light $n = 30$, moderate $n = 36$, severe $n = 5$), while 76 (54%) patients were diagnosed with altered D_{LCO} when using GLI reference equations (light $n = 49$, moderate $n = 29$, severe $n = 3$, $p = 0.0231$ for disease severity) (see **Figure 1**).

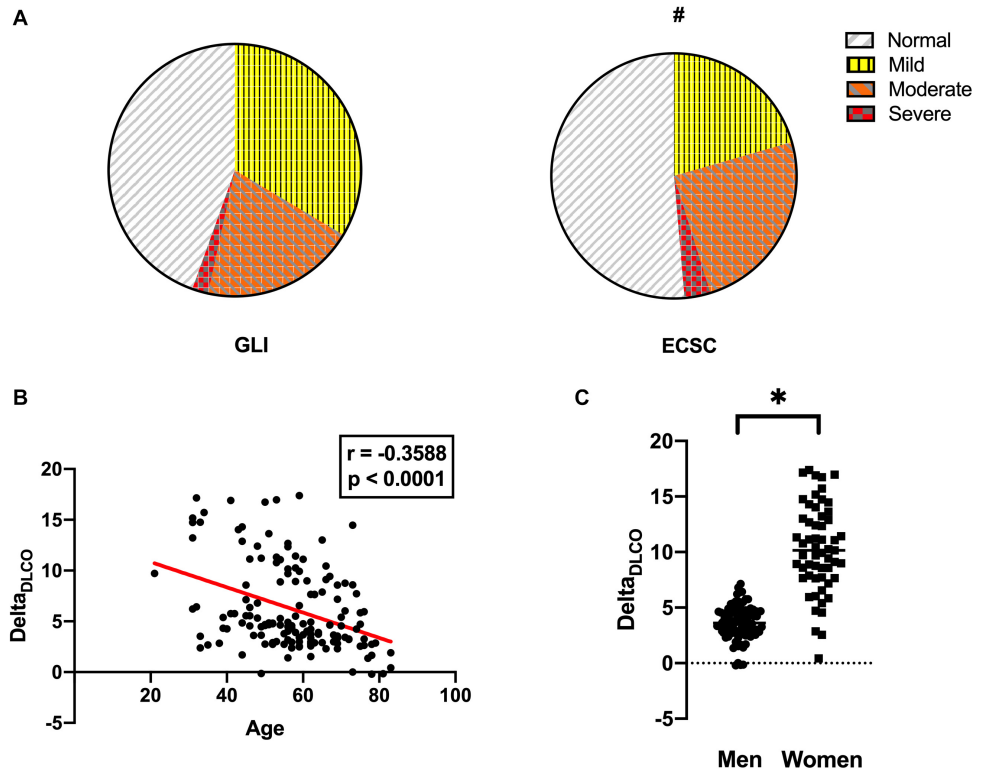


FIGURE 1 | Comparison between European Coal and Steel Community (ECSC) and Global Lung Function Initiative (GLI) reference equation in the diagnosis of diffusion capacity alteration. **(A)** Proportion of patients with normal D_{LCO}, mild, moderate, or severe diffusion alteration according to GLI (left) or ECSC (right) reference equations; $^{\#}p < 0.05$ (Mann–Whitney). **(B)** Spearman correlation showing the effect of age on $Delta_{DLCO}$ ($D_{LCO}\%_{predGLI} - D_{LCO}\%_{prcedECSC}$). **(C)** Effect of sex on $Delta_{DLCO}$; $^{*}p < 0.0001$.

We determined the difference in predicted value between GLI and ECSC 93: $Delta_{DLCO} = D_{LCO}\%_{predGLI} - D_{LCO}\%_{predECSC}$. There was an inverse correlation between age and $Delta_{DLCO}$ with a Spearman correlation coefficient $r = -0.3588$ (IC95 -0.4962; -0.039, $p < 0.0001$). There was a significant difference in $Delta_{DLCO}$ between men (median difference = 10) and women (median difference = 4, $p < 0.0001$).

Although more patients were diagnosed as having altered D_{LCO} with GLI, the severity of alteration was milder. Malinovschi et al. (2020) compared D_{LCO} (%pred) in 4,903 healthy never-smoking, middle-aged adults from the SCAPIS cohort. They found that the GLI LLN for D_{LCO} was lower than the estimated LLN by lambda–mu–sigma (LMS) method. Individuals with D_{LCO} above the GLI LLN but below the SCAPIS LLN had, to a larger extent, an increased respiratory burden. In chronic respiratory diseases, Wapenaar et al. (2019) showed that using GLI reference equations significantly enhances the number of patients who are eligible to clinical studies with a D_{LCO} threshold >30%. Brazzale et al. (2020) showed on a retrospective study on 33,863 D_{LCO} measures that, when using GLI equations, results were reclassified from abnormal to normal more frequently for younger adults and for female adults and that this effect was of different size depending on the D_{LCO} reference equation used (Crapo, Miller, or Roca). As for all lung function measurements (Bhatt et al., 2019), it is essential that appropriate reference values are used and that the criteria used to define altered D_{LCO} associate with clinical outcomes. This point is not trivial since authors do not always report the reference equations used for diagnosing abnormal D_{LCO} (Mo et al., 2020), and some authors use a fixed cutoff value of 80% (Mo et al., 2020), whereas others use the 5th centile or LLN (Frija-Masson et al., 2020). In addition, in our cohort, 49% of the subjects were Caucasian; thus, the prevalence obtained in other countries might not be the same.

Of note, the Global Lung Function Initiative very recently published an erratum on the reference equation for D_{LCO} that affected the predicted values for female adult and for the calculation of z-scores in female adults (Stanojevic et al., 2020). These modified equations are not implemented in most PFT software to date and thus cannot be used by clinicians, but the differences are minimal.

Current Knowledge on Diffusion Capacity Alteration After COVID-19

Limited data exist on pulmonary function in SARS-CoV-2 survivors. In the study by Mo et al. (2020), at hospital discharge, anomalies were noted in D_{LCO} (% pred) in 51 cases (47.2%) of the 110 patients. There was a significant difference in impaired diffusing capacity among the different groups of severity, which accounted for 30.4% in mild illness, 42.4% in pneumonia,

and 84.2% in severe pneumonia, respectively ($p < 0.05$). In the study by Frija-Masson et al. (2020), at 1 month after symptom onset, median D_{LCO} was 80 (Q1 70; Q3 92), but 30% (15/50) of the patients had altered diffusion capacity (defined as D_{LCO} < LLN). There was no difference in D_{LCO} (% pred values) between groups of CT extent but a significant difference in the proportion of abnormal values ($p = 0.0277$). Lower D_{LCO} (% predicted value) was significantly associated with older age (>50 years) ($p = 0.0351$); there was no significant difference between groups of clinical severity (i.e., oxygen requirement). In a study by Zhao et al. (2020a), among 55 patients evaluated at 3 months after symptom onset, 71% had abnormal chest CT, but only 16% of patients had D_{LCO} < 80% pred. Of note, in this study, only four patients had severe pneumonia (i.e., requiring oxygen), and four patients were included but had no radiological pneumonia. The higher prevalence of diffusion capacity alteration in our data is likely explained by the inclusion of 17% of patients who had required invasive ventilation. In the prospective study by Shah et al. (2020), 58% of the 60 included patients had abnormal D_{LCO} at 3 months, and 88% of them had abnormal chest CT.

Data are absent for patients with chronic respiratory diseases. Hu et al. (2020) reported that COPD increases all-cause mortality in patients with COVID-19, but no functional data during follow-up were available.

Altogether, these studies highlight the fact that more than half of the patients have altered D_{LCO} after SARS-CoV-2 infection and that lower D_{LCO} is related to older age and severe-to-extremely severe radiological pneumonia. Pre-SARS-CoV-2 pulmonary function was not available in published series, but most patients included were devoid of chronic respiratory diseases. In a recent meta-analysis on 378 survivors of MERS and SARS-CoV, Ahmed et al. (2020) report that D_{LCO} < 80% pred has a pooled estimate of 24.35 (95% confidence interval, 11.05–45.46) at 6 months. Despite a much smaller number of affected patients worldwide and the inclusion of only severe cases in the meta-analysis, this is markedly lower than in SARS-CoV-2 survivors.

ALTERATION OF D_{LCO}: VASCULAR DISEASE, FIBROSIS, OR BOTH?

Association Between Altered D_{LCO} and Restriction

In studies assessing pulmonary function at 1 and 3 months (Frija-Masson et al., 2020; Mo et al., 2020; Shah et al., 2020; Zhao et al., 2020a), altered D_{LCO} was the most common abnormality and was often accompanied by restrictive disorder. Restriction can result from reduction in chest wall compliance, reduction in lung compliance, inspiratory muscle weakness, or a combination thereof. Interestingly, any of these pathophysiological alterations may be present in survivors of severe COVID-19 due to COVID-specific extensive lung damage or myositis or complications of prolonged intensive care such as diaphragm dysfunction associated with critical illness myopathy (Petrof, 2018). In the study by Mo et al. (2020), 25% of patients had TLC < 80%, but the authors do not use LLN to diagnose restriction and do not report specifically patients with altered D_{LCO} and TLC; nonetheless, mean kCO (D_{LCO}/VA) was normal (92% pred value), and TLC and kCO were significantly lower in patients with severe pneumonia. In the study by Zhao et al. (2020a), altered D_{LCO} alone at 3 months was the most frequent pathological finding (16.36% of patients), but restriction and diffusion alteration were present in only 5.45% of patients. There was a significant correlation with initial D-dimer level, which suggests that vascular thrombosis and/or embolism may contribute to D_{LCO} reduction (Zhao et al., 2020a). In the data we present here, at 3 months, there was a significant difference in TLC ($p < 0.0001$) and D_{LCOGLI} ($p < 0.0001$) but not kCO for patients with residual ground glass opacities compared with normal CT at 3 months. There was a significant difference in TLC between patients with and without obesity ($p = 0.0167$) but not in D_{LCOGLI}. Radiological emphysema was present in a minority of patients and is unlikely to account for a significant proportion of altered D_{LCO}. This result suggests that alveolar lesions are a key determinant of reduced lung function. Thus, it is unclear if altered D_{LCO} up to 3 months after COVID-19 pneumonia reflects persistent alteration of the alveolar–capillary membrane, reduced lung volumes, or other mechanisms in COVID-19 survivors.

Autopsy Series and Case Reports

In the radiological case series by Zhao et al. (2020b), the most frequent feature during acute phase was ground glass opacities (GGO), either isolated GGO (86.1%) or mixed GGO and consolidation (64.4%). This was followed by vascular enlargement in the lesion (71.3%) and traction bronchiectasis (52.5%). At discharge, Wang et al. (2020) show that most patients present with consolidation of lesions, with fibrotic lesions remaining only in 12% of cases. Critically ill patients had more often consolidation and bilateral lung involvement (Qian et al., 2020). Unfortunately, most case series reporting radiological fibrosis after COVID-19 included a small number of patients, most who had required mechanical ventilation (either invasive or non-invasive), which in itself can cause lung injury (Fang et al., 2020; Huang et al., 2020).

Combet et al. (2020) report the case of a 38-year-old man who presented with extensive pulmonary honeycombing fibrosis in territories where GGO had been initially present, 10 days after symptom onset, and without invasive ventilation. Similarly, Schwensen et al. (2020) report the case of a 80 years old woman who had normal chest CT prior to infection and died of diffuse lung fibrosis.

Several histopathological series have been published (Magro et al., 2020; Menter et al., 2020). Although all reported a high prevalence of microthrombi and vascular lesions in deceased patients, the presence of alveolar damage was inconsistent. Polak et al. reviewed 129 cases of published lung samples (either full/partial autopsy or lung resection) and identified three main histological patterns: epithelial ($n = 110$, 85%), with reactive epithelial changes and diffuse alveolar damage (DAD); vascular ($n = 76$, 59%) with microvascular damage, (micro)thrombi, and acute fibrinous and organizing pneumonia;

and fibrotic (n = 28, 22%) with interstitial fibrosis. The epithelial and vascular patterns were present in all stages, whereas the fibrotic pattern started at 3 weeks of evolution. Patients could present with more than one pattern, either simultaneously or consecutively. Unfortunately, chest CT results were not reported; the presence of fibrosis was not associated with mechanical ventilation.

These differences could be explained by different inclusion criteria (deceased patients vs. lung sample), number of cases in the series, and difference in time from diagnosis to lung specimen. Indeed, most patients died after several days or weeks under ventilator support, which can lead to lung injury despite protective measures (Slutsky and Ranieri, 2013).

Vasculopathy, Fibrosis, or Both?

The key receptor to SARS-CoV-2 entry, angiotensin—converting enzyme (ACE)-2, is expressed on pneumocytes and macrophages, as well as on the surface of arterial endothelial and smooth muscle cells of the lungs (Hamming et al., 2004). Endothelial dysfunction induced by SARS-CoV-2 creates a favorable environment for thrombosis (Evans et al., 2020), which in turn can favor inflammation, representing the immunothrombosis model (Gaertner and Massberg, 2016). ACE-2 has been shown to be activated in acute lung injury and linked to acute respiratory distress syndrome (ARDS) severity (Orfanos et al., 2000; Jerng et al., 2006). Using a combined *in vitro* and *in silico* approach, Xu et al. (2020) showed that SARS-CoV-2 induces transcriptional signatures in human lung epithelial cells that promote lung fibrosis, such as TMPRSS2, ADAM metallopeptidase domain 17 (ADAM17), tissue inhibitor of metalloproteinase 3 (TIMP3), angiotensinogen, transforming growth factor beta 1 (TGFB1), connective tissue growth factor (CTGF), vascular endothelial growth factor A (VEGF A), and fibronectin.

Thus, is it possible to explain alteration of D_{LCO} by both fibrosis and vascular disease?

In infiltrative lung diseases (ILDs), Probst et al. (2020) recently reviewed the evidence of vascular involvement in fibrosis progression. In systemic sclerosis and idiopathic pulmonary fibrosis (IPF), there is an increase in vascular permeability even during early stages of the disease. There is also evidence for a vascular remodeling and a key role for the hematopoietic-vascular niche in fibrosis promotion (Cao et al., 2016).

As exerted by Eapen et al. (2020), endothelial-to-mesenchymal transition (EndMT) can occur when endothelial cells respond to injury and transform themselves in a more aggressive mesenchymal state. The authors point out that histopathological findings in fatal COVID-19 cases reveal important vascular changes that are compatible with EndMT and that this is consistent with the different receptors that facilitate SARS-CoV-2 entry in endothelial cells. The disruption of endothelial cells induced by EndMT facilitates cell migration and can trigger fibrosis.

Neutrophils extracellular traps (NETs) are DNA fibers decorated with proteins normally confined to granules, including antimicrobial molecules. They are implicated in lung damage by promoting differentiation and function of fibroblasts (*in vitro*) (Chrysanthopoulou et al., 2014), thrombosis, and can be formed in response to numerous infectious and non-infectious stimuli (Boeltz et al., 2019). The presence of NET has been confirmed in the lungs of patients with severe COVID-19, infiltrating airways, interstitium, and vascular compartment (Radermecker et al., 2020). This ubiquitous presence could result in vascular damage and fibrosis altogether, but all four patients had been under mechanical ventilation and died of respiratory failure, and these findings might not be generalized to all COVID-19 patients. Bendib et al. (2019) found NETs in bronchoalveolar lavage and blood of patients with ARDS but no significant relationship between bronchoalveolar lavage neutrophil extracellular trap concentrations and ventilator-free days.

CONCLUSION AND PERSPECTIVES

Alteration of diffusion capacity of the lung is frequent after SARS-CoV-2 infection, although its prevalence and severity depend on the reference equation used. The use of GLI reference equations should be strongly encouraged. It is still unclear if this alteration results from vascular disease (including thrombopathy), fibrotic sequelae, respiratory muscle weakness, or a combination of these factors.

This high prevalence of altered D_{LCO} will induce a high number of patients to follow after infection has resolved and put a pressure on pulmonologists. Giving a wide access to PFT will be crucial (Andrejak et al., 2020; Raghu and Wilson, 2020; Rovere Querini et al., 2020), but better knowledge on the natural history of COVID-19 could help selecting the patients who may benefit from close and repeated follow-up of D_{LCO}. Different countries might choose different follow-up algorithms, depending not only on their capacity to give access to full PFT (including FRC and D_{LCO}) or spirometry only, but also on the ongoing epidemics that will affect access to PFT and PFT procedures (Ers Group 9.1 and Ers Group 4.1, 2020; Rovere Querini et al., 2020; Wilson et al., 2020). To prevent overwhelming of PFT laboratories, chest CT and/or X-ray have been proposed as screening tools (George et al., 2020), but X-ray might not have a high sensitivity to detect sequelae. In addition, these algorithms often rely on persistent symptoms to decide further investigations, and it has been established that dyspnea is present in at least 50% of patients at 3 months, including those with mild initial symptoms (Garrigues et al., 2020; Goërtz et al., 2020). Tools assessing functional status and quality of life are of great importance when evaluating the cost of COVID-19 on patients' life (Klok et al., 2020). A common frame of surveillance and outcome measures is needed to have access to comparable data worldwide (Patel et al., 2020). The effect of COVID-19 on patients with chronic respiratory diseases needs to be assessed in multicentric cohorts. Finally, the effect of different treatments, particularly steroids, needs to be assessed.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, in respect of the GDPR.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of the French learned society for respiratory medicine – Société de Pneumologie de Langue Française. The patients/participants provided their non opposition to participate in this study.

AUTHOR CONTRIBUTIONS

JF-M: acquisition of data, manuscript writing, and statistics. CB, HB, LM, DP, and MF: acquisition of data, manuscript correction, and final approval. LP and M-Pd'O: manuscript writing and final approval. All authors contributed to the article and approved the submitted version.

REFERENCES

Ahmed, H., Patel, K., Greenwood, D. C., Halpin, S., Lewthwaite, P., Salawu, A., et al. (2020). Long-term clinical outcomes in survivors of severe acute respiratory syndrome and Middle East respiratory syndrome coronavirus outbreaks after hospitalisation or ICU admission: a systematic review and meta-analysis. *J. Rehabil. Med*. 52:jrm00063. doi: 10.2340/16501977-2694

Andrejak, C., Blanc, F. X., Costes, F., Crestani, B., Debieuvre, D., Perez, T., et al. (2020). [Guide for follow-up of patients with SARS-CoV-2 pneumonia. management proposals developed by the French-language Respiratory Medicine Society. Version of 10 may 2020]. *Rev. Mal. Respir*. 37, 505–510. doi: 10.1016/j.rmr.2020.05.001

Bendib, I., de Chaisemartin, L., Granger, V., Schlemmer, F., Maitre, B., Hüe, S., et al. (2019). Neutrophil extracellular traps are elevated in patients with pneumonia-related acute respiratory distress syndrome. *Anesthesiology* 130, 581–591. doi: 10.1097/ALN.0000000000002619

Bhatt, S. P., Balte, P. P., Schwartz, J. E., Cassano, P. A., Couper, D., Jacobs, D. R., et al. (2019). Discriminative accuracy of FEV1:FVC thresholds for COPD-related hospitalization and mortality. *JAMA* 321, 2438–2447. doi: 10.1001/jama.2019.7233

Boeltz, S., Amini, P., Anders, H. J., Andrade, F., Bilyy, R., Chatfield, S., et al. (2019). To NET or not to NET:current opinions and state of the science regarding the formation of neutrophil extracellular traps. *Cell Death Differ*. 26, 395–408. doi: 10.1038/s41418-018-0261-x

Brazzale, D. J., Seccombe, L. M., Welsh, L., Lanteri, C. J., Farah, C. S., and Ruehland, W. R. (2020). Effects of adopting the global lung function initiative 2017 reference equations on the interpretation of carbon monoxide transfer factor. *Eur. Respir. J*. 55:1901905. doi: 10.1183/13993003.01905-2019

Cao, Z., Lis, R., Ginsberg, M., Chavez, D., Shido, K., Rabbany, S. Y., et al. (2016). Targeting of the pulmonary capillary vascular niche promotes lung alveolar repair and ameliorates fibrosis. *Nat. Med*. 22, 154–162. doi: 10.1038/nm.4035

Chrysanthopoulou, A., Mitroulis, I., Apostolidou, E., Arelaki, S., Mikroulis, D., Konstantinidis, T., et al. (2014). Neutrophil extracellular traps promote differentiation and function of fibroblasts: NETs induce fibrosis via differentiation of fibroblasts. *J. Pathol*. 233, 294–307. doi: 10.1002/path.4359

Combet, M., Pavot, A., Savale, L., Humbert, M., and Monnet, X. (2020). Rapid onset honeycombing fibrosis in spontaneously breathing patient with COVID-19. *Eur. Respir. J*. 56:2001808. doi: 10.1183/13993003.01808-2020

Eapen, M. S., Lu, W., Gaikwad, A. V., Bhattarai, P., Chia, C., Hardikar, A., et al. (2020). Endothelial to mesenchymal transition: a precursor to post-COVID-19 interstitial pulmonary fibrosis and vascular obliteration? *Eur. Respir. J*. 56:2003167. doi: 10.1183/13993003.03167-2020

Ers Group 9.1 and Ers Group 4.1 (2020). *Recommendation from ERS Group 9.1 (Respiratory Function Technologists /Scientists)Lung Function Testing During COVID-19 Pandemic and Beyond*. Available Online at: https://ers.app.box.com/s/zs1uu88wy51monr0ewd990itoz4tsn2h (accessed May 6, 2020).

Evans, P. C., Rainger, G. E., Mason, J. C., Guzik, T. J., Osto, E., Stamataki, Z., et al. (2020). Endothelial dysfunction in COVID-19: a position paper of the ESC working group for atherosclerosis and vascular biology, and the ESC council of basic cardiovascular science. *Cardiovasc. Res*. 116, 2177–2184. doi: 10.1093/cvr/cvaa230

Fang, Y., Zhou, J., Ding, X., Ling, G., and Yu, S. (2020). Pulmonary fibrosis in critical ill patients recovered from COVID-19 pneumonia: preliminary experience. *Am. J. Emerg. Med*. 38, 2134–2138. doi: 10.1016/j.ajem.2020.05.120

Frija-Masson, J., Debray, M. P., Gilbert, M., Lescure, F. X., Travert, F., Borie, R., et al. (2020). Functional characteristics of patients with SARS-CoV-2 pneumonia at 30 days post-infection. *Eur. Respir. J*. 56:2001754. doi: 10.1183/13993003.01754-2020

Gaertner, F., and Massberg, S. (2016). Blood coagulation in immunothrombosis—At the frontline of intravascular immunity. *Semin. Immunol*. 28, 561–569. doi: 10.1016/j.smim.2016.10.010

Garrigues, E., Janvier, P., Kherabi, Y., Le Bot, A., Hamon, A., Gouze, H., et al. (2020). Post-discharge persistent symptoms and health-related quality of life after hospitalization for COVID-19. *J. Infect*. 81, e4–e6. doi: 10.1016/j.jinf.2020.08.029

George, P. M., Barratt, S. L., Condliffe, R., Desai, S. R., Devaraj, A., Forrest, I., et al. (2020). Respiratory follow-up of patients with COVID-19 pneumonia. *Thorax* 75, 1009–1016. doi: 10.1136/thoraxjnl-2020-215314

Goërtz, Y. M. J., Van Herck, M., Delbressine, J. M., Vaes, A. W., Meys, R., Machado, F. V. C., et al. (2020). Persistent symptoms 3 months after a SARS-CoV-2 infection: the post-COVID-19 syndrome? *ERJ Open Res*. 6, 00542–2020. doi: 10.1183/23120541.00542-2020

Hamming, I., Timens, W., Bulthuis, M., Lely, A., Navis, G., and van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. a first step in understanding SARS pathogenesis. *J. Pathol*. 203, 631–637. doi: 10.1002/path.1570

Hu, W., Dong, M., Xiong, M., Zhao, D., Zhao, Y., Wang, M., et al. (2020). Clinical courses and outcomes of patients with chronic obstructive pulmonary disease during the COVID-19 epidemic in Hubei, China. *Int. J. Chron. Obstruct. Pulmon. Dis*. 15, 2237–2248. doi: 10.2147/COPD.S265004

Huang, W., Wu, Q., Chen, Z., Xiong, Z., Wang, K., Tian, J., et al. (2020). The potential indicators for pulmonary fibrosis in survivors of severe COVID-19. *J. Infect*. doi: 10.1016/j.jinf.2020.09.027 Epub ahead of print.

Jerng, J. S., Yu, C. J., Wang, H. C., Chen, K. Y., Cheng, S. L., and Yang, P. C. (2006). Polymorphism of the angiotensin-converting enzyme gene affects the outcome of acute respiratory distress syndrome. *Crit. Care Med*. 34, 1001–1006. doi: 10.1097/01.CCM.0000206107.92476.39

Johnson, D. C. (2000). Importance of adjusting carbon monoxide diffusing capacity (DLCO) and carbon monoxide transfer coefficient (KCO) for alveolar volume. *Respir. Med*. 94, 28–37. doi: 10.1053/rmed.1999.0740

Jonhs Hopkins University of Medicine (2018). *New Cases of Covid-19 in World Countries*. Available online at: https://coronavirus.jhu.edu/data/new-cases (accessed February 21, 2018).

Klok, F. A., Boon, G. J. A. M., Barco, S., Endres, M., Geelhoed, J. J. M., Knauss, S., et al. (2020). The post-COVID-19 functional status scale: a tool to measure functional status over time after COVID-19. *Eur. Respir. J*. 56:2001494. doi: 10.1183/13993003.01494-2020

Magro, C., Mulvey, J. J., Berlin, D., Nuovo, G., Salvatore, S., Harp, J., et al. (2020). Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. *Transl. Res*. 220, 1–13. doi: 10.1016/j.trsl.2020.04.007

Malinovschi, A., Zhou, X., Bake, B., Bergström, G., Blomberg, A., Brisman, J., et al. (2020). Assessment of global lung function initiative (GLI) reference equations for diffusing capacity in relation to respiratory burden in the Swedish CArdioPulmonary bioImage Study (SCAPIS). *Eur. Respir. J*. 56:1901995. doi: 10.1183/13993003.01995-2019

Menter, T., Haslbauer, J. D., Nienhold, R., Savic, S., Hopfer, H., Deigendesch, N., et al. (2020). Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology* 77, 198–209. doi: 10.1111/his.14134

Mo, X., Jian, W., Su, Z., Chen, M., Peng, H., Peng, P., et al. (2020). Abnormal pulmonary function in COVID-19 patients at time of hospital discharge. *Eur. Respir. J*. 55:2001217. doi: 10.1183/13993003.01217-2020

Orfanos, S. E., Armaganidis, A., Glynos, C., Psevdi, E., Kaltsas, P., Sarafidou, P., et al. (2000). Pulmonary capillary endothelium-bound angiotensin-converting enzyme activity in acute lung injury. *Circulation* 102, 2011–2018. doi: 10.1161/01.CIR.102.16.2011

Patel, K., Straudi, S., Yee Sien, N., Fayed, N., Melvin, J. L., and Sivan, M. (2020). Applying the WHO ICF framework to the outcome measures used in the evaluation of long-term clinical outcomes in coronavirus outbreaks. *Int. J. Environ. Res. Public Health* 17:6476. doi: 10.3390/ijerph17186476

Pellegrino, R. (2005). Interpretative strategies for lung function tests. *Eur. Respir. J.* 26, 948–968. doi: 10.1183/09031936.05.00035205

Petrof, B. J. (2018). Diaphragm weakness in the critically Ill: basic mechanisms reveal therapeutic opportunities. *Chest* 154, 1395–1403. doi: 10.1016/j.chest.2018.08.1028

Probst, C. K., Montesi, S. B., Medoff, B. D., Shea, B. S., and Knipe, R. S. (2020). Vascular permeability in the fibrotic lung. *Eur. Respir. J.* 56:1900100. doi: 10.1183/13993003.00100-2019

Qian, S. Z., Hong, W. D., Lingjie, M., Chenfeng, L., Zhendong, F., and Pan, J. Y. (2020). Clinical characteristics and outcomes of severe and critical patients with 2019 novel coronavirus disease (COVID-19) in Wenzhou: a retrospective study. *Front. Med.* 7:552002. doi: 10.3389/fmed.2020.552002

Radermecker, C., Detrembleur, N., Guiot, J., Cavalier, E., Henket, M., d'Emal, C., et al. (2020). Neutrophil extracellular traps infiltrate the lung airway, interstitial, and vascular compartments in severe COVID-19. *J. Exp. Med.* 217:e20201012. doi: 10.1084/jem.20201012

Raghu, G., and Wilson, K. C. (2020). COVID-19 interstitial pneumonia: monitoring the clinical course in survivors. *Lancet Respir. Med.* 8, 839–842. doi: 10.1016/S2213-2600(20)30349-0

Revel, M. P., Parkar, A. P., Prosch, H., Silva, M., Sverzellati, N., Gleeson, F., et al. (2020). COVID-19 patients and the radiology department – advice from the European Society of Radiology (ESR) and the European Society of Thoracic Imaging (ESTI). *Eur. Radiol.* 30, 4903–4909. doi: 10.1007/s00330-020-06865-y

Rovere Querini, P., De Lorenzo, R., Conte, C., Brioni, E., Lanzani, C., Yacoub, M. R., et al. (2020). Post-COVID-19 follow-up clinic: depicting chronicity of a new disease. *Acta Biomed.* 91, 22–28. doi: 10.23750/abm.v91i9-S.10146

Schwensen, H. F., Borreschmidt, L. K., Storgaard, M., Redsted, S., Christensen, S., and Madsen, L. B. (2020). Fatal pulmonary fibrosis: a post-COVID-19 autopsy case. *J. Clin. Pathol.* doi: 10.1136/jclinpath-2020-206879 Epub ahead of print.

Shah, A. S., Wong, A. W., Hague, C. J., Murphy, D. T., Johnston, J. C., Ryerson, C. J., et al. (2020). A prospective study of 12-week respiratory outcomes in COVID-19-related hospitalisations. *Thorax* doi: 10.1136/thoraxjnl-2020-216308 Epub ahead of print.

Slutsky, A. S., and Ranieri, V. M. (2013). Ventilator-Induced Lung Injury. *N. Engl. J. Med.* 369, 2126–2136. doi: 10.1056/NEJMra1208707

Stanojevic, S., Brian, L. G., Cooper, B. G., Bruce, R. T., Kim, W. C., Richard, W. F., et al. (2020). Official ERS technical standards: global lung function initiative reference values for the carbon monoxide transfer factor for Caucasians. *Eur. Respir. J.* 56:1750010. doi: 10.1183/13993003.50010-2017

Stanojevic, S., Graham, B. L., Cooper, B. G., Thompson, B. R., Carter, K. W., Francis, R. W., et al. (2017). Official ERS technical standards: global lung function initiative reference values for the carbon monoxide transfer factor for Caucasians. *Eur. Respir. J.* 50:1700010. doi: 10.1183/13993003.00010-2017

Wang, C., Shi, B., Wei, C., Ding, H., Gu, J., and Dong, J. (2020). Initial CT features and dynamic evolution of early-stage patients with COVID-19. *Radiol. Infect. Dis.* 7, 195–203. doi: 10.1016/j.jrid.2020.08.002

Wapenaar, M., Miedema, J. R., Lammering, C. J., Mertens, F. W., and Wijsenbeek, M. S. (2019). The impact of the new global lung function initiative T_{LCO} reference values on trial inclusion for patients with idiopathic pulmonary fibrosis. *Eur. Respir. J.* 53:1801895. doi: 10.1183/13993003.01895-2018

Wilson, K. C., Kaminsky, D. A., Michaud, G., Sharma, S., Nici, L., Folz, R. J., et al. (2020). Restoring pulmonary and sleep services as the COVID-19 pandemic lessens: from an association of pulmonary, critical care, and sleep division directors and american thoracic society-coordinated task force. *Ann. Am. Thorac. Soc.* 17, 1343–1351. doi: 10.1513/AnnalsATS.202005-514ST

Xu, J., Xu, X., Jiang, L., Dua, K., Hansbro, P. M., and Liu, G. (2020). SARS-CoV-2 induces transcriptional signatures in human lung epithelial cells that promote lung fibrosis. *Respir. Res.* 21:182. doi: 10.1186/s12931-020-01445-6

Zhao, W., Zhong, Z., Xie, X., Yu, Q., and Liu, J. (2020b). Relation between chest CT findings and clinical conditions of coronavirus disease (COVID-19) pneumonia: a multicenter study. *AJR Am. J. Roentgenol.* 214, 1072–1077. doi: 10.2214/AJR.20.22976

Zhao, Y., Shang, Y., Song, W., Li, Q., Xie, H., Xu, Q., et al. (2020a). Follow-up study of the pulmonary function and related physiological characteristics of COVID-19 survivors three months after recovery. *EClinicalMedicine* 25:100463. doi: 10.1016/j.eclinm.2020.100463

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

BRIEF RESEARCH REPORT
published: 12 April 2021
doi: 10.3389/fphys.2021.578708

The Affinity of Hemoglobin for Oxygen Is Not Altered During COVID-19

Thomas Gille[1,2], Lucile Sesé[1,2†], Eric Aubourg[3†], Emmanuelle E. Fabre[4,5], Florence Cymbalista[5,6], Kayaththiry Caroline Ratnam[6], Dominique Valeyre[2,7], Hilario Nunes[2,7], Jean-Paul Richalet[2] and Carole Planès[1,2]*

[1] Service de Physiologie et Explorations Fonctionnelles, Hôpital Avicenne, GHUPSSD, Assistance Publique—Hôpitaux de Paris, Bobigny, France, [2] Inserm UMR 1272 "Hypoxie et Poumon," UFR SMBH Léonard de Vinci, Université Sorbonne Paris Nord, Bobigny, France, [3] CNRS, CEA, Astroparticule et Cosmologie, Université de Paris, Paris, France, [4] Laboratoire de Biochimie, Hôpital Avicenne, GHUPSSD, Assistance Publique—Hôpitaux de Paris, Bobigny, France, [5] Inserm UMR 978 ASIH, UFR SMBH Léonard de Vinci, Université Sorbonne Paris Nord, Bobigny, France, [6] Laboratoire d'Hématologie-Biologie, Hôpital Avicenne, GHUPSSD, Assistance Publique—Hôpitaux de Paris, Bobigny, France, [7] Service de Pneumologie, Centre de Référence Maladies Pulmonaires Rares, Hôpital Avicenne, GHUPSSD, Assistance Publique—Hôpitaux de Paris, Bobigny, France

OPEN ACCESS

Edited by:
Georges Leftheriotis,
Université Côte d'Azur, France

Reviewed by:
Bruno Chenuel,
Université de Lorraine, France
Anna Bogdanova,
University of Zurich, Switzerland

***Correspondence:**
Thomas Gille
thomas.gille@aphp.fr

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Red Blood Cell Physiology, a section of the journal Frontiers in Physiology

Received: 02 July 2020
Accepted: 16 March 2021
Published: 12 April 2021

Citation:
Gille T, Sesé L, Aubourg E, Fabre EE, Cymbalista F, Ratnam KC, Valeyre D, Nunes H, Richalet J-P and Planès C (2021) The Affinity of Hemoglobin for Oxygen Is Not Altered During COVID-19. Front. Physiol. 12:578708. doi: 10.3389/fphys.2021.578708

Background: A computational proteomic analysis suggested that SARS-CoV-2 might bind to hemoglobin (Hb). The authors hypothesized that this phenomenon could result in a decreased oxygen (O_2) binding and lead to hemolytic anemia as well. The aim of this work was to investigate whether the affinity of Hb for O_2 was altered during COVID-19.

Methods: In this retrospective, observational, single-center study, the blood gas analyses of 100 COVID-19 patients were compared to those of 100 non-COVID-19 patients. Fifty-five patients with carboxyhemoglobin (HbCO) $\geq$8% and 30 with sickle cell disease (SCD) were also included ("positive controls" with abnormal Hb affinity). P_{50} was corrected for body temperature, pH, and PCO_2.

Results: Patients did not differ statistically for age or sex ratio in COVID-19 and non-COVID-19 groups. Median P_{50} at baseline was 26 mmHg [25.2–26.8] vs. 25.9 mmHg [24–27.3], respectively ($p = 0.42$). As expected, P_{50} was 22.5 mmHg [21.6–23.8] in the high HbCO group and 29.3 mmHg [27–31.5] in the SCD group ($p < 0.0001$). Whatever the disease severity, samples from COVID-19 to non-COVID-19 groups were distributed on the standard O_2-Hb dissociation curve. When considering the time-course of P_{50} between days 1 and 18 in both groups, no significant difference was observed. Median Hb concentration at baseline was 14 $g.dl^{-1}$ [12.6–15.2] in the COVID-19 group vs. 13.2 $g.dl^{-1}$ [11.4–14.7] in the non-COVID-19 group ($p = 0.006$). Among the 24 COVID-19 patients displaying anemia, none of them exhibited obvious biological hemolysis.

Conclusion: There was no biological argument to support the hypothesis that SARS-CoV-2 could alter O_2 binding to Hb.

Keywords: COVID-19, SARS-CoV-2, hemoglobin-oxygen affinity, P50, gas exchange, gas transport, hemolysis, anemia

INTRODUCTION

In December 2019, a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in the Chinese city of Wuhan. The related coronavirus disease (COVID-19) rapidly spread worldwide during the following months, straining healthcare resources in many countries (Yu et al., 2020; Zhu et al., 2020). This pandemic urged the scientific community to quickly uncover and deliver information about the disease. Therefore, a substantial number of preprint articles have been made available, sparking a debate on whether they constitute reliable sources of scientific data (Smyth et al., 2020). Among them, an *in silico* modeling of molecular docking suggested that some structural and non-structural viral proteins might bind to hemoglobin (Hb) in several spots (Wenzhong and Hualan, 2020). The authors hypothesized that SARS-CoV-2 could dissociate iron ions from porphyrin, resulting in a decreased affinity of Hb for oxygen (O_2) and a decrease in O_2 binding. They also speculated that this mechanism could lead to hemolytic anemia, and that some by-products could participate in the pathophysiology of the disease. Indeed, an excess in free heme has previously been shown to promote oxidative and inflammatory stress (Wagener et al., 2020).

Although they were not supported by any experimental validation, such as *in vitro* biochemical interaction, nor any clinical observation, these conclusions were largely relayed in the media and social networks. One response on the ChemRxiv platform, identifying presumed flaws in the computational analysis, did not get as much audience (Read, 2020). Several academics called for research investigating the interaction between Hb and SARS-CoV-2 (Chowdhury and Anwar, 2020; Wagener et al., 2020). The aim of this work was therefore to investigate whether the affinity of Hb for O_2 was altered in COVID-19.

MATERIALS AND METHODS

Patient Selection

This retrospective, observational, single-center study compared 100 patients with COVID-19 and 100 control patients. The COVID-19 group (group 1) included patients with positive SARS-CoV-2 polymerase chain reaction (PCR) and at least one blood gas analysis (BGA) collected in Avicenne University Hospital, Bobigny, France, between 2020/03/16 and 2020/04/12, either in emergency room (ER), general ward or intensive care unit (ICU). One hundred patients were randomly selected, with a 1:4 stratification on the number of collected BGAs (one BGA vs. $\geq$2 BGAs) in order to favor the inclusion of patients with $\geq$2 samples, so that the time-course of P_{50} could be evaluated.

The non-COVID-19 group was a historical "negative control" (group 2), it included patients with at least one BGA collected between 2019/03/01 and 2019/04/30. One hundred unmatched patients were randomly selected, with the same stratification. For each patient in COVID-19 and non-COVID-19 groups, 1–5 BGAs were selected (see below).

Sample Selection

BGAs were made using an ABL90 FLEX or an ABL800 FLEX analyzer (Radiometer, Brønshøj, Denmark). Samples with fetal Hb (HbF) >20%, sickle Hb (HbS), or any technical problem (air bubbles, sample insufficiently shaken…) were discarded. To avoid a disproportion in the weight of each patient in the analysis, and to obtain samples collected at different levels of oxygen therapy, the number of samples was limited to 5 per patient in COVID-19 and non-COVID-19 groups, which were selected as follows: (a) first BGA in ER (if applicable); (b) first BGA in ward (if applicable); (c) first BGA in ICU (if applicable); (d) BGA after 8 $\pm$ 3 days of hospitalization or last BGA before death if the patient died before D_8; (e) BGA after 15 $\pm$ 3 days of hospitalization or last BGA before death if the patient died before D_{15}.

Assessment of Hb Affinity

P_{50} is the oxygen partial pressure when Hb is 50% saturated with O_2. It is negatively correlated with Hb affinity. For one BGA, a reliable value of P_{50} can be calculated when Hb saturation is <97% (BGAs with saturation $\geq$97% were not used for these analyses). To allow comparisons between samples, all P_{50} values were standardized for normal conditions (body temperature = 37°C; pH = 7.4; PCO_2 = 40 mmHg). The normal value of P_{50} in these conditions is 26.8 mmHg (West and Luks, 2016). The oxyhemoglobin (HbO_2) dissociation model was computed taking into account carboxyhemoglobin (HbCO) and methemoglobin (MetHb), using Hill's model corrected by Dash in the (p,s) space of Roughton (Hill, 1921; Roughton and Darling, 1944; Dash et al., 2016): s is the combined O_2 + carbon monoxide (CO) saturation ($fHbO_2$ + fHbCO)/(1-fMetHb), and p = PO_2 + M PCO where M is the Haldane ratio of affinities (Douglas et al., 1912). The curve was first displaced by all known effects (temperature, pH, PCO_2), and the extra p scaling to match the BGA was measured. The same scaling was applied to the model P_{50} (computed for O_2 saturation = 50%). This measured P_{50} was then scaled back to standard conditions using Dash's model.

A raise in 2,3-diphosphoglycerate (2,3-DPG) can induce a decrease in Hb affinity for O_2 (hence an increase in P_{50}). 2,3-DPG concentration ([2,3-DPG]) was not routinely measured in our patients, however factors modulating [2,3-DPG] were assessed, such as Hb concentration ([Hb]), age, phosphatemia, and history of heart failure (de Verdier and Garby, 1972; Purcell and Brozović, 1974). As hydroxychloroquine is known to provoke methemoglobinemia (Hall et al., 1986), the relation between hydroxychloroquine and MetHb level in the COVID-19 group was also assessed.

Model Validation

Two "positive control" groups for abnormal affinity were also used, to test if our model was able to detect clinically significant changes in Hb affinity in various conditions. All consecutive patients with HbCO $\geq$8%, starting from 2016/01/01, were included in the high HbCO group (group 3). One BGA per patient was selected. In this group, Hb was supposed to be

normal, but the presence of an unusual amount of CO was expected to stabilize Hb in its relaxed R-state and to provoke an increase in Hb affinity for O_2 (West and Luks, 2016).

Finally, the last group comprised 30 patients with homozygous HbSS sickle cell disease: SCD group (group 4). Data from all available BGAs with Hb saturation <97% were collected (from 2015/07/27 to 2020/12/08). The SCD group was used to assess if our model using Dash's equations was still valid with abnormal Hb, as HbS affinity for O_2 is decreased (Huynh-Moynot et al., 2011; Ribeil et al., 2017).

Assessment of Hemolysis in Anemic Patients

Among COVID-19 patients, if at least one BGA showed a [Hb] $\leq$ 11 g.dl^{-1}, blood smears and patient files were reviewed with a hemobiologist and the following data throughout the study period were gathered and analyzed: [Hb] on complete blood count (CBC), mean corpuscular volume (MCV), reticulocyte count, presence or absence of schistocytes, plasmatic concentrations of total and unconjugated bilirubin, lactate dehydrogenase (LDH), haptoglobin, ferritin, and C-reactive protein (CRP).

Statistical Analysis

Demographic and blood gas characteristics were compared between COVID-19 and non-COVID-19 groups using χ^2 test (qualitative variables), Mann-Whitney or unpaired *t*-test (quantitative variables, according to distribution). Differences between measured HbO_2 and predicted HbO_2 were assessed by unpaired *t* test. Before/after comparisons in the COVID-19 group (mechanical ventilation, [Hb]) were performed with paired *t*-test. Comparisons between $\geq$3 groups were assessed with Kruskal-Wallis test and Dunn's multiple comparison test. Spearman correlation coefficient (*r*) was employed to examine the relation between P_{50} and [Hb], age or phosphatemia. For P_{50} time-course, two-way ANOVA was performed. A $p < 0.05$ was considered significant. Prism® software was used (GraphPad Software Inc., San Diego, CA, United States).

RESULTS

Study Population

All 100 COVID-19 patients being hospitalized or at least seen in ER, none of them was asymptomatic. Fever, dyspnea, cough and other classical COVID-19 symptoms were common. In the non-COVID-19 group, the most frequent diagnoses were infection, airway disease (chronic obstructive pulmonary disease, asthma, bronchiectasis…), interstitial lung disease or heart failure (**Supplementary Table 1**). Patients in both COVID-19 and non-COVID-19 groups did not differ statistically for age or sex ratio. COVID-19 patients were significantly heavier and more frequently non-smokers. They required higher O_2 delivery at baseline (**Table 1**), and 80 finally necessitated O_2 therapy at some point.

Fifty-five patients had displayed a HbCO $\geq$ 8% since 2016 and were included in the high HbCO group (median HbCO level: 9.4% [8.6–12.6]). The reason for HbCO elevation was tobacco consumption in 26 (47.3%), CO poisoning in 15 (27.3%), and undetermined in the 14 others (25.4%). Thirty patients were included in the SCD group. One hundred and twenty-one BGAs were analyzed in the present study, among which 106 were collected in a context of vaso-occlusive crisis (VOC) and/or acute chest syndrome (ACS). Other indications were: respiratory infection without ACS (n = 7), scheduled health check (n = 6), thoracic pain without VOC (n = 2). Demographic characteristics are presented in **Supplementary Table 2**.

TABLE 1 | Demographic and blood gas characteristics at baseline.

	COVID-19 (n = 100)	Non-COVID-19 (n = 100)	p
Age (years)	62 [48–72]	66.5 [52–76]	NS
Sex			
Male	70	69	NS
Female	30	31	
Body mass index* (kg.m^{-2})	29.5 [26.1–31.3]	25.4 [21.9–29.9]	**0.0002**
Smoking history			
Never smoker	51	39	
Former smoker	28	36	**0.009**
Current smoker	4	16	
Not available	17	9	
Pack-years#	20 [11–50]	30 [16–50]	NS
Place of first sample			
Emergency room	84	67	
Ward	12	22	**0.017**
Intensive care unit	4	11	
Severity			
Ambient air	51	70	
Low dose O_2 (1-6 l.min^{-1})	35	21	**0.023**
High dose O_2 ($\geq$7 l.min^{-1} or ventilation)	14	9	
Blood gas variables			
Temperature (°C)	37.9 [37–38.7]	37 [36.5–37.1]	**<0.0001**
PO_2 (mmHg)	75.8 [65–93]	72.6 [60.2–84]	–
PCO_2 (mmHg)	35.7 [32–39.5]	38 [31.8–43.2]	–
pH	7.44 [7.41–7.47]	7.42 [7.38–7.46]	–
Hemoglobin (g.dl^{-1})	14 [12.6–15.2]	13.2 [11.4–14.7]	**0.006**
Oxyhemoglobin (%)	93.2 [90.4–95.5]	92.6 [88.6–94.4]	–
Oxygen content (ml.100 ml^{-1})	18.2 [16.4–20.1]	16.9 [14.1–19]	–
Carboxyhemoglobin (%)	0.9 [0.7–1.1]	1.3 [0.8–1.7]	–
Methemoglobin (%)	1.1 [1–1.2]	0.8 [0.6–1.1]	**<0.0001**
P_{50}‡ (mmHg)	26 [25.2–26.8]	25.9 [24–27.3]	NS

Data are presented as numbers, or medians and interquartile ranges between square brackets. Some blood gas variables were not statistically compared because of the patients receiving oxygen therapy. NS, non-significant.

**BMI: the number of available values was 70 and 76 for each group, respectively.*

#Tobacco consumption: the number of available values was 28/32 and 44/52 for each group, respectively.

‡P_{50}: the number of exploitable values (saturation < 97%) was 69 and 79 for each group, respectively.

Bold values are significant p-values (i.e., under 0.05).

Blood Gas Characteristics

Among COVID-19 patients, 51 were on ambient air at baseline. Blood gases were analyzed from arterial sample for 48 of them and venous sample for the other 3. In the non-COVID-19 group, 70 patients were on ambient air at baseline, with 59 arterial and 11 venous samples (**Table 2**). Despite a trend for lower PO_2 in the COVID-19 group, no statistical difference was seen for PO_2, PCO_2 or pH between COVID-19 and non-COVID-19 patients in ambient air. Median HbCO level was slightly, but significantly, lower in COVID-19 patients. On the contrary, median MetHb level was slightly, but significantly, higher in COVID-19 patients.

Hb Affinity in COVID-19 and Non-COVID-19 Groups

A total number of 253 samples were selected for the 100 COVID-19 patients throughout the study period, and 221 in the non-COVID-19 group. Twenty-three COVID-19 patients and 27 non-COVID-19 patients had only 1 BGA. Raw HbO_2 values (without standardization) in relation to PO_2 in both groups are presented in **Figure 1A**, while HbO_2 values standardized for normal conditions (Std-HbO_2) in COVID-19 and non-COVID-19 groups are presented in **Figures 1B,C**, respectively. In both groups, mean difference between measured Std-HbO_2 value and predicted HbO_2 given by the standard O_2-Hb dissociation curve was very low: $-0.3 \pm 0.7\%$ ($p = 0.73$) and $-1.1 \pm 0.9\%$ ($p = 0.21$), respectively. This low dispersion was observed at any given PO_2 and whatever the level of oxygen therapy. Importantly, median P_{50} at baseline was not different between COVID-19 group (26 mmHg [25.2–26.8]) and non-COVID-19 group (25.9 mmHg [24–27.3]; $p = 0.42$) (**Table 1**). As expected, it was significantly lower in the high HbCO group (22.5 mmHg [21.6–23.8]) and significantly higher in the SCD group (29.3 mmHg [27–31.5] ($p < 0.0001$ for all comparisons). No correlation was found between P_{50} and age or phosphatemia (all correlation coefficients $r < 0.15$) or history of heart failure ($p = 0.28$) in both COVID-19 and non-COVID-19 groups. In the COVID-19 group, median MetHb level was significantly higher in the subgroup of samples collected in patients having received hydroxychloroquine ($n = 74$): 1.5% [1.2–1.8] vs. 1.1% [1–1.3] in the absence of hydroxychloroquine ($n = 177$) ($p < 0.0001$). Median P_{50} in these two subgroups was 25.5 mmHg [24.9–26.5] vs. 26.1 mmHg [24.6–27.3], respectively ($p = 0.07$).

When considering P_{50} time-course between days 1 and 18, no significant difference was observed between COVID-19 and non-COVID-19 patients: no group effect nor time effect ($p = 0.72$) (**Figure 2**). Global P_{50} stability over time was similarly observed when considering only the most severe patients: eighteen COVID-19 patients necessitated mechanical ventilation, their median P_{50} was 25.7 mmHg [25.1–26] with mechanical ventilation and 26 mmHg [25.2–26.9] without ($p = 0.19$) (**Supplementary Figure 1**).

TABLE 2 | Characteristics of the arterial blood gas analyses collected in ambient air at baseline.

	COVID-19 ($n = 48$)	Non-COVID-19 ($n = 59$)	p
Temperature (°C)	37.8 [36.9–38.3]	37 [36.5–37]	**<0.0001**
PO_2 (mmHg)	71.5 [62.6–78.9]	76.1 [65.8–89.5]	NS
PCO_2 (mmHg)	35.7 [32.1–38.4]	35.7 [30.2–38.7]	NS
pH	7.44 [7.42–7.46]	7.43 [7.41–7.48]	NS
Hemoglobin ($g.dl^{-1}$)	14.5 [13.3–15.6]	13.4 [12.4–15]	**0.026**
Oxyhemoglobin (%)	91.9 [89.8–93.9]	93.3 [91–94.6]	NS
Oxygen content ($ml.100\ ml^{-1}$)	18.9 [16.9–20.6]	17.5 [15.9–19.2]	NS
Carboxyhemoglobin (%)	0.9 [0.7–1.2]	1.3 [0.8–2]	**0.002**
Methemoglobin (%)	1 [0.9–1.2]	0.7 [0.6–1]	**<0.0001**
P_{50}* (mmHg)	26.1 [25.4–26.7]	26 [24.6–27.3]	NS

Data are presented as medians and interquartile ranges between square brackets. NS, non-significant.
**P_{50}: the number of exploitable values (saturation < 97%) was 41 and 56 for each group, respectively.*
Bold values are significant p-values (i.e., under 0.05).

Hb Affinity in the High HbCO Group

The graphical representations of raw HbO_2 and Std-HbO_2 in relation to PO_2 show that Std-HbO_2 reached an upper limit of 92.6% because of the competitive binding of CO to Hb (**Supplementary Figures 2A,B**). Contrary to COVID-19 and non-COVID-19 groups, mean difference between measured Std-HbO_2 and predicted HbO_2 was important in the high HbCO group: $-8.6 \pm 2.1\%$ ($p = 0.0001$). Taking into account the combined saturation of Hb with O_2 and CO (Std-$SatO_2$ + CO), compared to the standard O_2-Hb dissociation curve, a shift in the relation between combined saturation and PO_2 was observed to the left, indicating a lower P_{50} and a greater Hb affinity for O_2 (**Supplementary Figure 2C**). At last, when the partial pressure in CO (PCO) was also taken into account, the median difference between measured combined saturation and predicted combined saturation was reduced to $-0.16 \pm 1.65\%$ ($p = 0.92$), indicating that our model was able to explain every kind of variation (**Supplementary Figure 2D**).

Hb Affinity in the SCD Group

In the same line, mean difference between measured Std-HbO_2 and predicted HbO_2 was important in the SCD group: $-5.3 \pm 4.1\%$ ($p < 0.0001$). As expected, a shift was observed to the right in the relation between Std-HbO_2 and PO_2, as compared to the standard O_2-Hb dissociation curve (**Supplementary Figure 3**), indicating that our model was able to detect clinically significant changes in Hb affinity even in a group of patients with abnormal Hb (here, a rise in P_{50} with decreased Hb affinity). Taking into account combined Hb saturation (Std-$SatO_2$ + CO) and PCO did not change those results. Of note, median P_{50} while taking hydroxycarbamide was significantly less elevated than without treatment: 28.2 mmHg [27–31.2] vs. 30 mmHg [26.9–31.9], respectively ($p = 0.014$). However, all samples were retained for analysis in the present study. On the other hand, the effect of transfusion on P_{50} could be assessed in a subgroup of 11 SCD patients: P_{50} decreased in only 7 of them after transfusion, and the mean difference in P_{50} before/after transfusion in the whole subgroup was -0.5 ± 1.7 mmHg ($p = 0.38$).

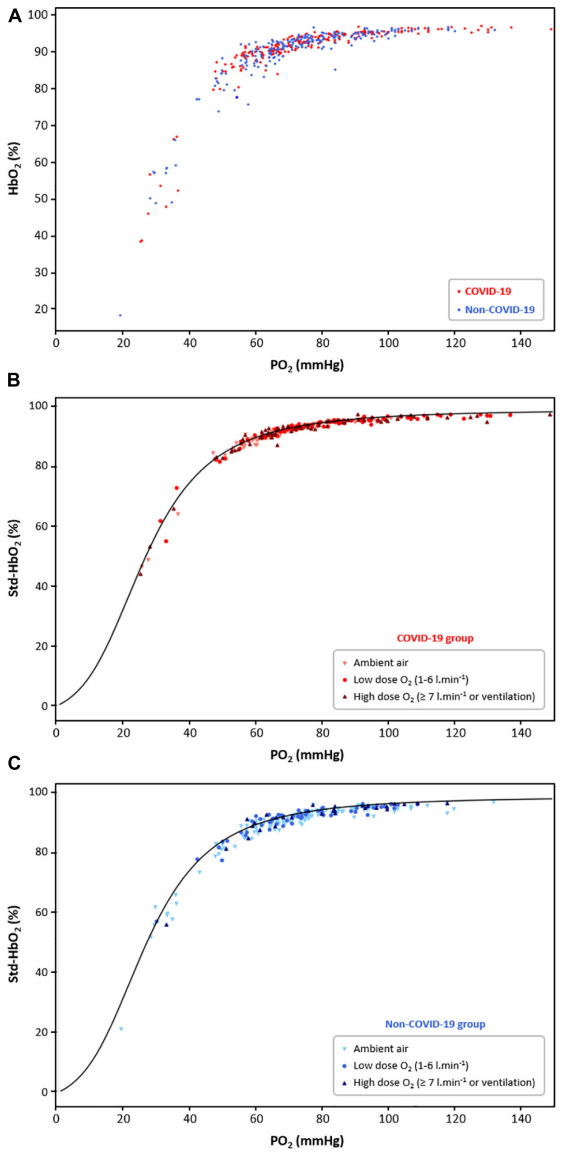


FIGURE 1 | (A) Raw oxyhemoglobin (HbO_2) in relation to PO_2 in the COVID-19 group (red dots, 100 patients, 253 samples) and the non-COVID-19 group (blue dots, 100 patients, 221 samples). **(B)** Standardized HbO_2 (Std-HbO_2) in relation to PO_2 in the COVID-19 group, according to the level of oxygen therapy (light red triangles: ambient air; medium red dots: O_2 between 1 and 6 $l.min^{-1}$; dark red triangles: $O_2 \geq 7$ $l.min^{-1}$ or ventilation). Measured HbO_2 was standardized for normal conditions (temperature = 37°C; pH = 7.4; PCO_2 = 40 mmHg) in order to compare it to the predicted HbO_2 given by the standard O_2-Hb dissociation curve, represented in black. **(C)** Std-HbO_2 for normal conditions in relation to PO_2 in the non-COVID-19 group, according to the level of oxygen therapy (light blue triangles: ambient air; medium blue dots: O_2 between 1 and 6 $l.min^{-1}$; dark blue triangles: $O_2 \geq 7$ $l.min^{-1}$ or ventilation).

Anemia and Hemolysis

Median [Hb] at baseline was significantly higher in the COVID-19 group than in the non-COVID-19 group (**Table 1**). Twenty-four COVID-19 patients displayed [Hb] $\leq$ 11 $g.dl^{-1}$ at some point. Among them, 17 exhibited no biological sign of hemolysis, and the cause of anemia was undetermined for the other 7 (due to limited retrospective data). Among biological variables related to anemia, inflammation markers (ferritin, CRP) were the only significant differences between anemic patients from COVID-19 to non-COVID-19 groups (**Supplementary Table 3**).

Among the 24 anemic COVID-19 patients, before/after comparison of P_{50} between highest and lowest [Hb] was possible in 16 of them. At highest [Hb] (mean: 12.9 $\pm$ 1.4 $g.dl^{-1}$), mean P_{50} value was 25.6 $\pm$ 0.9 mmHg; whereas it was 25.5 $\pm$ 1.2 mmHg at lowest [Hb] (mean: 9.3 $\pm$ 1.2 $g.dl^{-1}$) ($p = 0.6$) (**Supplementary Figure 4**). Taking the whole COVID-19 population, no significant correlation was found between P_{50} and [Hb] ($r = 0.19$; $p = 0.07$). Similarly, no correlation between these two variables was found in the SCD group either ($r = -0.06$; $p = 0.52$).

DISCUSSION

Comparing P_{50} values and the distribution of HbO_2/PO_2 in relation to the standard O_2-Hb dissociation curve in 100 COVID-19 patients and 100 non-COVID-19 patients, we found no argument to support the hypothesis that SARS-CoV-2 can be responsible for a clinically significant alteration of O_2 binding to Hb, neither at baseline nor later in the disease course. In contrast, we were able to identify a shift in the relation between PO_2 and HbO_2, and variations in P_{50} value, in 55 patients with HbCO $\geq$8% (increased Hb affinity) and 30 patients with sickle cell disease (decreased Hb affinity, even more when they were not treated by hydroxycarbamide). Moreover, no COVID-19 patients displayed hemolysis stigma.

Due to the huge number of BGA samples collected in our institution during the study period (3,706 in March-April 2019 and 5,346 between 2020/03/16 and 2020/04/12, regardless of the diagnosis), a random draw of patients had to be performed. Despite our 1:4 stratification on the number of collected samples, the COVID-19 group finally comprised 23 patients with only 1 BGA, whereas they were 27 in the non-COVID-19 group. This was due to two facts: for some patients with multiple BGAs, only one sample finally met our selection criteria; conversely, for some patients that had only one BGA during the predefined period, we could analyze other samples collected before or after. Eventually, we were able to compare 253 BGAs from 100 COVID-19 patients with positive SARS-CoV-2 PCR, to 221 samples from 100 non-COVID-19 controls, providing extensive information about blood gases and Hb affinity for O_2 in COVID-19.

Median P_{50} corrected for body temperature, pH and PCO_2 at baseline was 26 mmHg [25.2–26.8] in the COVID-19 group vs. 25.9 mmHg [24–27.3] in the non-COVID-19 group. These values are slightly lower than the normal theoretical value of 26.8 mmHg, which is, however, calculated for normal HbCO and MetHb levels (West and Luks, 2016). In our study, non-COVID-19 patients displayed a slightly, but significantly, higher median HbCO level, which was consistent with the greater proportion of smokers in this group. On the contrary, COVID-19 patients displayed a slightly, but significantly, higher median MetHb level, probably secondary to the use of hydroxychloroquine in some of

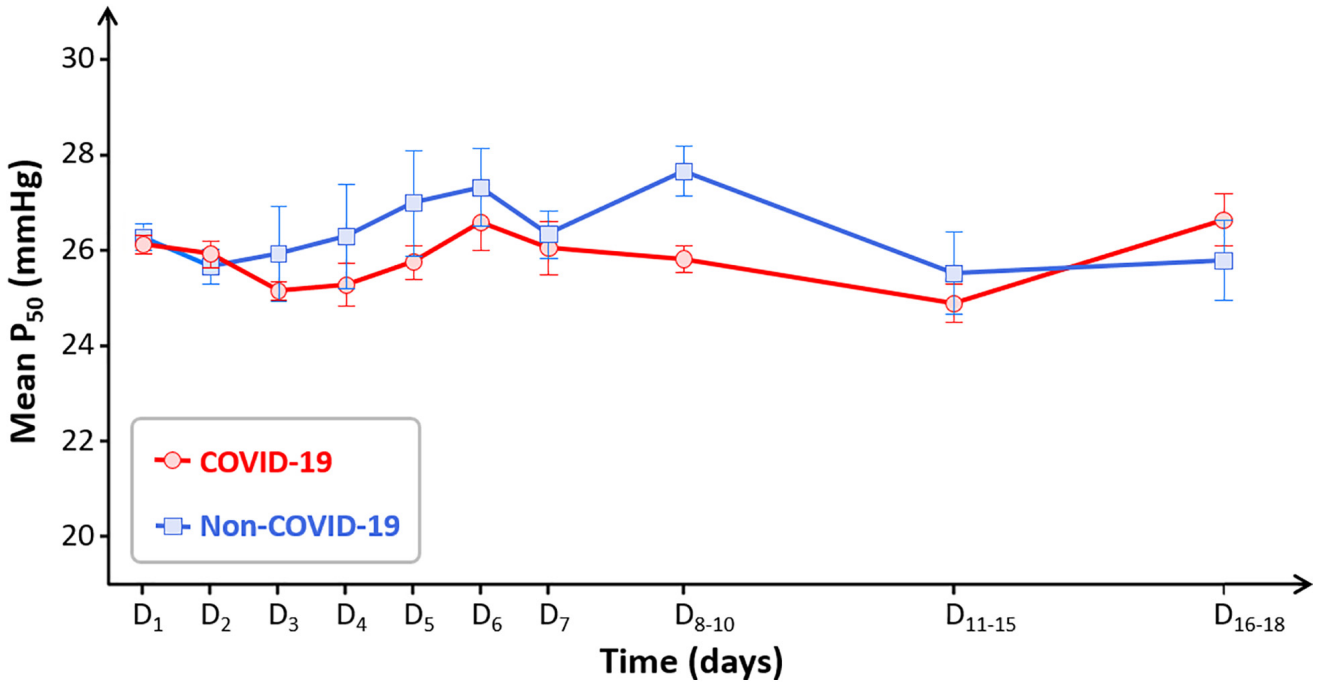


FIGURE 2 | Time-course of mean P_{50} in the COVID-19 group (red circles) and the non-COVID-19 group (blue squares). Some days were regrouped to have sufficient number of samples (D_{8-10}: n = 15 in the COVID-19 group and n = 10 in the non-COVID-19 group; D_{11-15}: n = 11 in both groups; D_{16-18}: n = 4 and n = 6, respectively). Data are presented as means and SE.

them, a drug which can potentially raise MetHb level (Hall et al., 1986). Both HbCO and MetHb are well known to increase Hb affinity for O_2 and decrease P_{50} (Douglas et al., 1912; Darling and Roughton, 1942; Roughton and Darling, 1944). Moreover, [2,3-DPG] can be modified in diverse conditions: chronic hypoxia, alkalosis, heart failure and anemia can increase [2,3-DPG]; while acidosis, blood transfusion, polycythemia, hypophosphatemia and greater age can decrease it (de Verdier and Garby, 1972; Purcell and Brozović, 1974). Because [2,3-DPG] was not routinely measured in our institution, and although the main confounding factors were assessed in the present work (pH, history of heart failure, phosphatemia, age), it is possible that some of our patients displayed a decreased [2,3-DPG]. This could be another possible explanation for P_{50} values lower than 26.8 mmHg in our cohort. Anyway, the clinical significance of the effects of 2,3-DPG variation on oxygen affinity is thought to be minimal (Macdonald, 1977). Another limit of our study is that P_{50} was calculated in a blood gas analyzer, i.e., not measured. The technique to directly measure P_{50} is longer and not routinely performed: it consists in the exposure of a blood sample to an increasing partial pressure of oxygen and subsequent deoxygenation with nitrogen gas in a Hemox-Analyzer. However, as stated in the manufacturer reference manual, ABL FLEX analyzers can estimate a reliable value of P_{50} from a blood sample with saturation <97%. We proceeded as would do the blood gas analyzer, but we calculated standardized P_{50} using the equations validated by Dash (Dash et al., 2016). Indeed, our model was able to identify pathological P_{50} values in our "positive control" groups, even in the presence of abnormal Hb: most P_{50} values were lower than normal in the HbCO group, with a median P_{50} of 22.5 mmHg [21.6–23.8]; on the contrary most P_{50} values were higher than normal in the SCD group, with a median P_{50} of 30 mmHg [26.9–31.9] in untreated patients and 28.2 mmHg [27–31.2] in the ones receiving hydroxycarbamide. Moreover, P_{50} calculation by blood gas analyzers is, under certain conditions, routinely used by some referral centers in the diagnostic approach of hemoglobins with high O_2 affinity (Orvain et al., 2017). Anyway, in the present study, the sample distribution of high HbCO and SCD groups was shifted from the standard O_2-Hb dissociation curve, indicating a modified Hb affinity, whatever the potential lack of precision of P_{50} calculation in our model compared to the gold-standard. On the contrary, no clinically significant change of Hb affinity could be observed in COVID-19 patients, whose samples were clearly distributed on the standard dissociation curve, as for the non-COVID-19 control group.

Our findings are in line with the results of a British study conducted in only 14 critically ill COVID-19 patients, and 11 age- and sex-matched controls (Daniel et al., 2020). Mean P_{50}, measured by Hemox-Analyzer, was not statistically different between both groups: 29 ± 2.3 vs. 28.5 ± 1.8 mmHg, respectively. The reasons why P_{50} values were higher than the normal theoretical value were not discussed. In another British study, mean P_{50} of 43 intubated and ventilated COVID-19 patients, retrospectively calculated from blood gas analyzer results, was 23.4 ± 3.13 mmHg, even significantly lower than a historical cohort of unmatched critically ill controls (24.6 ± 5.42 mmHg)

(Vogel et al., 2020). The authors hypothesized that those low values could be explained by reduced [2,3-DPG], and that for some reason it was even more reduced in COVID-19 patients. Another possible cause was the use of samples with saturation ≥97% to calculate P_{50}. At a cellular level, data are conflicting about the potential predisposition of impaired O_2 transport during COVID-19 (Park et al., 2020; Thomas et al., 2020); but, to date, there is no biological evidence to support the hypothesis of Wenzhong and Hualan that SARS-CoV-2 could be responsible for a clinically significant alteration of Hb affinity for O_2 (Wenzhong and Hualan, 2020). By the way, about 19% of COVID-19 patients are considered to display severe-to-critical pneumonia (Wu and McGoogan, 2020), with often profound hypoxemia which in no instance can be explained by altered Hb affinity (West and Luks, 2016).

Another claim in the preprint article of Wenzhong and Hualan (2020) was that SARS-CoV-2 could be responsible for hemolytic anemia. Indeed, potential causes of anemia are numerous and often intertwined in critically ill patients (hemodilution, iron deficiency by repeated blood sampling, surgical site bleeding or other invasive procedures, inflammation. . .) (Heming et al., 2011; Spinelli and Bartlett, 2016), particularly in such an inflammatory condition as COVID-19. In the present work, fever and dehydration could explain, at least in part, the higher median [Hb] in COVID-19 patients at baseline, compared to non-COVID-19 patients. Anyway, median [Hb] was normal at baseline, and although 24 COVID-19 patients later displayed anemia in the course of their disease, none of them exhibited obvious hemolysis. In a Chinese study comparing hematologic variables between critically ill COVID-19 and other COVID-19 patients not having required ICU, median [Hb] was normal at baseline in both groups, but the median [Hb] nadir was then lower in critically ill patients (11.1 $g.dl^{-1}$ [10.2–11.9] vs. 13.6 $g.dl^{-1}$ [12.7–15.1]) (Fan et al., 2020). In a literature review mostly analyzing data from Chinese centers, the authors stated that anemia was not a common laboratory finding in COVID-19 patients, but [Hb] tended to decline during hospitalization (Liu et al., 2020). At last, two meta-analyses showed that low [Hb] was associated with disease severity in COVID-19 (Alnor et al., 2020; Lippi and Mattiuzzi, 2020). Hemolysis was not mentioned in any of these articles. However, it cannot be excluded that occult intravascular hemolysis might occur at some level which could not be detected with classical biological signs, requiring more sensitive techniques such as detecting red blood cell microvesicles (Camus et al., 2015).

Several reports of acute hemolysis after SARS-CoV-2 infection were published, but not from direct viral action on Hb. Twelve patients presented with autoimmune hemolytic anemia (AIHA), among them 4 had B lymphoid malignancy, one had monoclonal gammapathy (Jensen et al., 2020; Lazarian et al., 2020; Lopez et al., 2020) and 2 had Evans syndrome (Li et al., 2020; Wahlster et al., 2020). Later, it was stated that AIHA could concern 12% of the subgroup of anemic COVID-19 patients (Algassim et al., 2021). Fourteen additional patients were described: five with paroxysmal nocturnal hemoglobinuria (Kulasekararaj et al., 2020; Pike et al., 2020) and 9 with previously undiagnosed glucose-6-phosphate dehydrogenase (G6PD) deficiency uncovered in context of acute hemolysis (Aguilar and Averbukh, 2020; Beauverd et al., 2020; De Franceschi et al., 2020; Kuipers et al., 2020; Maillart et al., 2020; Palmer et al., 2020; Sasi et al., 2020; Sgherza et al., 2020; Lopes et al., 2021). Indeed, infections are the most common trigger for hemolysis in G6PD-deficient individuals, and it is unclear if the use of chloroquine or hydroxychloroquine in these patients can worsen the phenomenon (Afra et al., 2020a,b).

In conclusion, the COVID-19 pandemic has greatly promoted preprint servers, with no less than 12,194 preliminary reports about COVID-19 hosted on arXiv platforms at the time of writing (MedRxiv, 2021). While it is a thrilling way to rapidly share information about the disease, the absence of conventional peer-review is at risk of spreading erroneous conclusions, sometimes amplified by the media and/or social networks (Smyth et al., 2020). The draft of Wenzhong and Hualan (2020) hypothesizing that SARS-CoV-2 could "attack" hemoglobin received quite a wide coverage and drew the public's attention, as well as some academics'. However, the present study found no biological argument to think that Hb affinity for O_2 is significantly altered during COVID-19, nor that COVID-19 can directly induce significant hemolytic anemia.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Comité Local d'Ethique pour la Recherche Clinique Avicenne-Jean Verdier-René Muret (CLEA), Hôpitaux Universitaires de Paris-Seine-Saint-Denis (HUPSSD), Assistance Publique—Hôpitaux de Paris (AP-HP), Bobigny, France (#CLEA-2020-129). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

TG, LS, and EA wrote the manuscript. TG, J-PR, and CP conceived and planned the study. EA processed the data (consistency checks, oxyhemoglobin dissociation model, P_{50} measurement, data standardization). EF extracted laboratory data. FC and KR reviewed the blood smears and files of anemic patients. TG performed the statistical analyses. All authors discussed the results and contributed to the final manuscript.

ACKNOWLEDGMENTS

We would like to deeply thank Prof. Ranjan K. Dash, for fruitful discussions and sharing his latest matlab model. Many thanks also to Rémi Letestu, Sylvain Le Jeune, and Amélie Beaugrand for their precious help and advice.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.578708/full#supplementary-material

Supplementary Figure 1 | P_{50} with and without mechanical ventilation in the COVID-19 group. P_{50} values were standardized for normal conditions (temperature=37°C; pH=7.4; PCO_2=40 mmHg). Before/after comparison was possible in 13 out of 18 COVID-19 patients having required mechanical ventilation, no significant difference was found (paired *t* test, p=0.38).

Supplementary Figure 2 | (A) Raw oxyhemoglobin (HbO_2) in relation to PO_2 in the high HbCO group (55 patients, 55 samples). **(B)** Standardized oxyhemoglobin (Std-HbO_2) in relation to PO_2. Measured HbO_2 was standardized for normal conditions (temperature=37°C; pH=7.4; PCO_2=40 mmHg) in order to compare it to the predicted HbO_2 given by the standard O_2-Hb dissociation curve, represented in black. **(C)** Standardized combined saturation for oxygen and carbon monoxide (Std-$Sat_{O2+\ CO}$) in relation to PO_2. **(D)** Std-$Sat_{O2+\ CO}$ in relation to combined partial pressure (PO_2+M PCO).

Supplementary Figure 3 | (A) Raw oxyhemoglobin (HbO_2) in relation to PO_2 in the SCD group (30 patients, 121 samples). **(B)** Standardized oxyhemoglobin (Std-HbO_2) in relation to PO_2. Measured HbO_2 was standardized for normal conditions (temperature=37°C; pH=7.4; PCO_2=40 mmHg) in order to compare it to the predicted HbO_2 given by the standard O_2-Hb dissociation curve, represented in black.

Supplementary Figure 4 | Evolution of P_{50} in 16 anemic COVID-19 patients between highest and lowest hemoglobin concentration [Hb]. P_{50} values were standardized for normal conditions (temperature=37°C.; pH=7.4; PCO_2=40 mmHg).

Supplementary Table 1 | Main diagnosis in the non-COVID-19 group.

Supplementary Table 2 | Demographic characteristics in high HbCO and SCD groups.

Supplementary Table 3 | Biological data related to anemia in patients from all groups with hemoglobin concentration $\leq$ 11 $g.dl^{-1}$.

REFERENCES

Afra, T. P., Nampoothiri, R. V., Razmi, T. M., and Hafi, N. A. B. (2020a). Linking hydroxychloroquine to hemolysis in a 'suspected' glucose-6-phosphate dehydrogenase deficient patient with COVID-19 infection – a critical appraisal. *Eur. J. Intern. Med.* 80, 101–102. doi: 10.1016/j.ejim.2020.07.001

Afra, T. P., Vasudevan Nampoothiri, R., and Razmi, T. M. (2020b). Doubtful precipitation of hemolysis by hydroxychloroquine in glucose-6-phosphate dehydrogenase-deficient patient with COVID-19 infection. *Eur. J. Haematol.* 105, 512–513. doi: 10.1111/ejh.13460

Aguilar, J., and Averbukh, Y. (2020). Hemolytic anemia in a glucose-6-phosphate dehydrogenase-deficient patient receiving hydroxychloroquine for COVID-19: a case report. *Perm. J.* 24:20.158. doi: 10.7812/TPP/20.158

Algassim, A. A., Elghazaly, A. A., Alnahdi, A. S., Mohammed-Rahim, O. M., Alanazi, A. G., Aldhuwayhi, N. A., et al. (2021). Prognostic significance of hemoglobin level and autoimmune hemolytic anemia in SARS-CoV-2 infection. *Ann. Hematol.* 100, 37–43. doi: 10.1007/s00277-020-04256-3

Alnor, A., Sandberg, M. B., Gils, C., and Vinholt, P. J. (2020). Laboratory tests and outcome for patients with COVID-19: a systematic review and meta-analysis. *J. Appl. Lab. Med.* 5, 1038–1049. doi: 10.1093/jalm/jfaa098

Beauverd, Y., Adam, Y., Assouline, B., and Samii, K. (2020). COVID-19 infection and treatment with hydroxychloroquine cause severe haemolysis crisis in a patient with glucose-6-phosphate dehydrogenase deficiency. *Eur. J. Haematol.* 105, 357–359. doi: 10.1111/ejh.13432

Camus, S. M., De Moraes, J. A., Bonnin, P., Abbyad, P., Le Jeune, S., Lionnet, F., et al. (2015). Circulating cell membrane microparticles transfer heme to endothelial cells and trigger vasoocclusions in sickle cell disease. *Blood* 125, 3805–3814. doi: 10.1182/blood-2014-07-589283

Chowdhury, S. F., and Anwar, S. (2020). Management of hemoglobin disorders during the COVID-19 pandemic. *Front. Med.* 7:306. doi: 10.3389/fmed.2020.00306

Daniel, Y., Hunt, B. J., Retter, A., Henderson, K., Wilson, S., Sharpe, C. C., et al. (2020). Haemoglobin oxygen affinity in patients with severe COVID-19 infection. *Br. J. Haematol.* 190, e126–e127. doi: 10.1111/bjh.16888

Darling, R. C., and Roughton, F. J. W. (1942). The effect of methemoglobin on the equilibrium between oxygen and hemoglobin. *Am. J. Physiol.* 137, 56–68. doi: 10.1152/ajplegacy.1942.137.1.56

Dash, R. K., Korman, B., and Bassingthwaighte, J. B. (2016). Simple accurate mathematical models of blood HbO2 and HbCO2 dissociation curves at varied physiological conditions: evaluation and comparison with other models. *Eur. J. Appl. Physiol.* 116, 97–113. doi: 10.1007/s00421-015-3228-3

De Franceschi, L., Costa, E., Dima, F., Morandi, M., and Olivieri, O. (2020). Acute hemolysis by hydroxycloroquine was observed in G6PD-deficient patient with severe COVD-19 related lung injury. *Eur. J. Intern. Med.* 77, 136–137. doi: 10.1016/j.ejim.2020.04.020

de Verdier, C. H., and Garby, L. (1972). 2,3-Diphosphoglycerate and respiratory function of hemoglobin. *Biochimie* 54, 613–617. doi: 10.1016/s0300-9084(72)80150-0

Douglas, C. G., Haldane, J. S., and Haldane, J. B. S. (1912). The laws of combination of hæmoglobin with carbon monoxide and oxygen. *J. Physiol.* 44, 275–304. doi: 10.1113/jphysiol.1912.sp001517

Fan, B. E., Chong, V. C. L., Chan, S. S. W., Lim, G. H., Lim, K. G. E., Tan, G. B., et al. (2020). Hematologic parameters in patients with COVID-19 infection. *Am. J. Hematol.* 95, E131–E134. doi: 10.1002/ajh.25774

Hall, A. H., Kulig, K. W., and Rumack, B. H. (1986). Drug- and chemical-induced methaemoglobinaemia. Clinical features management. *Med. Toxicol.* 1, 253–260. doi: 10.1007/BF03259842

Heming, N., Montravers, P., and Lasocki, S. (2011). Iron deficiency in critically ill patients: highlighting the role of hepcidin. *Crit. Care* 15, 1–7. doi: 10.1186/cc9992

Hill, A. V. (1921). The combinations of haemoglobin with oxygen and carbon monoxide, and the effects of acid and carbon dioxide. *Biochem. J.* 15, 577–586. doi: 10.1042/bj0150577

Huynh-Moynot, S., Moynot, J.-C., Commandeur, D., Deserts, M. D. D., Montelescaut, É, Kenane, N., et al. (2011). Drépanocytose : des aspects moléculaires à la pratique clinique. À propos d'un cas et revue de la littérature. *Ann. Biol. Clin.* 69, 679–684. doi: 10.1684/abc.2011.0633

Jensen, C. E., Wilson, S., Thombare, A., Weiss, S., and Ma, A. (2020). Cold agglutinin syndrome as a complication of Covid-19 in two cases. *Clin. Infect. Pract.* 7:100041. doi: 10.1016/j.clinpr.2020.100041

Kuipers, M. T., van Zwieten, R., Heijmans, J., Rutten, C. E., de Heer, K., Kater, A. P., et al. (2020). Glucose-6-phosphate dehydrogenase deficiency-associated hemolysis and methemoglobinemia in a COVID-19 patient treated with chloroquine. *Am. J. Hematol.* 95, E194–E196. doi: 10.1002/ajh.25862

Kulasekararaj, A. G., Lazana, I., Large, J., Posadas, K., Eagleton, H., Villajin, J. L., et al. (2020). Terminal complement inhibition dampens the inflammation during COVID-19. *Br. J. Haematol.* 190, e141–e143. doi: 10.1111/bjh.16916

Lazarian, G., Quinquenel, A., Bellal, M., Siavellis, J., Jacquy, C., Re, D., et al. (2020). Autoimmune haemolytic anaemia associated with COVID-19 infection. *Br. J. Haematol.* 190, 29–31. doi: 10.1111/bjh.16794

Li, M., Nguyen, C. B., Yeung, Z., Sanchez, K., Rosen, D., and Bushan, S. (2020). Evans syndrome in a patient with COVID-19. *Br. J. Haematol.* 190, e59–e61. doi: 10.1111/bjh.16846

Lippi, G., and Mattiuzzi, C. (2020). Hemoglobin value may be decreased in patients with severe coronavirus disease 2019. *Hematol. Transfus. Cell Ther.* 42, 116–117. doi: 10.1016/j.htct.2020.03.001

Liu, X., Zhang, R., and He, G. (2020). Hematological findings in coronavirus disease 2019: indications of progression of disease. *Ann. Hematol.* 99, 1421–1428. doi: 10.1007/s00277-020-04103-5

Lopes, D. V., Lazar Neto, F., Marques, L. C., Lima, R. B. O., and Brandão, A. A. G. S. (2021). Methemoglobinemia and hemolytic anemia after COVID-19 infection

without identifiable eliciting drug: a case-report. *IDCases* 23:e01013. doi: 10.1016/j.idcr.2020.e01013

Lopez, C., Kim, J., Pandey, A., Huang, T., and DeLoughery, T. G. (2020). Simultaneous onset of COVID-19 and autoimmune haemolytic anaemia. *Br. J. Haematol.* 190, 31–32. doi: 10.1111/bjh.16786

Macdonald, R. (1977). Red cell 2,3-diphosphoglycerate and oxygen affinity. *Anaesthesia* 32, 544–553. doi: 10.1111/j.1365-2044.1977.tb10002.x

Maillart, E., Leemans, S., Van Noten, H., Vandergraesen, T., Mahadeb, B., Salaouatchi, M. T., et al. (2020). A case report of serious haemolysis in a glucose-6-phosphate dehydrogenase-deficient COVID-19 patient receiving hydroxychloroquine. *Infect. Dis. (Lond)* 52, 659–661. doi: 10.1080/23744235.2020.1774644

MedRxiv (2021). *COVID-19 SARS-CoV-2 Preprints from MedRxiv and BioRxiv*. Available online at: https://connect.medrxiv.org/relate/content/181 (accessed January 10, 2021).

Orvain, C., Joly, P., Pissard, S., Badiou, S., Badens, C., Bonello-Palot, N., et al. (2017). Diagnostic approach to hemoglobins with high oxygen affinity: experience from France and Belgium and review of the literature. *Ann. Biol. Clin.* 75, 39–51. doi: 10.1684/abc.2016.1204

Palmer, K., Dick, J., French, W., Floro, L., and Ford, M. (2020). Methemoglobinemia in patient with G6PD deficiency and SARS-CoV-2 infection. *Emerg. Infect. Dis. J.* 26, 2279–2281. doi: 10.3201/eid2609.202353

Park, K. C., Donovan, K., McKechnie, S., Ramamurthy, N., Klenerman, P., and Swietach, P. (2020). Single-cell oxygen saturation imaging shows that gas exchange by red blood cells is not impaired in COVID-19 patients. *Br. J. Haematol.* 190, e229–e232. doi: 10.1111/bjh.17025

Pike, A., Muus, P., Munir, T., Mitchell, L., Arnold, L., Riley, K., et al. (2020). COVID-19 infection in patients on anti-complement therapy: the leeds national paroxysmal nocturnal haemoglobinuria service experience. *Br. J. Haematol.* 191, e1–e4. doi: 10.1111/bjh.17097

Purcell, Y., and Brozović, B. (1974). Red cell 2,3-diphosphoglycerate concentration in man decreases with age. *Nature* 251, 511–512. doi: 10.1038/251511a0

Read, R. (2020). Flawed methods in "COVID-19: attacks the 1-beta chain of hemoglobin and captures the porphyrin to inhibit human heme metabolism." *ChemRixv* [Preprint] doi: 10.26434/chemrxiv.12120912.v2

Ribeil, J.-A., Hacein-Bey-Abina, S., Payen, E., Magnani, A., Semeraro, M., Magrin, E., et al. (2017). Gene therapy in a patient with sickle cell disease. *N. Engl. J. Med.* 376, 848–855. doi: 10.1056/NEJMoa1609677

Roughton, F. J. W., and Darling, R. C. (1944). The effect of carbon monoxide on the oxyhemoglobin dissociation curve. *Am. J. Physiol.* 141, 17–31. doi: 10.1152/ajplegacy.1944.141.1.17

Sasi, S., Yassin, M. A., Nair, A. P., and Al Maslamani, M. S. (2020). A case of COVID-19 in a patient with asymptomatic hemoglobin d thalassemia and glucose-6-phosphate dehydrogenase deficiency. *Am. J. Case Rep.* 21:e925788. doi: 10.12659/AJCR.925788

Sgherza, N., Dalfino, L., Palma, A., Vitucci, A., Campanale, D., Grasso, S., et al. (2020). "Hemolysis, or not hemolysis, that is the question". Use of hydroxychloroquine in a patient with COVID-19 infection and G6PD deficiency. *Mediterr. J. Hematol. Infect. Dis.* 12:e2020076. doi: 10.4084/mjhid.2020.076

Smyth, A. R., Rawlinson, C., and Jenkins, G. (2020). Preprint servers: a 'rush to publish' or 'just in time delivery' for science? *Thorax* 75, 532–533. doi: 10.1136/thoraxjnl-2020-214937

Spinelli, E., and Bartlett, R. H. (2016). Anemia and transfusion in critical care: physiology and management. *J. Intensive Care Med.* 31, 295–306. doi: 10.1177/0885066615571901

Thomas, T., Stefanoni, D., Dzieciatkowska, M., Issaian, A., Nemkov, T., Hill, R. C., et al. (2020). Evidence of structural protein damage and membrane lipid remodeling in red blood cells from COVID-19 patients. *J. Proteome Res.* 19, 4455–4469. doi: 10.1021/acs.jproteome.0c00606

Vogel, D. J., Formenti, F., Retter, A. J., Vasques, F., and Camporota, L. (2020). A left shift in the oxyhaemoglobin dissociation curve in patients with severe coronavirus disease 2019 (COVID-19). *Br. J. Haematol.* 191, 390–393. doi: 10.1111/bjh.17128

Wagener, F. A. D. T. G., Pickkers, P., Peterson, S. J., Immenschuh, S., and Abraham, N. G. (2020). Targeting the heme-heme oxygenase system to prevent severe complications following COVID-19 infections. *Antioxidants* 9:540. doi: 10.3390/antiox9060540

Wahlster, L., Weichert-Leahey, N., Trissal, M., Grace, R. F., and Sankaran, V. G. (2020). COVID-19 presenting with autoimmune hemolytic anemia in the setting of underlying immune dysregulation. *Pediatr. Blood Cancer* 67:e28382. doi: 10.1002/pbc.28382

Wenzhong, L., and Hualan, L. (2020). COVID-19: attacks the 1-beta chain of hemoglobin and captures the porphyrin to inhibit human heme metabolism. *ChemRxiv* [Preprint] doi: 10.26434/chemrxiv.11938173.v8

West, J. B., Luks, A. M. (2016). "Gas transport by the blood: how gases are moved to and from the peripheral tissues," in *West's Respiratory Physiology: The Essentials* (Philadelphia, PA: Wolters Kluwers), 94–115.

Wu, Z., and McGoogan, J. M. (2020). Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in china: summary of a report of 72 314 cases from the chinese center for disease control and prevention. *JAMA* 323, 1239–1242. doi: 10.1001/jama.2020.2648

Yu, J., Chai, P., Ge, S., and Fan, X. (2020). Recent understandings toward coronavirus disease 2019 (covid-19): from bench to bedside. *Front. Cell Dev. Biol.* 8:476. doi: 10.3389/fcell.2020.00476

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., et al. (2020). A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.* 382, 727–733. doi: 10.1056/NEJMoa2001017

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

PERSPECTIVE
published: 20 April 2021
doi: 10.3389/fphys.2021.624528

Supplemental Learning in Respiratory Physiology for Healthcare Professionals Towards Successful Treatment of COVID-19

Helen Wallace[1]* *and Robert Angus*[2]

[1] Department of Women and Children's Health, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, United Kingdom, [2] School of Medicine, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, United Kingdom

The immunological and pathophysiological response to COVID-19 can cause severe respiratory impairment affecting gas exchange and lung mechanics. Such was the scale of the respiratory support needed during the first wave of the pandemic, that recruitment of non-respiratory clinical staff was essential to help deal with the growing number of cases. It quickly became apparent that it was vital to rapidly equip these healthcare professionals with appropriate physiological knowledge and practical skills if therapies were to be applied effectively. Furthermore, the unravelling of unusual clinical features of COVID-19, further highlighted a need for knowledge of long-established principles of respiratory physiology. An online digital educational resource, or "respiratory learning tool kit" was developed with interactive material including visualisations, animations, and pathophysiological examples to facilitate understanding. The learning outcomes were centred on physiological principles, essential for understanding the pathophysiology relating to COVID-19, and management and treatment. Topics included principles of gas exchange, gas transport, homeostasis and central control of respiration. These basic physiological principles were linked to pathophysiology and clinical skills around oxygen administration and non-invasive supports such as Continuous Positive Airway Pressure (CPAP). From the degree of engagement and evaluation comments, it was clear that the resource successfully achieved its aim—to increase physiological knowledge and its practical understanding, enabling healthcare professionals to practice with confidence in such an uncertain environment.

Keywords: education, respiratory, physiology, clinical, COVID-19, pathophysiology, treatment

OPEN ACCESS

Edited by:
Adriana Castello Costa Girardi, University of São Paulo, Brazil

Reviewed by:
Camilo Lellis-Santos, Universidade Federal de São Paulo, Brazil
Rose Anna Riel Banal, University of the City of Manila, Philippines

***Correspondence:**
Helen Wallace
hwallace@liverpool.ac.uk

Specialty section:
This article was submitted to Respiratory Physiology, a section of the journal Frontiers in Physiology

Received: *31 October 2020*
Accepted: *25 March 2021*
Published: *20 April 2021*

Citation:
Wallace H and Angus R (2021) Supplemental Learning in Respiratory Physiology for Healthcare Professionals Towards Successful Treatment of COVID-19. Front. Physiol. 12:624528. doi: 10.3389/fphys.2021.624528

INTRODUCTION

On the 11th March 2020, the World Health Organisation declared coronavirus disease 19 (COVID-19) a pandemic, meaning the illness had spread worldwide. Coronavirus disease 2019 is a viral disease caused by a coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Chan et al., 2020). A mammoth effort has been made to characterise COVID-19, and while

this knowledge is evolving weekly, less than a year in, the transmission, pathogenesis, clinical course and even longer term consequences are already described (Frazer, 2020; Muge et al., 2020).

In COVID-19 infection, the immunological response with its pathophysiological consequences can cause severe respiratory compromise affecting gas exchange and lung mechanics. This has resulted in a large number of people needing oxygen therapy or some degree of respiratory support during the pandemic, presenting major challenges to expert respiratory and critical care teams and acute clinicians. The scale of the pandemic was such that nurses, doctors and paramedical staff from outside areas of respiratory expertise moved to supplement these teams. It followed that there was an urgent need to understand the best modalities of support for people, and to solve issues of minimising aerosolisation and the potential for cross infection.

Training was an urgent need; in addition to practical clinical skills around oxygen administration and non-invasive supports such as Continuous Positive Airway Pressure (CPAP), an understanding of lung physiology and the optimisation of function with intervention was critical. While some clinicians such as theatre and anaesthetic teams had appropriate skills and knowledge, many of the clinicians assisting had nothing more than their pre-qualification education to fall back on. These clinicians needed the appropriate physiological knowledge as well as practical skills if therapies were to be applied effectively and patients properly monitored. In this article, we report on a "respiratory learning tool kit" that was developed to support healthcare professionals who found themselves in the unfamiliar territory of respiratory critical care. We outline key physiological principles and relate them to the pathophysiology that occurs as a result of COVID-19 infection. We then go on to demonstrate how this knowledge feeds into decisions on management and treatment of COVID-19 patients and how the toolkit provided the essential knowledge required.

Key Physiological Principles Relating to Pathophysiology of COVID-19

One of the manifestations of COVID-19 disease consists of pulmonary injury and entry of fluid into alveolar spaces. The resulting effect on the respiratory system can only be completely understood by re-visiting first principles on gas exchange—the process of gas exchange including relevant gas laws and diffusion, and how the respiratory system is designed to maximise this process under normal conditions (see **Supplementary Material**). Understanding the concept of gas diffusion gradients and the oxygen (O_2) alveolar-arterial (A-a) gradient is vital when faced with problem-solving clinical scenarios and when decision making on choosing the appropriate methods to improve lung mechanics and gas exchange. Of course, when considering the above, carbon dioxide (CO_2) is also an integral part of gas exchange, and the regulation of CO_2 levels and blood pH is vital to homeostasis and normal physiology. Moving on from gas exchange itself to O_2 transport provides understanding on how O_2 is delivered to the tissues. Here, the relationship between O_2 and haemoglobin and knowledge of the O_2 dissociation curve is key to understanding the dynamics of O_2 availability and the thresholds that maintain normal physiological processes.

Work of breathing and compliance are important determinants of a patient's respiratory course and symptomatology. Underlying physiological principles include the mechanics of breathing in relation to Boyle's law; the airflow, resistance and pressure gradients across the lung; and lung volumes and capacities. Integral to the understanding of lung mechanics is the central control of breathing, and how ventilation is modulated under different conditions including feedback loops from central and peripheral chemoreceptors. Local regulation of ventilation and perfusion and ventilation-perfusion rate is another key determinant of O_2 and CO_2 levels in the blood.

In addition to the pathophysiology, basic physiological principles must be applied in order to understand how different therapies can be used to treat respiratory failure. Patients with COVID-19 mainly present with type 1 respiratory failure with low O_2 levels and normal or low CO_2. The World Health Organisation (WHO) interim guidance indicates that 15% of patients require oxygen therapy, and 5% need ITU support with modalities such as continuous positive airways pressure (CPAP) or invasive ventilation (WHO, 2020). When arterial O_2 is low, the O_2 gradient becomes important to determine the source of the hypoxemia and O_2 therapy would be the most effective treatment. In instances where standard O_2 therapy is insufficient to prevent respiratory distress, other more intensive treatments would need to be considered. Another interesting observation in COVID-19 patients, is that the proning technique can be successfully used to improve lung mechanics and gas exchange. In this technique, functional alveoli are recruited more effectively in ventilated patients.

What also became clear in certain patients with COVID-19 was that clinical picture and apparent recovery often did not follow the type of patterns that clinicians routinely see with acute respiratory infection. For example, with basic bed side observations such as respiratory rate or pulse, patients often appeared well with no signs of respiratory distress; and yet when measured had remarkably low O_2 saturations. Indeed, in the popular press it was reported that patients with "remarkably low O_2 saturation, seemingly incompatible with life and clearly at risk, were using their mobile phones" (Levitan, 2020). This phenomenon has been reported across the globe in patients with COVID-19 and has been referred to as silent hypoxemia (Chandra et al., 2020). Unlike most other acute respiratory diseases, oxygen levels can fall dangerously low, without initially causing respiratory distress. By the time symptoms manifest, patients will have become in need of urgent intervention. Hypoxemia, is pre-dominantly due to the compromise of alveolar spaces or possibly micro-emboli associated with COVID-19 (Muge et al., 2020), without an apparent early change in lung mechanics. Other pathophysiological mechanisms to explain the lack of dyspnoea, include the reduced response of the respiratory centres in the brain to low O_2, particularly in elderly patients (Kronenberg and Drage, 1973; Peterson et al., 1981) and also in the presence of comorbidities like diabetes where responses are blunted (O'Donnell et al., 1988; Nishimura et al., 1989; Weisbrod et al., 2005). Again, referring back to key

physiological principles, the sensitivity of respiratory centres to minute changes in CO_2, even small increases in $PaCO_2$ will result in large increases in ventilation. Carbon dioxide levels are often normal or low in patients with COVID-19, so the body will not be alerted that there is a problem. Altered central and peripheral chemoreceptor response could be postulated, with the SARS-CoV2 ACE2 receptor cells being found centrally (Xia and Lazartigues, 2008) and in the carotid body (Fung, 2015). Further potential mechanisms include the disproportionate chemoreceptor stimulation to oxygen saturation due to temperature-induced shifts to the right of the O_2 dissociation curve; high temperature or fever is a pre-dominant feature of COVID-19. Finally, viral effects on blood vessel constriction through ACE2 receptor binding may contribute to hypoxemia by causing dysregulation of ventilation-perfusion coupling. Recognition of "silent" hypoxemia has emphasised the importance of physiological measurement, together with monitoring, to inform decisions on treatment and management. Understanding the underlying physiological mechanisms and how they are modified will serve to enhance patient care (Tobin et al., 2020).

Emerging evidence indicates that patients may experience respiratory symptoms months after contracting COVID-19 (Frazer, 2020). Predictions can also be made from the 2003 outbreak of severe acute respiratory syndrome (SARS). However many patients recovering from COVID-19 are suffering from pre-existing disease so they may demonstrate more severe long-term effects. Knowledge of lung physiology will be important in helping patients understand some post COVID features, where in some, the lung injury leads to fibrosis, altered compliance and impairment of gas exchange.

Respiratory Learning Tool Kit

We were presented with the challenge of facilitating the acquisition of effective physiological knowledge and understanding, which could not be achieved by simply referring to standard textbooks or the plethora of often-unreliable information on the internet. We achieved this by developing a digital educational resource, that includes peer reviewed explanations of key physiological principles using visualisation and animations to facilitate understanding (Hwang et al., 2012; see **Supplementary Material**). Evidence has indicated that after testing knowledge, online eLearning for undergraduates in healthcare professions was equivalent or possibly superior to traditional learning (George et al., 2014). The digital physiology educational material was initially developed by the authors, as a supplement to the traditional didactic teaching of challenging key concepts for physiology and medical students. The digital resource was based on different learning approaches with a common structure, including learning through acquisition, investigation and practice (Laurillard, 2012). These core physiological principles were then linked to pathophysiological scenarios; explaining how disease processes can alter lung function and lung mechanics leading to respiratory failure, by giving clinical examples. The resource is also interactive, testing knowledge and understanding of the user as one progresses through each tutorial. This knowledge can then be applied to key features of COVID-19 as mentioned above. This is key to understanding the clinical review, assessment, monitoring and management of respiratory patients, some of whom may be at risk of developing respiratory insufficiency. The physiological learning outcomes included in the tool kit are detailed below:

- Define and describe the principles of gas exchange i.e., diffusion and the gas laws, and the properties of the alveolar-circulatory interface
- Explain the concept of homeostasis with reference to the respiratory system
- Explain the role of lungs in maintaining acid/base balance and explain how the kidneys interact with the lungs in acid-base regulation
- Know normal values of arterial blood gases and be able to interpret their basic values
- Discuss the central control of ventilation and the role of the medulla and pons in its control
- Describe how other brain regions including cerebral cortex, cerebellum, limbic system and hypothalamus can modulate ventilation
- Describe and explain the importance of how sensory input modulates ventilation including feedback loops from peripheral chemoreceptors and central chemoreceptors

Given the context, physiological principles were taught alongside the clinical teaching on therapeutics; physiologists, together with physicians, nurses and physiotherapists shaped and delivered the material. In the context of a pandemic of a novel organism, continuing education of healthcare professionals is vital for adequate prevention and management of disease. For example, CPAP and other aerosol generating procedures can generate small particles that travel and persist in the atmosphere. In order to reduce the risk of viral spread to staff and patients, treatment is delivered via a well-fitted full facemask, with a separate exhalation valve. Cohorting patients positive for COVID-19 is also carried out, and staff follow PPE guidelines meticulously when treating patients.

Engagement and Evaluation of the Respiratory Tool Kit

To achieve the largest impact, the respiratory learning toolkit was put online, and enabled and curated by the third party charitable provider of education, "Education for Health." The toolkit went live in July as the first wave of the pandemic was settling. Five Hundred and seventy three health care professionals accessed the website and utilised the content in the first 8 weeks. More detailed data obtained from 161 users, showed that 24 users completed the toolkit, while the remaining 137 did not complete the course but were noted to spend significant periods of time on the toolkit. It was possible to complete the toolkit in 1 day and the average time spent on the learning resource was 6.35 h $\pm$ 1.76 [(SEM) n = 161]. Users accessed the toolkit an average of 2.69 times $\pm$ 0.2, ranging between 1 and 14 times. One hundred users accessed the toolkit on the same day, with the remaining 61 users revisiting on a different day ranging from within 2 days to 5 months after the first access date. Feedback was invited after

completion of the toolkit and was presented as a 5 point Likert scale as shown in **Table 1**. Levels of evaluation included "strongly disagree" through to "strongly agree". The questions included, "The online session increased my knowledge and understanding of the subject," and "I will be able to apply what I learned in my work." Results are presented from the 26 users who evaluated the course. As can be seen in **Table 1**, none of the respondents scored "disagree" or "strongly disagree." Overall, the number of people who completed the tool kit was extremely encouraging and more than expected. Of note, the time of day the toolkit was accessed varied; with earliest access noted to be at 03:49, median time of 14:28 and latest access at 22:47. This illustrates the flexible accessibility of the online learning tool. The high level of engagement and preliminary feedback indicates that the toolkit was very well received. Questions inviting free textual comments are shown in **Table 2**. Individual comments were extremely positive showing appreciation for both the physiological and clinical aspects of the course.

DISCUSSION

The main objective in the development of this learning resource was to provide health care professionals with an immediate and easy access to a respiratory learning tool kit. Such was the observed lack of knowledge in the current pandemic, that there is an urgent need for the learning material. It must be emphasised that due to the circumstances surrounding the production of the learning material, the above initiative was not primarily intended as a full blown research study as reflected in the limited data obtained. There was no formal assessment of learners at the end of the course and no formal credit for the learning; the low numbers that completed the evaluation may reflect that. This is also a common outcome in other reported technology enhanced learning programs for healthcare professionals (Nicoll et al., 2018). Studies often provide insufficient data to support transferability or direct future learning programs, and it is difficult to ascertain whether e-learning positively affects patient outcomes (Vaona et al., 2018). Nevertheless, the data available from this respiratory learning toolkit clearly demonstrate a high level of engagement, and indications from evaluations suggest that the toolkit enhanced the knowledge and facilitated the understanding of the users in respiratory physiology (**Tables 1**, **2**). As demonstrated from the large numbers who accessed and engaged in the tool kit, it was clear that the main aim was successfully achieved. Interestingly, although only a small proportion of users completed the toolkit, the average time spent on the resource was significant. This indicates that the users utilised content that was beneficial to themselves. Of further note, are the data showing the pattern of engagement, wherein a significant number of users re-visited the toolkit over a 5 month period. This suggests that users benefitted from the long term accessibility of this online platform, re-visiting the toolkit to consolidate their knowledge and understanding. Future studies will include more rigorous analysis of the self-assessment element of the toolkit to generate evidence of understanding. Further encouragement on the completion of the evaluation survey will also provide more information on the opinions and experience of users. It would be useful to determine which elements of the toolkit were utilised more than others.

The global COVID-19 pandemic requires a response from all aspects of life sciences. As described here, providing

TABLE 1 | Responses to evaluation questions.

Question	Neutral	Agree	Strongly agree	No answer
The online session increased my knowledge and understanding of the subject	3	10	12	1
I will be able to apply what I learned in my work	3	9	10	4
Total	6	19	22	5

TABLE 2 | Free text comments to evaluation question.

Question	Response
Additional comments, this includes something you think could be added to/changed about the online content	"I think everything was up to date considering knowledge was limited at time of development of this course" "I feel very strongly that all staff working with acute Covid 19 patients should complete this course and it should be free to all Trusts to facilitate this. The quality of information is excellent and will go some way to ensuring that there is parity of knowledge across the NHS" "Short quizzes at the end of each section to test knowledge/learning" "Enjoyable course—easily completed in 1 day
Please tell us something you learned that you plan to put into practice in your work	"Correct management of the NIV patient, when to step up and step down patients to manage them effectively" "Acute Use of NIV and CPAP safely" "Learned a lot about NIV and respiratory care in relation to COVID-19" "More detailed questions to ask when assessing patients with breathlessness. I will be able to incorporate this into my patient assessments" "I will take a more active role in the setting up of patients on NIV/CPAP, ensuring correct settings, circuits etc." "Understanding difference between BIPAP/CPAP" "Being clearer on the physiology behind respiration" "Always provide evidence based best practiced care to patients" "Looking more closely at blood gases"

a combination of practical and physiological understanding acquired from the learning toolkit gives clinicians a foundational knowledge to practice with confidence in such an uncertain environment. As an example, silent hypoxia is described in some articles as "baffling." With reflection, that need not be the case if one considers what is known about physiological response changes with age, temperature and comorbidities, in particularly diabetes. Severe acute respiratory syndrome coronavirus 2 may also have a direct effect on nerve function such as chemoreceptors, given the ACE2 receptor distribution and the effect of the virus on the olfactory nerve. The effect of coronavirus on chemoreceptors would indeed be a valuable research question that could partly explain the delayed pulmonary manifestation in patients. That said, the pattern of COVID-19 was something that needed to be described, learned and applied as effective management are evolved and researched. It is a credit to life sciences and medicine the way this has happened and continues.

This collaboration of university staff, clinicians and the charity team allowed the provision of a digital resource showing a high degree of engagement; it was easily accessible on any device, anytime, anywhere. Going forward, effective assessment and evaluation tools must be included in online learning tools to demonstrate knowledge acquisition and the overall degree of learner satisfaction. Further studies are also needed to assess the effects of technology enhanced learning on patient outcomes. Learning resources applying core physiological principles and practical application in this context can be expanded to a wider audience including post-graduate students, medical students and translational physiologists. The proper understanding of known physiology and pathophysiological responses should enhance patient management, focus research questions and certainly remove a degree of the "bafflingly."

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

HW completed the physiological aspects of the respiratory toolkit, including animations, slides, audio and learning outcomes, and contributed to the writing of the manuscript. RA oversaw all aspects of the clinical content of the respiratory toolkit including identifying learning outcomes, and contributed to writing the manuscript. Both authors contributed to the article and approved the submitted version.

FUNDING

The toolkit was supported by Education for Health: Dr. Linda Edwards Chief Executive, Ann Saxon Director of Learning Design and Quality, Preeti Minhas Head of Respiratory, Fiona Kilburn Senior IT Manager and Stephen Ryan, Director of Innovations, http://www.EducationforHealth.org.

ACKNOWLEDGMENTS

We would also like to thank Andrew Evans Think Philanthropy, https://www.thinkphilanthropy.com, Karen Ward, Consultant Physiotherapist Liverpool University Hospitals NHS Foundation Trust, Verity Ford, Principal Physiotherapist Liverpool University Hospitals NHS Foundation Trust and Han Duijvendak, Producer/Director, HANDSTAND Films, http://www.handstand-uk.eu. Moreover, the design and development of the physiology animations was supported by Dr. Rebecca Hamm and the Centre for Educational Development and Support (CEDS), University Of Liverpool.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.624528/full#supplementary-material

REFERENCES

Chan, J. F., Yuan, S., Kok, K. H., To, K. K., Chu, H., Yang, J., et al. (2020). A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 395, 514–523. doi: 10.1016/S0140-6736(20)30154-9

Chandra, A., Chakraborty, U., Pal, J., and Karmakar, P. (2020). Silent hypoxia: a frequently overlooked clinical entity in patients with COVID-19. *BMJ Case Rep. CP* 13:e237207. doi: 10.1136/bcr-2020-237207

Frazer, E. (2020). Long term respiratory complications of covid-19. *BMJ* 370:m3001. doi: 10.1136/bmj.m3001

Fung, M. L. (2015). Expressions of angiotensin and cytokine receptors in the paracrine signaling of the carotid body in hypoxia and sleep apnea. *Respir. Physiol. Neurobiol.* 209, 6–12. doi: 10.1016/j.resp.2014.09.014

George, P. P., Papachristou, N., and Belisario, J. M. (2014). Online eLearning for undergraduates in health professions: A systematic review of the impact on knowledge, skills, attitudes and satisfaction. *JGH* 4, 1–17. doi: 10.7189/jogh.04.010406

Hwang, I., Tam, M., Lam, S., and Lam, P. (2012). Review of Use of Animation as a Supplementary Learning Material of Physiology Content in Four Academic Years. *Electr. J. e-Learning* 10, 368–377.

Kronenberg, R. S., and Drage, C. W. (1973). Attenuation of the ventilatory and heart rate responses to hypoxia and hypercapnia with aging in normal men. *J. Clin. Invest.* 52, 1812–1819. doi: 10.1172/jci107363

Laurillard, D. (2012). *Teaching as a Design Science: Building Pedagogical Patterns for Learning and Technology*. London: Routledge.

Levitan, R. (2020). *The infection that's silently killing coronavirus patients*. New York, NY: The New York Times.

Muge, C., Krutika, K., Jason, K., and Malik, P. (2020). Virology, transmission, and pathogenesis of SARS-CoV-2. *BMJ* 371:m3862. doi: 10.1136/bmj.m3862

Nicoll, P., MacRury, S., and Smyth, K. (2018). Evaluation of technology-enhanced learning programs for healthcare professionals: systematic review. *J. Med. Internet Res.* 20:e131. doi: 10.2196/jmir.9085

Nishimura, M., Miyamoto, K., Suzuki, A., Yamamoto, H., Tsuji, M., Kishi, F., et al. (1989). Ventilatory and heart rate responses to hypoxia and hypercapnia in patients with diabetes mellitus. *Thorax* 44, 251–257. doi: 10.1136/thx.44.4.251

O'Donnell, C. R., Friedman, L. S., Russomanno, J. H., and Rose, R. M. (1988). Diminished perception of inspiratory-resistive loads in insulin-dependent diabetics. *N. Engl. J. Med.* 319, 1369–1373. doi: 10.1056/nejm198811243192102

Peterson, D. D., Pack, A. I., Silage, D. A., and Fishman, A. P. (1981). Effects of aging on ventilatory and occlusion pressure responses to hypoxia and hypercapnia. *Am. Rev. Respir. Dis.* 124, 387–391.

Tobin, M. J., Laghi, F., and Jubran, A. (2020). Why COVID-19 Silent Hypoxemia Is Baffling to Physicians. *Am. J. Respir. Crit. Care Med.* 202, 356–360. doi: 10.1164/rccm.202006-2157cp

Vaona, A., Banzi, R., Kwag, K. H., Rigon, G., Cereda, D., Pecoraro, V., et al. (2018). E-learning for health professionals. *Cochrane Database Syst. Rev.* 1:CD011736. doi: 10.1002/14651858.CD011736.pub2

Weisbrod, C. J., Eastwood, P. R., O'Driscoll, G., and Green, D. J. (2005). Abnormal ventilatory responses to hypoxia in Type 2 diabetes. *Diabet. Med.* 22, 563–568. doi: 10.1111/j.1464-5491.2005.01458.x

WHO (2020). *Clinical management of COVID-19: interim guidance*. Geneva: WHO.

Xia, H., and Lazartigues, E. (2008). Angiotensin-converting enzyme 2 in the brain: properties and future directions. *J. Neurochem.* 107, 1482–1494. doi: 10.1111/j.1471-4159.2008.05723

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

PERSPECTIVE
published: 29 June 2021
doi: 10.3389/fphys.2021.628288

How and to What Extent Immunological Responses to SARS-CoV-2 Shape Pulmonary Function in COVID-19 Patients

Pierantonio Laveneziana[1,2†], Christian Straus[1,2†] and Silke Meiners[3†]*

[1] Assistance Publique - Hôpitaux de Paris (AP-HP), Groupe Hospitalier Universitaire APHP-Sorbonne Université, Sites Pitié-Salpêtrière, Saint-Antoine et Tenon, Service des Explorations Fonctionnelles de la Respiration, de l'Exercice et de la Dyspnée (Département R3S), Paris, France, [2] Sorbonne Université, INSERM, UMRS 1158 Neurophysiologie Respiratoire Expérimentale et Clinique, Paris, France, [3] Comprehensive Pneumology Center (CPC), University Hospital, Ludwig-Maximilians University, Helmholtz Zentrum München, Member of the German Center for Lung Research (DZL), Munich, Germany

COVID-19 is a disease caused by a new coronavirus SARS-CoV-2, primarily impacting the respiratory system. COVID-19 can result in mild illness or serious disease leading to critical illness and requires admission to ICU due to respiratory failure. There is intense discussion around potential factors predisposing to and protecting from COVID-19. The immune response and the abnormal respiratory function with a focus on respiratory function testing in COVID-19 patients will be at the center of this Perspective article of the Frontiers in Physiology Series on "The Tribute of Physiology for the Understanding of COVID-19 Disease." We will discuss current advances and provide future directions and present also our perspective in this field.

Keywords: COVID-19, immune response, respiratory function testing, respiratory physiology, diffusing capacity for lung carbon monoxide

OPEN ACCESS

Edited by:
Silvia Demoulin-Alexikova, Université de Lorraine, France

Reviewed by:
Barbro N. Melgert, University of Groningen, Netherlands
Hari H. Subramanian, Boston Scientific, United States

****Correspondence:***
Pierantonio Laveneziana
pierantonio.laveneziana@aphp.fr

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Respiratory Physiology, a section of the journal Frontiers in Physiology

Received: *12 November 2020*
Accepted: *31 May 2021*
Published: *29 June 2021*

Citation:
Laveneziana P, Straus C and Meiners S (2021) How and to What Extent Immunological Responses to SARS-CoV-2 Shape Pulmonary Function in COVID-19 Patients. Front. Physiol. 12:628288. doi: 10.3389/fphys.2021.628288

INTRODUCTION

COVID-19 is a disease caused by a new coronavirus SARS-CoV-2, primarily impacting the respiratory system. COVID-19 can result in mild illness or serious disease leading to critical illness and requires admission to ICU due to respiratory failure.

A major unresolved conundrum is the large spectrum of clinical presentations of patients with COVID-19, ranging from asymptomatic infections or symptomatic mild infections with fever, headache or mild respiratory symptoms (like cough or sore throat) and malaise in 80–85% of patients to flu-like illness and viral pneumonia. Within the "pneumonia phenotype" we also have a large clinical and pathophysiological spectrum that extends from only minor opacification with near normal chest radiographs and mild hypoxemia (in ∼80% of hospitalized patients). Some of these patients develop an acute respiratory failure with severe hypoxemia of quick progression to a phenotype presenting with greater hypoxemia and higher respiratory rates (∼15% of hospitalized patients) to severe diseases manifestations. Seriously ill patients develop severe hypoxemia requiring mechanical ventilation. Their CT scans document edema in the lower lobes, Angio-CTs detect multiple ground-glass opacities often showing micro-embolic lesions and lung ultrasonography that are consistent with interstitial injury with B lines (white lung). This latter phenotype is compatible with an organizing pneumonia with hypoxic vasoconstriction associated

with severe hypoxemia (∼2/3 of patients requiring mechanical ventilation). The last phenotype, less common than the previous one, represents an advanced stage with associated acute lung injury requiring mechanical ventilation (Rello et al., 2020). A subset of severe COVID-19 patients also present with coagulation defects with elevated levels of D-dimers and fibrinogen suggesting thrombotic microangiopathy and vasculopathy in the gas-exchange networks and systemically (Huertas et al., 2020; Iba et al., 2020; Vinayagam and Sattu, 2020). This latter phenotype suggests a combination of respiratory and vascular dysfunction in the lungs of severely ill COVID-19 patients which was confirmed in several pathological studies recently (Ackermann et al., 2020; Potus et al., 2020). The particular feature of SARS-CoV2 to induce both respiratory and vascular dysfunction has been established in the past year (Del Turco et al., 2020; Varga et al., 2020).

Increasing evidence suggests that these diverse clinical phenotypes might be explained by the immunological failure to control and restrict SARS-CoV2 infection of the lung. Failure and skewing of the adaptive immune system, promiscuous infection of epithelial (pneumocytes), endothelial as well as immune cells, coagulation defects and uncontrolled neutrophilic activation potentially govern the impact of COVID-19 on respiratory function and clinical phenotypes (Ackermann et al., 2020; Casadevall and Pirofski, 2020; Del Turco et al., 2020; Gordon et al., 2020a; Tay et al., 2020; **Figure 1**). An increased understanding of the immunological dysfunction underlying the different clinical phenotypes of COVID-19 survivors impacts the management of clinical and pathophysiological consequences of this disease. The immune response and the abnormal respiratory function with a focus on respiratory function testing in COVID-19 patients will be at the heart of this Perspective article of the Frontiers in Physiology Series on "The Tribute of Physiology for the Understanding of COVID-19 Disease." We will discuss current advances and provide future directions and present also our perspective in this field.

RESPIRATORY IMMUNOLOGY

SARS-CoV-2 infection primarily targets the respiratory tract. For the virus to effectively enter the host cell it requires the membrane expression of angiotensin-converting enzyme-2 (ACE2) together with cofactors, such as transmembrane serine protease 2 (TMPRSS2), and furin (Hoffmann et al., 2020; Lukassen et al., 2020; Sungnak et al., 2020; Wang et al., 2020). A plethora of studies analyzed expression of these factors in lung cells using single cell RNA sequencing (scRNA seq), *in situ* hybridization as well as immunohistochemistry profiling (Hikmet et al., 2020; Hou et al., 2020; Lukassen et al., 2020; Sungnak et al., 2020; Ziegler et al., 2020). Despite some surprising controversies, the emerging view is that ciliated airway cells and alveolar type 2 epithelial cells are the primary targets for SARS-CoV-2.

Upon virus infection, cells directly respond to the infectious agents by activating a protective type I interferon program. This includes the secretion of type I interferons (IFN I) that serve to initiate, amplify, and/or sustain host inflammatory responses (Martin et al., 1997; Yoshikawa et al., 2009). In particular, monocytes/macrophages and natural killer (NK) cells are IFN-responsive cells of the innate immune system. They play a major role in raising an efficient T cell-mediated adaptive immune response and thereby determine the outcome of virus infections (Frieman et al., 2008; Lazear et al., 2019). NK cells directly kill virally infected cells via their spontaneous cytolytic activity and activate the innate and adaptive immune system via secreting a variety of soluble mediators. Monocytes have specialized roles in the human lung (Landsman et al., 2007; Bassler et al., 2019; Kulikauskaite and Wack, 2020). Tissue resident macrophages, such as alveolar macrophages (AM) of the lung play a major role in early innate immunity against infections and environmental challenges (Kopf et al., 2015; Wynn and Vannella, 2016; Watanabe et al., 2019). Together with patrolling monocytes these macrophages engulf apoptotic cells and can cross-present engulfed antigen to T cells eliciting adaptive immune responses (Kopf et al., 2015; Thomas et al., 2015). Activation of cytotoxic $CD8^+$ T cell responses is essential for fast clearing of infected cells and maintaining long-term suppression of viral infections via immunological memory (Zuniga et al., 2015; Schmidt and Varga, 2018). This type I interferon triggered immune activation allows confinement of the virus infection in a time (usually within 3 days) and spatial manner and development of adaptive immune cell memory (Schmidt and Varga, 2018).

SARS-CoV2 effectively suppresses type I interferon responses in infected cells. Several SARS-CoV and SARS-CoV2 encoded viral proteins have been demonstrated to interfere with the IFN I signaling pathway (Kopecky-Bromberg et al., 2007; Shi et al., 2014, 2019; Gordon et al., 2020b)[1]. The infected cells of the respiratory epithelium thus fail to launch a robust IFN-I response to SARS-CoV2 but at the same produce exuberant inflammatory cytokines which disrupts a balanced anti-viral innate and adaptive immunity (Angka et al., 2020; Blanco-Melo et al., 2020; Schultze and Aschenbrenner, 2021). Failure of the adaptive immune system to confine the SARS-CoV2 infection to the upper respiratory tract within the first days post infection may contribute to the observed biphasic disease course in patients that develop COVID-19 pneumonia (Jesenak et al., 2020; Tay et al., 2020). Indeed, in the blood, the number of adaptive immune cells and their functionality is reduced in severely ill patients (Zhou et al., 2020). In particular, lymphopenia is the most consistent laboratory abnormality in severe COVID-19–infected patients. Progressive lymphodepletion and signs of T cell exhaustion are observed in patients who clinically deteriorate with severe COVID-19 (Diao et al., 2020; Zhang et al., 2020). In contrast, reappearance of effector T cells associates with recovery from the disease (Odak et al., 2020). Moreover, lungs from patients who succumbed to SARS-CoV2 show extensive cellular immune infiltrates with macrophages representing a prominent cell type (Barton et al., 2020; Ruscitti et al., 2020; Tian et al., 2020b; Schultze and Aschenbrenner, 2021). These data strongly indicate that a defective type I interferon response at the onset of infection is key to the observed uncontrolled immune responses, such as

[1]https://www.biorxiv.org/content/10.1101/2020.06.17.156455v1

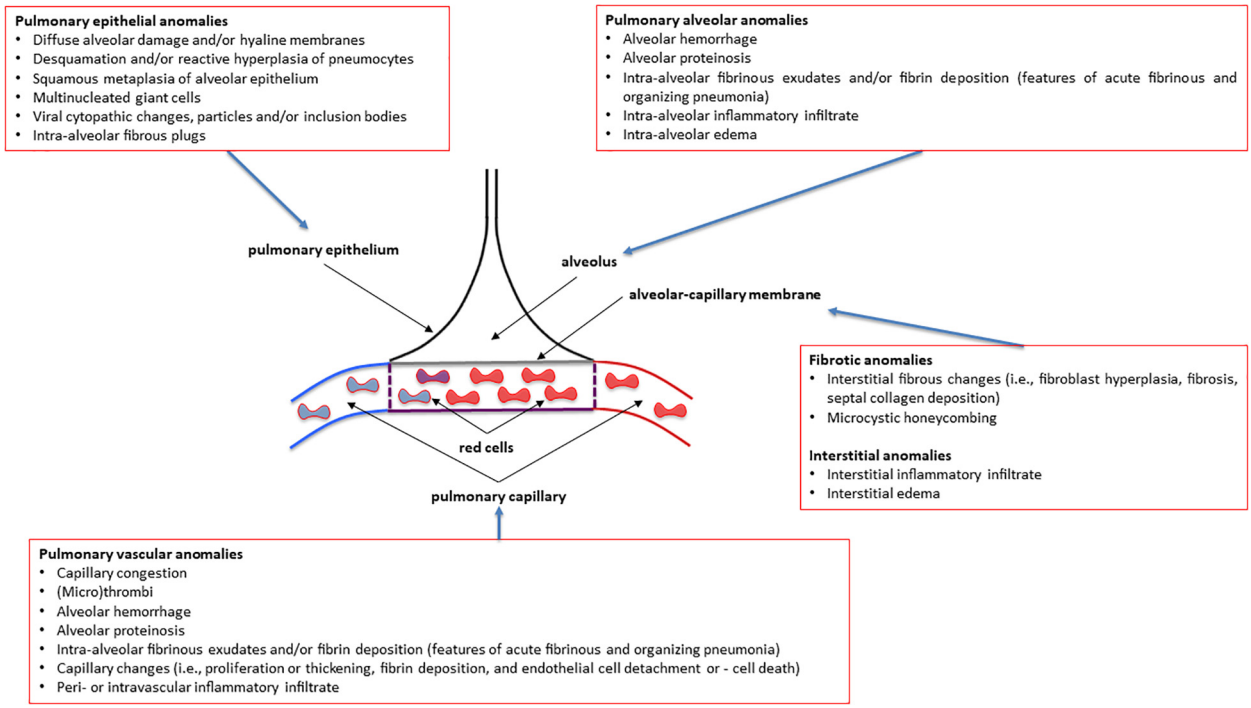


FIGURE 1 | Overview of the most common pulmonary pathology findings observed in post-mortem patients affected by various degrees of severity of COVID-19 (coronavirus disease 2019). See the text for more details and explanations. This is an original figure, no permission to reproduce is required.

excessive cytokine production ("cytokine storm") and impaired protective T cell responses (Gordon et al., 2020b; Tay et al., 2020). This scenario might involve an imbalanced activation of NK and T cells as described for severe influenza infections (Frank and Paust, 2020).

Defective local and temporal confinement of SARS-CoV2 virus replication due to impaired type I interferon responses in the primarily infected lung epithelial cells may result in a spill-over of the infection into the vascular system of the lung. Such spreading of the infection will be fostered by excessive inflammatory signaling in the alveolus that causes disruption of the basement membrane, leakiness of alveolar capillaries and massive immune cell recruitment. SARS-CoV2 is able to infect endothelial and immune cells (Ackermann et al., 2020; Huertas et al., 2020; Iba et al., 2020; Potus et al., 2020)[2] suggesting some promiscuity in terms of host cell selectivity (tropism) as suggested previously as a general feature of Coronoviruses (Hulswit et al., 2016). The tropism of a virus is defined as its ability to infect specific cell types, organs or species. This capacity depends on the expression of receptors for viral entry, e.g., ACE2 for SARS-CoV2, and cofactors, such as TMPRSS2 and furin, but also on the permissiveness of the cell to allow virus replication and support productive infection. Accordingly, viral tropism is determined by multiple viral and host cell factors (Adler et al., 2017). Not only more and more host factors are identified that fine-tune SARS-CoV2 entry and replication in host cells (Hou et al., 2020), but also new receptors are being uncovered, such as for example neuropilin-1, which is highly expressed in fibroblasts, brain and endothelial cells (Cantuti-Castelvetri et al., 2020)[3]. These factors might determine the rather broad tropism of SARS-CoV2 as observed in autopsied lungs of COVID-19 patients (Ackermann et al., 2020; Huertas et al., 2020; Iba et al., 2020; Potus et al., 2020) and in *ex vivo* models and cells (Hui et al., 2020). Promiscuity of the SARS-CoV2 with regard to host cell tropism together with unconfined infection resulting in high local virus load might then result in aberrant infection of endothelial and recruited immune cells in addition to inflammatory activation of these cells.

Damaged respiratory epithelial cells and pulmonary endothelial dysfunction activate platelets and formation of intravascular microthrombi (Connors and Levy, 2020; Del Turco et al., 2020). These pathophysiological changes will contribute to impaired hypoxemic vasoconstriction and the clinical phenotype of "happy hypoxemia" in COVID-19 patients (Dhont et al., 2021). If the immune system doesn't gain control, endothelial dysfunction and coagulation defects might spread systemically causing vasculitis, disseminated intravascular coagulopathy (DIC) and immunothrombosis (Engelmann and Massberg, 2013; Jackson et al., 2019). This scenario is fully supported by the recent study on SARS-CoV2 infected Macaques that developed severe vascular disease and pulmonary thrombosis (Aid et al., 2020). Defective coagulation has been observed in severe COVID-19 patients as evidenced by thrombocytopenia, elevated levels of D-Dimer and of fibrin/fibrinogen degradation products (for an overview see Iba et al., 2020).

[2]https://doi.org/10.1101/2020.09.25.20195818

[3]www.proteinatlas.org

How can we relate the above-described immune dysregulation in severely ill COVID-19 patients to altered lung pathology and respiratory function? As the presence of D-Dimer correlates with reduced lung function and DLCO, one could envision that coagulation defects in the lung capillaries and the formation of microthrombi contribute to impaired gas exchange in COVID-19 patients with severe disease (**Figure 1**; Zhao et al., 2020). Gas exchange will also be hampered by extracellular matrix deposition upon repair of the damaged respiratory epithelium in COVID-19 survivors (**Figure 1**). Fibrotic remodeling of the lung might be driven by skewing of adaptive T cell responses toward impaired regulatory T cell ($CD4^+$ Treg) function and increased Th17 differentiation as observed in severely ill COVID-19 patients (De Biasi et al., 2020; Zhang and Zhang, 2020). Th17 is a well-known $CD4^+$ T cell subset causally involved in organ fibrosis including the lung (Barron and Wynn, 2011; Way et al., 2013). Along these lines, reduced numbers of $CD4^+$ Tregs have been observed in severe cases of COVID-19 together with increased levels of cytotoxic follicular helper cells and cytotoxic T helper cells (Meckiff et al., 2020). An additional line of evidence suggests the involvement of inflammatory neutrophils that persist in the lung. Recent data from autopsies of deceased COVID-19 patients demonstrated prominent activation of neutrophils in the lung with extracellular NET formation and alterations in extracellular matrix deposition together with multiorgan dysfunction (Schurink et al., 2020; Wu et al., 2020). Of note, in some patients the viral infection load of the diseased lung tissue was minimal suggesting that the immune system was unleashed at some point independent of the virus infection (Casadevall and Pirofski, 2020; Wu et al., 2020). An unpublished preprint suggests that neutrophil NET formation promotes the transition of lung epithelial cells toward a mesenchymal phenotype that might contribute to fibrotic lung remodeling[4].

PHYSIOLOGY AND PATHOPHYSIOLOGY OF ABNORMAL PULMONARY FUNCTION VARIABLES AS OBSERVED IN COVID-19 SURVIVORS

Altered lung diffusion capacity is the most common anomaly followed by restrictive ventilatory defect. This section attempts to describe the physiology and pathophysiology that underlies the three most common abnormal pulmonary function variables observed in COVID-19 survivors: TLCO, TLCO/VA and Total Lung Capacity. A particular focus will be paid on highlighting the difference between TLCO and TLCO/VA and on what is important about having a greater decline in TLCO than in TLCO/VA, and how this feeds back to lung pathology.

The lung transfer (or diffusing) capacity for carbon monoxide (CO) [TLCO or DLCO; TLCO being more commonly used in North-America whereas DLCO being more commonly used in Europe] reflects the capacity of CO transfer from the environment to the pulmonary capillary blood and represents the most clinically practical standard methodology to assess the gas exchange in the lung. In this review we will use the term TLCO. KCO, the transfer or diffusion coefficient is the rate constant for CO uptake from alveolar gas and is impacted mostly by the thickness and area of the alveolar capillary membrane, the volume of blood circulating in pulmonary capillaries coupling ventilated alveoli and the concentration and properties of hemoglobin in the alveolar capillaries blood (**Figures 2A**,**B**). KCO and the alveolar volume (VA) are the two main factors that determine TLCO (**Figures 2A**,**B**). From a mathematical standpoint, KCO can be calculated as TLCO/VA under BTPS conditions (Body Temperature, ambient Pressure, Saturated with water vapor). It should be noted that TLCO/VA is not a simple ratio as the relationship between lung volume and CO uptake is certainly less than 1:1 (Hughes and Pride, 2012). The use of KCO has recently been recommended instead of TLCO/VA, as TLCO/VA may be interpreted that TLCO can be normalized for VA (Graham et al., 2017).

A low TLCO is not exclusively determined by reduced VA (Nusair, 2020), and residual interstitial anomalies (Chen et al., 2020; Mo et al., 2020; Qin et al., 2021) and pulmonary vascular anomalies (i.e., abnormal capillary-alveolar units) (Morris et al., 2021) may play a fundamental role and this could be also the case in COVID-19 survivors (**Figures 2A**,**B**). This holds true as the interpretation of low TLCO must consider the complex relationship between VA, TLCO and KCO, and may inopportunely exclude the presence of abnormal gas exchange in the lung (**Figures 2A**,**B**). To prove this point, we can use data from "severe pneumonia" COVID-19 related patients discussed in this review to model according to Hughes and Pride (2012) what TLCO and KCO responses would be expected if VA was diminished as a consequence of either suboptimal alveolar expansion or due to loss of alveolar units while having a normal expansion in communicating alveoli. We would then observe two trajectories: the first one is that the decline in TLCO would be largely greater than expected if a decrease in VA was the unique anomaly, regardless of the mechanism behind the diminished VA; the second one is that a decrease in VA due to either above-mentioned mechanism would be associated with an augmentation in KCO, which would be contrary to the diminished KCO observed in many of the discharged patients with severe COVID-19. Therefore, the decrease in KCO may suggest that loss of alveolar units is not sufficient to determine the observed alteration in TLCO. Thus, while the anomalies in TLCO observed in "severe pneumonia" COVID-19 related patients explored in several studies may be partially explained by diminished VA, the decrease in KCO measured together with the diminished VA also implies that abnormal gas exchange in the lung occurs. Now, the question arises as whether this is due to anomaly of the alveolar-capillary barrier or to abnormal pulmonary blood volume. Unfortunately, this cannot be easily determined based on data presented in these studies. Lung fibrosis associated with acute respiratory distress syndrome in COVID-19 patients, would likely alter alveolar-capillary units, giving rise to loss of alveolar units and altered gas exchange in the lung. The consequence would be a decrease in both VA and KCO (for that diminished VA). There is mounting

[4]https://doi.org/10.1101/2020.11.09.374769

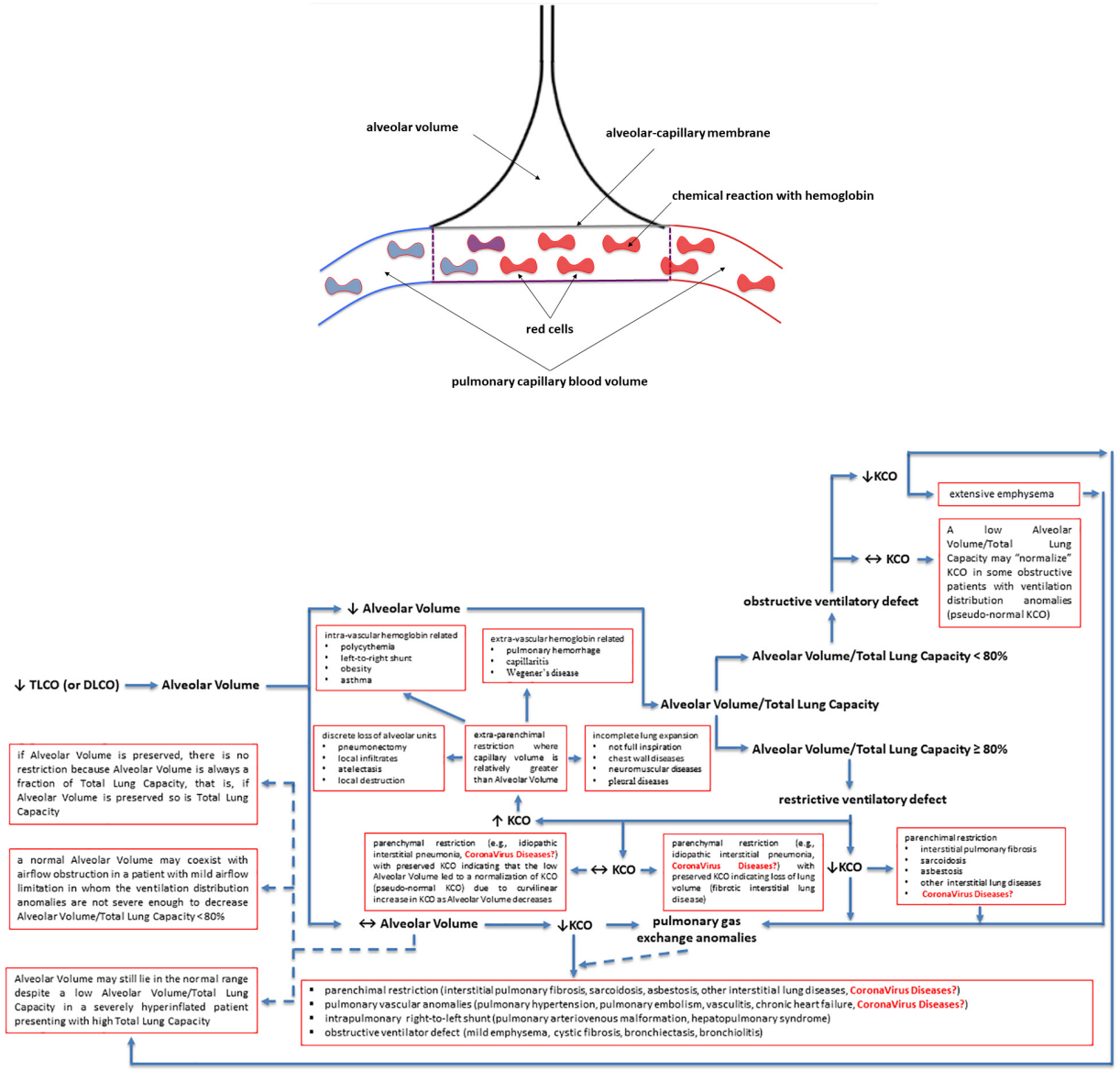


FIGURE 2 | Factors contributing to a decreased lung transfer (or diffusing) capacity for carbon monoxide (CO) (TLCO or DLCO) and the algorithm that allows physiologists and clinicians to unravel its mechanisms. If TLCO (or DLCO) is reduced, the next step is to check whether the Alveolar Volume is preserved or reduced. If Alveolar Volume is diminished, the next step is to check whether the Alveolar Volume/Total Lung Capacity ratio is low (<80%) due to ventilation maldistribution secondary to an obstructive ventilatory defect or is preserved (≥80%) due to restrictive ventilatory defect, associated or not with impaired pulmonary gas exchange. If Alveolar Volume is preserved, please follow the arrows in the algorithm to get some explanations and to see whether the KCO is reduced and there are pulmonary gas exchange anomalies associated with. KCO, transfer or diffusion coefficient (KCO = TLCO/Alveolar Volume or DLCO/Alveolar Volume); Coronavirus diseases is written in red as potential yet not fully understood mechanisms explaining the TLCO or DLCO anomalies observed in Coronavirus diseases, such as COVID-19 (caused by SARS-CoV-2), SARS (caused by SARS-CoV-1) and MERS (caused by MERS-CoV); SARS, Severe Acute Respiratory Syndrome; MERS, Middle East Respiratory Syndrome; CoV, coronavirus; COVID-19, coronavirus disease 2019. See the text for more details and explanations. This is an original figure, no permission to reproduce is required.

evidence for impaired pulmonary hemodynamics in COVID-19 patients (Potus et al., 2020), including vascular pruning, decreased pulmonary blood volume and abnormal pulmonary blood volume distribution as measured via high resolution CT (Lins et al., 2020; Morris et al., 2021). **Figures 2A,B** shows that a decrease in KCO may develop in the context of alveolar-capillary damage, microvascular pathology, or anemia. Factors responsible for a reduced VA are numerous and may include decreased alveolar expansion, alveolar damage or loss, or inspired gas maldistribution in the context of obstructive ventilator defect. Therefore, when KCO turns normal, in the presence of a low TLCO, it is associated with reduced VA,

thus indicating a restrictive ventilator defect (see below and **Figures 2A**,**B**). This is because only the functional alveolar units have been sampled thereby providing an erroneous picture toward more preserved areas of the lungs (**Figures 2A**,**B**). It should be noted that if VA is preserved, there is no restrictive ventilatory defect because VA is always a fraction of Total Lung Capacity, i.e., if VA is preserved so is Total Lung Capacity (**Figures 2A**,**B**). To conclude and for the sake of clarity: the same TLCO may occur with various combinations of VA and KCO, each suggesting different abnormal respiratory conditions. It is difficult to interpret which one plays the predominant role because both diminished alveolar volume and KCO concur to the pathogenesis of altered lung diffusion capacity. TLCO gives a global evaluation of gas exchange in the lung, while the alveolar-capillary membrane diffusing capacity only depends on molecular diffusion of the membranes. We would thus need more refined techniques capable of measuring more specifically the alveolar-capillary membrane. These could include measurement of TLCO with inhaled gas mixtures containing two or three different oxygen fractions, or combined TLCO and diffusing capacity measurements of the lung for nitric oxide (DLNO). Such sophisticated analysis would shed light on the precise mechanisms of reduced TLCO in COVID-19 survivors and allows distinguishing between interstitial and pulmonary capillary anomalies (see "Future directions, perspectives and conclusions" section).

The second most common abnormality in COVID-19 survivors is a restrictive ventilatory defect. A restrictive ventilatory defect is defined by a pathologically decreased total lung capacity. If caused by parenchymal lung disease, restrictive ventilator defect is accompanied by reduced gas transfer, which may be marked clinically by desaturation after exercise or even at rest (see the above paragraph).

Total lung capacity is the greatest volume of gas in the lungs achieved after maximal voluntary inspiration. It depends on the static balance between the outward forces generated by inspiratory muscles during a maximal inspiratory effort and the inward elastic forces of the chest wall and lung. It is the lung that normally contributes the most to the elastic recoil forces of the respiratory system at total lung capacity. At total lung capacity, these two sets of forces are equal and opposite in sign. The decrease in total lung capacity usually reflects the reduced lung volumes either because of an alteration in lung parenchyma or because of a disease of the pleura, chest wall, or neuromuscular apparatus that may affect the pressure-generating capacity of the inspiratory muscles or the compliance of the lung or the compliance of the chest wall. Interstitial lung anomalies as such those observed in some forms of COVID-19 (Polak et al., 2020) may result in a restrictive ventilatory defect (**Figures 1**, **2**).

ABNORMAL RESPIRATORY FUNCTION IN COVID-19 PATIENTS

Respiratory function testing has been performed in COVID-19 survivors at the time of hospital discharge and weeks after hospital discharge. This seems an important issue when dealing with COVID-19 survivors as these respiratory function testing anomalies may have a huge impact on the management, independency and quality of life of these patients as well as on the healthcare systems.

At the Time of Hospital Discharge

In the Fumagalli' study 13 patients with COVID-19 pneumonia were enrolled and the authors found that at the time of clinical recovery, 10 out of 13 patients presented with a restrictive pattern measured at spirometry: forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) were lower compared to lower limit of normality values, while FEV_1/FVC was higher compared to the upper limit of normality values. These results obtained in a very small sample size should be taken with caution as measure of Total Lung Capacity, preferably with plethysmography, was not included and the diagnosis of restrictive pattern was made exclusively on the reduced FVC, which is questionable and not acceptable (Pellegrino, 2005). In addition, TLCO measurement was not employed; this would have permitted a better understanding of the origin and the quality of pulmonary gas exchange damage.

In the Mo' study 110 patients with COVID-19 infection were enrolled, which included 24 cases of mild illness, 67 cases of pneumonia and 19 cases of severe pneumonia (Mo et al., 2020). Spirometry, plethysmography and TLCO tests were performed on the day of or one day before hospital discharge. The authors found that 47% of their patients had anomalies in TLCO, 25% in TLC, 14% in FEV_1, 9% in FVC, 4.5% in the FEV_1/FVC ratio and 7% in small airway function. The most interesting observation was the significant difference in impaired TLCO among the different groups of severity, which accounted for 30% in mild illness, 42% in pneumonia and 84% in severe pneumonia, respectively ($p < 0.05$). This trend of the gradual decrease in level of TLCO among patients was identical with the varying degree of severity. Of note, in 50% of the TLCO -impaired patients, the TLCO corrected for alveolar volume (TLCO/VA) was still within the normal range, which might indicate that TLCO decrease was more than the TLCO/VA in recovered subjects. In addition, the value of TLC as% of predicted in severe pneumonia cases was much less than that of pneumonia or mild illness, suggesting higher impairment of lung volume in severe cases. No significant difference among the discharged survivors with different severity in regard to other ventilatory defects (e.g., reduced FEV_1/FVC) was observed.

These two studies, strongly suggest that respiratory function needs to be carefully investigated in COVID-19 patients, as it was already done for other atypical pneumonia, such as severe influenza A (H1N1) pneumonia (Hsieh et al., 2018). This is because the lung is the most affected organ by COVID-19 and previous other atypical pneumonia, with anomalies that include diffuse alveolar epithelium destruction, capillary damage/bleeding, hyaline membrane formation, alveolar septal fibrous proliferation, and pulmonary consolidation.

In Discharged Patients

In the same study by Fumagalli and co-workers FVC was still lower than the lower limit of normality after 6 weeks from hospital discharge (Fumagalli et al., 2020). Again here, these results obtained in a very small sample size should be taken with caution as measure of TLC was not included and the diagnosis of restrictive pattern was made exclusively on the reduced FVC, which is questionable and not acceptable (Pellegrino, 2005). Another study by Huang et al. (2020) performed respiratory function testings in 57 COVID-19 patients after 30 days of hospital discharged and found anomalies in 75% of them; 10, 9, 44, 12, and 53% of enrolled patients had FVC, FEV_1, FEV_1/FVC ratio, TLC, and TLCO values less than 80% of predicted values, respectively, whereas 49 and 23% of patients presented with maximum static inspiratory and expiratory pressure (PImax and PEmax, respectively) values less than 80% of the corresponding predicted values. Compared with non-severe cases (n = 40), severe patients (n = 17) showed higher incidence of TLCO impairment (76 vs. 43%, p = 0.019), and significantly lower percentage of predicted TLC. Of note, only 11% of patients showed obstructive and 12% restrictive ventilatory defects (Huang et al., 2020). What is also striking yet surprising is that a small percentage of patients with no residual imaging abnormalities presented with a slight decrease in TLCO. Similar to this study, Frija-Masson et al. (2020) observed abnormal lung function in more than 50% of COVID-19 patients after 30 days of hospital discharge. Almost one third of these patients had decreased TLCO values indicating that these patients have lung vascular damage which coincides with data from Huang et al. (Huang et al., 2020).

On the contrary, Rogliani et al. (2020) have recently pointed out that hospitalized patients with mild-to-moderate forms of COVID-19 are not at risk of developing pulmonary fibrosis. In their study, patients were enrolled within two months from hospital discharged and authors found that FEV1 and FVC, both expressed as % predicted, were in the normal range. Here again, these results should be taken with caution as neither measurement of TLC nor of TLCO was included in the study.

Few studies have explored pulmonary function in COVID-19 survivors at 3 (Cortés-Telles et al., 2021; Qin et al., 2021) and 4 (Anastasio et al., 2021) months after hospital discharge. All these studies showed alteration in TLCO (in more than 50% of patients), in total lung capacity (in more than 10% of patients), in pressure generating capacity of respiratory muscles (in less than 40–50% of patients) but to a much lesser extent alterations in the airway functions (in less than 10% of patients). The results of these studies converged to the conclusion that the worst the lung involvement during SARS-COV-2 infection (in those patients who developed acute respiratory distress syndrome or those who required invasive mechanical ventilation) the worst the impairment in pulmonary function after 3–4 months especially in terms of TLCO and the less the likelihood to improve pulmonary function over time. Accordingly, respiratory rehabilitation and gradual physical activity immediately after hospital-discharge should be encouraged as it can slow down or improve respiratory function, such as total lung capacity and TLCO, quality of life and anxiety in these fragile patients (Liu et al., 2020).

In conclusion, several mechanisms, sequential or not, may occur and explain the damages induced by SARS-CoV2 infections of the lungs. They include the microvascular damages with interstitial thickening with clear lungs on radiology exams along with a severe hypoxemia (McGonagle et al., 2020; Tian et al., 2020a), the development of alveolar injury inducing a gradual loss of the alveolar spaces (Tian et al., 2020a), and last but not least the diminished alveolar volume that may be explained by changes in mechanical properties of the lungs and the chest wall and by dysfunction of respiratory muscles after critical illness. These anomalies can be temporary or responsible for a potential long lasting pulmonary parenchymal dysfunction post-COVID-19 (Spagnolo et al., 2020). Given these interplays, two hypotheses on reduced TLCO can be proposed in COVID-19 survivors: (1) a reduced TLCO with normal TLCO/VA may be in favor of definitive alveolar loss/destruction, with no optimistic perspectives of recovering; (2) a reduced TLCO with diminished LCO/VA may be in favor of alveolar lesions (pulmonary capillary and/or membrane anomalies) that are still evolving, with the optimistic perspective of some and at least partial recovery. We should therefore follow-up COVID-19 survivors to see whether they are able to recover from their DLCO anomalies. Few studies have explored some "predictors" for lung function decline, especially for TLCO. Pulmonary interstitial damage (inferred to by the Chest CT total severity score), the development of acute respiratory distress syndrome, and vascular damage (inferred to by the high D-dimer levels at the time of hospital admission) have been pointed out as potential predictors for lung function decline, especially for TLCO but also for TLC (Morris et al., 2021; Qin et al., 2021).

SPECIFIC FEATURES OF RESPIRATORY DYSFUNCTION IN COVID-19 COMPARED TO OTHER VIRAL PNEUMONIA (SARS, MERS, AND INFLUENZA A H1N1)

The observations on anomalies in respiratory function, especially in DLCO, in more than 50% of the COVID-19 survivors raise the question of a potential progression toward lung fibrosis in some patients. Interestingly, the greater decline in TLCO compared to TLCO/VA suggests that impaired diffusion across the membrane may be more causative for pulmonary dysfunction than reduced lung volume. Previous studies have demonstrated that patients that recovered from coronavirus pneumonia still have damaged lungs. Impaired lung function was common and lasted for months or even years. In follow-up studies on rehabilitating SARS patients lasting from half a year to 3 years, impaired TLCO was the most common anomaly, ranging from 15 to 44%, followed by reduced TLC, ranging from 5 to 11% (Hui et al., 2005a,b; Ngai et al., 2010). Park et al. (2018) showed that 37% of MERS survivors still presented with an impairment of TLCO, but normal TLC at 12 months. In addition, pulmonary function improved significantly in the first 3 months but with no further significant improvement from 3 to 6 months after discharge

among survivors to severe influenza A (H1N1) pneumonia (Hsieh et al., 2018). Some other studies showed a complete normalization of pulmonary function 6 months after H1N1-related ARDS (Toufen et al., 2011). On the contrary, about 80% of survivors to ARDS not provoked by influenza A H1N1 had reduced diffusing capacity, 20% had airway obstruction, and 20% had restrictive pattern 12 months after recovery (Orme et al., 2003). These data are discordant with preliminary follow-up results on COVID-19 survivors highlighting the greater and persistent decline of pulmonary function (TLCO and total lung capacity) in COVID-19 survivors compared with SARS, MERS, and influenza A (H1N1) survivors.

Studies on lung function in COVID-19 survivors at 6 and 12 months from hospital discharge are thus urgently needed in order to monitor the long-term effect of COVID-19 infection on the respiratory system in patients with severe-to-extremely-severe pneumonia. A prediction would be that at least at 6 months from hospital discharge these patients may still present with an abnormal TLCO and, to lesser extent, a restrictive ventilatory defect.

FUTURE DIRECTIONS, PERSPECTIVES, AND CONCLUSION

Immunological understanding of early as well as chronic immune responses might be helpful for future stratification of surviving COVID-19 patients with chronic respiratory impairment. In our opinion, potential future directions and perspectives are as follows:

- Pathological and lung function evidence for a vascular component of severe COVID-19 patients which has long-lasting consequences should be explored.
- Immunological evidence on deranged adaptive immune function that may drive fibrotic lung diseases and evidence for impaired diffusion capacity in survivors of severe COVID-19 needs to be evaluated.
- More attention should be paid to COVID-19 survivors presenting with impaired (minor or not) diffusion capacity and perhaps with persistent dyspnea but with no other associated anomalies in chest or CT scan imaging. Techniques capable of measuring more specifically the alveolar-capillary membrane, such as measurement of TLCO including inhaled gas mixtures containing two or three different oxygen fractions or combined TLCO and diffusing capacities of the lung for nitric oxide (DLNO) measurements, are welcome to shed light on the precise mechanisms of reduced TLCO in COVID-19 survivors particularly in distinguishing between interstitial and pulmonary capillary anomalies.
- More particularly, two hypotheses on reduced TLCO could be tested in COVID-19 survivors: (1) a reduced TLCO with normal TLCO/VA may be in favor of definitive alveolar loss/destruction, with no optimistic perspectives of recovering; (2) a reduced TLCO with diminished TLCO/VA may be in favor of alveolar lesions (pulmonary capillary and/or membrane anomalies) that are still evolving, with potential and optimistic perspective of some recovering, at least partial. We should therefore follow-up COVID-19 survivors to see whether they are able to recover from their DLCO anomalies.
- A long-lasting follow-up in terms of respiratory function testing is proposed for COVID-19 survivors as results from literature are conflicting as to whether these patients may fully recover or even develop pulmonary sequelae.

This combined perspective on basic immunological responses and physiological abnormalities might foster a better understanding of the disease course and may also shape future stratification of patients and treatment options.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al. (2020). Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N. Engl. J. Med.* 383, 120–128. doi: 10.1056/nejmoa2015432

Adler, B., Sattler, C., and Adler, H. (2017). Herpesviruses and their host cells: a successful liaison. *Trends Microbiol.* 25, 229–241. doi: 10.1016/j.tim.2016.11.009

Aid, M., Busman-Sahay, K., Vidal, S. J., Maliga, Z., Bondoc, S., Starke, C., et al. (2020). Vascular disease and thrombosis in SARS-CoV-2-infected rhesus macaques. *Cell* 183, 1354–1366.e13. doi: 10.1016/j.cell.2020.10.005

Anastasio, F., Barbuto, S., Scarnecchia, E., Cosma, P., Fugagnoli, A., Rossi, G., et al. (2021). Medium-term impact of COVID-19 on pulmonary function, functional capacity and quality of life. *Eur. Respir. J.* 2004015. doi: 10.1183/13993003.04015-2020

Angka, L., Market, M., Ardolino, M., and Auer, R. C. (2020). Is innate immunity our best weapon for flattening the curve? *J. Clin. Invest.* 130, 3954–3956. doi: 10.1172/jci140530

Barron, L., and Wynn, T. A. (2011). Fibrosis is regulated by Th2 and Th17 responses and by dynamic interactions between fibroblasts and macrophages. *Am. J. Physiol. Gastrointest. Liver Physiol.* 300, G723–G728. doi: 10.1152/ajpgi.00414.2010

Barton, L. M., Duval, E. J., Stroberg, E., Ghosh, S., and Mukhopadhyay, S. (2020). COVID-19 autopsies, Oklahoma, USA. *Am. J. Clin. Pathol.* 153, 725–733. doi: 10.1093/ajcp/aqaa062

Bassler, K., Schulte-Schrepping, J., Warnat-Herresthal, S., Aschenbrenner, A. C., and Schultze, J. L. (2019). The myeloid cell compartment—cell by cell. *Annu. Rev. Immunol.* 37, 269–293. doi: 10.1146/annurev-immunol-042718-041728

Blanco-Melo, D., Nilsson-Payant, B. E., Liu, W.-C. C., Uhl, S., Hoagland, D., Møller, R., et al. (2020). Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell* 181, 1036–1045.e9. doi: 10.1016/j.cell.2020.04.026

Cantuti-Castelvetri, L., Ojha, R., Pedro, L. D., Djannatian, M., Franz, J., Kuivanen, S., et al. (2020). Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science* 370, 856–860. doi: 10.1126/science.abd2985

Casadevall, A., and Pirofski, L. A. (2020). In fatal COVID-19, the immune response can control the virus but kill the patient. *Proc. Natl. Acad. Sci. U.S.A.* 117, 30009–30011. doi: 10.1073/pnas.2021128117

Chen, R., Gao, Y., Chen, M., Jian, W., Lei, C., Zheng, J., et al. (2020). Impaired pulmonary function in discharged patients with COVID-19: more work ahead. *Eur. Respir. J.* 56:2002194. doi: 10.1183/13993003.02194-2020

Connors, J. M., and Levy, J. H. (2020). COVID-19 and its implications for thrombosis and anticoagulation. *Blood* 135, 2033–2040. doi: 10.1182/BLOOD.2020006000

Cortés-Telles, A., López-Romero, S., Figueroa-Hurtado, E., Pou-Aguilar, Y. N., Wong, A. W., Milne, K. M., et al. (2021). Pulmonary function and functional capacity in COVID-19 survivors with persistent dyspnoea. *Respir. Physiol. Neurobiol.* 288:103644. doi: 10.1016/j.resp.2021.103644

De Biasi, S., Meschiari, M., Gibellini, L., Bellinazzi, C., Borella, R., Fidanza, L., et al. (2020). Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. *Nat. Commun.* 11:3434. doi: 10.1038/s41467-020-17292-4

Del Turco, S., Vianello, A., Ragusa, R., Caselli, C., and Basta, G. (2020). COVID-19 and cardiovascular consequences: is the endothelial dysfunction the hardest challenge? *Thromb. Res.* 196, 143–151. doi: 10.1016/j.thromres.2020.08.039

Dhont, S., Derom, E., van Braeckel, E., Depuydt, P., and Lambrecht, B. N. (2021). Conceptions of the pathophysiology of happy hypoxemia in COVID-19. *Respir. Res.* 22:12. doi: 10.1186/s12931-021-01614-1

Diao, B., Wang, C., Tan, Y., Chen, X., Liu, Y. Y., Ning, L., et al. (2020). Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Front. Immunol.* 11:827. doi: 10.3389/fimmu.2020.00827

Engelmann, B., and Massberg, S. (2013). Thrombosis as an intravascular effector of innate immunity. *Nat. Rev. Immunol.* 13, 34–45. doi: 10.1038/nri3345

Frank, K., and Paust, S. (2020). Dynamic natural killer cell and T cell responses to influenza infection. *Front. Cell. Infect. Microbiol.* 10:425. doi: 10.3389/fcimb.2020.00425

Frieman, M., Heise, M., and Baric, R. (2008). SARS coronavirus and innate immunity. *Virus Res.* 133, 101–112. doi: 10.1016/j.virusres.2007.03.015

Frija-Masson, J., Debray, M.-P., Gilbert, M., Lescure, F.-X., Travert, F., Borie, R., et al. (2020). Functional characteristics of patients with SARS-CoV-2 pneumonia at 30 days post-infection. *Eur. Respir. J.* 56:2001754. doi: 10.1183/13993003.01754-2020

Fumagalli, A., Misuraca, C., Bianchi, A., Borsa, N., Limonta, S., Maggiolini, S., et al. (2020). Pulmonary function in patients surviving to COVID-19 pneumonia. *Infection* 49, 153–157. doi: 10.1007/s15010-020-01474-9

Gordon, D. E., Hiatt, J., Bouhaddou, M., Rezelj, V. V., Ulferts, S., Braberg, H., et al. (2020a). Comparative host-coronavirus protein interaction networks reveal pan-viral disease mechanisms. *Science* 370:eabe9403. doi: 10.1126/science.abe9403

Gordon, D. E., Jang, G. M., Bouhaddou, M., Xu, J., Obernier, K., White, K. M., et al. (2020b). A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* 583, 459–468. doi: 10.1038/s41586-020-2286-9

Graham, B. L., Brusasco, V., Burgos, F., Cooper, B. G., Jensen, R., Kendrick, A., et al. (2017). 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. *Eur. Respir. J.* 49:1600016. doi: 10.1183/13993003.00016-2016

Hikmet, F., Méar, L., Edvinsson, Å, Micke, P., Uhlén, M., and Lindskog, C. (2020). The protein expression profile of ACE2 in human tissues. *Mol. Syst. Biol.* 16:e9610. doi: 10.15252/msb.20209610

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181, 271–280.e8. doi: 10.1016/j.cell.2020.02.052

Hou, Y. J., Okuda, K., Edwards, C. E., Martinez, D. R., Asakura, T., Dinnon, K. H., et al. (2020). SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. *Cell* 182, 429–446.e14. doi: 10.1016/j.cell.2020.05.042

Hsieh, M.-J., Lee, W.-C., Cho, H.-Y., Wu, M.-F., Hu, H.-C., Kao, K.-C., et al. (2018). Recovery of pulmonary functions, exercise capacity, and quality of life after pulmonary rehabilitation in survivors of ARDS due to severe influenza A (H1N1) pneumonitis. *Influenza Other Respir. Viruses* 12, 643–648. doi: 10.1111/irv.12566

Huang, Y., Tan, C., Wu, J., Chen, M., Wang, Z., Luo, L., et al. (2020). Impact of coronavirus disease 2019 on pulmonary function in early convalescence phase. *Respir. Res.* 21:163. doi: 10.1186/s12931-020-01429-6

Huertas, A., Montani, D., Savale, L., Pichon, J., Tu, L., Parent, F., et al. (2020). Endothelial cell dysfunction: a major player in SARS-CoV-2 infection (COVID-19)? *Eur. Respir. J.* 56:2001634. doi: 10.1183/13993003.01634-2020

Hughes, J. M. B., and Pride, N. B. (2012). Examination of the carbon monoxide diffusing capacity (D <scp>l</scp> $_{CO}$) in relation to its K <scp>co</scp> and V <scp>a</scp> components. *Am. J. Respir. Crit. Care Med.* 186, 132–139. doi: 10.1164/rccm.201112-2160CI

Hui, D. S., Joynt, G. M., Wong, K. T., Gomersall, C. D., Li, T. S., Antonio, G., et al. (2005a). Impact of severe acute respiratory syndrome (SARS) on pulmonary function, functional capacity and quality of life in a cohort of survivors. *Thorax* 60, 401–409. doi: 10.1136/thx.2004.030205

Hui, D. S., Wong, K. T., Ko, F. W., Tam, L. S., Chan, D. P., Woo, J., et al. (2005b). The 1-year impact of severe acute respiratory syndrome on pulmonary function, exercise capacity, and quality of life in a cohort of survivors. *Chest* 128, 2247–2261. doi: 10.1378/chest.128.4.2247

Hui, K. P. Y., Cheung, M. C., Perera, R. A. P. M., Ng, K. C., Bui, C. H. T., Ho, J. C. W., et al. (2020). Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. *Lancet Respir. Med.* 8, 687–695. doi: 10.1016/S2213-2600(20)30193-4

Hulswit, R. J. G., de Haan, C. A. M., and Bosch, B.-J. (2016). Coronavirus spike protein and tropism changes. *Adv. Virus Res.* 96, 29–57. doi: 10.1016/bs.aivir.2016.08.004

Iba, T., Connors, J. M., and Levy, J. H. (2020). The coagulopathy, endotheliopathy, and vasculitis of COVID-19. *Inflamm. Res.* 69, 1181–1189. doi: 10.1007/s00011-020-01401-6

Jackson, S. P., Darbousset, R., and Schoenwaelder, S. M. (2019). Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. *Blood* 133, 906–918. doi: 10.1182/blood-2018-11-882993

Jesenak, M., Brndiarova, M., Urbancikova, I., Rennerova, Z., Vojtkova, J., Bobcakova, A., et al. (2020). Immune parameters and COVID-19 infection – associations with clinical severity and disease prognosis. *Front. Cell. Infect. Microbiol.* 10:364. doi: 10.3389/fcimb.2020.00364

Kopecky-Bromberg, S. A., Martínez-Sobrido, L., Frieman, M., Baric, R. A., and Palese, P. (2007). Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. *J. Virol.* 81, 548–557. doi: 10.1128/jvi.01782-06

Kopf, M., Schneider, C., and Nobs, S. P. (2015). The development and function of lung-resident macrophages and dendritic cells. *Nat. Immunol.* 16, 36–44. doi: 10.1038/ni.3052

Kulikauskaite, J., and Wack, A. (2020). Teaching old dogs new tricks? The plasticity of lung alveolar macrophage subsets. *Trends Immunol.* 41, 864–877. doi: 10.1016/j.it.2020.08.008

Landsman, L., Varol, C., and Jung, S. (2007). Distinct differentiation potential of blood monocyte subsets in the lung. *J. Immunol.* 178, 2000–2007. doi: 10.4049/jimmunol.178.4.2000

Lazear, H. M., Schoggins, J. W., and Diamond, M. S. (2019). Shared and distinct functions of type I and type III interferons. *Immunity* 50, 907–923. doi: 10.1016/j.immuni.2019.03.025

Lins, M., Vandevenne, J., Thillai, M., Lavon, B. R., Lanclus, M., Bonte, S., et al. (2020). Assessment of small pulmonary blood vessels in COVID-19 patients using HRCT. *Acad. Radiol.* 27, 1449–1455. doi: 10.1016/j.acra.2020.07.019

Liu, K., Zhang, W., Yang, Y., Zhang, J., Li, Y., and Chen, Y. (2020). Respiratory rehabilitation in elderly patients with COVID-19: a randomized controlled study. *Complement. Ther. Clin. Pract.* 39:101166. doi: 10.1016/j.ctcp.2020.101166

Lukassen, S., Chua, R. L., Trefzer, T., Kahn, N. C., Schneider, M. A., Muley, T., et al. (2020). SARS -CoV-2 receptor ACE 2 and TMPRSS 2 are primarily expressed in bronchial transient secretory cells. *EMBO J.* 39:e105114. doi: 10.15252/embj.20105114

Martin, L. D., Rochelle, L. G., Fischer, B. M., Krunkosky, T. M., and Adler, K. B. (1997). Airway epithelium as an effector of inflammation: molecular regulation

of secondary mediators. *Eur. Respir. J.* 10, 2139–2146. doi: 10.1183/09031936.97.10092139

McGonagle, D., O'Donnell, J. S., Sharif, K., Emery, P., and Bridgewood, C. (2020). Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia. *Lancet Rheumatol.* 2, e437–e445. doi: 10.1016/S2665-9913(20)30121-1

Meckiff, B. J., Ramírez-Suástegui, C., Fajardo, V., Chee, S. J., Kusnadi, A., Simon, H., et al. (2020). Imbalance of regulatory and cytotoxic SARS-CoV-2-reactive CD4+ T cells in COVID-19. *Cell* 183, 1340–1353.e16. doi: 10.1016/j.cell.2020.10.001

Mo, X., Jian, W., Su, Z., Chen, M., Peng, H., Peng, P., et al. (2020). Abnormal pulmonary function in COVID-19 patients at time of hospital discharge. *Eur. Respir. J.* 55:2001217. doi: 10.1183/13993003.01217-2020

Morris, M. F., Pershad, Y., Kang, P., Ridenour, L., Lavon, B., Lanclus, M., et al. (2021). Altered pulmonary blood volume distribution as a biomarker for predicting outcomes in COVID-19 disease. *Eur. Respir. J.* 2004133. doi: 10.1183/13993003.04133-2020

Ngai, J. C., Ko, F. W., Ng, S. S., To, K.-W., Tong, M., and Hui, D. S. (2010). The long-term impact of severe acute respiratory syndrome on pulmonary function, exercise capacity and health status. *Respirology* 15, 543–550. doi: 10.1111/j.1440-1843.2010.01720.x

Nusair, S. (2020). Abnormal carbon monoxide diffusion capacity in COVID-19 patients at time of hospital discharge. *Eur. Respir. J.* 56:2001832. doi: 10.1183/13993003.01832-2020

Odak, I., Barros-Martins, J., Bošnjak, B., Stahl, K., David, S., Wiesner, O., et al. (2020). Reappearance of effector T cells is associated with recovery from COVID-19. *EBioMedicine* 57:102885. doi: 10.1016/j.ebiom.2020.102885

Orme, J., Romney, J. S., Hopkins, R. O., Pope, D., Chan, K. J., Thomsen, G., et al. (2003). Pulmonary function and health-related quality of life in survivors of acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 167, 690–694. doi: 10.1164/rccm.200206-542OC

Park, W. B., Jun, K. I., Kim, G., Choi, J.-P., Rhee, J.-Y., Cheon, S., et al. (2018). Correlation between pneumonia severity and pulmonary complications in Middle East respiratory syndrome. *J. Korean Med. Sci.* 33:e169. doi: 10.3346/jkms.2018.33.e169

Pellegrino, R. (2005). Interpretative strategies for lung function tests. *Eur. Respir. J.* 26, 948–968. doi: 10.1183/09031936.05.00035205

Polak, S. B., Van Gool, I. C., Cohen, D., von der Thüsen, J. H., and van Paassen, J. (2020). A systematic review of pathological findings in COVID-19: a pathophysiological timeline and possible mechanisms of disease progression. *Mod. Pathol.* 33, 2128–2138. doi: 10.1038/s41379-020-0603-3

Potus, F., Mai, V., Lebret, M., Malenfant, S., Breton-Gagnon, E., Lajoie, A. C., et al. (2020). Novel insights on the pulmonary vascular consequences of COVID-19. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 319, L277–L288. doi: 10.1152/ajplung.00195.2020

Qin, W., Chen, S., Zhang, Y., Dong, F., Zhang, Z., Hu, B., et al. (2021). Diffusion capacity abnormalities for carbon monoxide in patients with COVID-19 at three-month follow-up. *Eur. Respir. J.* 2003677. doi: 10.1183/13993003.03677-2020

Rello, J., Storti, E., Belliato, M., and Serrano, R. (2020). Clinical phenotypes of SARS-CoV-2: implications for clinicians and researchers. *Eur. Respir. J.* 55:2001028. doi: 10.1183/13993003.01028-2020

Rogliani, P., Calzetta, L., Coppola, A., Puxeddu, E., Sergiacomi, G., D'Amato, D., et al. (2020). Are there pulmonary sequelae in patients recovering from COVID-19? *Respir. Res.* 21:286. doi: 10.1186/s12931-020-01550-6

Ruscitti, P., Berardicurti, O., Iagnocco, A., and Giacomelli, R. (2020). Cytokine storm syndrome in severe COVID-19. *Autoimmun. Rev.* 19:102562. doi: 10.1016/j.autrev.2020.102562

Schmidt, M. E., and Varga, S. M. (2018). The CD8 T cell response to respiratory virus infections. *Front. Immunol.* 9:678. doi: 10.3389/fimmu.2018.00678

Schultze, J. L., and Aschenbrenner, A. C. (2021). COVID-19 and the human innate immune system. *Cell* 184, 1671–1692. doi: 10.1016/j.cell.2021.02.029

Schurink, B., Roos, E., Radonic, T., Barbe, E., Bouman, C. S. C., de Boer, H. H., et al. (2020). Viral presence and immunopathology in patients with lethal COVID-19: a prospective autopsy cohort study. *Lancet Microbe* 1, e290–e299. doi: 10.1016/s2666-5247(20)30144-0

Shi, C. S., Nabar, N. R., Huang, N. N., and Kehrl, J. H. (2019). SARS-coronavirus open reading frame-8b triggers intracellular stress pathways and activates NLRP3 inflammasomes. *Cell Death Discov.* 5:101. doi: 10.1038/s41420-019-0181-7

Shi, C.-S., Qi, H.-Y., Boularan, C., Huang, N.-N., Abu-Asab, M., Shelhamer, J. H., et al. (2014). SARS-coronavirus open reading frame-9b suppresses innate immunity by targeting mitochondria and the MAVS/TRAF3/TRAF6 signalosome. *J. Immunol.* 193, 3080–3089. doi: 10.4049/jimmunol.1303196

Spagnolo, P., Balestro, E., Aliberti, S., Cocconcelli, E., Biondini, D., Casa, G. D., et al. (2020). Pulmonary fibrosis secondary to COVID-19: a call to arms? *Lancet Respir. Med.* 8, 750–752. doi: 10.1016/S2213-2600(20)30222-8

Sungnak, W., Huang, N., Bécavin, C., Berg, M., Queen, R., Litvinukova, M., et al. (2020). SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat. Med.* 26, 681–687. doi: 10.1038/s41591-020-0868-6

Tay, M. Z., Poh, C. M., Rénia, L., MacAry, P. A., and Ng, L. F. P. (2020). The trinity of COVID-19: immunity, inflammation and intervention. *Nat. Rev. Immunol.* 20, 363–374. doi: 10.1038/s41577-020-0311-8

Thomas, G., Tacke, R., Hedrick, C. C., and Hanna, R. N. (2015). Nonclassical patrolling monocyte function in the vasculature. *Arterioscler. Thromb. Vasc. Biol.* 35, 1306–1316. doi: 10.1161/ATVBAHA.114.304650

Tian, S., Hu, W., Niu, L., Liu, H., Xu, H., and Xiao, S.-Y. (2020a). Pulmonary pathology of early-phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. *J. Thorac. Oncol.* 15, 700–704. doi: 10.1016/j.jtho.2020.02.010

Tian, S., Xiong, Y., Liu, H., Niu, L., Guo, J., Liao, M., et al. (2020b). Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. *Mod. Pathol.* 33, 1007–1014. doi: 10.1038/s41379-020-0536-x

Toufen, C. Jr., Costa, E. L. V., Hirota, A. S., Li, H. Y., Amato, M. B. P., and Carvalho, C. R. R. (2011). Follow-up after acute respiratory distress syndrome caused by influenza a (H1N1) virus infection. *Clinics* 66, 933–937. doi: 10.1590/S1807-59322011000600002

Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., et al. (2020). Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 395, 1417–1418. doi: 10.1016/S0140-6736(20)30937-5

Vinayagam, S., and Sattu, K. (2020). SARS-CoV-2 and coagulation disorders in different organs. *Life Sci.* 260:118431. doi: 10.1016/j.lfs.2020.118431

Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., et al. (2020). Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell* 181, 894–904.e9. doi: 10.1016/j.cell.2020.03.045

Watanabe, S., Alexander, M., Misharin, A. V., and Budinger, G. R. S. (2019). The role of macrophages in the resolution of inflammation. *J. Clin. Invest.* 129, 2619–2628. doi: 10.1172/JCI124615

Way, E. E., Chen, K., and Kolls, J. K. (2013). Dysregulation in lung immunity - the protective and pathologic Th17 response in infection. *Eur. J. Immunol.* 43, 3116–3124. doi: 10.1002/eji.201343713

Wu, M., Chen, Y., Xia, H., Wang, C., Tan, C. Y., Cai, X., et al. (2020). Transcriptional and proteomic insights into the host response in fatal COVID-19 cases. *Proc. Natl. Acad. Sci. U.S.A.* 117, 28336–28343. doi: 10.1073/pnas.2018030117

Wynn, T. A., and Vannella, K. M. (2016). Macrophages in tissue repair, regeneration, and fibrosis. *Immunity* 44, 450–462. doi: 10.1016/j.immuni.2016.02.015

Yoshikawa, T., Hill, T., Li, K., Peters, C. J., and Tseng, C.-T. K. (2009). Severe acute respiratory syndrome (SARS) coronavirus-induced lung epithelial cytokines exacerbate SARS pathogenesis by modulating intrinsic functions of monocyte-derived macrophages and dendritic cells. *J. Virol.* 83, 3039–3048. doi: 10.1128/JVI.01792-08

Zhang, M., and Zhang, S. (2020). T cells in fibrosis and fibrotic diseases. *Front. Immunol.* 11:1142. doi: 10.3389/fimmu.2020.01142

Zhang, X. X. X., Tan, Y., Ling, Y., Lu, G., Liu, F., Yi, Z., et al. (2020). Viral and host factors related to the clinical outcome of COVID-19. *Nature* 583, 437–440. doi: 10.1038/s41586-020-2355-0

Zhao, Y. M., Shang, Y. M., Song, W. B., Li, Q. Q., Xie, H., Xu, Q. F., et al. (2020). Follow-up study of the pulmonary function and related physiological characteristics of COVID-19 survivors three months after recovery. *EClinicalMedicine* 25:100463. 10.1016/j.eclinm.2020.100463

Zhou, R., To, K. K. W., Wong, Y. C., Liu, L., Zhou, B., Li, X., et al. (2020). Acute SARS-CoV-2 infection impairs dendritic cell and T cell responses. *Immunity* 53, 864–877.e5. doi: 10.1016/j.immuni.2020.07.026

Ziegler, C. G. K., Allon, S. J., Nyquist, S. K., Mbano, I. M., Miao, V. N., Tzouanas, C. N., et al. (2020). SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. *Cell* 181, 1016–1035.e19. doi: 10.1016/j.cell.2020.04.035

Zuniga, E. I., Macal, M., Lewis, G. M., and Harker, J. A. (2015). Innate and adaptive immune regulation during chronic viral infections. *Annu. Rev. Virol.* 2, 573–597. doi: 10.1146/annurev-virology-100114-055226

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership