frontiers | Research Topics

Reviews in pulmonary medicine 2022

Edited by
Bruno Guedes Baldi and Shuibang Wang

Published in
Frontiers in Medicine

ISSN 1664-8714
ISBN 978-2-8325-4154-8
DOI 10.3389/978-2-8325-4154-8

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

Reviews in pulmonary medicine 2022

Topic editors

Bruno Guedes Baldi — University of São Paulo, Brazil

Shuibang Wang — National Institutes of Health (NIH), United States

Citation

Baldi, B. G., Wang, S., eds. (2023). *Reviews in pulmonary medicine 2022*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4154-8

Table of contents

frontiers | Frontiers in Medicine

TYPE Editorial
PUBLISHED 04 December 2023
DOI 10.3389/fmed.2023.1296581

OPEN ACCESS

EDITED BY
Simon D. Pouwels,
University Medical Center Groningen, Netherlands

REVIEWED BY
Lei Wang,
University Medical Center Groningen, Netherlands
Mugdha M. Joglekar,
University Medical Center Groningen, Netherlands, in collaboration with reviewer LW

*CORRESPONDENCE
Shuibang Wang
✉ swang@cc.nih.gov
Bruno Guedes Baldi
✉ bruno.guedes2@terra.com.br

RECEIVED 18 September 2023
ACCEPTED 22 November 2023
PUBLISHED 04 December 2023

CITATION
Wang S and Baldi BG (2023) Editorial: Reviews in pulmonary medicine 2022.
Front. Med. 10:1296581.
doi: 10.3389/fmed.2023.1296581

Editorial: Reviews in pulmonary medicine 2022

Shuibang Wang[1]* and Bruno Guedes Baldi[2]*

[1]Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, MD, United States, [2]Pulmonary Division, Heart Institute (InCor), University of São Paulo Medical School, São Paulo, Brazil

KEYWORDS

lung disease, lung cancer, chronic obstructive pulmonary disease (COPD), ARDS, cell senescence and apoptosis, lymphangioleiomyomatosis (LAM)

Editorial on the Research Topic
Reviews in pulmonary medicine 2022

Lung diseases are a major cause of morbidity and mortality worldwide. In recent years, there have been significant advances in our understanding of the mechanisms of lung diseases, as well as the development of new treatments. The collection of *Reviews in Pulmonary Medicine 2022* reviewed several lung diseases and their treatments.

Sepsis-related acute respiratory distress syndrome (ARDS) is a life-threatening condition that occurs when inflammation in the lungs leads to fluid buildup and breathing problems. It is a major cause of death in sepsis patients, with a fatality rate of 30–40% (Gong et al.). Currently, there is no cure for ARDS. The main treatment is supportive care, such as mechanical ventilation and fluid management. However, there are ongoing research efforts to develop new treatments that target the underlying mechanisms of ARDS. Some of the potential targets for ARDS treatment include inflammatory mediators, such as cytokines and chemokines, endothelial cell dysfunction, epithelial cell injury, disruption of VE-cadherin, alveolar macrophage activation, neutrophil apoptosis, and excessive production of reactive oxygen species (ROS) (Gong et al.). To gain new insights into ARDS pathogenesis and to identify and develop new therapeutic targets, biomarkers of the disease have been actively sought. These biomarkers, such as the soluble form of the receptor for advanced glycation end-products (sRAGE) and angiopoietin-2 (ANG2), are believed to be markers of type I alveolar epithelial cell injury and lung endothelial barrier dysfunction, respectively. High levels of sRAGE and ANG2 are also associated with an increased risk for ARDS and severity of the disease. The precise role of sRAGE in ARDS remains a topic of ongoing debate. A prevailing view suggests that sRAGE plays a protective anti-inflammatory role by acting as a decoy receptor, preventing RAGE ligands from binding to membrane-bound RAGE. However, opposing arguments propose that elevated sRAGE levels may instead result from overstimulation of cell surface RAGE, leading to downstream inflammatory signaling (1). In contrast, elevated ANG2 levels are believed to directly contribute to the increased risk of developing ARDS (2). Experimental evidence suggests that reducing blood ANG2 or blocking its signaling can improve survival in animal models (3).

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide. It is characterized by the destruction of the airways and alveoli and caused by smoking and air pollution. COPD is incurable, and its pathogenesis is complex and not fully understood. Multiple factors and their interactions, including persistent inflammation, oxidative stress, and protease/antiprotease imbalance, are known factors contributing to the pathogenesis of COPD. In addition, most recent studies suggest that ferroptosis also plays an

important role in the pathogenesis of COPD, and it may be a target for new therapies (Meng et al.). Different from apoptosis and necroptosis, ferroptosis is a recently discovered form of cell death that is mediated by phospholipid peroxidation through free iron-mediated Fenton reactions in the presence of ROS. Cigarette smoke exposure can induce ferroptosis in lung epithelial cells by increasing the levels of iron and ROS in the lungs, leading to cell death and tissue damage. Ferroptosis can also be triggered by the depletion of glutathione (GSH), an antioxidant that protects cells from ROS damage and detoxifies lipid peroxides. Deficiency or inhibition of *SLC7A11* (solute carrier family 7 member 11), *GPX4* (glutathione peroxidase 4), and *NFE2L2* (NFE2 like BZIP transcription factor 2, also known as *NRF2*) genes, which direct generation of GSH, superoxide dismutase, catalase, heme oxygenase, and other antioxidants, exacerbate ferroptosis in COPD. Therefore, targeting ferroptosis by inducing these genes and/or by removing excessive iron from the body may be a promising new approach for the treatment of COPD.

Lung cancer (LC) is the leading cause of cancer death worldwide, with about 1.8 million deaths in 2020 as estimated by the International Agency for Research on Cancer. Therapies such as chemotherapy, radiation, and their combination, can be effective at initial treatment of LC, but they often have relapse of the disease that then manifests in a chemotherapy-resistant form. In recent years, there has been a growing interest in a new approach to LC therapy called immunogenic cell death (ICD) (Xu et al.). ICD is a form of regulated cell death in which damage-associated molecular patterns (DAMPs) and tumor-associated antigens (TAAs) are released. These molecules activate dendritic cells to present tumor antigens to T cells, which then kill the LC cells. In this way, the dying cancer cells are transformed into a therapeutic vaccine that can boost the body's defenses specifically against LC and provide long-term effects to the patients. To date, the DAMPs that have been mechanistically linked to ICD include ATP, CALR (calreticulin), HMGB1 (high mobility group box 1), Type I IFN (interferon), and ANXA1 (annexin A1). Since doxorubicin was identified as the first ICD inducer in 2005 (4), different therapeutic agents have been found to initiate ICD in LC. For example, crizotinib, a targeted therapy used to treat LC patients with *ALK* (ALK receptor tyrosine kinase) gene rearrangements, can induce ICD in *ALK*-positive LC cells. Lurbinectedin, another potent ICD inducer, was approved by the US Federal Drug Administration in 2020 for the treatment of relapsed small-cell LC (5). To kill cancer cells more effectively and achieve better results of LC treatment, different ICD inducers are being used or are on clinical trials in combination with immune checkpoint inhibitors, such as pembrolizumab, which targets PD-1 (programmed death protein 1)/PD-L1 (programmed death ligand 1) pathway (5).

In addition to sepsis-related ARDS, COPD, and LC, the collection of reviews also includes other pulmonary diseases, such as interstitial lung diseases (ILDs), including rare lymphangioleiomyomatosis (LAM), and cryptogenic organizing pneumonia (COP), portopulmonary hypertension (PoPH), eosinophilic granulomatosis with polyangiitis (EGPA), pleural effusion, and obstructive sleep apnea. Clinical features, pathogenesis, diagnosis, current treatment options and future perspectives for these diseases have been discussed in detail. For instance, research has discovered that LAM is linked to mutations in the *TSC2* or *TSC1* gene, which trigger hyperactivation of the mTORC1 signaling pathway and subsequent LAM cell senescence (Bernardelli et al.). Cellular senescence, characterized by a permanent proliferation arrest, anti-apoptosis, and proinflammatory phenotype, is also observed in COPD (6), LC (7), idiopathic pulmonary fibrosis (8), and pulmonary arterial hypertension (9). Therefore, hindering senescence and eliminating senescent cells using mTORC1 inhibitors and senolytic drugs could be potential therapeutic strategies for LAM and these lung diseases.

In summary, lung diseases continue to be a significant global health concern. Researchers have been focusing on the discovery of genetic susceptibility, the molecular mechanism of disease, various biomarkers, immune dysfunction, and environmental/lifestyle triggers. Advancements in these research areas have facilitated the development of targeted therapies, early diagnosis and disease monitoring, personalized treatment, and potential preventive strategies. Gene therapy and gene editing, though still in the early stages of development, hold promise for correcting genetic defects that contribute to lung diseases. Mesenchymal stem cell-based therapies could also offer the possibility of regenerating damaged lung tissue and restoring lung function. Additionally, artificial intelligence and machine learning tools can enable researchers and clinicians to analyze vast amounts of data, process images, and identify patterns and associations, thereby accelerating progress in these research efforts.

Author's note

The opinions expressed in this article are the authors' own and do not represent any position or policy of the National Institutes of Health, the Department of Health and Human Services, or the United States Government.

Author contributions

SW: Writing – original draft, Writing – review & editing. BB: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. SW's research study was funded by intramural funds from the National Institute of Health Clinical Center.

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 author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Erusalimsky JD. The use of the soluble receptor for advanced glycation-end products (sRAGE) as a potential biomarker of disease risk and adverse outcomes. *Redox Biol.* (2021) 42:101958. doi: 10.1016/j.redox.2021.101958

2. Gallagher DC, Parikh SM, Balonov K, Miller A, Gautam S, Talmor D, et al. Circulating angiopoietin 2 correlates with mortality in a surgical population with acute lung injury/adult respiratory distress syndrome. *Shock.* (2008) 29:656–61. doi: 10.1097/SHK.0b013e31815dd92f

3. Lomas-Neira JL, Heffernan DS, Ayala A, Monaghan SF. Blockade of endothelial growth factor, angiopoietin-2, reduces indices of ards and mortality in mice resulting from the dual-insults of hemorrhagic shock and sepsis. *Shock.* (2016) 45:157–65. doi: 10.1097/SHK.0000000000000499

4. Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J Exp Med.* (2005) 202:1691–701. doi: 10.1084/jem.20050915

5. Kepp O, Zitvogel L, Kroemer G. Lurbinectedin: an FDA-approved inducer of immunogenic cell death for the treatment of small-cell lung cancer. *Oncoimmunology.* (2020) 9:1795995. doi: 10.1080/2162402X.2020.1795995

6. Houssaini A, Breau M, Kebe K, Abid S, Marcos E, Lipskaia L, et al. Activation Of The Mtor Pathway Drives Lung Cell Senescence And Emphysema In Chronic Obstructive Pulmonary Disease (COPD). *Am J Resp Crit Care.* (2017) 195:PA3921. doi: 10.1183/1393003.congress-2017.PA3921

7. Haston S, Gonzalez-Gualda E, Morsli S, Ge JF, Reen V, Calderwood A, et al. Clearance of senescent macrophages ameliorates tumorigenesis in KRAS-driven lung cancer. *Cancer Cell.* (2023) 41:1242. doi: 10.1016/j.ccell.2023.05.004

8. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, et al. Cellular senescence mediates fibrotic pulmonary disease. *Nat Commun.* (2017) 8:14532. doi: 10.1038/ncomms14532

9. Born E, Lipskaia L, Breau M, Houssaini A, Beaulieu D, Marcos E, et al. Eliminating senescent cells can promote pulmonary hypertension development and progression. *Circulation.* (2023) 147:650–66. doi: 10.1161/CIRCULATIONAHA.122.058794

frontiers | Frontiers in Medicine

SYSTEMATIC REVIEW
published: 11 April 2022
doi: 10.3389/fmed.2022.847146

Efficacy and Safety of Pleural Cryobiopsy vs. Forceps Biopsy for Evaluation of Undiagnosed Pleural Effusion: A Systematic Review and Meta-Analysis

Mohan Giri[1†], *Haiyun Dai*[1†], *Shuliang Guo*[1*], *Yishi Li*[1], *Lin He*[2] *and Rongjuan Zhuang*[1]

[1] Department of Pulmonary and Critical Care Medicine, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China, [2] Department of Respiratory and Critical Care Medicine, Fuling Central Hospital, Chongqing, China

OPEN ACCESS

Edited by:
Laurent Pierre Nicod,
University of Lausanne, Switzerland

Reviewed by:
Gunnar N. Hillerdal,
Karolinska University Hospital, Sweden
Masaki Fujita,
Fukuoka University, Japan

***Correspondence:**
Shuliang Guo
guosl999@sina.com

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: 01 January 2022
Accepted: 21 March 2022
Published: 11 April 2022

Citation:
Giri M, Dai H, Guo S, Li Y, He L and Zhuang R (2022) Efficacy and Safety of Pleural Cryobiopsy vs. Forceps Biopsy for Evaluation of Undiagnosed Pleural Effusion: A Systematic Review and Meta-Analysis. Front. Med. 9:847146. doi: 10.3389/fmed.2022.847146

Background: Pleural cryobiopsy is a novel technique for the diagnosis of pleural pathologies. However, the safety and feasibility of this modality compared to standard forceps for pleural biopsy has not been fully elucidated. This systematic review and meta-analysis aims to establish the efficacy and safety of cryobiopsy for evaluation of undiagnosed pleural effusion.

Methods: For this systematic review and meta-analysis, we searched PubMed, Embase, Scopus, and Web of science databases up to December 16, 2021 to identify relevant articles. We included randomized controlled trials, cohort studies, retrospectives studies and case series that compared pleural cryobiopsy and forceps biopsy. A qualitative assessment was performed using the QUADAS-2 tool.

Results: Of the 365 articles identified by our search, 15 studies were eligible for inclusion. The specimen sizes obtained with cryobiopsy were significantly larger compared with forceps biopsy (Standard mean difference 1.16; 95 % CI: 0.51–1.82; $P < 0.01$). Furthermore, the cryobiopsy tissue specimens were deeper (OR 2.68; 95 % CI: 1.39–5.16; $P < 0.01$) and qualitatively better with less crush artifacts (OR 0.06; 95 % CI: 0.01–0.26; $P < 0.01$). There was no significant difference in diagnostic yield (OR 1.32; 95 % CI: 0.79–2.21; $P = 0.29$) and mild to moderate bleeding events (OR 1.21; 95 % CI: 0.64–2.29; $P = 0.57$) between pleural cryobiopsy and forceps biopsy. No publication bias was observed among these studies.

Conclusions: Compared to flexible forceps biopsy pleural cryobiopsy obtained larger and deeper tissue specimens with less crush artifacts but does not show superiority for diagnostic yield. Further studies are still needed to verify these findings.

Keywords: pleural effusion, pleural cryobiopsy, forceps biopsy, meta-analysis, pleuroscopy

INTRODUCTION

The accurate diagnosis of pleural effusion is challenging and undiagnosed pleural effusion is frequently encountered in about 10–20 % cases, even after thoracentesis and closed pleural biopsy (1, 2). Medical thoracoscopy or video-assisted thoracoscopic surgery (VATS) performed using rigid or semi-rigid thoracoscope plays vital role for evaluating undiagnosed pleural effusion (3). Traditionally rigid pleuroscopy has been the procedure of choice as it offers larger pleural biopsy specimens with greater ease than semi-rigid pleuroscopy (4). However, in resource-limited settings rigid pleuroscopy may not be available and is more expensive than semi-rigid pleuroscopy. Although, the pleural biopsy specimens obtained during semi-rigid thoracoscopy are smaller but it is widely available and has good sensitivity (91%) and specificity (100%) in the diagnosis of exudative pleural effusion (5). Procuring adequate samples with sufficient depth from thickened or fibrosed pleura remain the most important limitation of semi-rigid pleuroscopy. Therefore, there is an immense need of alternative technology that could allow adequate biopsies of fibrotic pleura with larger specimen size to enhance the diagnostic yield. In recent years, cryotechnology has emerged as a promising tool for treating benign and malignant lung diseases (6). In addition to the therapeutic purpose cryotechnology is widely used for diagnostic purposes in interstitial lung disease, lung tumors, and in determination of lung rejection in transplant patients (7–9). Cryoprobe-based therapy is based on the Joule–Thomson effect whereby a liquefied gas exits at a high flow expands rapidly resulting in very low temperature at the tip of cryoprobe. Furthermore, the development of cryoadhesion or cryorecanalization has revolutionized the field of bronchology and introduced cryobiopsy (CB) as a promising sampling technique. The specimens obtained by cryobiopsy are larger and better-preserved with less crush artifact than traditional forceps biopsy (10, 11).

Several studies have compared the diagnostic yield, specimen size, bleeding severity, tissue depth of pleural CB and forceps biopsy (FB), but the results of these studies have been heterogeneous (12–21). Furthermore, most of these studies were conducted either with small populations or retrospectively. A recent meta-analysis by Shafiq et al. reported diagnostic yield of 96.5% for pleural cryobiopsy and 93.1% for forceps biopsy (22). However, in that meta-analysis authors pooled the results of only seven observational studies and failed to include randomized crossover study (23) that compared pleural cryobiopsy and forceps biopsy for the diagnosis of pleural effusions. In addition, they omitted pooled analysis of many efficacy (such as specimen size, biopsy death and crush artifacts) and safety endpoints (i.e., bleedings severity). Similarly, meta-analysis by Rial et al. (24) only pooled data of diagnostic yield and showed that pleural cryobiopsy was not superior to forceps biopsies. However, no meta-analysis, to date, has examined the efficacy of pleural cryobiopsy specifically for the specimen size harvested in subjects with undiagnosed pleural effusion. We therefore conducted a systemic review and meta-analysis to assess the efficacy and safety of cryobiopsy vs. forceps biopsy for evaluation of undiagnosed pleural effusion and attempted to ascertain whether there are variability in efficacy and safety endpoints with the two techniques. Our meta-analysis comparing the efficacy and safety of the pleural cryobiopsy vs. forceps biopsy is the largest to date, as we included more than twice the number of patients included in any previous meta-analysis (22, 24).

METHODS

The reporting of current meta-analysis was in accordance with the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (25).

Search Strategy and Selection Criteria

The PubMed, Embase, Scopus, and Web of Sciences databases were searched for relevant articles. The last search was performed on December 16, 2021. The following search strategy (Cryobiopsy OR Cryoprobe biopsy OR Forceps biopsy OR pleural cryobiopsy) AND (Pleura OR Pleural effusion OR Pleural biopsy OR Pleuroscopy OR Thoracoscopy) was employed to identify all relevant studies. The full search strategies for all databases are available in **Supplementary Appendix 1**. We assessed all of the references of selected articles to include additional studies.

The inclusion criteria were as follows: (1) Study population: Studies in which adult patients undergoing pleural biopsy either by pleuroscopy or by thoracoscopy; (2) Comparative studies: Studies that compare cryobiopsy and forceps biopsy; (3) Outcome included overall diagnostic yield, bleeding severity, specimen size, crush artifacts, and depth of specimen. Randomized controlled trials, cohort studies, retrospectives studies and case series were included. Exclusion criteria were studies with <5 subjects, non-comparatives studies, and review articles. We performed electronic search without any time and language restrictions.

Data Extraction

All duplicate studies were excluded by using by EndNote X 8.0 software. The two investigators (M.G. and H.Y.D.) who performed the literature search also independently extracted the data from included studies. Disagreements were resolved with a third investigator. Using a standardized data extraction form two independent reviewers abstracted the data. The extracted data included first author, year of publication, age, percent male, type of study, specimen size, diagnostic rate, bleeding severity, depth of the tissue and presence of artifacts. For continuous outcome such as specimen size, we abstracted mean and standard deviation. When only median and range were reported in studies, we calculated mean and standard deviation according to the Wan et al. (26).

Types of Outcome Measures

The primary outcome was standardized mean difference (SMD) of sample size obtained by cryobiopsy vs. forceps biopsy. Secondary outcomes were diagnostic yield, biopsy depth, crush artifacts, and bleeding severity for these two types of biopsy methods.

Quality Assessment

Two authors (M.G. and H.Y.D.) independently assessed the quality of individual studies using Quality Assessment of Diagnostic Accuracy Studies 2 score (QUADAS-2) tool (27). Disagreements among the reviewers were discussed and resolved during a consensus meeting. Based on the QUADAS-2 tool, each article was evaluated for risk of bias in 4 domains: (1) patient selection, (2) index test, (3) reference standard, (4) flow and timing. For each domain, the risk of bias and concerns about applicability (which also include patient selection, index test and reference standard) were analyzed and rated as low, high or unclear risk. Interrater agreement of QUADAS-2 ratings were assessed using Cohen kappa statistic.

Statistical Analysis of Data

All statistical analysis were performed with the R Statistical Software Package 4.1.0. Odds ratio (OR) with 95 % confidence interval (CI) was calculated for dichotomous data and standard mean difference (SMD) with corresponding 95% CI for continuous data. Statistical significance was set at $P < 0.05$. I^2 and *P*-values were calculated to assess the heterogeneity among the included studies. A *P*-value < 0.1 and I^2 value $>50\%$ indicated substantial heterogeneity across studies. Due to the wide variation in institutional protocols for performing pleural cryobiopsy as there is no standardized methodology to perform it, we conducted all the analysis using a random-effect model. Publication bias was assessed using funnel plots and the Egger's test or the Harbord's test. Sensitivity analysis was performed to evaluate the influence of each study on the overall effect size by using the leave-one-out method (i.e., by removing one study at a time).

RESULTS

Description of Included Studies

Figure 1 shows the details of study selection process. Of the 361 records identified from literature search and four potentially eligible studies from additional source. After title and abstract screening, we assessed 18 full text articles, of which 15 were included in the meta-analysis. Three studies were excluded because they were conference article with abstract only. All studies were published after 2015. Among those 15 studies, eight prospective studies (12, 15, 17, 20, 21, 28–30), four retrospective studies (13, 18, 19, 31), two cases series (14, 16) and one randomized controlled trial (23) were analyzed, comprising 1061 biopsies (555 cryobiopsies and 506 forceps biopsies). Cryobiopsy of pleura was obtained in most patients (in 11 out of 15 studies) using cryoprobe 2.4 mm diameter. Detailed characteristics of the included studies are shown in **Table 1**.

Quality Assessment

Summary of QUADAS-2 assessments of included studies is summarized in **Supplementary Figure 1**. The quality of included studies was generally fair. A higher risk of bias was mainly due to inappropriate patient selection and index test, and only few studies scored high risk in quality assessment (14, 16, 17, 20, 31). In most of studies, there was low or unclear risk of bias. The Interrater agreement for quality assessment of included studies between both reviewers was very good, with Cohen kappa being 88.3 %.

Primary Outcome

Specimen Size

A total of 12 studies were included in the pooled analysis of specimen size (**Supplementary Table 1**). The results of meta-analysis showed that the pooled standardized mean difference (SMD) for specimen size with cryobiopsy vs. forceps biopsy was 1.16 (95 % CI: 0.51–1.82; $P < 0.01$) (**Figure 2**). The above value indicated that the biopsies from cryobiopsy were significantly larger than specimens obtained by forceps biopsy. The heterogeneity was significant ($I^2 = 90\%$, $P < 0.01$). No evidence of publication bias was detected by the Egger's test ($P = 0.57$) and visual inspection of funnel plot (**Supplementary Figure 2**). The sensitivity analysis showed that no single study significantly affected the final pooled estimates of specimen size (**Supplementary Figure 3**).

Secondary Outcomes

Diagnostic Yield

Overall, 15 studies compared diagnostic rate of pleural cryobiopsy with forceps biopsy. The pooled diagnostic yield of cryobiopsy was 94.1 % (522/555) and forceps biopsy was 91.3 % (462/506). Compared with forceps biopsy, cryobiopsy was not associated with a significant increase in diagnostic rate (OR 1.32; 95 % CI: 0.79–2.21; $P = 0.29$) in patients with unexplained pleural effusion (**Figure 3**). The heterogeneity was not significant ($I^2 = 5\%$; $P = 0.39$). The visual inspection of funnel plot showed roughly symmetrical distribution of studies (**Supplementary Figure 4**). However, the Harbord test did not show evidence of publication bias ($P = 0.38$). With respect to diagnostic rate, the direction and magnitude of the pooled ORs did not vary substantially with leave-one-out method, showing robustness of our finding (**Supplementary Figure 5**).

Crush Artifacts and Biopsy Depth

Crush artifact in pleural biopsy specimens can make pathological interpretation very challenging. Five studies reported data on crush artifacts (**Supplementary Table 2**). Compared with forceps biopsy, cryobiopsy specimens tended to be artifacts that were less crushed and had better tissue integrity (OR 0.04; 95 % CI: 0.01–0.26; $P < 0.01$) (**Figure 4**). This effect size was robust in the leave-one-out sensitivity analysis (**Supplementary Figure 6**). Visual inspection of funnel plot (**Supplementary Figure 7**) and the Harbord test did not show evidence of publication bias ($P = 0.83$). Biopsy depth was reported in seven studies (**Supplementary Table 3**). Pleural cryobiopsy was highly successful in obtaining deeper tissue (up to the pleural fat or deeper) than that of forceps biopsy (OR 2.68; 95 % CI: 1.39–5.16; $P < 0.01$) (**Supplementary Figure 8**). There was moderate heterogeneity among the studies ($I^2 = 48\%$; $P = 0.07$). Visual inspection of the funnel plot (**Supplementary Figure 9**) did not show asymmetry and the Harbord test ($P > 0.05$) also revealed no significant publication bias. Sensitivity analysis using

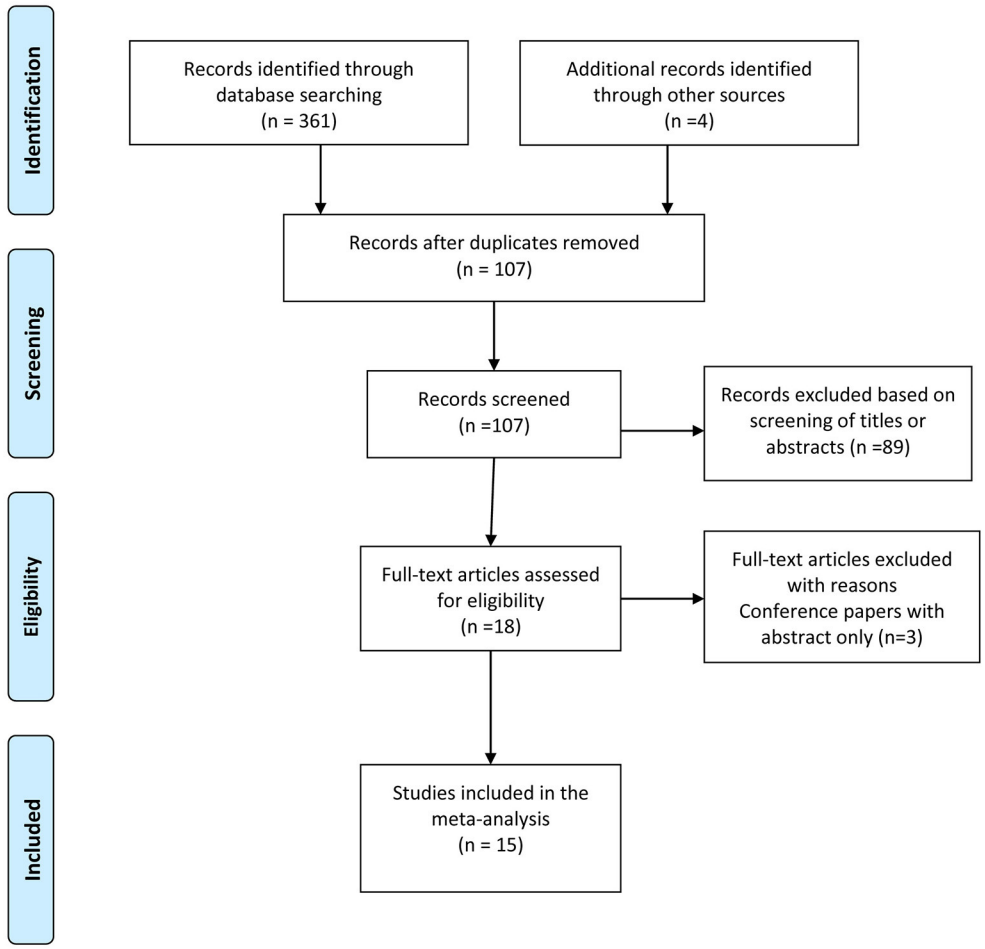

FIGURE 1 | PRISMA Flow chart of study selection process.

the leave-one-out approach showed that our result was robust (**Supplementary Figure 10**).

Bleeding Severity

Ten studies evaluated bleeding severity (**Table 2**) of which six studies contributed to the pooled analysis of bleeding event. Mild to moderate bleeding events were not significantly different between cryobiopsy and forceps biopsy group (OR 1.21; 95 % CI: 0.64–2.29; $P = 0.57$) (**Supplementary Figure 11**). There was no evidence of heterogeneity among pooled studies ($I^2 = 0\%$, $P = 0.92$). Funnel plot showed relatively symmetrical plot (**Supplementary Figure 12**) and no publication bias was detected by the Harbord test ($P > 0.05$). Furthermore, sensitivity analysis indicated that the results were robust (**Supplementary Figure 13**).

DISCUSSION

Pleural biopsy is indicated for unexplained pleural effusion that remains undiagnosed even after radiological imaging and pleural fluid analysis. Pleural cryobiopsy is evolving as the new technique for diagnosing exudative pleural effusion, when the diagnosis has remained elusive despite one or two thoracentesis. In fact, only limited studies have been conducted that compared pleural cryobiopsy and forceps based pleural biopsy. In the present meta-analysis of 15 articles involving 1061 biopsies with undiagnosed pleural effusion who underwent pleural cryobiopsy and forceps biopsy, we found that efficacy/safety outcome of specimen size, biopsy depth and crush artifacts differ significantly between cryobiopsy and forceps biopsy. However, diagnostic yield and bleeding events were similar between the groups.

This meta-analysis showed that the specimens obtained using cryobiopsy were significantly larger in size than the forceps biopsy specimens (SMD = 1.16; 95% CI: 0.51–1.82; $P < 0.01$). Previous pooled analysis of seven studies evaluating cryobiopsy and forceps biopsy also documented that biopsy size harvested by cryobiopsy were significantly larger than forceps biopsy (SMD = 0.867; 95% CI: 0.427–1.308; $P < 0.001$) (32). However, this systematic review and meta-analysis was only published as conference abstract. Conference abstracts often lack rigorous peer review, conclusions drawn from the results presented in such abstract may be biased or imprecise. In our study larger

TABLE 1 | Characteristics of included studies.

References	Study type	Selection criteria	Age[a]	Cryoprobe size (mm)	CB diagnostic rate (%)	FB diagnostic rate (%)
Ahmed et al. (31)	Retrospective	Undiagnosed exudative pleural effusion	54 ± NR	3	23/30 (76.7)	23/30 (76.7)
Baess et al. (28)	Prospective	Undiagnosed exudative pleural effusion	53.6 ± 15.1	2.4	24/24 (100)	24/24 (100)
Chen et al. (12)	Prospective	Unexplained unilateral pleural effusion	64.8 (22–92)	1.9	91/92 (98.9)	84/92 (91.3)
Dhooria et al. (23)	Randomized controlled	Undiagnosed exudative pleural effusion	53 (39–65)	2.4	39/50 (78)	38/50 (76)
El Sayad (29)	Prospective	Undiagnosed exudative pleural effusion	CB: 55.5 ± 10.9 FB: 52.92 ± 8.45	2.8	26/26 (100)	25/25 (100)
Ismail et al. (30)	Prospective	Undiagnosed exudative pleural effusion	62.92 ± 14.64	2.4	50/50 (100)	50/50 (100)
Lee et al. (13)	Retrospective	Undiagnosed pleural effusion	64.4 (55.4–76.4)	1.9	25/28 (89.3)	15/17 (88.2)
Maturu (14)	Case series	Undiagnosed exudative pleural effusion	50 (29-61)	2.4	6/6 (100)	3/4 (75)
Muhammad (15)	Prospective	Undiagnosed exudative pleural effusion	51.03 ± 7.518	2.4	30/30 (100)	30/30 (100)
Nakai et al. (16)	Case series	Undiagnosed pleural effusion	67.6 ± 6.15	2.4	5/5 (100)	1/5 (20)
Pathak et al. (17)	Prospective	Undiagnosed exudative pleural effusion	69 ± 11	2.4	10/10 (100)	10/10 (100)
Rozman et al. (21)	Prospective	Undiagnosed exudative pleural effusion	61 (33–83)	2.4	14/15 (93.3)	15/15 (100)
Thomas et al. (18)	Retrospective	Undiagnosed pleural effusion	72 (47–89)	2.4	20/22 (90)	20/22 (90)
Tousheed et al. (19)	Retrospective	Undiagnosed exudative pleural effusion	54.51 ± 14.99	2.4	86/87 (99)	50/52 (96.1)
Wurps et al. (20)	Prospective	Undiagnosed exudative pleural effusion	67.5 ± 13.5	2.4	73/80 (91.3)	74/80 (92.5)

CB, cryobiopsy; FB, forceps biopsy; NR, Not reported.
[a]*Values are mean ± SD or mean (range).*

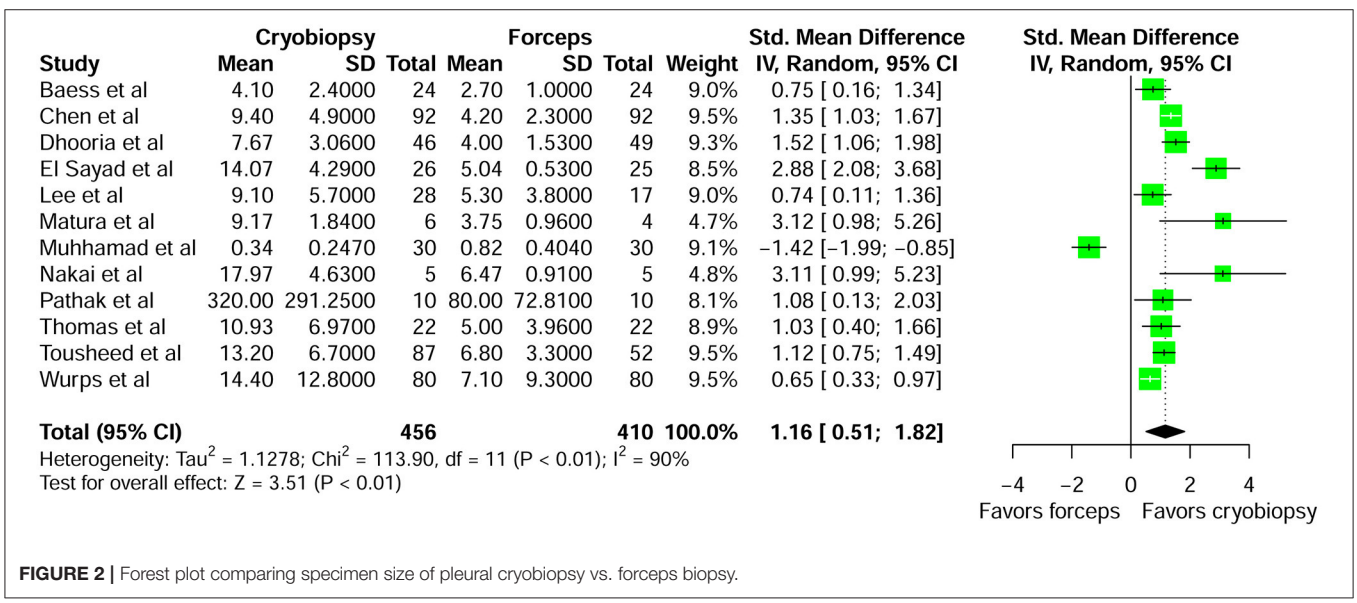

FIGURE 2 | Forest plot comparing specimen size of pleural cryobiopsy vs. forceps biopsy.

samples with sufficient extrapleural fat tissue or pleural tissue might have helped in a confident histological diagnosis of the pleural effusion in patients with thickened or fibrosed pleura. Cryobiopsy is a promising technique because both biopsy size and quality contribute to diagnostic yield. In a recent meta-analysis, Shafiq et al. (22) demonstrated that larger pleura samples were obtained through cryobiopsy than through forceps biopsy. However, they just performed qualitative analysis of specimen size and failed to perform the pooled analysis. Similarly, another meta-analysis has focused exclusively on diagnostic yield and omitted pooled analysis of other efficacy and safety end points (24). Cryobiopsy is performed using several different variations of technique across centers, this variability in institutional protocols for performing the procedure might be the reason for significant heterogeneity for biopsy size across included studies.

Our systematic review and meta-analysis revealed that the diagnostic yield of pleural cryobiopsy is comparable to that of traditional pleural biopsy using flexible forceps for undiagnosed

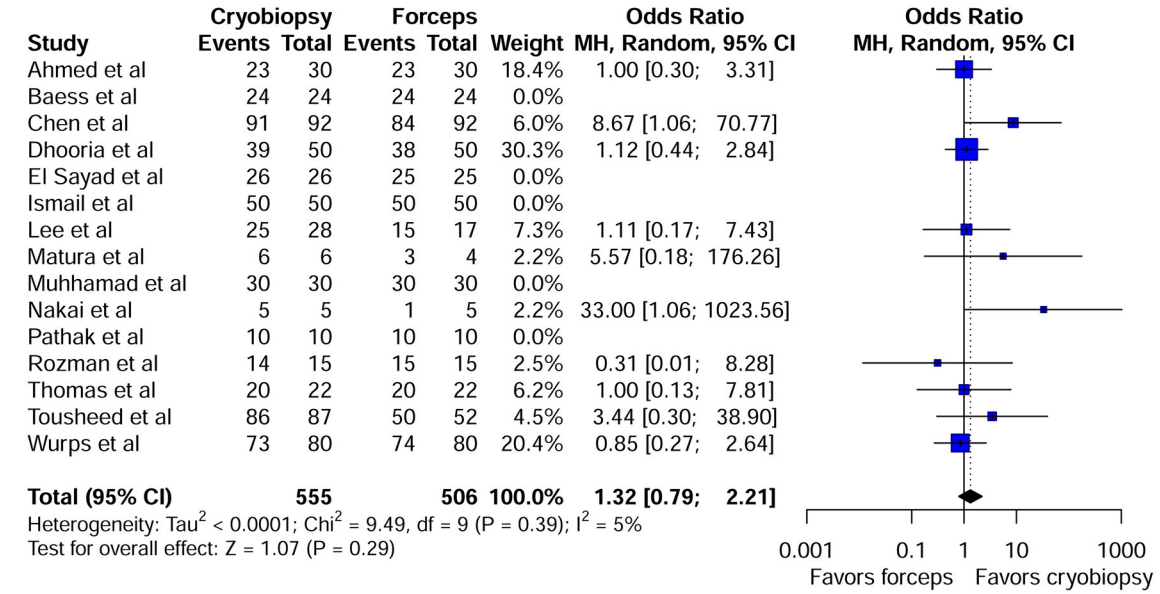

FIGURE 3 | Forest plot comparing diagnostic yield of pleural cryobiopsy vs. forceps biopsy.

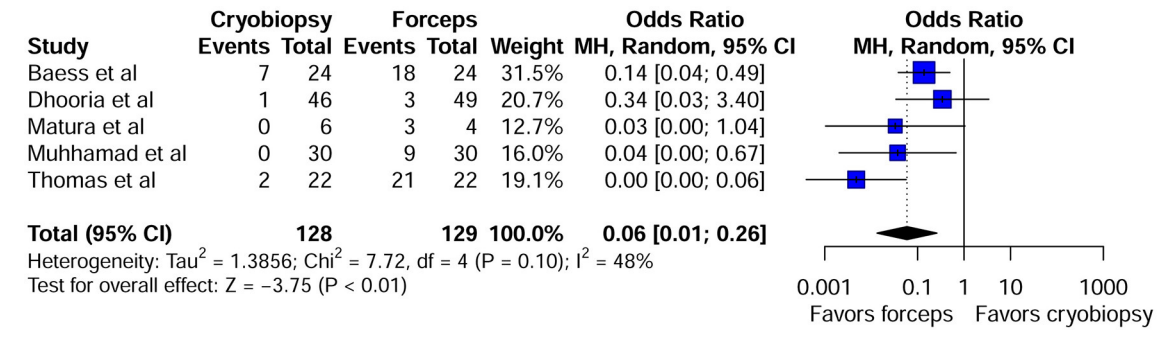

FIGURE 4 | Forest plot of crush artifacts for pleural cryobiopsy vs. forceps biopsy.

pleural effusion (OR 1.32; 95 % CI: 0.79–2.21; $P = 0.29$). In a recent study, Chen et al. (12) showed that cryobiopsy during semi-rigid pleuroscopy was a safe technique with a higher diagnostic yield than forceps biopsy for the diagnosis of exudative pleural effusion (EPE). Similarly, Touseed et al. (19) revealed that diagnostic yield was 99% with cryobiopsy and 96% with forceps biopsy, however there was no significant difference in the diagnostic yield between these two techniques. The first randomized trial that has compared the yield of the two techniques found that diagnostic rate of pleural effusion with cryobiopsy was higher than forceps biopsy (CB: 78.0% Vs FFB 76 %) even though there was no statistically significant difference (23). On the other hand, in patients undergoing pleural biopsy using flexible forceps, followed by a flexible cryoprobe introduced through the pleuroscope, Thomas et al. (18) found that diagnostic yield achieved with cryobiopsies was similar to the yield of forceps biopsy. However, there was no significant improvement in diagnostic yield by combining FB with the CB in this small cohort. Similarly, a recent meta-analysis by Shafiq et al. (22) demonstrated that pleural cryobiopsy is a safe method with similar diagnostic value, comparable to flexible forceps biopsy. However, they just included seven observational studies in their meta-analysis, which was less than the number of our included studies. Furthermore, we included the first ever randomized crossover study that compared pleural cryobiopsy and flexible forceps biopsy in subjects undergoing medical thoracoscopy for the diagnosis of pleural effusions (23). Despite its remarkable ability to harvest significantly deeper and larger specimens with less crush artifact, pleural cryobiopsy does not show superiority for diagnostic yield over forceps biopsy. Given that both techniques produced more than a 90% diagnostic rate, larger numbers of cases should be evaluated in future studies to find a statistically significant difference. Importantly, cryobiopsies will produce better results in cases

TABLE 2 | Qualitative analysis of bleeding severity.

Author	Definition of bleeding severity	Number of patients in CB, *n* (%)	Number of patients in FB, *n* (%)
Ahmed et al.	Mild bleeding	0/30 (0)	1/30 (3.33)
Baess et al.	NR	NR	NR
Chen et al.	Nil: slight, self-limited	84/92 (91.3)	86/92 (93.5)
	Mild: requiring vasoactive drug (adrenaline) injection	8/92 (8.7)	6/92 (6.5)
	Moderate to severe: requiring electrocautery or APC intervention	0/92 (0)	0/92 (0)
Dhooria et al.	Minimal:self-limited ooze	46/46 (100)	49/49 (100)
	Mild: requiring prolonged suctioning	0/46 (0)	0/49 (0)
	Major: requiring blood transfusion, causing hemodynamic instability or ICU admission	0/46 (0)	0/49 (0)
Ismail et al.	NR	NR	NR
El Sayad et al.	Self-limited	16/26 (61.5)	15/25 (60)
	Mild bleeding	10/26 (38.5)	10/25 (40)
	Moderate to severe	0/26 (0)	0/25 (0)
Lee et al.	No bleeding	25/28 (89.3)	16/17 (94.1)
	Mild: self-limiting	3/28 (10.7)	1/17 (5.9)
	Moderate: electrocautery application for hemostasis	0 (0)	0 (0)
	Severe: intravenous resuscitation, blood transfusion, and surgical or radiological interventions required	0 (0)	0 (0)
Matura et al.	NR	0/6	NR
Muhhamad et al.	NR	NR	NR
Nakai et al.	Mild bleeding	1/5 (20)	0/5 (0)
Pathak et al.	NR	NR	NR
Rozman et al.	Slight: self-limited	42/42 (100)	NR
	Moderate: electrocautery intervention	0/42 (0)	NR
	Severe: interruption of the procedure, chest tube drainage and iv resuscitation	NR	NR
Thomas et al.	Nil bleeding	17/22 (77.3)	18/22 (81.8)
	Mild: self-limiting	5/22 (22.7)	4/22 (18.2)
	Moderate: electrocautery application for hemostasis	0/22 (0)	0/22 (0)
	Severe: intravenous resuscitation, blood transfusion and/or surgical or radiological interventions	0/22 (0)	0/22 (0)
Tousheed et al.	Minimal bleeding	87/87 (100)	NR
Wurps et al.	Moderate to severe bleeding	0/6 (0)	0/6 (0)

CB, cryobiopsy; FB, forceps biopsy; APC, argon plasma coagulation; NR, Not reported; ICU, intensive care unit.

with thickened and sclerotic pleura (where a forceps biopsy can be difficult), but forceps biopsies are adequate in the vast majority of cases. Additionally, in areas with a higher prevalence of asbestos-related pleural disease, cryobiopsy may play an important role in achieving a diagnosis. Although a rigid thoracoscope can obtain larger biopsy specimens from thickened pleura, its maneuverability in the pleural space is limited.

The definition of bleeding severity varied across studies. In most of the studies, slight or self-limited bleeding was reported which was similar between cryobiopsy and forceps biopsy groups (12, 13, 18, 21, 23). Our pooled analysis revealed that there were no significant differences in the mild to moderate bleeding events between the cryobiopsy and forceps biopsy groups. No severe bleeding was reported that required blood transfusion, causing hemodynamic instability or ICU admission in the individual studies included in this meta-analysis. More RCTs assessing bleeding severity in standardized way are required to draw further conclusion regarding the safety of these biopsy methods. Histopathological diagnosis of malignant mesothelioma is particularly challenging due to the presence of diffusely thickened or fibrotic pleura (33). The role of pleural cryobiopsy is paramount in this regard as the higher number of deep biopsies containing fatty tissue should enable to detect mesothelioma more accurately compared to forceps biopsy. Pooled analysis of biopsy depth in our meta-analysis also revealed that the cryobiopsy was able to obtain biopsies containing fatty tissue or deeper layer than forceps biopsy. The study performed by Shafiq et al. (22) incorporated a similar result but they did not perform pooled analysis of biopsy depth. The presence of artifacts in histological sections obtained by flexible forceps biopsy is a very common finding

and represents a potentially major pitfall for the pathological diagnosis of pleural disease (34, 35). Our pooled result showed that in comparison with forceps biopsy, crush artifacts were minimal with cryobiopsy. In line with our study Shafiq et al. (22) also reported fewer instances of crush artifacts with cryobiopsy but they just performed qualitative analysis regarding artifacts. This systematic review and meta-analysis has several limitations. First, the small number of studies identified, the studies were mostly small in sample size and retrospective in design. Second, study by Nakai et al. (16) included five patients with pleural effusion, and in this study they investigated the utility of cryoprobe and conventional biopsy in the diagnosis of malignant pleural mesothelioma, so its representativeness for undiagnosed pleural effusion may be open to doubt. Third, there was pronounced variation in institutional protocols for performing pleural cryobiopsy as there is no standardized methodology to perform it. Fourth, operator's skills in performing biopsy procedure was not reported by majority of the studies. Fifth, the risk of bleeding is not robustly reported in many published studies and the definition of bleeding with cryobiopsy and forceps biopsy was not uniform. Well designed, larger multi-center randomized trials and prospective studies are warranted to provide more evidence for efficacy and safety of pleural cryobiopsy.

CONCLUSIONS

In conclusion, despite the limitations noted, compared with forceps biopsy, cryobiopsy is relatively safe procedure with larger artifact-free specimen but does not offer high diagnostic yield. However, no meaningful conclusion can be drawn regarding severe bleeding events. Direct comparison of cryobiopsy and forceps biopsy through multi-center randomized, controlled trial would be valuable to verify our findings.

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

MG, HD, and SG: conceptualization and project administration. MG, SG, HD, and YL: data curation and review and editing. MG, HD, YL, SG, and LH: formal analysis. MG, HD, and RZ: investigation. SG and MG: supervision. SG, MG, RZ, LH, and YL: validation. MG, YL, RZ, and SG: visualization. MG, LH, RZ, and SG: writing. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by Research projects on key disease prevention and control technologies in Chongqing (Project number: 2019ZX002) and Chongqing Pulmonary Nodule office.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.847146/full#supplementary-material

REFERENCES

1. Light RW. Clinical practice. Pleural effusion. *N Engl J Med.* (2002) 346:1971–7. doi: 10.1056/NEJMcp010731
2. Poe RH, Israel RH, Utell MJ, Hall WJ, Greenblatt DW, Kallay MC. Sensitivity, specificity, and predictive values of closed pleural biopsy. *Arch Intern Med.* (1984) 144:325–8. doi: 10.1001/archinte.1984.00350140139020
3. Lee P, Mathur PN, Colt HG. Advances in thoracoscopy: 100 years since Jacobaeus. *Respiration.* (2010) 79:177–86. doi: 10.1159/000268617
4. Lee P, Colt HG. Pleuroscopy in 2013. *Clin Chest Med.* (2013) 34:81–91. doi: 10.1016/j.ccm.2012.11.003
5. Agarwal R, Aggarwal AN, Gupta D. Diagnostic accuracy and safety of semirigid thoracoscopy in exudative pleural effusions: a meta-analysis. *Chest.* (2013) 144:1857–67. doi: 10.1378/chest.13-1187
6. Tomic R, Podgaetz E, Andrade RS, Dincer HE. Cryotechnology in diagnosing and treating lung diseases. *J Bronchology Interv Pulmonol.* (2015) 22:76–84. doi: 10.1097/LBR.0000000000000103
7. Wälscher J, Gro B, Eberhardt R, Heussel CP, Eichinger M, Warth A, et al. Transbronchial Cryobiopsies for Diagnosing Interstitial Lung Disease: Real-Life Experience from a Tertiary Referral Center for Interstitial Lung Disease. *Respiration.* (2019) 97:348–54. doi: 10.1159/000493428
8. Imabayashi T, Uchino J, Yoshimura A, Chihara Y, Tamiya N, Kaneko Y, et al. Safety and Usefulness of Cryobiopsy and Stamp Cytology for the Diagnosis of Peripheral Pulmonary Lesions. *Cancers (Basel).* (2019) 11:410. doi: 10.3390/cancers11030410
9. Montero MA, de Gracia J, Culebras Amigo M, Mugnier J, Álvarez A, Berastegui C, et al. The role of transbronchial cryobiopsy in lung transplantation. *Histopathology.* (2018) 73:593–600. doi: 10.1111/his.13656
10. Pajares V, Puzo C, Castillo D, Lerma E, Angeles Montero M, Ramos-Barbōn D, et al. Diagnostic yield of transbronchial cryobiopsy in interstitial lung disease: a randomized trial. *Respirology.* (2014) 19:900–6. doi: 10.1111/resp.12322
11. Schuhmann M, Bostanci K, Bugalho A, Warth A, Schnabel PA, Herth FJF, et al. Endobronchial ultrasound-guided cryobiopsies in peripheral pulmonary lesions: a feasibility study. *Eur Respir J.* (2014) 43:233–9. doi: 10.1183/09031936.00011313
12. Chen CH, Cheng WC, Wu BR, Chen CY, Chen WC, Liao WC, et al. Feasibility and Safety of Pleuroscopic Cryobiopsy of the Pleura: a Prospective Study. *Can Respir J.* (2018) 2018:6746470. doi: 10.1155/2018/6746470
13. Lee CS, Li SH, Chang CH, Chung FT, Chiu LC, Chou CL, et al. Comparison of Diagnostic Yield and Safety between Semirigid Pleuroscopic Cryobiopsy and Forceps Biopsy for Undiagnosed Pleural Effusion. *Can Respir J.* (2019) 2019:5490896. doi: 10.1155/2019/5490896
14. Maturu VN, Sehgal IS, Dhooria S, Bal A, Aggarwal AN, Behera D, et al. Pleuroscopic cryobiopsy: case series and systematic review. *J Bronchology Interv Pulmonol.* (2015) 22:e11–3. doi: 10.1097/LBR.0000000000000193
15. Muhammad R, Hussein S, Mohammad M, Ahmed M, Ali G. Thoracoscopic pleural cryobiopsy versus conventional forceps biopsy in diagnosis of exudative pleural effusion of unknown etiology. *Egypt J Bronchol.* (2019) 13:162–9. doi: 10.4103/ejb.ejb_72_18
16. Nakai T, Matsumoto Y, Sasada S, Tanaka M, Tsuchida T, Ohe Y, et al. Cryobiopsy during flex-rigid pleuroscopy: an emerging alternative biopsy

method in malignant pleural mesothelioma. A comparative study of pathology. *Jpn J Clin Oncol.* (2019) 49:559–66. doi: 10.1093/jjco/hyz032
17. Pathak V, Shepherd RW, Hussein E, Malhotra R. Safety and Feasibility of Pleural Cryobiopsy Compared to Forceps Biopsy During Semi-rigid Pleuroscopy. *Lung.* (2017) 195:371–5. doi: 10.1007/s00408-017-9998-0
18. Thomas R, Karunarathne S, Jennings B, Morey S, Chai SM, Lee YCG, et al. Pleuroscopic cryoprobe biopsies of the pleura: a feasibility and safety study. *Respirology.* (2015) 20:327–32. doi: 10.1111/resp.12441
19. Tousheed SZ, Manjunath PH, Chandrasekar S, Murali Mohan BV, Kumar H, Hibare KR, et al. Cryobiopsy of the Pleura: An Improved Diagnostic Tool. *J Bronchology Interv Pulmonol.* (2018) 25:37–41. doi: 10.1097/LBR.0000000000000444
20. Wurps H, Schönfeld N, Bauer TT, Bock M, Duve C, Sauer R, et al. Intra-patient comparison of parietal pleural biopsies by rigid forceps, flexible forceps and cryoprobe obtained during medical thoracoscopy: a prospective series of 80 cases with pleural effusion. *BMC Pulm Med.* (2016) 16:98. doi: 10.1186/s12890-016-0258-5
21. Rozman A, Camlek L, Marc Malovrh M, Kern I, Schönfeld N. Feasibility and safety of parietal pleural cryobiopsy during semi-rigid thoracoscopy. *Clin Respir J.* (2016) 10:574–8. doi: 10.1111/crj.12256
22. Shafiq M, Sethi J, Ali MS, Ghori UK, Saghaie T, Folch E. Pleural cryobiopsy: a systematic review and meta-analysis. *Chest.* (2020) 157:223–30. doi: 10.1016/j.chest.2019.09.023
23. Dhooria S, Bal A, Sehgal IS, Prasad KT, Muthu V, Aggarwal AN, et al. Pleural cryobiopsy versus flexible forceps biopsy in subjects with undiagnosed exudative pleural effusions undergoing semirigid thoracoscopy: a crossover randomized trial (COFFEE Trial). *Respiration.* (2019) 98:133–41. doi: 10.1159/000497212
24. Rial MB, Rodríguez IL, Roibás CM, Fernández VL, Delgado MN, Barreira ÁS, et al. Diagnostic Yield and Safety of Pleural Cryobiopsy during Medical Thoracoscopy to Diagnose Pleural Effusion. A Systematic Review and Meta-Analysis. *Arch Bronconeumol (English Ed.* (2020) 56:784–91. doi: 10.1016/j.arbr.2020.10.003
25. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* (2009) 6:e1000097. doi: 10.1371/journal.pmed.1000097
26. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol.* (2014) 14:135. doi: 10.1186/1471-2288-14-135
27. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med.* (2011) 155:529–36. doi: 10.7326/0003-4819-155-8-201110180-00009
28. Baess AI, Hassanein EG, Emara MAS, Eshmawey HA. Modified thoracoscopic pleural cryo-biopsy in diagnosis of exudative pleural effusion of undetermined etiology. *Egypt J Bronchol.* (2021) 15:21. doi: 10.1186/s43168-021-00069-8
29. El Sayed T, Mansour A, Ali R, El Hadidy T. Efficacy and safety of thoracoscopic cryobiopsy in patients with undiagnosed exudative pleural effusion. *Egypt J Chest Dis Tuberc.* (2021) 70:541–6. doi: 10.4103/ecdt.ecdt_22_21
30. Elwakil IS, Abo Elsaad RA, Abdelkmabo OMAR, Tealeb ASM. Comparison of Parietal Pleural Biopsies by Rigid Forceps and Cryoprobe during Medical Thoracoscopy in Patients with Exudative Pleural Effusion. *Med J Cairo Univ.* (2020) 88:367–74. doi: 10.21608/mjcu.2020.93998
31. Ahmed MM, AlSharkawy SH, Shoukri AM, AbouBakr YT. Evaluation of safety and diagnostic yield of pleural cryobiopsies during thoracoscopy. *Egypt J Bronchol.* (2019) 13:63–6. doi: 10.4103/ejb.ejb_48_18
32. Cheng WC, Shen MF, Chen C-Y, Tu C-Y, Liao W-C. Feasibility and safety of pleuroscopic cryobiopsy of the pleura compared to forceps biopsy: a systemic review and meta-analysis. In: *B70. Progress in Novel Diagnostics and Interventions American Thoracic Society International Conference Abstracts*. American Thoracic Society. p. A4179–A4179. doi: 10.1164/ajrccm-conference.2020.201.1_MeetingAbstracts.A4179
33. Husain AN, Colby T V, Ordóñez NG, Allen TC, Attanoos RL, Beasley MB, et al. Guidelines for Pathologic Diagnosis of Malignant Mesothelioma 2017 Update of the Consensus Statement From the International Mesothelioma Interest Group. *Arch Pathol Lab Med.* (2018) 142:89–108. doi: 10.5858/arpa.2017-0124-RA
34. Griff S, Ammenwerth W, Schönfeld N, Bauer TT, Mairinger T, Blum TG, et al. Morphometrical analysis of transbronchial cryobiopsies. *Diagn Pathol.* (2011) 6:53. doi: 10.1186/1746-1596-6-53
35. Franke KJ, Theegarten D. Hann von Weyhern C, Nilius G, Brueckner C, Hetzel J, et al. Prospective controlled animal study on biopsy sampling with new flexible cryoprobes versus forceps: evaluation of biopsy size, histological quality and bleeding risk. *Respiration.* (2010) 80:127–32. doi: 10.1159/000287251

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 | Frontiers in Medicine

REVIEW
published: 03 May 2022
doi: 10.3389/fmed.2022.877765

Telehealth Technology Application in Enhancing Continuous Positive Airway Pressure Adherence in Obstructive Sleep Apnea Patients: A Review of Current Evidence

*Benjamin Ka Seng Thong[1,2], Grace Xin Yun Loh[1,2], Jia Jan Lim[1,2], Christina Jia Liang Lee[1,2], Shu Ning Ting[1,2], Hong Peng Li[1] and Qing Yun Li[1]**

[1] Department of Respiratory and Critical Care Medicine, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [2] Shanghai Jiao Tong University School of Medicine, Shanghai, China

Obstructive sleep apnea (OSA) is a common type of sleep-disordered breathing associated with multiple comorbidities. Continuous positive airway pressure (CPAP) is the first choice for moderate-severe OSA but poor compliance brings a great challenge to its effectiveness. Telehealth interventions ease the follow-up process and allow healthcare facilities to provide consistent care. Fifth-generation wireless transmission technology has also greatly rationalized the wide use of telemedicine. Herein, we review the efficacy of the telehealth system in enhancing CPAP adherence. We recommend applying telemonitoring in clinical practice and advocate the development of a biopsychosocial telemedicine model with the integration of several interventions. Big databases and promising artificial intelligent technologies make clinical decision support systems and predictive models based on these databases possible.

Keywords: sleep apnea syndromes, eHealth, telemedicine, compliance, CPAP, telemonitoring

OPEN ACCESS

Edited by:
Zhongxing Zhang, Clinic and Care Center Barmelweid, Switzerland

Reviewed by:
Corrado Pelaia, Magna Graecia University, Italy
Tarja Saaresranta, University of Turku, Finland

****Correspondence:***
Qing Yun Li
liqingyun68@hotmail.com

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *17 February 2022*
Accepted: *05 April 2022*
Published: *03 May 2022*

Citation:
Thong BKS, Loh GXY, Lim JJ, Lee CJL, Ting SN, Li HP and Li QY (2022) Telehealth Technology Application in Enhancing Continuous Positive Airway Pressure Adherence in Obstructive Sleep Apnea Patients: A Review of Current Evidence. Front. Med. 9:877765. doi: 10.3389/fmed.2022.877765

INTRODUCTION

Obstructive sleep apnea (OSA) is a common type of sleep-disordered breathing caused by pharyngeal collapse during sleep (1). It is estimated that approximately 1 billion adults aged 30–69 years suffer from OSA worldwide (2). Several studies revealed comorbidities linked with OSA including cardiovascular, cerebrovascular, metabolic diseases, cancer, etc. (3). It is also associated with reduced quality of life (4), excessive daytime somnolence, elevated risk of accidents (5, 6), and sudden cardiac death (7). Since the 1980s, continuous positive airway pressure (CPAP) established as first-line therapy for moderate to severe OSA (8). However, low adherence to CPAP brings a great challenge to clinical practice (9–11). Several strategies are employed to promote adherence and enhance the efficacy of CPAP, i.e., multiple educational interventions, behavioral therapies, CPAP device modifications, etc. (12). During the past two decades, telehealth technology has been applied to the CPAP field, easing the follow-up process and allowing healthcare personnel to deliver more consistent care. Telehealth is defined as the application of telecommunications and digital communication technologies to deliver and facilitate health services (13). Telehealth adopted mobile health, video, audio, digital images, and telemonitoring (TM) to provide clinical and non-clinical services (14). The 2015 guidelines for telemedicine utilization published by the American Academy of Sleep Medicine (AASM) promoted telehealth technology development, and the

coronavirus disease (COVID-19) pandemic has expedited internet-based home telemedicine for the diagnosis and treatment of OSA (15). Recent updates from AASM advocated the provision of high-quality sleep care through telehealth interventions and suggested a significant role of telehealth in maintaining the continuity of sleep health (16). Fifth-generation (5G) wireless transmission technology has increased the possibility of wider telemedicine applications. Herein, we aim to review the efficacy of the telehealth system on CPAP adherence and propose the possibility for future developments.

FACTORS ASSOCIATED WITH CONTINUOUS POSITIVE AIRWAY PRESSURE ADHERENCE

Patient characteristics such as age (17, 18), gender (17, 19), race (20), and smoking status (21) could affect adherence, although these factors were not consistent determinants of CPAP adherence (12). For example, the adherence to CPAP positively correlated with age was reported in a retrospective study (17), but a large cohort study showed a negative correlation with age, particularly in those aged >75 years old, which may be due to the body sickness, sleeping time, or sleep quality of the elderly can affect the adherence of CPAP (18). Smokers caused decreased CPAP adherence compared to non-smokers (21), which is attributed to those smokers being more susceptible to upper airway discomfort, the greater severity of OSA, and as a result, less likely to take advice from healthcare providers (21).

The severity of OSA or the symptoms affects CPAP compliance. Apnea-hypopnea index (AHI), oxygen desaturation index (ODI), and the severity of excessive daytime sleepiness are positively related to adherence (21–23). Further, side effects of CPAP may influence adherence, like mucosal drying, difficult nasal breathing, claustrophobia, etc. (24, 25). Additionally, patients' psychosocial factors such as the internal locus of control (26), mental health, personality (27–29), and availability of social support (30) can affect adherence. Patients with greater locus control can overcome the side effect to achieve better adherence (26). Also, social support and bed partner is vital in improving CPAP compliance. The sleeping partner could give feedback and advice on the patients' symptomatic improvements thus may help to improve the adherence (30). Moreover, those who are motivated to resolve their problems tend to have better adherence (27–29).

The higher income and education level is associated with increased adherence. The socio-economic status is positively correlated with health knowledge and accessibility to healthcare services (31). Medical cost, insufficient time, and transport issues concerning patients reduce CPAP adherence. Thus, CPAP adherence is determined by multifactor and should be individualized and closely monitored to address non-compliance and tolerance when prescribed (12, 25).

APPLICATION OF TELEMONITORING ON THE CONTINUOUS POSITIVE AIRWAY PRESSURE ADHERENCE

Effects of Tele-Monitoring on the Obstructive Sleep Apnea Outcome

TM is a subset of telehealth. Real-time feedback reduces clinical care time and ameliorates physiological health account for the positive short-term TM effect significantly (32–36). TM significantly improves psychological health score of Short Form-12 in TM after 6 months (p = 0.05) and also shows a higher positive change in score than standard group (SG) which (9.26 ± 2.09 vs. 0.73 ± 1.78, p = 0.003) (37, 38). Additionally, TM possessed significant clinical improvements and reduced side effects, and improved the tidal volume (TG = 9.4 mL/kg vs. SG = 8.7 mL/kg, p = 0.022) (39) and the blood pressure (systolic blood pressure reduces by 7.4 mmHg and diastolic blood pressure reduces by 4.1 mmHg) (40). It mitigates disease severity shown by improvements on the Epworth Sleepiness Scale (TG ranges from 3.7 to 4.58, SG ranges from 6.05 to 6.1) (38, 40, 41) and reduces residual AHI (TG = 1.3 ± 1.0 vs. SG = 3.2 ± 3.8, p = 0.04) (36). With a combination of patient engagement tools, TM even reduces treatment termination by 2.8–5.6% and reduces mask leakage [(TG = 16.9 L/min vs. SG = 19.4 L/min, $p < 0.0001$) and (TG = 2.7 ± 4.0 L/min vs. SG = 4.1 ± 5.3 L/min, $p < 0.001$)] in two respective studies (32, 33, 42).

TM system decreases patients' burden of visits by enabling flexible timing and reduced traveling times greatly (36). It also reduces costs by €47.32–153.34 compared to inpatient care (43, 44). However, the greater costs of the advanced device and no reimbursement for telemedicine services from most insurance companies are still barriers to patients of lower socioeconomic status (45). Thus, insurance coverage for telemedicine and subsidizing CPAP devices might solve the patients' financial concerns and secure long-term benefits. TM able to reduce nursing time but there is increased workload in CPAP technicians (36, 44, 46). Since OSA is associated with multiple comorbidities, we notice a distinct lack of studies exploring the associations between TM effects on those with disability.

Effects of the Telemonitoring in Enhancing Continuous Positive Airway Pressure Adherence

TM system transmits patients' data to healthcare providers (HPs), enabling HPs to track CPAP usage and adherence at specific time intervals, i.e., 1, 3, and 6 months and more (**Table 1**) (35, 38, 40, 47–51). Optimally, the HPs will contact the patients through phone calls, messages, email, or by using the automatic feedback system to reinforce knowledge or encourage patients (40, 47, 48). The controlled pilot study and retrospective study demonstrate TM significantly enhanced CPAP mean daily usage hours and median CPAP usage after 1 month (36, 50), although a randomized controlled trial (RCT) finds no significant effect of TM in this 1 month (49).

TABLE 1 | Summary of telemonitoring studies.

References	Country	Study design (follow-up), *N* (men)	Mean age ± SD (Yrs)	Intervention vs. comparison (system/device)	Adherence criteria	Major findings
Murase et al. (47)	Japan	RCT (6M), *N* = 483 (407)	TM-group: 60 ± 11 Yrs 3M-group: 60 ± 13 Yrs 1M-group: 61 ± 12 Yrs	(TM-group, *N* = 161): Follow-up every 3 months + monthly telemedicine 3 months-group (3M-group, *N* = 166): Follow-up every 3 months 1 month-group (1M-group, *N* = 156): monthly follow-up Device: S9 or AirSense 10; Resmed Corp or REMstar Auto System One or DreamStation; Philips Corp.	% days with ≥4 h/night of CPAP use ≥70%	CPAP adherence: TM-group: 76.6–79.5%, $p < 0.01$ 1M-group: 76.2–78.4%, $p = 0.03$ 3M-group: 75.6–74.4%, $p = 0.24$
Rattray et al. (46)	Indianapolis	Prospective, mixed-methods (3M), *N* = 90 (84)	IG: 54.9 ± 13.9 Yrs CG: 56.2 ± 15.5 Yrs	IG (*N* = 38): Telesleep quality improvement program (with AirView, ResMed) CG (*N* = 52): Usual care Device: AirSense-10; ResMed	≥ 4 h/night for >70% of nights	PAPadherence: IG vs. CG: 32 vs. 23%, $p = 0.470$
Pepin et al. (38)	French	RCT (6M), *N* = 306 (226)	Median age: Total: 61.3 Yrs IG: 60.8 Yrs CG: 61.8 Yrs	IG (*N* = 157): CPAP initiation educational program + multimodal telemonitoring CG (*N* = 149): CPAP initiation educational program	Not reported	CPAP compliance: IG vs. CG: 5.28 vs. 4.75 h, $p = 0.05$
Mansell et al. (39)	United Kingdom	Longitudinal within-group repeated measures, *N* = 52 (21)	Total = 62 Yrs	All participants were monitored *via* the modem technology and Encore Anywhere (Philips Respironics) system	% days used >4 h	Increased patient compliance from 90 to 96% ($p = 0.007$), and a change in tidal volumes (9.4 vs. 8.7 mL/kg/ideal body weight, $p = 0.022$).
Turino et al. (44)	Lleida, Spain	Prospective randomized controlled study (3M), *N* = 100 (77)	IG: 56 ± 13 Yrs CG: 54 ± 12 Yrs	IG (*N* = 52): Standard care + CPAP equipped with mobile 2G (GSM/GPRS) technology capable CG (*N* = 48): Instruction session + follow-up Device: AirSense 10; ResMed, Martinsried, Germany	Use of CPAP for ≥4 h/day	Compliance: ($p = 0.627$) CG vs. IG: 4.9 vs. 5.1 h/night
Malhotra et al. (33)	United States	Retrospective Study (90 days), *N* = 128,037	IG: 51.8 ± 13.0 Yrs CG: 52.2 ± 13.4 Yrs	IG (*N* = 42679): Receive myAir (provide real-time feedback and coaching to patients based on their data within AirView) CG (*N* = 85358): Did not use the patient engagement tool	≥4 h/night on at least 70% of nights	IG (87.3%) achieving adherence criteria while CG (70.4%), $p < 0.001$ Average therapy usage was 5.9 (IG) vs. 4.9 h/night in the matched CG, $p < 0.001$
Hwang et al. (48)	Southern California	RT (3M), *N* = 556 (325)	Total: 50.5 ± 12.1 Yrs Usual care: 51.9 ± 13.1 Yrs Tel-Ed: 50.3 ± 11.8 Yrs Tel-TM: 48.8 ± 11.8 Yrs Tel-Both: 50.7 ± 11.7 Yrs	Usual care (*N* = 129): Usual care alone Tel-Ed (*N* = 164): Usual care + telemedicine web-based education Tel-TM (*N* = 125): Usual care + CPAP telemonitoring with automated feedback messaging based on usage data for 90 days Tel-Both (*N* = 138): Usual care + both telemedicine-based education and telemonitoring with feedback messaging Device: AirSense 10; ResMed Corp	≥4 h/night on ≥70% of days	Medicare adherence: Usual care vs. Tel-TM 53.5 vs. 65.5% (Odds ratio 1.7, $p = 0.003$) Usual care vs. Tel-Both: 53.5 vs. 73.2% (Odds ratio 2.4, $p = 0.001$) Average daily used: Usual care vs. Tel-TM: 3.8 vs. 4.4 h, $p < 0.001$ Usual care vs. Tel-Both = 3.8 vs. 4.8 h, $p < 0.001$
Anttalainen et al. (36)	Finland	Retrospective Study (1 year), *N* = 111 (IG: 72%, CG: 70.5%)	IG: 53.9 ± 12.2 Yrs CG: 56.4 ± 11.8 Yrs	IG (*N* = 50): CPAP with Restraxx TM online system CG (*N* = 61): CPAP Device: S9 Elite (ResMed, Sydney, Australia), ResTraxx Online (ResMed, Sydney, Australia) database	>4 h per day	Mean CPAP adherence (IG vs. CG: 6.4 vs. 6.1 h; $p = 0.63$) was good in both groups at 1-year follow-up.
Kotzian et al. (51)	Vienna, Austria	RCT (3M, 12M), *N* = 33 (23)	IG: 62.9 ± 5.3 Yrs CG: 61.8 ± 5.3 Yrs	IG (*N* = 17): Standard care + data monitoring CG (*N* = 16): Standard care Device: AirSense 10 AutoSet CPAP (Resmed)	≥4 h/night	Mean adherence to PAP uses all days: (3M) CG vs. IG: 299 vs. 375 min per day, $p = 0.017$ (12M) CG vs. IG: 307 vs. 352 min per day, $p = 0.204$

(Continued)

TABLE 1 | (Continued)

References	Country	Study design (follow-up), *N* (men)	Mean age ± SD (Yrs)	Intervention vs. comparison (system/device)	Adherence criteria	Major findings
Fernandes et al. (49)	Lisbon, Portugal	RCT (4 weeks), *N* = 51 (42)	Total: 54.0 ± 12.6 Yrs IG1: 56.3 ± 12.1 Yrs IG2: 53.5 ± 11.7 Yrs CG: 52.3 ± 15.2 Yrs	IG1 (Phone-call care, *N* = 18): Received regular phone calls (to assess self-reported adherence and address any issues regarding the patient's clinical status or treatment) IG2 (Telemonitored clinical care, *N* = 12): All CPAP devices were fitted with a ResTraxx wireless transmitter (ResMed) to allow remote data collection of adherence or data were collected and transmitted to the computer server CG (Usual clinical care, *N* = 21): Only attended the follow-up for clinical assessment and data collection Device: AutoSet Spirit S8 flow generator unit (ResMed, San Diego, CA)	≥4 h/night for >70% of nights	Mean: Adherence: IG1 vs. IG2 vs. CG: 3.9 vs. 5.0 vs. 5.1 h/day, $p > 0.05$
Fields et al. (37)	United States	Prospective, parallel-group randomized pilot study (3M), *N* = 34 (32)	Total = 53.2 ± 14.8 Yrs IG = 46.7 ± 13.1 Yrs CG = 58.2 ± 14.4 Yrs	CG (*N* = 20): In-person instruction from experienced sleep therapists IG (*N* = 14): No in-person set-up instruction, according to the instructional DVD and brochure Device: Type 3 portable monitor (Embletta Gold; Embla, Inc., Broomfield, CO) (Automatic Positive Airway Pressure)	Mean daily minutes of PAP use over 3M	The mean days of usage: CG vs. IG: 54 vs. 65 days
Hoet et al. (35)	Brussels, Belgium	RT (3M), *N* = 46 (IG: 17%, CG: 57%)	IG: 59 ± 13 Yrs CG: 54 ± 14 Yrs	CG (*N* = 23): Received written instructions and were able to contact the sleep unit (with a telephone call or visit) as often as needed IG (*N* = 23): Usual care + the T4P (SRETT medical, France) TM unit was added to the CPAP that allows practitioners to obtain data *via* the transmission Device: S9 or Airsense 10 from Resmed or DreamStation from Philips	≥4 h per night on ≥70% of nights	Mean duration of use at 3M: ($p = 0.018$) IG vs. CG: 5.7 vs. 4.2 h/night Mean of the total number of hours used: ($p = 0.034$) IG vs. CG: 507 vs. 387 h Compliance between 6 weeks and 3M: IG, $p = 0.003$ CG, $p = 0.03$
Schoch et al. (52)	Eastern Switzerland	RCT (6M), *N* = 169 (27)	Median age: IG: 55 Yrs CG: 57 Yrs	IG (*N* = 82): Instruction session + CPAP machine (was coupled to a telemetry device that regularly transmitted the acquired data to a secure online depository) + follow-up (at 1 and 6M after CPAP initiation) CG (*N* = 87): Instruction session + CPAP machine + follow-up (at 1 and 6M after CPAP initiation) Device: Automated CPAP devices (ICON + AUTO; Fisher & Paykel)	≥4 h/night	Percentage of nights with CPAP use: IG vs. CG: 92 vs. 88.2%, $p = 0.565$ Average nightly use: IG vs. CG: 5.6 vs. 4.8 h, $p = 0.663$
Nilius et al. (40)	Germany	RT (6M), *N* = 75 (55)	IG: 58.6 ± 9.3 Yrs CG: 55.4 ± 10.4 Yrs	IG (*N* = 38): Based on telemonitoring, telephone calls, and remote interventions CG (*N* = 37): Standard practice Device: Positive pressure device (ICON, Fisher and Paykel healthcare, New Zealand)	>4 h/night	Daily usage: CG vs. IG: 2.1 vs. 4.4 h/night, $p < 0.001$ Days used > 4 h: CG vs. IG: 27.5 vs. 57.3%, $p < 0.001$
Frasnelli et al. (50)	Eastern Switzerland	Controlled pilot study (1M), *N* = 223 (IG: 76%, CG: 78%)	IG: 55 Yrs CG: 55 Yrs	IG (*N* = 113): Telemetry-triggered interventions (S9 wireless module, ResTraxx) CG (*N* = 110): Home therapy (S9 AutoSet/AutoSet Spirit II/Somnobalance/RemStar Auto Aflex)	≥4 h per night	The median CPAP use: $p < 0.05$ IG vs. CG: 5.3 vs. 4.6 h/night The median days of usage: $p = 0.023$ IG vs. CG: 28 vs. 27 days

(Continued)

TABLE 1 | (Continued)

References	Country	Study design (follow-up), *N* (men)	Mean age ± SD (Yrs)	Intervention vs. comparison (system/device)	Adherence criteria	Major findings
Woehrle et al. (42)	Germany	Randomized, controlled clinical trials (1 year), *N* = 6,802 (5,070)	IG: 59 ± 13 Yrs CG: 59 ± 13 Yrs	IG (*N* = 3,401): PAP + AirView (a cloud-based remote monitoring system) CG (*N* = 3,401): PAP + healthcare provider, sleep laboratory and/or treating physician	Not reported	At 1-year, the overall therapy termination rate was significantly lower (5.4 vs. 11.0%; $p < 0.001$), and time to therapy termination was significantly longer (348 vs. 337 days; $p < 0.05$) in the IG
Woehrle et al. (32)	Germany	Retrospective study (180 days), *N* = 1,000 (880)	IG: 56 ± 13 Yrs CG: 55 ± 12 Yrs	IG (*N* = 500): Telemonitoring and patient engagement tool (AirView + myAir) CG (*N* = 500): telemonitoring alone (AirView; proactive care)	Termination rate	Therapy termination occurred less often in the IG ($p < 0.001$).

SD, standard deviation; Yrs, years; M, month; IG, intervention group; CG, comparison group; TM, telemedicine; RT, randomized trial; RCT, randomized controlled trial.

Randomized trials illustrate CPAP adherence improves after 3-month of TM (35, 48). A shorter time to the first technical intervention might be associated with better compliance (35). Researchers have further confirmed that the combination of TM and education is more effective than TM alone (48). Notably, some prospective studies did not find significant effects at 3 months follow up, which may be attributed to the small sample sizes (37, 44, 46).

TM improves CPAP adherence at 6-month follow-up (38, 40, 47). TM significantly improves CPAP compliance [telemonitor group (TG) = 57.3 ± 34.5% vs. standard group (SG) = 27.5 ± 32.5%, $p = 0.00025$] and daily CPAP usage time (TG = 4.4 ± 2.5 h/night vs. SG = 2.1 ± 2.2 h/night, $p = 0.000063$) (40). A RCT observed a positive effect of multimodal TM in CPAP compliance compared to the usual care group (TG = 5.28 ± 2.23 h vs. SG = 4.75 ± 2.50 h, $p = 0.05$) (38). TM increases the percentage of days with good adherence from 76.6 ± 24.2% to 79.5 ± 22.0% ($p < 0.01$) after 6 months compared to baseline (47). Contrastingly, a RCT reported that TM did not improve overall adherence after 6 months, which might be due to the "ceiling effect" (52).

Finally, the current evidence has not confirmed the long-term effect on CPAP adherence at 1-year follow-up (36, 51). High dropout rates and telemonitoring during the habituation stage may account for the insignificant results (51). Compared to the titration stage, TM during the habituation stage might be another reason that leads to insignificant results (36). Although there are no significant improvements, TM still possesses a non-inferiority therapeutic effect compared to usual care after 1 year. We postulate that personality traits are a vital factor determining the length of effective TM intervention based on other researches (28) and suggest future research on interventions to secure long-term telemonitoring effects.

It should be noticed that most of the recent findings show equivocal effects similar to a meta-analysis which demonstrates a significantly higher mean difference of 0.79 h of CPAP compliance in the TM group for short-term follow-up (<3 months), but not for long-term follow up (45). Although the long-term effects remain contentious, we still recommend including telemonitoring in current clinical practice due to its multiple benefits and non-inferiority compared to usual care.

Various Telemonitoring Approaches Showed Different Effects for Continuous Positive Airway Pressure Adherence

Several TM approaches are used to improve adherence to CPAP (**Table 2**).

Telephone: Health coaching through the phone improves CPAP usage in the past 30 days ($p = 0.03$) (53). The researchers call the patients in the intervention group three times in 30 days to identify and assist them in immediate problem-solving (53). The HPs checked CPAP usage, understood the obstacles, and provide patients with different solutions. A designed list of responses used to communicate helps solve issues more effectively in contrast to a study showing the negative result (49, 53). These well-designed interventions might account for the positive effect. In addition, other studies determine that positively framed message increases CPAP total use hours in week-2 but the effect diminishes in week-6 (54).

Text message: Text messages for reminding about CPAP use significantly improve overall compliance in the first 7 days, but the effect fades at 30 days (55). Other research utilizing email and automated message methods shows no significant effect on CPAP adherence (56). The different content of responses from HPs in these studies might account for the differing results. The faded effect might be due to reduced motivation, which could possibly be addressed by a lack of interesting content, videos, or further information on CPAP therapy (54).

Apps: During the past 5 years, a total of three new apps ("SleepMapper"; "MyPathway"; "Appnea") have been reported in detail (57–59). The apps allow HPs to provide real-time feedback and education, it also allowed patients to self-monitor and receive a personalized prescription for CPAP usage. "SleepMapper" significantly increases any CPAP usage at night (SG = 55.5 ± 24.0% vs. "SleepMapper" group = 78.0 ± 22.0%, $p < 0.001$) and usage of CPAP > 4 h (SG = 37.0 ± 25.0% vs. "SleepMapper" group = 54.0 ± 27.0%, $p = 0.02$) compared to the standard care group (57). Compared to new CPAP

TABLE 2 | Summary of telehealth approaches.

References	Country	Study design (follow-up), *N* (men)	Mean age ± SD (Yrs)	Intervention and comparison	Sleep assessment	Adherence criteria	Major findings
Telephone							
Willard-Grace et al. (53)	United States	RT (30 days), *N* = 131 (88)	Total: 49.1 ± 12.1 Yrs IG: 48.5 ± 11.6 Yrs CG: 49.5 ± 12.5 Yrs	IG: Health coaching group (*N* = 56) The health coach will receive a list of patients in the health coaching group and contact them by telephone during the study. CG: Usual care group (*N* = 76)	Not reported	≥4 h/night	The proportion using CPAP device at any time in the past 30 days between IG and CG (%): 55.4 vs. 41.3%, $p = 0.03$. The number of hours used on average over the past 30 days between IG and CG: 2.1 ± 2.8 h vs. 1.8 ± 2.7 h, $p = 0.04$.
Pengo et al. (54)	United Kingdom	Prospective randomized study, (6 weeks), *N* = 112 (84)	IG: Positively framed message group: 46.7 ± 12.2 Yrs Negatively framed message group: 47.1 ± 11.7 Yrs CG: 53.5 ± 12.5 Yrs	IG: Positively framed message group (*N* = 26) Method: The patient receives the positively framed message during phone calls. Negatively framed message group (*N* = 37) Method: The patient receives the negatively framed message during phone calls. CG: Standard care group (*N* = 39)	(i) have both a 4% ODI ≥ 5 events/hour and typical symptoms of OSA (Epworth Sleepiness Scale) > 10 points. (ii) 4% ODI > 15 events/hour	> 4 h/night	The CPAP total hours used among IG (positively framed message group and negatively framed message group and CG (mean ± SD): (i) At week-2, 53.7 ± 31.4 h, 35.6 ± 27.4 h, and 40.8 ± 33.5 h, respectively, $p < 0.05$. (ii) At week-6, there was no significant difference among the 3 groups ($p = 0.679$)
Text message							
Munafo et al. (56)	United States	Randomized, prospective, non-blinded study (90 days), *N* = 138 (95)	IG: 52.3 ± 10.6 Yrs CG: 50.0 ± 11.7 Yrs	IG: Telehealth group (*N* = 69): The patients will log in to the U-sleep website and if one of the following intervention points had been triggered, the patient will receive notifications *via* automated text/email: (i) No CPAP data for 2 consecutive days (ii) CPAP usage <4 h for 3 consecutive nights (iii) CPAP usage met Medicare criteria for adherence CG: Standard of care group (*N* = 69):	Not reported	≥4 h/night	Thedaily usage of CPAP between IG and CG (mean ± SD): 5.1 ± 1.9 vs. 4.7 ± 2.1, $p = 0.24$. The percentage of the amount of patients' days CPAP used for more than 4 h between IG and CG (mean ± SD): 70.2 ± 26.7 vs. 63.3 ± 28.5, $p = 0.17$. The number of minutes coaching required per patient between IG and CG (mean ± SD): 23.9 ± 26.3 vs. 58.3 ± 25.0, $p < 0.001$.
Kataria et al. (55)	United States	RCT (30 days), *N* = 19 (not mentioned)	Not reported	IG: Reminder group: The patient received education at the first visit and a nightly text message as a reminder. CG: Standard-of-care group	Not reported	≥4 h/night.	Themean overall PAP compliance percentage between IG and CG (%): (i) At first 7 days (%): 83.9% vs. 55.4%, $p = 0.04$. (ii) At 30 days (%): 58.9% vs. 36.9%, $p = 0.22$.
APPs							
Hostler et al. (57)	United States	CCT (11 weeks), *N* = 61	IG: 44.5 ± 11.3 Yrs CG: 42.1 ± 6.8 Yrs	IG: SleepMapper group (*N* = 30) Method: The patient receives a standard education + follow-up + SleepMapper application. CG: Standard care group (*N* = 31)	AHI ≥ 5.0 events/hour	>4 h/night for at least 70% of nights	Thepercentage of any CPAP usage at night between IG and CG (mean ± SD): 78.0 ± 22.0% vs. 55.5 ± 24.0%, $p < 0.001$. The percentage usage of CPAP which > 4 h between IG and CG (mean ± SD): 54.0 ± 27.0% vs. 37.0 ± 25.0%, $p = 0.02$.

(Continued)

TABLE 2 | (Continued)

References	Country	Study design (follow-up), *N* (men)	Mean age ± SD (year)	Intervention and comparison	Sleep assessment	Adherence criteria	Major findings
Isetta et al. (82)	Spain	RT (6 weeks), *N* = 60 (47)	Total: 56 ± 10 Yrs IG: 56 ± 9 Yrs CG: 54 ± 12 Yrs	APPnea IG: Regular users (*N* = 38) (APPnea use > 66% of all days) Method: (i) Every day, APPnea will send a message to ask the patient to answer three yes/no questions about OSA treatment. (ii) Once a week, patients will be required to provide their body weight. (iii) APPnea possesses the recommendation section about CPAP use and a good lifestyle. (iv) Global summaries of the questionnaire answers are available to the patient in graphical format weekly. CG: Non-regular users (*N* = 22) (APPnea use <66% of all days)	Not reported	Not reported	The mean hours of CPAP use between IG and CG (mean ± SD): 5.5 ± 1.6 h/day vs. 5.0 ± 1.5 h/day. The regular use of "APPnea" can improve CPAP usage.
Baltaxe et al. (59)	Spain	RCT (3 months), *N* = 67 (38)	IG: 68 ± 15.8 Yrs CG: 65 ± 14.7 Yrs	IG: MyPathway group (*N* = 33) The patient receives face-to-face motivational intervention + follow-up through the MyPathway app. CG: Usual care group (*N* = 34)	Not reported	Not reported	No significant difference in the number of hours used per day ($p = 0.28$). However, the patients showed a high acceptance of the "MyPathway" app (mean score of 7.5/10 on the questionnaire) and agreed that it was easy to use (mean score of 8.5/10 on the questionnaire)

SD, standard deviation; Yrs, years; IG, intervention group; CG, comparison group; CCT, controlled clinical trial; RT, randomized trial; RCT, randomized controlled trial.

patients, "Appnea" significantly improves CPAP adherence who frequently used when compared to rarely used patients (5.6 ± 1.4 vs. 4.3 ± 1.3 h/night, $p = 0.008$) (58). In contrast, although participants were satisfied with the "MyPathway" app, there was no significant modification in PAP adherence. Almost a quarter of patients were 70–79 years and senior patients are perhaps less motivated in learning and utilizing new technology, thus resulting in negative outcomes (59). Limitations of current studies are the small sample sizes and the short study duration (57, 58).

Despite inconclusive results, mobile apps, phone calls, and text messages still possess some advantages. Apps remain the most promising approach. Future app development should continue to focus on user-friendly, online educational programs, troubleshooting models, and real-time access to CPAP usage and AHI data for self-monitoring. They can encourage patients to maintain a healthy lifestyle and adhere to given therapy with minimal effort from HPs (60). The required smartphones or tablet devices are usually affordable (61). For telephone calls and text messages which displayed short-term effects, additional modulations are required to enhance adherence. We postulate that a convenient troubleshooting model and active approach are vital in determining the success of the intervention. Actively assisting users to troubleshoot problems shows better results than passively waiting for calls (59). Maintaining long-term compliance with CPAP therapy is challenging. Adherence to CPAP may decline over time exacerbated by the negative attitudes toward the treatment and insufficient support from family members and the healthcare team (62).

Application of Tele-Education in Improving Continuous Positive Airway Pressure Adherence

Tele-education is the application of technologies to provide distance learning. Patients' negative perceptions of the benefit and health value of CPAP are the common causes of poor CPAP adherence (63, 64). Patients' lack of confidence in the therapeutic effect of CPAP results in poor adherence (65, 66). Therefore, education is vital in enhancing patients' perception of CPAP therapy. Tele-education is a well-known alternative model to educate patients. The modes of tele-education include slide shows, videos, audio, and web-based learning (67). Therefore, patients can watch the educational material as soon as they are

TABLE 3 | Summary of tele-education studies.

References	Country	Study design (follow-up), *N* (men)	Mean age ± SD (Yrs)	Intervention vs. comparison	Length of CPAP use	Outcomes
Bakker et al. (71)	United States	RCT (6M), *N* = 83 (55)	Total: 63.9 ± 7.4 Yrs IG: 63.8 ± 8.3 Yrs CG: 63.9 ± 7.4 Yrs	IG: CPAP + standardized motivational enhancement delivered by a psychologist during two appointments and six phone calls over 32 weeks (*N* = 41) CG: CPAP (*N* = 42) Duration: 32 weeks	Not reported	The brief motivational enhancement significantly increased the average nightly use of CPAP time by 99 min more than the CPAP-only group ($p = 0.003$) after 6 months. (IG: 4.4 h/night vs. CG: 3.3 h/night)
Guralnick et al. (68)	United States	RCT (30 days), *N* = 212 (95)	IG: 54.1 Yrs CG: 50.3 Yrs	IG: Watched video which included information to increase knowledge about the consequences of untreated severe OSA and the importance of CPAP adherence + usual care (*N* = 99) CG: Usual care (*N* = 113) Length of video: 4 min and available online	Not reported	No differences in CPAP adherence at 30 days (3.3, 95% Confidence interval 2.8–3.8 h/day video education; vs. 3.5, 95% Confidence interval 3.1 to 4.0 h/day usual care; $p = 0.44$) or during the 30 days after the sleep clinic visit. (IG: 3.3 h/night vs. CG: 3.5 h/night)
Hwang et al. (48)	United States	RCT (3M), *N* = 734 (349)	Total: 49.1 ± 12.5 Yrs IG: 49.1 ± 12.2 Yrs CG: 50.2 ± 12.7 Yrs	IG: Two educational programs (1) Watch a video about the pathophysiology of OSA and the information about CPAP before the CPAP therapy. Duration of education session: 15 min. (2) Email about the instructions to use CPAP during the first week of intervention. (*N* = 380) CG: CPAP (*N* = 354)	>4 h/night for at least 70% of nights	Telemedicine-based education did not significantly improve CPAP adherence but did increase clinic attendance for OSA evaluation. ($p > 0.05$) (average usage on all days, IG: 5.1 ± 2.5 h/night vs. CG: 4.6 ± 2.5 h/night)
Dharmakulaseelan et al. (69)	Canada	Randomized Feasibility Study (6M), *N* = 48 (30)	IG: 71 Yrs CG: 66.0 Yrs	IG: Educational pamphlet and slideshow. Content of education: risk factors, symptoms, consequences, and treatment of poststroke/transient ischemic attack OSA, good sleep hygiene practices + usual care (*N* = 25) CG: Usual care (*N* = 23) Length of slideshow: 5 min	>4 h/night for at least 70% of nights or ≥28 h/week	No significant difference in mean hours of CPAP use at the 6M follow-up. (IG: 36.4 h/week, vs. CG: 41.9 h/week)

M, months; SD, standard deviation; Yrs, years; IG, intervention group; CG, comparison group; RCT, randomized controlled trial.

available and shorten clinical care time (36). The recent COVID-19 pandemic has also accelerated the development of tele-education.

A few studies have investigated the effect of tele-education (**Table 3**). RCTs found educational videos have no significant improvement in CPAP adherence (48, 68). Although patients understood the given slideshow and educational booklet, there was no significant improvement in mean hours of CPAP (69). The Health Belief Model states that a change in health behavior is primarily due to health perception instead of knowledge (66). Confidence in the therapeutic effect is a key factor that alters health practice (66). Therefore, non-continuous tele-education alone is insufficient (48, 68, 69). Most studies had a small sample size (68, 69) and a short period of intervention (48, 68, 69), which may also influence the outcome. No examination of patients' knowledge, attitude, and practice pre-and post-study is also a limitation of existing educational studies.

Educational intervention with motivation enhancement significantly improves CPAP adherence (70). Motivation enhancement is a behavioral intervention based on the principle of the motivational interview (71). Patients had a one-on-one conversation session with a psychologist, watched an educational video, and received follow-up phone calls from the psychologist (71). This intervention significantly increased the average nightly use of CPAP time by 99 min more than the CPAP-only group ($p = 0.003$) after 6 months (71).

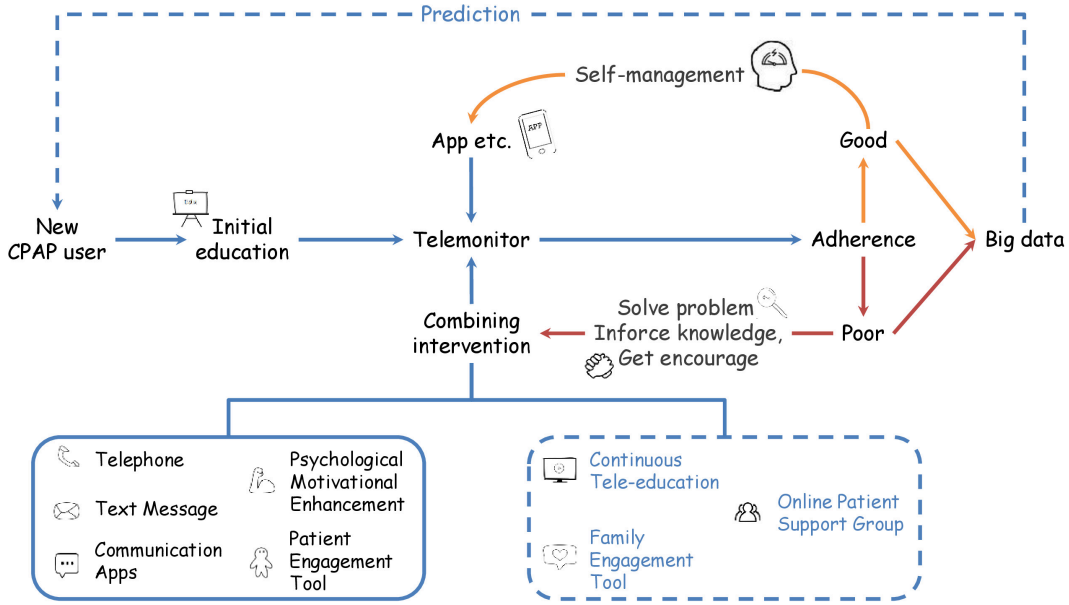

FIGURE 1 | Flowchart for future telehealth application. New CPAP users receive education and initiate a self-management approach initially. Then, healthcare providers remotely monitor CPAP usage data and identify the patient's adherence occasionally. For the patient with good adherence, healthcare providers administrate an app for the patient to self-manage. Patients with insufficient adherence should join combining interventions. Currently available interventions include telephone, text message, psychological motivational enhancement, and patient engagement tools. Future studies on continuous tele-education, family engagement tools, and online patient support groups are required. Collection of adherence data able to build big data. Training artificial intelligence with big data might help to predict adherence of new CPAP users. Blue words and dotted lines represent future interventions.

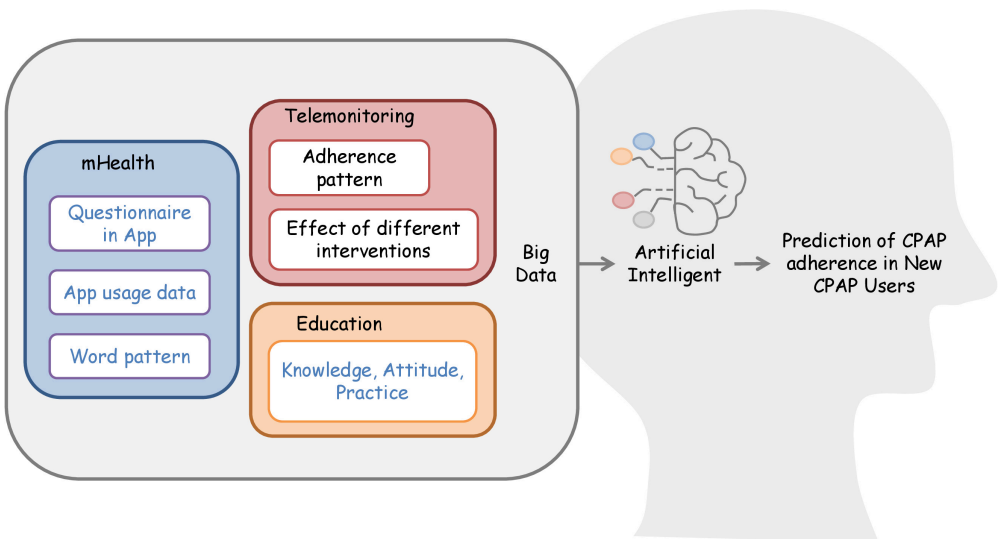

FIGURE 2 | Integration of big data and artificial intelligence for the development of the predictive model. Collection of adherence data from telemonitoring able to generate adherence patterns database. Future studies can use the database to continue work on the artificial intelligence model to predict CPAP adherence. Researchers can also consider building biopsychosocial databases by retrieving data such as psychological, social, and clinical questionnaires in-app. Developers can collect app usage data and word patterns used in telecommunication apps to train the artificial intelligence model to predict CPAP adherence. For education, researchers could work on a prediction model based on the knowledge, attitude, and practice of CPAP users. The blue words represent future intervention.

The development of tele-technology concerning OSA patients' psychology and social life remains scarce. Multiple healthcare-related parties should consider the implementation of tele-technology in the advancement of the biopsychosocial model. Since a recent study found group CPAP education enhanced acceptance of therapy, future studies can investigate educational interventions with peer support through social app platforms (72). Integration of continuous tele-education and online

motivational interviews might maximize the positive effect on patients' health behavior by enhancing their confidence level about the therapy (65, 71). We also suggest the inclusion of a polysomnography chart in the educational video, as one RCT found that this increased the mean usage hours (SG = 4.2 ± 2.5 h/night, interventional group = 5.2 ± 2.1 h/night, p = 0.027) and the compliance (SG = 68.3%, interventional group = 86.5%, p = 0.021) (73).

Future of Telehealth Systems for Enhancing Continuous Positive Airway Pressure Adherence

As individual treatments show heterogeneous results, we would like to propose an individualized telehealth model integrating all the interventions shown in **Figure 1**. Firstly, a new CPAP user should receive educational material in the form of a video, slideshow, or booklet. Then, adherence data should be transmitted to the cloud database, enabling HPs to analyze CPAP adherence. Patients with good compliance will use the apps for self-management and contact HPs when necessary. If HPs review the usage data periodically, then patients with poor adherence could receive the advice and combinations of interventions. Currently available interventions to combine included text message, telephone, motivational enhancement, and patient engagement tools. Developers and researchers could consider including family engagement tools (74), online patient support groups using telecommunication apps, and continuous tele-education in future research.

Moreover, the collection of adherence data from CPAP devices into a database also facilitates big data development (35, 38, 40, 47–51). The application of cloud based-data allows an overview of real-world data and investigation of CPAP adherence patterns (75, 76). Besides, big data shows the effectiveness of different interventions with minimal effort required, compared to the traditional observational studies (33). An analysis of AirView alone compares adherence patterns among various countries (N = 4,181,490) and supports the positive effects of a patient engagement tool (77). Big data from German homecare providers also reveals several predictors of poor CPAP adherence and investigated the effect of shifting therapy (78). The current benefits of big data analysis imply the worthiness of building more cloud databases and collecting various types of data.

Recently, some studies have developed artificial intelligence models to predict adherence (79–81). A model predicting the next-30-day adherence phenotype achieved the highest sensitivity (90%), specificity (96%), and accuracy (95%) (79). However, the model for 6-month adherence had a sensitivity ranging between 71 and 77% and a specificity ranging between 69 and 72% (80). These promising results encourage future studies to use big data to develop clinical prediction models to identify patients with likely poor adherence. Additionally, future research could collect various types of data to integrate the biopsychosocial model into clinical prediction models. For example, responses from clinical and psychological questionnaires, the app usage data, and word patterns of patients' responses can build a database to train more artificial intelligence models. Investigation of knowledge, attitude, and practice of CPAP adherence also assists in the generation of these prediction models (**Figure 2**).

CONCLUSION

Telemonitoring has been proven to improve the compliance of CPAP, resulting in the reduction of disease severity and side effects, and enhancement of social and psychological support. It also improves the quality of life and slightly alleviates the economic burden. We support the application of telemonitoring in CPAP management and following up. As tele-education alone is insufficient in improving CPAP adherence. Thus, we call for a bio-psycho-social care model integrating multiple interventions to promote better care for CPAP therapy.

AUTHOR CONTRIBUTIONS

BT, GL, JL, and QL: conception and design of the work. BT, GL, JL, CL, ST, HL, and QL: drafting of the manuscript and final approval for publication. BT, GL, JL, HL, and QL: critically revision for the content. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (82070089 and 81770084), the Key Research Program of Shanghai Science and Technology Commission (18140903600), and the National Key R&D Program of China (2018YFC1311900).

REFERENCES

1. Turino C, Bertran S, Gavalda R, Teixido I, Woehrle H, Rue M, et al. Characterization of the CPAP-treated patient population in catalonia. *PLoS One.* (2017) 12:e0185191. doi: 10.1371/journal.pone.0185191
2. Benjafield AV, Ayas NT, Eastwood PR, Heinzer R, Ip MSM, Morrell MJ, et al. Estimation of the global prevalence and burden of obstructive sleep apnoea: a literature-based analysis. *Lancet Respir Med.* (2019) 7:687–98. doi: 10.1016/s2213-2600(19)30198-5
3. Bonsignore MR, Baiamonte P, Mazzuca E, Castrogiovanni A, Marrone O. Obstructive sleep apnea and comorbidities: a dangerous liaison. *Multidiscip Respir Med.* (2019) 14:8. doi: 10.1186/s40248-019-0172-9
4. Moyer CA, Sonnad SS, Garetz SL, Helman JI, Chervin RD. Quality of life in obstructive sleep apnea: a systematic review of the literature. *Sleep Med.* (2001) 2:477–91. doi: 10.1016/S1389-9457(01)00072-7
5. Garbarino S, Guglielmi O, Sanna A, Mancardi GL, Magnavita N. Risk of occupational accidents in workers with obstructive sleep apnea: systematic review and meta-analysis. *Sleep.* (2016) 39:1211–8. doi: 10.5665/sleep.5834
6. Tregear S, Reston J, Schoelles K, Phillips B. Obstructive sleep apnea and risk of motor vehicle crash: systematic review and meta-analysis. *J Clin Sleep Med.* (2009) 5:573–81. doi: 10.5664/jcsm.27662
7. Gami AS, Olson EJ, Shen WK, Wright RS, Ballman KV, Hodge DO, et al. Obstructive sleep apnea and the risk of sudden cardiac death: a longitudinal

study of 10,701 adults. *J Am Coll Cardiol.* (2013) 62:610–6. doi: 10.1016/j.jacc.2013.04.080
8. Patil SP, Ayappa IA, Caples SM, Kimoff RJ, Patel SR, Harrod CG. Treatment of adult obstructive sleep apnea with positive airway pressure: an American academy of sleep medicine clinical practice guideline. *J Clin Sleep Med.* (2019) 15:335–43. doi: 10.5664/jcsm.7640
9. Naik S, Al-Halawani M, Kreinin I, Kryger M. Centers for medicare and medicaid services positive airway pressure adherence criteria may limit treatment to many medicare beneficiaries. *J Clin Sleep Med.* (2019) 15:245–51. doi: 10.5664/jcsm.7626
10. Weaver TE, Grunstein RR. Adherence to continuous positive airway pressure therapy: the challenge to effective treatment. *Proc Am Thorac Soc.* (2008) 5:173–8. doi: 10.1513/pats.200708-119MG
11. Rotenberg BW, Murariu D, Pang KP. Trends in CPAP adherence over twenty years of data collection: a flattened curve. *J Otolaryngol Head Neck Surg.* (2016) 45:43. doi: 10.1186/s40463-016-0156-0
12. Mehrtash M, Bakker JP, Ayas N. Predictors of continuous positive airway pressure adherence in patients with obstructive sleep apnea. *Lung.* (2019) 197:115–21. doi: 10.1007/s00408-018-00193-1
13. NEJM Catalyst. *What Is Telehealth?*. Waltham, MA: NEJM Catalyst (2018). doi: 10.1056/CAT.18.0268
14. Physicians AAoF. *What's the Difference Between Telemedicine and Telehealth?*. (2022). Available online at: https://www.aafp.org/news/media-center/kits/telemedicine-and-telehealth.html#:~{}:text=Telehealth%20is%20different%20from%20telemedicine,to%20remote%20non%2Dclinical%20services (accessed July 25, 2021).
15. Singh J, Badr MS, Diebert W, Epstein L, Hwang D, Karres V, et al. American academy of sleep medicine (AASM) position paper for the use of telemedicine for the diagnosis and treatment of sleep disorders. *J Clin Sleep Med.* (2015) 11:1187–98. doi: 10.5664/jcsm.5098
16. Shamim-Uzzaman QA, Bae CJ, Ehsan Z, Setty AR, Devine M, Dhankikar S, et al. The use of telemedicine for the diagnosis and treatment of sleep disorders: an American academy of sleep medicine update. *J Clin Sleep Med.* (2021) 17:1103–7. doi: 10.5664/jcsm.9194
17. Woehrle H, Graml A, Weinreich G. Age- and gender-dependent adherence with continuous positive airway pressure therapy. *Sleep Med.* (2011) 12:1034–6. doi: 10.1016/j.sleep.2011.05.008
18. Martinez-Garcia MA, Valero-Sanchez I, Reyes-Nunez N, Oscullo G, Garcia-Ortega A, Gomez-Olivas JD, et al. Continuous positive airway pressure adherence declines with age in elderly obstructive sleep apnoea patients. *ERJ Open Res.* (2019) 5:00178–2018. doi: 10.1183/23120541.00178-2018
19. Anttalainen U, Saaresranta T, Kalleinen N, Aittokallio J, Vahlberg T, Polo O. CPAP adherence and partial upper airway obstruction during sleep. *Sleep Breath.* (2007) 11:171–6. doi: 10.1007/s11325-007-0102-5
20. Billings ME, Auckley D, Benca R, Foldvary-Schaefer N, Iber C, Redline S, et al. Race and residential socioeconomics as predictors of CPAP adherence. *Sleep.* (2011) 34:1653–8. doi: 10.5665/sleep.1428
21. Jacobsen AR, Eriksen F, Hansen RW, Erlandsen M, Thorup L, Damgard MB, et al. Determinants for adherence to continuous positive airway pressure therapy in obstructive sleep apnea. *PLoS One.* (2017) 12:e0189614. doi: 10.1371/journal.pone.0189614
22. Campos-Rodriguez F, Martinez-Alonso M, Sanchez-de-la-Torre M, Barbe F, Spanish Sleep N. Long-term adherence to continuous positive airway pressure therapy in non-sleepy sleep apnea patients. *Sleep Med.* (2016) 17:1–6. doi: 10.1016/j.sleep.2015.07.038
23. McArdle N, Devereux G, Heidarnejad H, Engleman HM, Mackay TW, Douglas NJ. Long-term use of CPAP therapy for sleep apnea/hypopnea syndrome. *Am J Respir Crit Care Med.* (1999) 159:1108–14. doi: 10.1164/ajrccm.159.4.9807111
24. Singhal P, Joshi Y, Singh G, Kulkarni S. Study of factors affecting compliance of continuous positive airway pressure (CPAP) in obstructive sleep apnea-hypopnea syndrome (OSAHS). *Eur Respir J.* (2016) 48:A2362. doi: 10.1183/13993003.congress-2016.PA2362
25. Ghadiri M, Grunstein RR. Clinical side effects of continuous positive airway pressure in patients with obstructive sleep apnoea. *Respirology.* (2020) 25:593–602. doi: 10.1111/resp.13808
26. Wild MR, Engleman HM, Douglas NJ, Espie CA. Can psychological factors help us to determine adherence to CPAP? A prospective study. *Eur Respir J.* (2004) 24:461–5. doi: 10.1183/09031936.04.00114603
27. Copur AS, Erik Everhart D, Zhang C, Chen Z, Shekhani H, Mathevosian S, et al. Effect of personality traits on adherence with positive airway pressure therapy in obstructive sleep apnea patients. *Sleep Breath.* (2018) 22:369–76. doi: 10.1007/s11325-017-1559-5
28. Cayanan EA, Bartlett DJ, Chapman JL, Hoyos CM, Phillips CL, Grunstein RR. A review of psychosocial factors and personality in the treatment of obstructive sleep apnoea. *Eur Respir Rev.* (2019) 28:190005. doi: 10.1183/16000617.0005-2019
29. Law M, Naughton M, Ho S, Roebuck T, Dabscheck E. Depression may reduce adherence during CPAP titration trial. *J Clin Sleep Med.* (2014) 10:163–9. doi: 10.5664/jcsm.3444
30. Ye L, Malhotra A, Kayser K, Willis DG, Horowitz JA, Aloia MS, et al. Spousal involvement and CPAP adherence: a dyadic perspective. *Sleep Med Rev.* (2015) 19:67–74. doi: 10.1016/j.smrv.2014.04.005
31. Simon-Tuval T, Reuveni H, Greenberg-Dotan S, Oksenberg A, Tal A, Tarasiuk A. Low socioeconomic status is a risk factor for CPAP acceptance among adult OSAS patients requiring treatment. *Sleep.* (2009) 32:545–52. doi: 10.1093/sleep/32.4.545
32. Woehrle H, Arzt M, Graml A, Fietze I, Young P, Teschler H, et al. Effect of a patient engagement tool on positive airway pressure adherence: analysis of a German healthcare provider database. *Sleep Med.* (2018) 41:20–6. doi: 10.1016/j.sleep.2017.07.026
33. Malhotra A, Crocker ME, Willes L, Kelly C, Lynch S, Benjafield AV. Patient engagement using new technology to improve adherence to positive airway pressure therapy: a retrospective analysis. *Chest.* (2018) 153:843–50. doi: 10.1016/j.chest.2017.11.005
34. Pépin JL, Tamisier R, Hwang D, Mereddy S, Parthasarathy S. Does remote monitoring change OSA management and CPAP adherence? *Respirology.* (2017) 22:1508–17. doi: 10.1111/resp.13183
35. Hoet F, Libert W, Sanida C, Van den Broecke S, Bruyneel AV, Bruyneel M. Telemonitoring in continuous positive airway pressure-treated patients improves delay to first intervention and early compliance: a randomized trial. *Sleep Med.* (2017) 39:77–83. doi: 10.1016/j.sleep.2017.08.016
36. Anttalainen U, Melkko S, Hakko S, Laitinen T, Saaresranta T. Telemonitoring of CPAP therapy may save nursing time. *Sleep Breath.* (2016) 20:1209–15. doi: 10.1007/s11325-016-1337-9
37. Fields BG, Behari PP, McCloskey S, True G, Richardson D, Thomasson A, et al. Remote ambulatory management of veterans with obstructive sleep apnea. *Sleep.* (2016) 39:501–9. doi: 10.5665/sleep.5514
38. Pepin JL, Jullian-Desayes I, Sapene M, Treptow E, Joyeux-Faure M, Benmerad M, et al. Multimodal remote monitoring of high cardiovascular risk patients with OSA initiating CPAP a randomized trial. *Chest.* (2019) 155:730–9. doi: 10.1016/j.chest.2018.11.007
39. Mansell SK, Cutts S, Hackney I, Wood MJ, Hawksworth K, Creer DD, et al. Using domiciliary non-invasive ventilator data downloads to inform clinical decision-making to optimise ventilation delivery and patient compliance. *BMJ Open Respir Res.* (2018) 5:e000238. doi: 10.1136/bmjresp-2017-000238
40. Nilius G, Schroeder M, Domanski U, Tietze A, Schafer T, Franke KJ. Telemedicine improves continuous positive airway pressure adherence in stroke patients with obstructive sleep apnea in a randomized trial. *Respiration.* (2019) 98:410–20. doi: 10.1159/000501656
41. Aalaei S, Rezaeitalab F, Tabesh H, Amini M, Afsharisaleh L, Mostafavi SM, et al. Factors affecting patients' adherence to continuous positive airway pressure therapy for obstructive sleep apnea disorder: a multi-method approach. *Iran J Med Sci.* (2020) 45:170–8. doi: 10.30476/ijms.2019.45785
42. Woehrle H, Ficker JH, Graml A, Fietze I, Young P, Teschler H, et al. Telemedicine-based proactive patient management during positive airway pressure therapy: impact on therapy termination rate. *Somnologie (Berl).* (2017) 21:121–7. doi: 10.1007/s11818-016-0098-9
43. Lugo VM, Garmendia O, Suarez-Girón M, Torres M, Vázquez-Polo FJ, Negrín MA, et al. Comprehensive management of obstructive sleep apnea by telemedicine: clinical improvement and cost-effectiveness of a virtual sleep

unit. A randomized controlled trial. *PLoS One.* (2019) 14:e0224069. doi: 10.1371/journal.pone.0224069
44. Turino C, de Batlle J, Woehrle H, Mayoral A, Castro-Grattoni AL, Gómez S, et al. Management of continuous positive airway pressure treatment compliance using telemonitoring in obstructive sleep apnoea. *Eur Respir J.* (2017) 49:1601128. doi: 10.1183/13993003.01128-2016
45. Chen C, Wang J, Pang L, Wang Y, Ma G, Liao W. Telemonitor care helps CPAP compliance in patients with obstructive sleep apnea: a systemic review and meta-analysis of randomized controlled trials. *Ther Adv Chronic Dis.* (2020) 11:2040622320901625. doi: 10.1177/2040622320901625
46. Rattray NA, Khaw A, McGrath M, Damush TM, Miech EJ, Lenet A, et al. Evaluating the feasibility of implementing a telesleep pilot program using two-tiered external facilitation. *BMC Health Serv Res.* (2020) 20:357. doi: 10.1186/s12913-020-05164-y
47. Murase K, Tanizawa K, Minami T, Matsumoto T, Tachikawa R, Takahashi N, et al. A randomized controlled trial of telemedicine for long-term sleep apnea continuous positive airway pressure management. *Ann Am Thorac Soc.* (2020) 17:329–37. doi: 10.1513/AnnalsATS.201907-494OC
48. Hwang D, Chang JW, Benjafield AV, Crocker ME, Kelly C, Becker KA, et al. Effect of telemedicine education and telemonitoring on continuous positive airway pressure adherence. The tele-osa randomized trial. *Am J Respir Crit Care Med.* (2018) 197:117–26. doi: 10.1164/rccm.201703-0582OC
49. Fernandes M, Antunes C, Martinho C, Carvalho J, Abreu T, Oliveira A, et al. Evaluation of telemonitoring of continuous positive airway pressure therapy in obstructive sleep apnoea syndrome: telepap pilot study. *J Telemed Telecare.* (2019) 27:353–8. doi: 10.1177/1357633x19875850
50. Frasnelli M, Baty F, Niedermann J, Brutsche MH, Schoch OD. Effect of telemetric monitoring in the first 30 days of continuous positive airway pressure adaptation for obstructive sleep apnoea syndrome – a controlled pilot study. *J Telemed Telecare.* (2016) 22:209–14. doi: 10.1177/1357633x15598053
51. Kotzian ST, Saletu MT, Schwarzinger A, Haider S, Spatt J, Kranz G, et al. Proactive telemedicine monitoring of sleep apnea treatment improves adherence in people with stroke– a randomized controlled trial (hopes study). *Sleep Med.* (2019) 64:48–55. doi: 10.1016/j.sleep.2019.06.004
52. Schoch OD, Baty F, Boesch M, Benz G, Niedermann J, Brutsche MH. Telemedicine for continuous positive airway pressure in sleep apnea. A randomized, controlled study. *Ann Am Thorac Soc.* (2019) 16:1550–7. doi: 10.1513/AnnalsATS.201901-013OC
53. Willard-Grace R, Wolf J, Huang B, Lewis E, Su G. Pilot of brief health coaching intervention to improve adherence to positive airway pressure therapy. *Jt Comm J Qual Patient Saf.* (2020) 46:631–9. doi: 10.1016/j.jcjq.2020.08.011
54. Pengo MF, Czaban M, Berry MP, Nirmalan P, Brown R, Birdseye A, et al. The effect of positive and negative message framing on short term continuous positive airway pressure compliance in patients with obstructive sleep apnea. *J Thorac Dis.* (2018) 10:S160–9. doi: 10.21037/jtd.2017.07.110
55. Kataria LV, Sundahl CA, Skalina LM, Shah M, Pfeiffer MH, Balish MS, et al. Annie: the veterans health administration's personalized text message application promotes compliance with positive airway pressure. *Sleep.* (2017) 40:A196–A. doi: 10.1093/sleepj/zsx050.525
56. Munafo D, Hevener W, Crocker M, Willes L, Sridasome S, Muhsin M. A telehealth program for CPAP adherence reduces labor and yields similar adherence and efficacy when compared to standard of care. *Sleep Breath.* (2016) 20:777–85. doi: 10.1007/s11325-015-1298-4
57. Hostler JM, Sheikh KL, Andrada TF, Khramtsov A, Holley PR, Holley AB. A mobile, web-based system can improve positive airway pressure adherence. *J Sleep Res.* (2017) 26:139–46. doi: 10.1111/jsr.12476
58. Isetta V, Torres M, González K, Ruiz C, Dalmases M, Embid C, et al. A new mhealth application to support treatment of sleep apnoea patients. *J Telemed Telecare.* (2017) 23:14–8. doi: 10.1177/1357633x15621848
59. Baltaxe E, Embid C, Aumatell E, Martínez M, Barberan-Garcia A, Kelly J, et al. Integrated care intervention supported by a mobile health tool for patients using noninvasive ventilation at home: randomized controlled trial. *JMIR Mhealth Uhealth.* (2020) 8:e16395. doi: 10.2196/16395
60. Rathbone AL, Prescott J. The use of mobile apps and SMS messaging as physical and mental health interventions: systematic review. *J Med Internet Res.* (2017) 19:e295. doi: 10.2196/jmir.7740
61. Clough BA, Casey LM. The smart therapist: a look to the future of smartphones and mhealth technologies in psychotherapy. *Prof Psychol Res Pr.* (2015) 46:147–53. doi: 10.1037/pro0000011
62. Brostrom A, Nilsen P, Johansson P, Ulander M, Stromberg A, Svanborg E, et al. Putative facilitators and barriers for adherence to CPAP treatment in patients with obstructive sleep apnea syndrome: a qualitative content analysis. *Sleep Med.* (2010) 11:126–30. doi: 10.1016/j.sleep.2009.04.010
63. Scharf SM, Seiden L, DeMore J, Carter-Pokras O. Racial differences in clinical presentation of patients with sleep-disordered breathing. *Sleep Breath.* (2004) 8:173–83. doi: 10.1007/s11325-004-0173-5
64. Engleman HM, Wild MR. Improving CPAP use by patients with the sleep apnoea/hypopnoea syndrome (Sahs). *Sleep Med Rev.* (2003) 7:81–99. doi: 10.1053/smrv.2001.0197
65. Chuang SC, Cheng YH, Chang CJ, Chiang YT. The impact of self-confidence on the compromise effect. *Int J Psychol.* (2013) 48:660–75. doi: 10.1080/00207594.2012.666553
66. Rosenstock IM, Strecher VJ, Becker MH. Social learning theory and the health belief model. *Health Educ Q.* (1988) 15:175–83. doi: 10.1177/109019818801500203
67. Curran VR. Tele-education. *J Telemed Telecare.* (2006) 12:57–63. doi: 10.1258/135763306776084400
68. Guralnick AS, Balachandran JS, Szutenbach S, Adley K, Emami L, Mohammadi M, et al. Educational video to improve CPAP use in patients with obstructive sleep apnoea at risk for poor adherence: a randomised controlled trial. *Thorax.* (2017) 72:1132–9. doi: 10.1136/thoraxjnl-2017-210106
69. Dharmakulaseelan L, Kirolos N, Kamra M, Armesto-Heys A, Bouthillier C, Runions S, et al. Educating stroke/Tia patients about obstructive sleep apnea after stroke: a randomized feasibility study. *J Stroke Cerebrovasc Dis.* (2019) 28:104317. doi: 10.1016/j.jstrokecerebrovasdis.2019.104317
70. Patil SP, Ayappa IA, Caples SM, Kimoff RJ, Patel SR, Harrod CG. Treatment of adult obstructive sleep apnea with positive airway pressure: an American academy of sleep medicine systematic review, meta-analysis, and grade assessment. *J Clin Sleep Med.* (2019) 15:301–34. doi: 10.5664/jcsm.7638
71. Bakker JP, Wang R, Weng J, Aloia MS, Toth C, Morrical MG, et al. Motivational enhancement for increasing adherence to CPAP: a randomized controlled trial. *Chest.* (2016) 150:337–45. doi: 10.1016/j.chest.2016.03.019
72. Lettieri CJ, Walter RJ. Impact of group education on continuous positive airway pressure adherence. *J Clin Sleep Med.* (2013) 9:537–41. doi: 10.5664/jcsm.2742
73. Saraç S, Afçar G, Oruç Ö, Topçuoğlu ÖB, Saltürk C, Peker Y. Impact of patient education on compliance with positive airway pressure treatment in obstructive sleep apnea. *Med Sci Monit.* (2017) 23:1792–9. doi: 10.12659/msm.902075
74. Ventola CL. Mobile devices and apps for health care professionals: uses and benefits. *P T.* (2014) 39:356–64.
75. Cistulli PA, Armitstead J, Pepin JL, Woehrle H, Nunez CM, Benjafield A, et al. Short-term CPAP adherence in obstructive sleep apnea: a big data analysis using real world data. *Sleep Med.* (2019) 59:114–6. doi: 10.1016/j.sleep.2019.01.004
76. Patel SR, Bakker JP, Stitt CJ, Aloia MS, Nouraie SM. Age and sex disparities in adherence to CPAP. *Chest.* (2021) 159:382–9. doi: 10.1016/j.chest.2020.07.017
77. Drager LF, Malhotra A, Yan Y, Pépin JL, Armitstead JP, Woehrle H, et al. Adherence with positive airway pressure therapy for obstructive sleep apnea in developing versus developed countries: a big data study. *J Clin Sleep Med.* (2020) 17:703–9. doi: 10.5664/jcsm.9008
78. Woehrle H, Arzt M, Graml A, Fietze I, Young P, Teschler H, et al. Predictors of positive airway pressure therapy termination in the first year: analysis of big data from a German homecare provider. *BMC Pulm Med.* (2018) 18:186. doi: 10.1186/s12890-018-0748-8
79. Hevener W, Beine B, Woodruff J, Munafo D, Fernandez C, Rusk S, et al. Using AI to predict future CPAP adherence and the impact of behavioral and technical interventions. *Sleep.* (2020) 43:A243–A. doi: 10.1093/sleep/zsaa056.632
80. Araujo M, Kazaglis L, Bhojwani R, Iber C, Khadanga S, Srivastava J. Machine learning to predict pap adherence and compliance in tele-health management. *Sleep.* (2018) 41:A400–1. doi: 10.1093/sleep/zsy061.1077
81. Araujo M, Kazaglis L, Iber C, Srivastava J. A data-driven approach for continuous adherence predictions in sleep apnea therapy

management. In: Baru C, Huan J, Khan L, Hu XT, Ak R, Tian Y, editors. *2019 IEEE International Conference on Big Data*. (Piscataway, NJ: IEEE) (2019). p. 2716–25. doi: 10.1109/BigData47090.2019.9006476

82. Isetta V, Torres M, Gonzalez K, Ruiz C, Dalmases M, Embid C, et al. A new mhealth application to support treatment of sleep apnoea patients. *J Telemed Telecare.* (2017) 23:14–8. doi: 10.1177/1357633X15621848

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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

SYSTEMATIC REVIEW
published: 06 May 2022
doi: 10.3389/fmed.2022.868316

Clinical Effects of Rehabilitation on Balance in People With Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-Analysis

María Belén Canales-Díaz[1†], Carolina Olivares-Valenzuela[1†], Amanda Ramírez-Arriagada[1†], Carlos Cruz-Montecinos[1,2], Jordi Vilaró[3,4], Rodrigo Torres-Castro[1,3,5,6] and Rodrigo Núñez-Cortés[1,3]*

[1] Department of Physical Therapy, Faculty of Medicine, University of Chile, Santiago, Chile, [2] Section of Research, Innovation and Development in Kinesiology, Kinesiology Unit, San José Hospital, Santiago, Chile, [3] International Physiotherapy Research Network (PhysioEvidence), Barcelona, Spain, [4] Blanquerna School of Health Sciences, Global Research on Wellbeing, Universitat Ramon Llull, Barcelona, Spain, [5] Department of Pulmonary Medicine, Hospital Clínic, University of Barcelona, Barcelona, Spain, [6] Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain

OPEN ACCESS

Edited by:
Shinichiro Morishita,
Fukushima Medical University, Japan

Reviewed by:
Felipe González-Seguel,
Universidad del Desarrollo, Chile
Stéphanie Saxer,
University Hospital Zürich, Switzerland

***Correspondence:**
Rodrigo Torres-Castro
klgorodrigotorres@gmail.com

[†]These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: 02 February 2022
Accepted: 05 April 2022
Published: 06 May 2022

Citation:
Canales-Díaz MB, Olivares-Valenzuela C, Ramírez-Arriagada A, Cruz-Montecinos C, Vilaró J, Torres-Castro R and Núñez-Cortés R (2022) Clinical Effects of Rehabilitation on Balance in People With Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-Analysis. Front. Med. 9:868316. doi: 10.3389/fmed.2022.868316

Background: Patients with chronic obstructive pulmonary disease (COPD) have systemic damage secondary to the primary pulmonary impairment, expressed in impaired peripheral musculature and a deficit in postural control compared to healthy subjects. This study aimed to determine the effects of rehabilitation on balance in patients with COPD.

Methods: An exhaustive search was conducted in four databases (Pubmed, Cochrane Library, EMBASE, Web of Science). Articles with a population of COPD receiving rehabilitation (therapeutic exercise, pulmonary rehabilitation, or physical therapy modalities) in an outpatient setting were included. Two independent reviewers selected and assessed the study quality. The risk of bias was assessed with the Cochrane Risk of Bias Tool for Randomized Controlled Trials.

Results: A total of eight studies involving 284 patients were included in the qualitative synthesis. The meta-analysis showed an overall result in favor of balance training for the Berg Balance Scale (mean difference 3.91 points; 95% CI: 1.51 to 6.31; $P = 0.001$), Timed Up and Go test (mean difference −1.58 s; 95% CI: −2.63 to −0.53; $P = 0.003$) and Unipedal stance test (mean difference 3.56 s, 95% CI: 2.58 to 4.54; *P*).

Conclusion: This meta-analysis revealed that rehabilitation improve static and dynamic balance in patients with COPD.

Systematic Review Registration: PROSPERO ID: CRD42020218367.

Keywords: exercise, postural control, risk of fall, rehabilitation, chronic obstructive pulmonary disease (COPD)

INTRODUCTION

Non-communicable diseases kill more than 40 million people each year, accounting for 71% of deaths worldwide, of which respiratory diseases are the third most prevalent cause (1). Chronic Obstructive Pulmonary Disease (COPD) is the fourth leading cause of death in the world and the World Health Organization (WHO) estimates that it will be the third by the year 2030 (1). The COPD is defined as a common, preventable, and treatable disease characterized by respiratory symptoms and persistent airflow limitation due to airway or alveolar abnormalities, usually caused by significant exposure to noxious particles or gases (2). There is sufficient evidence to state that COPD patients have systemic damage secondary to the primary pulmonary impairment, which is expressed in an impairment of peripheral musculature and a deficit of postural control compared to healthy subjects of the same age (3).

Musculoskeletal dysfunction in COPD is associated with different factors, including nutritional alterations, inflammation, oxidative stress, drugs, and the presence of different comorbidities (4). On the other hand, physical deconditioning caused by exertional dyspnea leads to a more sedentary lifestyle, generating greater respiratory and peripheral muscle mass loss (5). In this context, muscle weakness, physical inactivity, and limited mobility are associated with more significant deterioration of postural control in people with COPD, which is associated with increased mortality, less independence, a poorer quality of life, and a higher risk of falling (3, 6). Regarding the latter, it has been reported that the history of falls in people with COPD could range from 33 to 50% (7–10). In routine practice, different clinical tests can be used to predict the fall risk in patients with COPD. For example, the Timed Up and Go test has high reliability and predictive validity for falls in older people (11), and the Berg Balance Scale (BBS) has been identified as useful in successfully identifying individuals at risk for falls (12).

The traditional approach to treating this disease has been based on alleviating and/or improving respiratory symptomatology (13). Nevertheless, in recent decades, pulmonary rehabilitation protocols have been modified to provide more comprehensive and functional care to patients, focusing on increasing participation, minimizing health care costs, increasing exercise tolerance, improving quality of life, decreasing hospitalizations, and reducing mortality (14). In addition, balance training has been installed as a new treatment target in COPD patients to prevent falls (15).

A recent meta-analysis found that people with COPD have reduced balance compared to healthy subjects, which may be related to reduced muscle strength, physical activity, and exercise capacity (16). Although pulmonary rehabilitation has shown promising results in improving exercise capacity (17), outcomes related to balance have been little studied. Therefore, this study aimed to determine the effects of rehabilitation on static and functional balance in people with COPD.

METHODS

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations (18). The protocol was previously registered in the PROSPERO International Prospective Register of Systematic Reviews (CRD42020218367) in November 2020.

Eligibility Criteria

Inclusion criteria were based on PICO: P) Population: Adults with a confirmed diagnosis of COPD based on Global Obstructive Lung Disease (GOLD) criteria (2); I) Intervention: outpatient rehabilitation programs (e.g., therapeutic exercise, pulmonary rehabilitation, or physical therapy interventions); C (Comparison): conventional treatment, usual care or active controls (e.g., education) or no intervention; O (Outcome): The included studies should evaluate static or dynamic balance using clinical tests [i.e., Berg Balance Scale (BBS), Time up and go (TUG), Unipedal Stance Test (UST), Balance Evaluation Systems Test (BESTest), or similar]. Randomized controlled trials (RCTs), controlled intervention studies, and before and after (Pre-Post) studies were included. Patients with neurological conditions or patients with acute exacerbation of COPD in the last 4 weeks were excluded.

Search Strategy

An exhaustive search was conducted in the following databases: PubMed, Cochrane Library, EMBASE and Web of Science, with the keywords divided into four domains: (1) Population: chronic obstructive pulmonary disease OR COPD; (2) Intervention: rehabilitative interventions OR pulmonary rehabilitation OR treatment outcome OR physical therapy modalities OR physical therapy interventions; (3) Outcomes: postural balance OR accidental falls OR risk of falls. (4) Condition: adults OR elderly. No temporary or language filters were included. The search was performed on titles, abstracts, and keywords. The selected terms will be combined using Boolean logical operators (OR, AND, NOT). All references were analyzed using Rayyan web software (19). An additional hand search of the references included in the selected studies and in the previous systematic reviews was performed.

Selection of Studies

First, two independent reviewers (COV and ARA) screened the studies by title and abstract according to the eligibility criteria. A third reviewer (RNC.) resolved discrepancies, and references considered not relevant were discarded. After this selection, full-text articles were accessed to assess compliance with the eligibility criteria. Any discrepancies were resolved by consensus in consultation with a third reviewer (RNC). The exclusion criteria were: (1) Wrong study design: Letters to the editor, editorial, review articles, and *in vivo* and *in vitro* studies were excluded (including the type of wrong publication); (2) Wrong population: we excluded non-outpatients or patients with non-stable diseases; (3) Wrong outcome: Measurement of balance by instrumental tests (e.g., posturography).

Data Extraction

A Microsoft Excel (Microsoft® Excel 2010, Microsoft Corporation, Seattle, USA) table was designed for data extraction. Data extraction was performed in duplicate using a standardized form that included the following data: author, year of publication, country of origin, study design, number of patients, number of men and women, age, forced expiratory volume in the first second in percent predicted values (FEV_1%pred), intervention (frequency, follow-up), results and conclusions. Disagreements were resolved by a third reviewer (RNC). If any relevant data were not included in the article, the authors were contacted by e-mail to obtain the information.

Methodological Quality Assessment

Risk of bias assessment of the included studies was performed using the Cochrane Risk for bias (RoB) tool for randomized clinical trials and ROBINS-I for non-randomized clinical trials (20). Three reviewers (COV, ARA, MCD) performed the assessment independently, and if there were discrepancies or disagreements between the reviewers' judgments, a fourth reviewer (RNC) was consulted.

Data Synthesis and Analysis

RevMan 5.3 software (The Cochrane Collaboration, Oxford, UK) was used for meta-analysis and generation of a forest plot that showed combined estimates with a 95% confidence interval. The mean difference of the results and the standard deviation were pooled for each study comparing an experimental intervention with a control group. Then, a random effects model with inverse variance (IV) method was used to obtain the combined effect measures for each primary outcome. This choice of weight minimizes the imprecision (uncertainty) of the pooled effect estimate. Statistical heterogeneity was assessed by I^2 and classified as could be unimportant (I^2 = 0-40%), moderate (I^2 = 30-60%), substantial (I^2 = 50-90%) or considerable (I^2 = 75-100%) (20).

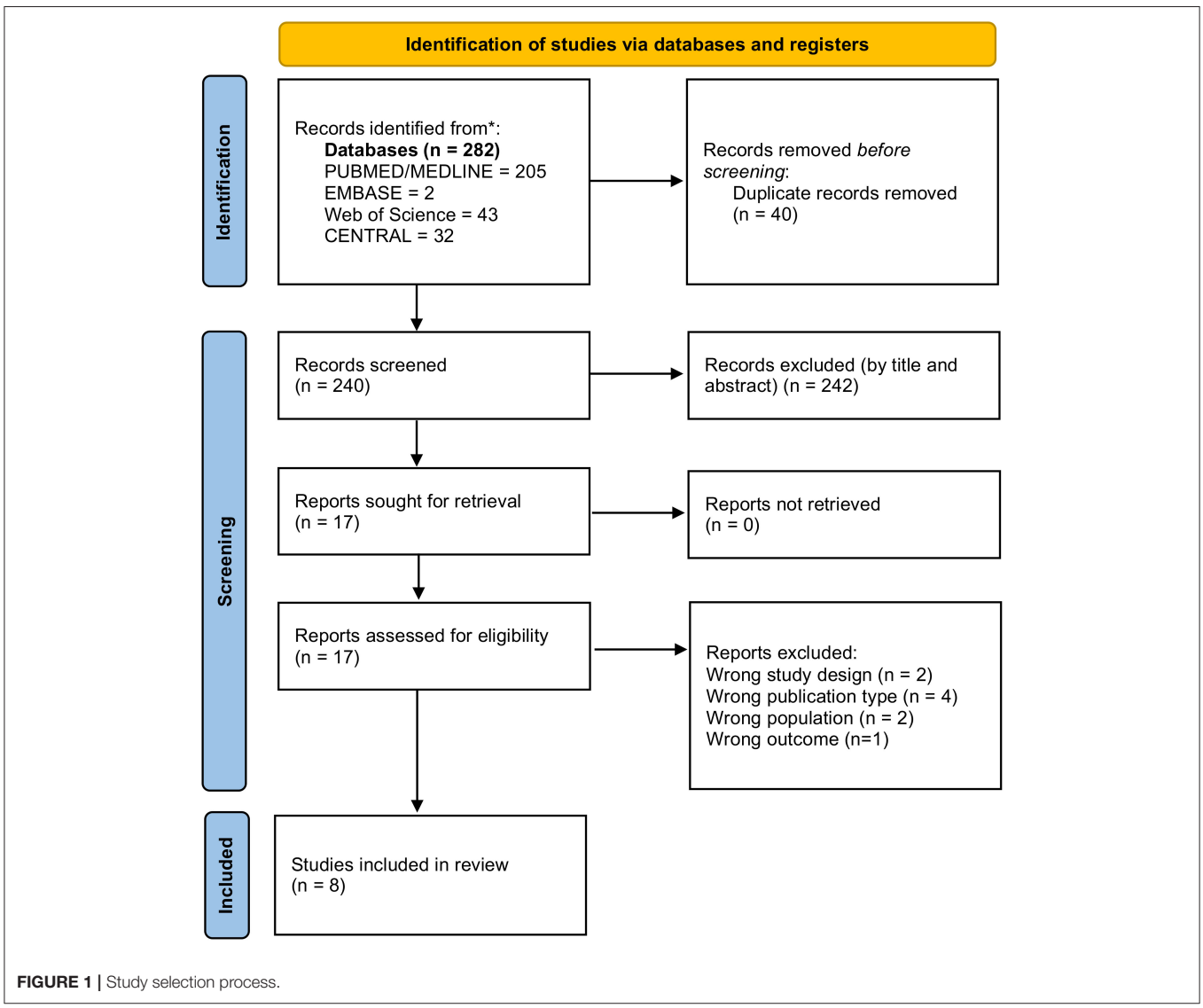

FIGURE 1 | Study selection process.

TABLE 1 | Characteristics of the studies.

References	Country	Design	*n* (M/F)	Age (years)	FEV_1 (% Pred)	BMI (kg/m^2)	Follow-up
Jácome et al. (27)	Portugal	Before-After	Total: 26 (16/10)	Total: 67.8 ± 10.3	Total: 83.8 ± 6.4	Total: 28.7 ± 5.0	12 weeks
Marques et al. (26)	Portugal	Before-After	Total: 22 (13/9)	Total: 68.0 ± 11.8	Total: 72.2 ± 22.3	Total: 28.4 ± 6.0	12 weeks
Mkacher et al. (23)	Tunisia	RCT	Total: 68 (68/0) CG: 33 (33/0) IG: 35 (35/0)	IG: 58.2 ± 4.3 CG: 61.2 ± 3.2	IG: 39.4 ± 10.3 CG: 38.6 ± 8.6	IG: 24.1 ± 3.8 CG: 25.2 ± 2.6	24 weeks
Rinaldo et al. (22)	Italy	RCT	Total: 24 (24/0) IG: 12 (12/0) CG: 12 (12/0)	IG: 66.2 ± 4.2 CG: 66.1 ± 4.5	IG: 60.1 ± 24.3 CG: 72.2 ± 18.8	IG: 29.9 ± 4.4 CG: 28.4 ± 5.7	42 weeks
Mekki et al. (21)	Tunisia	RCT	Total: 45 (45/0) IG:25 (25/0) CG: 20 (20/0)	IG: 59.6 ± 4.8 CG: 59.5 ± 3.1	IG: 57.7 ± 14.4 CG: 57.1 ± 10.2	IG: 25.6 ± 0.7 CG: 25.6 ± 0.5	24 weeks
Mounir et al. (28)	Egypt	RCT	Total: 48 (48/0) IG:24 (24/0) CG:24 (24/0)	IG: 63.1 ± 1.7 CG: 62.4 ± 1.6	IG: 63.6 ± 5.6 CG: 61.6 ± 8.5	IG: 24.8 ± 2.2 CG: 24.9 ± 2.4	8 weeks
Suresh et al. (25)	United Arab Emirates	RCT	Total: 20 (15/5) IG: 10 (8/2) CG: 10 (7/3)	IG: 55.2 ± 3.4 CG: 55.2 ±- 4.6	NR	NR	16 weeks
de Castro et al. (24)	Brazil	RCT	Total: 31 (18/13) CG: 17 (9/8) IG: 14 (9/5)	CG: 64 ± 8 IG: 65 ± 8	CG: 48 ± 17% IG: 51 ± 15%	IG: 28 ± 5 CG: 27 ± 4	12 weeks

BMI, Body mass index; CG, Control group; F, Female; IG, Intervention group; FEV_1%pred, Forced expiratory volume in the first second in percent predicted values; M, Male; NR, Not reported; RCT, Randomized controlled trial.

RESULTS

Study Selection

The initial search yielded 282 articles of interest, then 40 duplicates were eliminated and 225 were excluded in the screening of titles and abstracts. Of the 17 articles evaluated in the full-text screening, four were eliminated due to type of publication, two due to design, two due to study population, and one due to outcome. Finally, a total of eight articles were included in the qualitative synthesis (21–28). A detailed diagram of the selection process of these articles is presented in **Figure 1**.

Characteristics of the Included Studies

The included articles were published between 2014 and 2020, of which six corresponded to randomized clinical trials (21–25, 28) one non-randomized clinical trials (26), and one quasi-experimental study (27). Of the total number of articles selected, three were from Europe, two from Africa, two from Asia and one from South America (**Table 1**). All were published in English.

Participants

In total, 284 patients with COPD were enrolled in the included studies. The sample size was between 20 (25) and 68 (23) participants, with mean age varied between 55.2 ± 3.4 and 68.0 ± 11.8 years. Four studies included only male subjects with COPD (21–23, 28). Overall, most of the included patients were male ($n = 247$, 87%). The BMI ranged from 24.1 ± 3.8 to 29.9 ± 4.4 and FEV1%pred ranged from 38.6 ± 8.6% to 83.8 ± 6.4%. Total follow-up time ranged from 8 to 24 weeks.

Summary of Results

Seven of the selected studies included combined treatment protocols (21–26, 28), while only one study had an isolated intervention protocol (27). Regarding the intervention performed, can be classified into the following categories: balance exercises (22, 23, 25, 27, 28), strength training (21–28), endurance or walking exercises (21, 22, 24–28), aquatic exercises (24), and exercises with neuromuscular electrical stimulation (21). In addition, three articles included education and psychosocial support sessions (22, 23, 27). **Table 2** summarizes the different therapeutic interventions (frequency, intensity, time and type, follow-up), results, and conclusions used in each of the included studies.

Berg Balance Scale

Four studies reported changes in balance using the BBS comparing the results with a control group (21, 23, 25, 28). The overall result of the meta-analysis was in favor of the experimental group [mean difference 3.91 points (95% CI: 1.51 to 6.31; $P = 0.001$)] (**Figure 2**). Heterogeneity between studies was considerable ($I^2 = 95\%$).

Timed Up and Go

Six studies reported changes in balance using the TUG, of which three studies were included in the meta-analysis (21, 23,

TABLE 2 | Synthesis of interventions and results.

References	*n*	Intervention program	Frequency	Results	Conclusion
Jácome et al. (27)	26	PR program with exercise training: Endurance training (walking) at 60–80% of the average speed achieved during the 6MWT (20 min); Strength training including seven exercises (2 sets of 10 repetitions) of the major upper and lower limb muscle groups using free weights and ankle weights (15 min); Psychoeducation (90 min w/session); Balance training (5 min); Psychoeducation (one session/week, 90 min).	PR: 3 sessions/week, 60 min each	Significant effects on TUG: 7.8 vs. 6.7 seconds ($P < 0.001$, ES 0.8).	The PR program was effective in improving dyspnea, functional balance, muscle strength, exercise tolerance and cardiovascular endurance in patients with mild COPD.
Marques et al. (26)	22	Endurance, Strength and Balance exercise training + psychosocial support and education (60 min each session): Warm-up (5-10 min); Endurance: walking at 60-80% of HR obtained in 6MWT (20 min); Strength: 7 exercises of 2 sets of 10 repetitions for upper and lower extremities with 50–85% of 10 RM (15 min); Balance: static and dynamic exercises, using postures that gradually reduce the base, dynamic movements that disturb the center of gravity, tension of postural muscle groups, and dynamic movements with secondary tares decreasing the base of support (5 min); Return to calm.	Exercise: 3 times a week for 12 weeks	Significant post-PR improvements in TUG score (mean change -1.7 ± 1.4 s; $P = 0.001$; effect size = 1.249).	PR with a specific balance training component had a large effect on functional balance in COPD patients.
Mkacher et al. (23)	68 CG: 33 IG: 35	IG: PR + Balance training. CG: PR only Balance: duration of 30 min. Four types of exercise: posture exercise, transitions, walking exercises and functional strength. PR: twice daily supervised exercise training, daily breathing exercises, self-management education, psychological and social support.	3 days a week for 24 weeks.	Significant differences between groups were observed in TUG ($P < 0.01$), Tinetti ($P < 0.01$), BBS ($P < 0.01$) and Unipedal Stance Test scores. ($P < 0.05$).	Balance training incorporated into PR has significant improvements in balance test scores in COPD patients.
Rinaldo et al. (22)	24 IG: 12 CG: 12	IG: Physical activity education program with a progressive increase in the pace of physical activity in three modalities: aerobic classes with flexibility and balance exercises, Nordic walking or non-weight bearing exercises in circuit training. CG: Structured exercise program (traditional). Self-monitored intensity. The protocol included aerobic and strength exercises for 60 min. Endurance: 30 min of cycling or treadmill at modified Borg intensity 3-4; Strength: 4 sets of legs, arms and trunk at 50-80% of 1RM, load was adjusted cad 3 or 4 weeks according to results. Each session ends with flexibility and balance exercises.	IG: 60 min session, 3 times per week, for 28 weeks. CG: Prescribed program for 14 weeks.	Balance control improved markedly in both groups after training but was not maintained at follow-up.	Both programs can effectively and safely improve health-related parameters in COPD patients.
Mekki et al. (21)	45 IG:25 CG:20	IG: neuromuscular electrical stimulation + PR. CG: PR only PR: Warm-up (5-10 min), joint movement, stretching and low-intensity exercise, breathing techniques; Endurance: 45 min on cycle ergometer. 60-70% effort of max. HR obtained in 6MWT; Strength: 15 min. four exercises of 2 sets of 10 repetitions for upper and lower limbs; Return to calm. 45 min. Neuromuscular electrical stimulation (20 min) for quadriceps femoris, triceps suralis and bilateral hamstring. Applied current: biphasic symmetrical rectangular pulses of 400 μs with a frequency of 50 Hz. With intensity ranging from 15 to 60 mA.	3 times a week for 24 weeks.	In IG, TUG and BBS values are significantly higher than CG ($P = 0.02$, $P = 0.01$, respectively); Improved mid-lateral center of pressure displacement in I ($P < 0.001$).	Neuromuscular electrical stimulation added to PR improves physical tolerance and balance compared to PR alone.

(Continued)

TABLE 2 | Continued

References	*n*	Intervention program	Frequency	Results	Conclusion
Mounir et al. (28)	48 IG: 24 CG: 24	IG: balance training + PR CG: PR only Balance: Functional strength exercise (e.g., heel raise, toe raise, walking on toes, step-ups in all directions, squats, and core strength on ball); Stance exercise (e.g., tandem, narrow, one leg stance, and stand on uneven surfaces) with open eyes (each exercise 30 s) and then with eyes closed (each exercise 15 s); Transition exercise; Gait training. PR: Endurance training based on 60–80% of 6MWT speed achieved; Strength exercise using Thera band and weights (3 sets 8 repetitions each), 50–75% of 1 RM, repeated at week 4; daily breathing exercises.	IG: 25-30 minutes (total session), three times a week (every other day). CG: 25-30 minutes (total session), three times per week (day after day).	Significant increase in the BBS and BESTest after treatment in both groups, with a percentage of improvement in the control group was 5.01 and 9.15%, respectively, whereas in the study group was 16.04 and 25.46%, respectively.	Addition of balance training to PR program was more effective in improving balance in elderly patients with COPD.
Suresh et al. (25)	20 IG: 10 CG: 10	IG: Balance Training + PR. CG: PR only PR: 60 min. Endurance: Borg 5-6 for dyspnea or fatigue, walking; Strength: biceps, triceps, deltoids, quadriceps, hip flexors, extensors and abductors, 10-15 reps and Borg. Balance training: 15-20 min. circuit (standing exercises, transitions, ambulatory and functional exercises for balance).	3 days a week for 8 weeks.	Significant differences ($p < 0.05$) between groups before and after intervention for BBS and TUG	PR with or without balance training in subjects with moderate COPD produces statistically and clinically significant effects on balance, exercise tolerance, health-related quality of life, and risk of falls.
de Castro et al. (24)	31 CG: 17 IG: 14	IG: Aquatic training in the pool at 33° (water level: 1 m). CG: Land training. Endurance training: cycling and walking with sound stimuli. Cycling according to perceived exertion (Borg 4-6); Strength training: quadriceps, biceps and triceps (70% of 1RM, fully submerged segment).	3 days a week (60 min. session) for 12 weeks.	Aquatic training positively affected functional balance (TUG: mean difference of −1.17 s, 95%CI: −1.93 to −0.41, $P = 0.006$). In contrast, the static balance remained unchanged in both groups.	Functional balance improved after three months of high-intensity exercise training performed in water. However, non-specific training independent of the environment appears insufficient to improve static balance.

6MWT, 6-min walking test; BBS, Berg Balance Scale; BESTest, The Balance Evaluation Systems Test; COPD, chronic obstructive pulmonary disease; CG, control group; ES, Effect size; HR, Heart frequency; IG, intervention group; PR, Pulmonary rehabilitation; RM, repetition maximum; SGRQ, St George Respiratory Questionnaire; TUG, Timed up and Go.

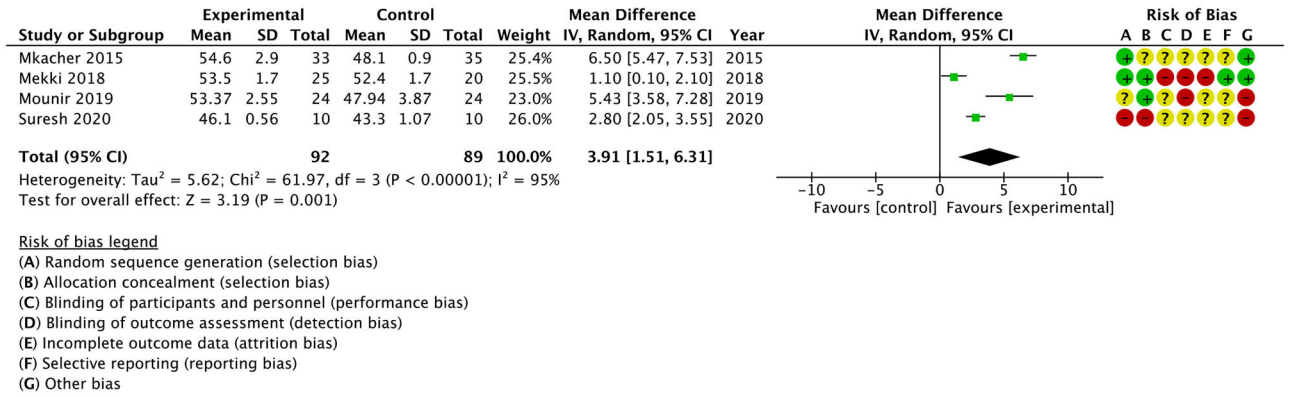

FIGURE 2 | Effects of rehabilitation interventions on the Berg balance scale. Each study considered in the meta-analysis corresponds to a point estimate, which is bounded by a 95% CI.

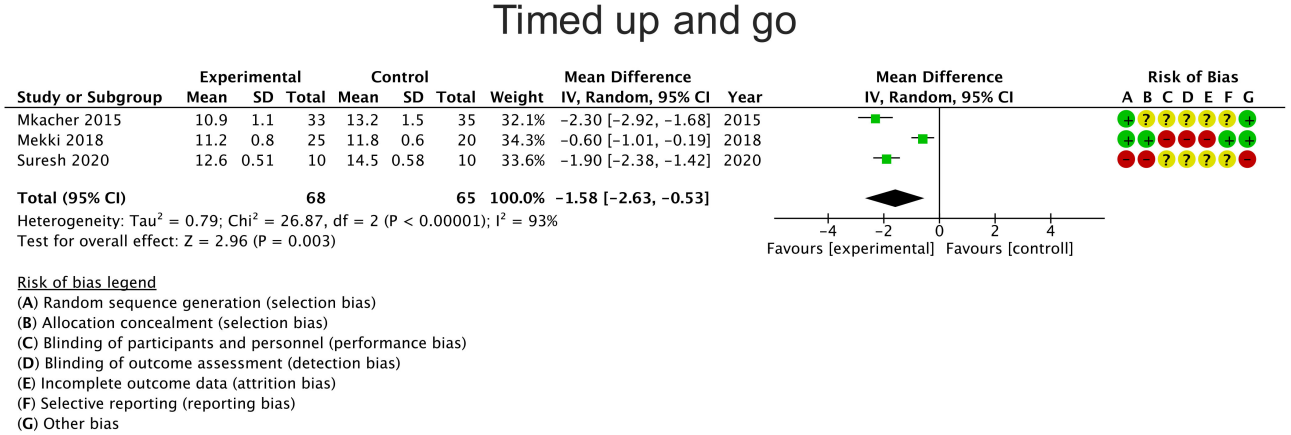

FIGURE 3 | Effects of rehabilitation interventions on the Test timed up and go. Each study considered in the meta-analysis corresponds to a point estimate, which is bounded by a 95% CI.

25) as they compared the intervention with a control group. The overall result of the meta-analysis was in favor of the experimental group [mean difference −1.58 s (95% CI: −2.63 to −0.53; $P = 0.003$)] (**Figure 3**). Heterogeneity between studies was considerable ($I^2 = 93\%$).

Other Balance Measures

Four studies reported changes in balance using the UST of which two studies were included in the meta-analysis (23, 25) as they compared the intervention with a control group. The overall result of the meta-analysis was in favor of the experimental group [mean difference 3.56 s (95% CI: 2.58 to 4.54; *P*)] (**Figure 4**). Heterogeneity between studies was moderate ($I^2 = 41\%$). On the other hand, two studies used the Activities-Specific Balance Confidence (ABC) scale (23, 25), one study used the Balance Evaluation Systems Test (BESTest) (28) and one study used the Tinetti test score (23). These studies reported a significant improvement in balance compared to the control group.

Risk of Bias

Regarding the random sequence generation domain, only one study was found to be at high risk (25). In the allocation concealment domain, one study presented high risk (25) and two were uncertain (23, 24). Two studies presented high risk for blinding of participants and personnel (21, 24) while the rest of the studies were uncertain (22, 23, 25, 28). Two studies presented high risk for blinding of outcome assessment domain (21, 28) and three studies had a high risk of bias due to incomplete outcome data (21, 22, 24). None of the studies presented a high risk of bias in the selective reporting domain, but most of the judgment was uncertain (22, 23, 25, 28). Two studies presented high risk for other sources of bias (25, 28). **Figure 5** shows the summary of each Risk of Bias domain.

DISCUSSION

This systematic review collected information regarding physical therapy interventions and protocols focused on balance

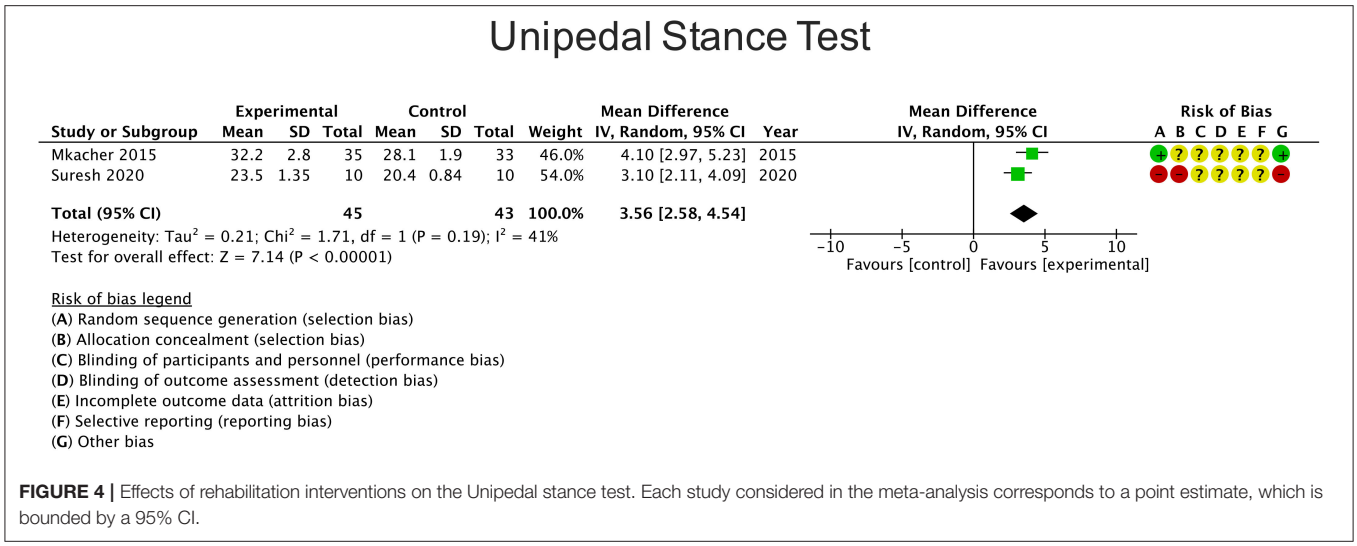

FIGURE 4 | Effects of rehabilitation interventions on the Unipedal stance test. Each study considered in the meta-analysis corresponds to a point estimate, which is bounded by a 95% CI.

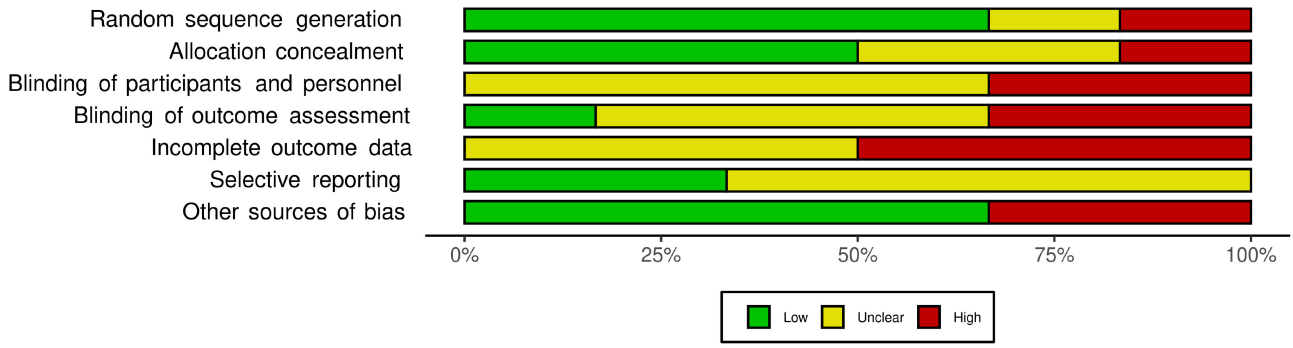

FIGURE 5 | Summary of the risk of bias assessment using the Cochrane Risk for bias (RoB) tool.

training and postural control in COPD patients. Most of the studies that added balance training found significant changes for balance improvement in COPD patients compared to conventional treatments.

Different interventions have been proposed in the current literature to improve balance, including strength training (21–28) endurance or walking exercises (21, 22, 24–28), aquatic exercises (24), exercises with neuromuscular electrical stimulation (21), and specific balance training in addition to traditional treatments (22, 23, 25, 26, 28). Significant improvements obtained in balance were evaluated with different clinical tests for functional balance (TUG) (21, 23, 25), static balance (UST) (22–25), or both (BBS and BESTestest) (21, 23, 25).

The minimum clinically important difference (MCID) with respect to TUG was recently established between 0.9 and 1.4 s (29). Therefore, our results indicate that balance training had an overall significant and clinically relevant effect for this outcome (mean difference −1.58 s). Regarding BBS, although the overall effect obtained from the meta-analysis (mean difference 3.91 points) was larger than the minimum detectable change described in the literature (3.49 points) (30), anchor-based MCID estimates range from 3.5 to 7.1 for BBS (31), making it difficult to estimate whether this change was clinically relevant. Regarding UST, the overall effect obtained from the meta-analysis (mean difference 3.56 s) was lower than the minimal detectable change (4.03 s) established in patients with COPD (30). The unipedal stance training protocols are effective for promoting balance gains in healthy adults (32). However, in COPD patients future studies are needed to establish the effectiveness regarding volume, frequency, and potential progressions of unipedal stance exercise protocols' (32).

These results agree with two similar systematic reviews; Delbressine et al. (33) found that exercise-based interventions have the potential to improve balance in COPD patients and pulmonary rehabilitation combined with balance training showed greater benefits. Chuatrakoon et al. (34) also found that available RCTs suggest that exercise interventions (e.g., cycling, Tai Chi) can improve balance performance in COPD patients, both in the outpatient and inpatient settings. However, in contrast to both reviews (33, 34), our study performed a quantitative synthesis of the effects of rehabilitation interventions on balance. This allows objective data to be obtained on the clinical relevance and accuracy of the differences between the proposed rehabilitation interventions and their comparators. In

addition, our meta-analysis only included balance assessment by clinical testing in the outpatient setting, excluding studies that incorporated instrumental assessment or evaluations in the inpatient setting. Therefore, our results could be extrapolated to a primary health care context, where community-based rehabilitation appears as a possibility for constant treatment that allows permanent control, and at the same time, promotes the autonomy of individuals concerning their pathology (35).

The various clinical tests used indicate that an alteration in static or functional balance can increase the risk of falls in patients with COPD. In particular, this population presents an even higher risk of falls due to musculoskeletal disorders and age. Furthermore, the pharmacological treatment of COPD includes corticosteroids (2), and it has been reported that this group of drugs favors the production of osteoclasts and a decrease in osteocytes, leading to an increased risk of osteopenia or osteoporosis (36, 37). Therefore, the management of these risk factors must be comprehensive.

Given that falls are associated with an increased risk of all-cause mortality in patients with COPD (38), improving balance and preventing falls should become a priority treatment goal in these patients. In this context, we know that balance can be influenced by many factors, including muscle strength and cognitive aspects (39). In this context, new approaches to assess and improve postural control have been proposed in the literature, such as dual-task training (40–42). This proposed intervention (e.g., secondary tasks, counting backwards) was considered in only two of the included studies (23, 26), with a large effect on functional balance in COPD patients. Therefore, pulmonary rehabilitation programs should include all these aspects in future research.

This study has some limitations. In general, the sample size included in the studies was relatively small. In addition, most of the studies had a high risk of bias in some of the domains evaluated, the most critical being the incomplete reporting of results, blinding of the patient and staff, and blinding of the evaluator. Finally, despite the significant improvements obtained in meta-analyses for the overall effect size in each outcome, we observed substantial to considerable statistical heterogeneity, which could be attributed to the few studies included and the low sample size.

CONCLUSION

Our results revealed that rehabilitation improve static and dynamic balance in patients with COPD. Due to the small sample size in this meta-analysis and considering the high prevalence of falls in people with COPD, future large-scale randomized controlled trials are needed to evaluate different exercise protocols' efficacy to prevent falls.

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

CC-M and RN-C: conception and design. MC-D, CO-V, AR-A, RT-C, and RN-C: acquisition, analysis, and interpretation of data. MC-D, CO-V, AR-A, and RN-C: manuscript writing. CC-M, JV, and RT-C: critical revision of the manuscript. All authors read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.868316/full#supplementary-material

REFERENCES

1. World Health Organization: WHO. *Noncommunicable diseases.* (2021). Available online at: https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases (accessed December 21, 2021).
2. Singh D, Agusti A, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease: the GOLD science committee report 2019. *Eur Respir J.* http://paperpile.com/b/kPRi0d/u5cu (2019) 53:1900164. doi: 10.1183/13993003.00164-2019
3. Porto EF, Castro AAM, Schmidt VGS, Rabelo HM, Kümpel C, Nascimento OA, et al. Postural control in chronic obstructive pulmonary disease: a systematic review. *Int J Chron Obstruct Pulmon Dis.* http://paperpile.com/b/kPRi0d/ZTpH (2015) 10:1233–9. doi: 10.2147/COPD.S63955
4. Gea J, Pascual S, Casadevall C, Orozco-Levi M, Barreiro E. Muscle dysfunction in chronic obstructive pulmonary disease: update on causes and biological findings. *J Thorac Dis.* (2015) 7:E418–38. doi: 10.3978/j.issn.2072-1439.2015.08.04
5. Agustí AGN, Sauleda J, Morlá M, Miralles C, Busquets X. Disfunción muscular esquelética de la EPOC. Mecanismos celulares. *Archivos de Bronconeumología.* (2001) 37:197-205. doi: 10.1016/S0300-2896(01)75049-1
6. Roig M, Eng JJ, Road JD, Reid WD. Falls in patients with chronic obstructive pulmonary disease: a call for further research. *Respir Med.* (2009) 103:1257–69. doi: 10.1016/j.rmed.2009.03.022
7. Beauchamp MK, Hill K, Goldstein RS, Janaudis-Ferreira T, Brooks D. Impairments in balance discriminate fallers from non-fallers in COPD. *Respir Med.* (2009) 103:1885–91. doi: 10.1016/j.rmed.2009.06.008
8. Tudorache E, Oancea C, Avram C, Fira-Mladinescu O, Petrescu L, Timar B. Balance impairment and systemic inflammation in chronic obstructive pulmonary disease. *Int J Chronic Obstruct Pulmon Dis.* (2015) 10:1847-52. doi: 10.2147/COPD.S89814
9. Oliveira CC, McGinley J, Lee AL, Irving LB, Denehy L. Fear of falling in people with chronic obstructive pulmonary disease. *Respir Med.* (2015) 109:483–9. doi: 10.1016/j.rmed.2015.02.003
10. Porto EF, Pradella CO, Rocco CM, Chueiro M Jr, Maia RQ, Ganança FF, et al. Comparative postural control in COPD patients and healthy individuals during dynamic and static activities. *J Cardiopulm Rehabil Prev.* (2017) 37:139–45. doi: 10.1097/HCR.0000000000000246
11. Okumiya K, Matsubayashi K, Nakamura T, Fujisawa M, Osaki Y, Doi Y, et al. The timed "up and go" test is a useful predictor of falls in community-dwelling older people. *J Am Geriatr Soc.* (1998) 46:928–30. doi: 10.1111/j.1532-5415.1998.tb02737.x

12. Lajoie Y, Gallagher SP. Predicting falls within the elderly community: comparison of postural sway, reaction time, the Berg balance scale and the Activities-specific Balance Confidence (ABC) scale for comparing fallers and non-fallers. *Arch Gerontol Geriatr.* (2004) 38:11–26. doi: 10.1016/S0167-4943(03)00082-7
13. Beauchamp MK, Janaudis-Ferreira T, Parreira V, Romano JM, Woon L, Goldstein RS, et al. A randomized controlled trial of balance training during pulmonary rehabilitation for individuals with COPD. *Chest.* (2013) 144:1803–10. doi: 10.1378/chest.13-1093
14. Balkissoon R, Lommatzsch S, Carolan B, Make B. Chronic obstructive pulmonary disease: a concise review. *Med Clin North Am.* (2011) 95:1125–41. doi: 10.1016/j.mcna.2011.08.009
15. Spruit MA, Singh SJ, Garvey C, ZuWallack R, Nici L, Rochester C, et al. An official American Thoracic Society/European Respiratory Society statement: key concepts and advances in pulmonary rehabilitation. *Am J Respir Crit Care Med.* (2013) 188:e13–64. doi: 10.1164/rccm.201309-1634ST
16. Loughran KJ, Atkinson G, Beauchamp MK, Dixon J, Martin D, Rahim S, et al. Balance impairment in individuals with COPD: a systematic review with meta-analysis. *Thorax.* (2020) 75:539–46. doi: 10.1136/thoraxjnl-2019-213608
17. Chen H, Li P, Li N, Wang Z, Wu W, Wang J. Rehabilitation effects of land and water-based aerobic exercise on lung function, dyspnea, and exercise capacity in patients with chronic obstructive pulmonary disease: A systematic review and meta-analysis. *Medicine.* (2021) 100:e26976. doi: 10.1097/MD.0000000000026976
18. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. *PLoS Med.* (2009) 6:e1000097. doi: 10.1371/journal.pmed.1000097
19. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev.* (2016) 5:210. doi: 10.1186/s13643-016-0384-4
20. Higgins JPT, Thomas J. *Cochrane Handbook for Systematic Reviews of Interventions*. John Wiley & Sons (2019). p. 728.
21. Mekki M, Paillard T, Sahli S, Tabka Z, Trabelsi Y. Effect of adding neuromuscular electrical stimulation training to pulmonary rehabilitation in patients with chronic obstructive pulmonary disease: randomized clinical trial. *Clin Rehabil.* (2019) 33:195–206. doi: 10.1177/0269215518791658
22. Rinaldo N, Bacchi E, Coratella G, Vitali F, Milanese C, Rossi A, et al. Effects of combined aerobic-strength training vs. fitness education program in COPD patients. *Int J Sports Med.* (2017) 38:1001–8. doi: 10.1055/s-0043-112339
23. Mkacher W, Mekki M, Tabka Z, Trabelsi Y. Effect of 6 months of balance training during pulmonary rehabilitation in patients with COPD. *J Cardiopulm Rehabil Prev.* (2015) 35:207–13. doi: 10.1097/HCR.0000000000000109
24. de Castro LA, Felcar JM, de Carvalho DR, Vidotto LS, da Silva RA, Pitta F, et al. Effects of land- and water-based exercise programmes on postural balance in individuals with COPD: additional results from a randomised clinical trial. *Physiotherapy.* (2020) 107:58–65. doi: 10.1016/j.physio.2019.08.001
25. Suresh Babu Reddy A, Nataraj Madagondapalli Srinivasan T, Kumar A, Vinod Babu K, Alaparthi GK, Bairapareddy KC. Effect of balance training on health related quality of life in patients with chronic obstructive pulmonary disease (COPD). *Indian J Public Health Res Dev.* (2020) 11:03. doi: 10.26452/ijrps.v12i1.4179
26. Marques A, Jácome C, Cruz J, Gabriel R, Figueiredo D. Effects of a pulmonary rehabilitation program with balance training on patients with COPD. *J Cardiopulm Rehabil Prev.* (2015) 35:154–8. doi: 10.1097/HCR.0000000000000097
27. Jácome C, Marques A. Impact of pulmonary rehabilitation in subjects with mild COPD. *Respir Care.* (2014) 59:1577-82. doi: 10.4187/respcare.03091
28. Mounir KM, Elserty NS, Obaya HE. Balance training: its influence on pulmonary rehabilitation. *Bull Facul Phys Ther.* (2019) 24:8-13. doi: 10.4103/bfpt.bfpt_14_18
29. Mesquita R, Wilke S, Smid DE, Janssen DJ, Franssen FM, Probst VS, et al. Measurement properties of the timed up and go test in patients with COPD. *Chron Respir Dis.* (2016) 13:344–52. doi: 10.1177/1479972316647178
30. Mkacher W, Tabka Z, Trabelsi Y. Minimal detectable change for balance measurements in patients with COPD. *J Cardiopulm Rehabilit Prev.* (2017) 37:223-8. doi: 10.1097/HCR.0000000000000240
31. Beauchamp MK, Harrison SL, Goldstein RS, Brooks D. Interpretability of change scores in measures of balance in people with COPD. *Chest.* (2016) 149:696–703. doi: 10.1378/chest.15-0717
32. Marcori AJ, Monteiro PHM, Oliveira JA, Doumas M, Teixeira LA. Single leg balance training: a systematic review. *Percept Mot Skills.* (2022) 129:232–52. doi: 10.1177/00315125211070104
33. Delbressine JM, Vaes AW, Goërtz YM, Sillen MJ, Kawagoshi A, Meijer K, et al. Effects of exercise-based interventions on fall risk and balance in patients with chronic obstructive pulmonary disease: a systematic review. *J Cardiopulm Rehabil Prev.* (2020) 40:152–63. doi: 10.1097/HCR.0000000000000513
34. Chuatrakoon B, Ngai SPC, Sungkarat S, Uthaikhup S. Balance impairment and effectiveness of exercise intervention in chronic obstructive pulmonary disease-a systematic review. *Arch Phys Med Rehabil.* (2020) 101:1590–602. doi: 10.1016/j.apmr.2020.01.016
35. Besoain-Saldaña Á, Sanhueza JR, Hizaut MM, Rojas VC, Ortega GH, Aliaga-Castillo V. Community-based rehabilitation (CBR) in primary care centers in Chile. *Rev Saude Publica.* (2020) 54:38. doi: 10.11606/s1518-8787.2020054001999
36. Lehouck A, Boonen S, Decramer M, Janssens W. COPD, bone metabolism, and osteoporosis. *Chest.* (2011) 139:648–57. doi: 10.1378/chest.10-1427
37. Sutter SA, Stein EM. The skeletal effects of inhaled glucocorticoids. *Curr Osteoporos Rep.* (2016) 14:106–13. doi: 10.1007/s11914-016-0308-1
38. Yohannes AM, Raue PJ, Kanellopoulos D, McGovern A, Sirey JA, Kiosses DN, et al. Predictors of all-cause mortality in patients with severe copd and major depression admitted to a rehabilitation hospital. *Chest.* (2016) 149:467–73. doi: 10.1378/chest.15-0529
39. McLay R, O'Hoski S, Beauchamp MK. Role of muscle strength in balance assessment and treatment in chronic obstructive pulmonary disease. *Cardiopulm Phys Ther J.* (2019) 30:1. doi: 10.1097/CPT.0000000000000093
40. Ozsoy I, Ozsoy G, Karti C, Buyukturan B, Yilmaz F, Buyukturan O, et al. Cognitive and motor performances in dual task in patients with chronic obstructive pulmonary disease: a comparative study. *Irish J Med Sci.* (2021) 190:723-30. doi: 10.1007/s11845-020-02357-6
41. Van Hove O, Cebolla AM, Andrianopoulos V, Leduc D, Guidat PA, Feipel V, et al. The influence of cognitive load on static balance in chronic obstructive pulmonary disease patients. *Clin Respir J.* (2021) 15:351–7. doi: 10.1111/crj.13307
42. Heraud N, Alexandre F, Gueugnon M, Davy C, Tremey E, Oliver N, et al. Impact of chronic obstructive pulmonary disease on cognitive and motor performances in dual-task walking. *COPD.* (2018) 15:277–82. doi: 10.1080/15412555.2018.1469607

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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 14 November 2022
DOI 10.3389/fmed.2022.1043859

OPEN ACCESS

EDITED BY
Shuibang Wang,
National Institutes of Health (NIH),
United States

REVIEWED BY
Francesco Forfori,
University of Pisa, Italy
Sanda Predescu,
Rush University Medical Center,
United States

*CORRESPONDENCE
Chuanzhu Lv
lvchuanzhu677@126.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 14 September 2022
ACCEPTED 31 October 2022
PUBLISHED 14 November 2022

CITATION
Gong H, Chen Y, Chen M, Li J, Zhang H, Yan S and Lv C (2022) Advanced development and mechanism of sepsis-related acute respiratory distress syndrome. *Front. Med.* 9:1043859. doi: 10.3389/fmed.2022.1043859

Advanced development and mechanism of sepsis-related acute respiratory distress syndrome

Huankai Gong[1,2†], Yao Chen[3†], Meiling Chen[1,2†], Jiankang Li[1,2], Hong Zhang[1,2], Shijiao Yan[4,5] and Chuanzhu Lv[2,4,6*]

[1]Emergency and Trauma College, Hainan Medical University, Haikou, China, [2]Key Laboratory of Emergency and Trauma of Ministry of Education, Hainan Medical University, Haikou, China, [3]Department of Oncology, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China, [4]Research Unit of Island Emergency Medicine, Chinese Academy of Medical Sciences (No. 2019RU013), Hainan Medical University, Haikou, China, [5]School of Public Health, Hainan Medical University, Haikou, China, [6]Emergency Medicine Center, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, China

The introduction of the Sepsis 3.0 guidelines in 2016 improved our understanding of sepsis diagnosis and therapy. Personalized treatment strategies and nursing methods for sepsis patients are recommended in the "Save Sepsis Campaign" in 2021. However, mortality in sepsis patients remains high. Patients with sepsis-related acute respiratory distress syndrome account for around 30% of them, with fatality rates ranging from 30 to 40%. Pathological specimens from individuals with sepsis-related ARDS frequently demonstrate widespread alveolar damage, and investigations have revealed that pulmonary epithelial and pulmonary endothelial injury is the underlying cause. As a result, the purpose of this work is to evaluate the mechanism and research progress of pulmonary epithelial and pulmonary endothelial damage in sepsis-related ARDS, which may provide new directions for future research, diagnosis, and therapy.

KEYWORDS

sepsis, acute respiratory distress syndrome - ARDS, epithelial injury, endothelial injury, mechanism, biomarkers

Introduction

Sepsis, one of the most prevalent complications in the ICU, has a high fatality rate due to its complicated molecular underpinnings. Sepsis was described in 2016 as a "life-threatening organ failure produced by an unbalanced host response to infection" (1). Sepsis is frequently characterized by a dysregulated host response to invading pathogens; this systemic inflammatory response can result in disseminated intravascular coagulation, multiple organ dysfunction syndromes (MODS), and mortality (2). With the incremental development of sepsis diagnosis, treatment, and management over the last several decades, and the ongoing updating of recommendations, the mortality rate of sepsis has significantly declined (about 20–30 percent) (3). However, early detection of sepsis, prevention of multiple organ failure, and improved prognosis remain pressing concerns.

Sepsis frequently causes organ dysfunction and damage, such as acute kidney injury (AKI), acute lung injury (ALI), and ALI can be exacerbated by acute respiratory distress syndrome (ARDS). As a result, ARDS is often regarded as a deadly consequence of severe sepsis, with sepsis accounting for around 32% of all cases. The major histological hallmark of ARDS is severe diffuse alveolar damage, which is frequently driven by endothelial dysfunction and local inflammation. As a diverse illness, ARDS frequently manifests as sudden exacerbations of non-cardiogenic pulmonary edema, severe hypoxemia, and the requirement for mechanical ventilation (4, 5).

The focus of this review is on the pathophysiology and current research on sepsis-associated ARDS. It also goes through the biomarkers that play a role in sepsis-related ARDS, which may provide new directions for future research, diagnosis, and therapy.

The potential mechanisms of sepsis-related ARDS

A significant pathogenic characteristic of ARDS is the damage to vascular endothelial (VE) cells, alveolar epithelial cells, and epigenetics. However, the complex pathways underlying sepsis-related ARDS remain unknown. We have illustrated some potential mechanisms in Figure 1.

VE injury

Although the nature and mechanism of endothelial injury in ARDS remain unknown, new research suggests that it is linked to inflammatory responses, VE-cadherin alteration, apoptosis, or other cell death pathways (such as pyroptosis and autophagy), and oxidative stress.

Inflammatory responses

In patients with sepsis, the immune system is usually in disequilibrium. Antigen-presenting cells activate a variety of signaling pathways between immune cells under external and internal stimulations, resulting in the release of inflammatory mediators such as interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor-alpha (TNF-α), and the release of pro-inflammatory signals accelerates the vascular endothelial dysfunction, which, in turn, promotes the inflow of inflammatory cells (such as neutrophils, macrophages, monocytes, lymphocytes, and lymphocytes), forming a vicious pro-inflammatory cycle, which ultimately aggravates and amplifies lung or systemic inflammation (6, 7). Therefore, pathogen-derived inflammatory mediators and activated immune cells not only trigger immunological responses but also cause host cell harm in sepsis.

The activation and destruction of endothelial cells can result in the production of pro-inflammatory signaling molecules (such as platelet-activating factor, angiopoietin 2, tumor necrosis factor, VE growth factor, inflammasome product IL-1, and others) and the accumulation of leukocytes, resulting in the recruitment of neutrophils and macrophages, activation of alveolar epithelial cells, and effector T cells (5, 8), Leukocyte aggregation is most commonly seen in the form of neutrophil-platelet aggregates, which have complicated thrombo-inflammatory properties (9). This can lead to increased protein permeability in the pulmonary vascular system, which can lead to hypovolemia and multiple organ failure. Neutrophil extracellular traps (NETs), for example, have been linked to the disruption of alveolar-capillary and epithelial barriers in recent research on acute lung damage and ARDS, as well as having inflammatory effects on the lung and other organs (10).

In addition, alveolar macrophages (AM) can cooperate with other immune cells to regulate lung inflammation, and AM cell death plays an important role in the development process of lung inflammation (11–14). On one hand, a variety of proinflammatory cytokines (e.g., inflammasomes and IL-1 β) can activate or amplify the lung injury response of macrophages, T cells, and other immune cells (15–17). On the other hand, AM cell death or pyroptosis promotes neutrophil migration into the lungs, increases the concentration of cytokines (e.g., IL-6, TNF-α, and IL-1β) in the alveoli, and aggravates lung injury (18). Thus, the interaction between inflammation and cell death is expected to further affect and accelerate the progression of ARDS.

In addition, the bacterial endotoxin lipopolysaccharide (LPS), which is a typical activator of sepsis-induced lung injury, can activate the overexpression and release of a variety of pro-inflammatory proteins, resulting in severe cellular or organ damage.

Disruption of VE-cadherin

The synergistic effect of VE-cadherin and endothelial receptor kinase (TIE2), which is regulated by VE protein tyrosine phosphatase, ensures the integrity of VE cells (VE-PTP) (4, 19, 20). Several variables influence and regulate the activity of VE-cadherin and the stability of adhesive junctions, including cytoskeletal interactions, GTPases, phosphorylation, and dephosphorylation (21). Dissociation of VE-PTP from VE-cadherin has been linked to enhanced alveolar-capillary permeability in inflammatory acute lung damage, as well as endothelial cell junction relaxation and inflammatory alveolar protein leakage, according to research (9, 22). The inflammatory response can destabilize VE-cadherin by releasing a range of small molecules (such as angiopoietin 2 and VE growth factor). Furthermore, actin filaments that generate tension and actin stress fibers that generate tension work together to influence the stability of VE cell connections (23, 24). Loss

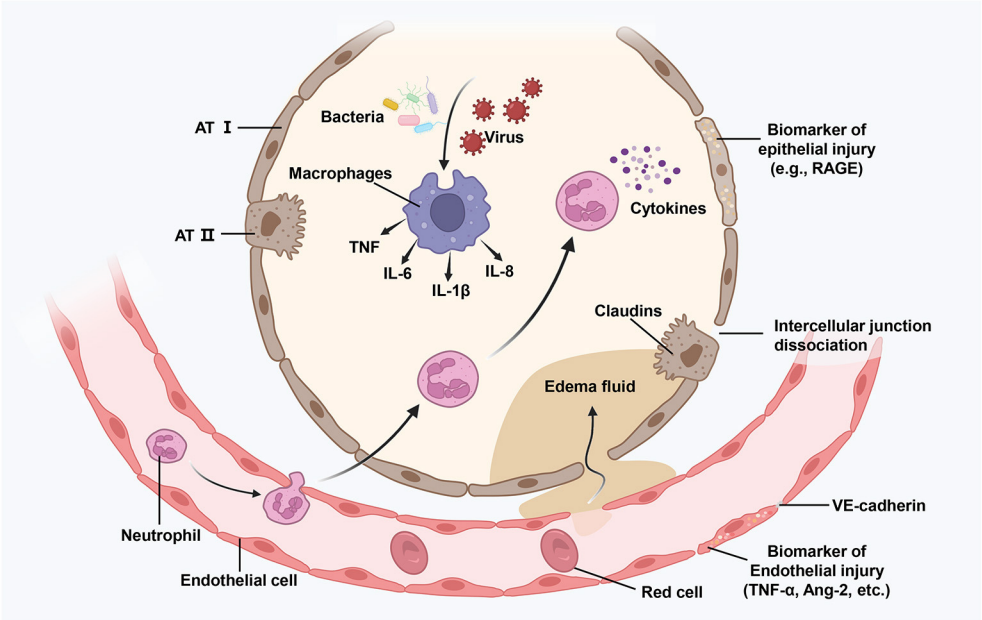

FIGURE 1
Illustration of an injured alveolus. Various damage factors (such as attack by bacteria and viruses) can directly or indirectly cause damage to the distal alveolar structure and the related microvascular regions. During the exudative phase, alveolar macrophages get activated, resulting in the release of powerful pro-inflammatory mediators and chemokines (such as TNF, IL-6, and IL-8) that promote the accumulation of neutrophils and monocytes. Activated neutrophils (such as cytokines) further promote damage by releasing toxic mediators. The resulting damage causes the loss of barrier functions as well as interstitial an intra-alveolar flooding. ATI, alvcolar type I cell; TNG, tumor necrosis factor; IL-6, interlcukin-6; Ang-2, angiopoietin 2; RAGE, receptor for advance glycation end-products.

of intercellular adhesion during actomyosin contraction causes gaps to emerge between endothelial cells. These mechanisms combine to promote endothelial and epithelial permeability, which contributes to edematous fluid buildup and hypoxemia.

Apoptosis or other cell death pathways

The clinical significance of neutrophil apoptosis sensitivity in the etiology of ARDS is unknown. ARDS has been linked to the influx of neutrophils into the alveoli in several studies. Apoptotic neutrophils amass in the alveoli as a result of decreased AM proliferation, resulting in secondary necrosis and the release of inflammatory mediators (25, 26). In addition, the buildup of a significant number of neutrophils plays a key role in the release of additional inflammatory mediators and pro-inflammatory factors throughout the ALI process (27).

Necroptosis has been studied extensively as a crucial contributor to apoptosis in ARDS. Under the stimulation of a death signal, receptor-interacting protein kinase 1 (RIPK1) and RIPK3 govern necrotic cell death, which can be suppressed by necrostatin-1 (NEC-1) (28, 29). The presence of HMGB1 in bronchoalveolar lavage of patients with acute lung damage generally indicates necroptosis. Similarly, numerous intracellular bacteria and viruses elicit necroptosis in the lungs and have a role in sepsis-induced ARDS development (30).

Pyroptosis plays a role in the pathophysiology of ARDS as well. Lipopolysaccharide, which is primarily mediated by the cysteine protease (Caspase) family, affects endothelial cell pyroptosis in animal models (such as Caspase-1, Caspase-4, Caspase-11). Gasdermin D can be cleaved by activated Caspase-1 to generate the N-terminus or C-terminus of Gasdermin D, which is a direct executive protein of pyroptosis. Gasdermin D's N-terminus attaches to phospholipid proteins on the cell membrane, creating a hole that allows a flood of inflammatory substances to escape the cell (31).

In conclusion, apoptosis overexpression is important in the development of acute lung damage and ARDS.

Oxidative stress

Oxidative stress is frequently associated with ARDS. A large number of cytokines and inflammatory cells can be released during the inflammatory response of sepsis, and a large number of reactive oxygen species (ROS) can be generated through the oxidative stress response, causing varying degrees of damage to the structure and function of cells, such as mitochondrial damage (32). As per a past report, when cells are exposed to bacteria, leukocyte respiration increases, which kills the pathogens by producing ROS, superoxide, hydrogen peroxide, and hydroxyl radicals (33). NADPH oxidase (NOX) is an

enzyme that uses NADPH to catalyze the reduction of oxygen to produce superoxide (34). NOX is commonly referred to as a "professional ROS producer". Currently, seven NOX isoforms are known, namely, NOX1, NOX2, NOX3, NOX4, NOX5, Duox1, and Duox2 (35). Among these, only NOX1, NOX2, and NOX4 are expressed in the vasculature, and all of them have been implicated in ROS-mediated vascular diseases (36). The ROS produced by LPS exposure has been demonstrated to be NOX1-dependent in macrophages and NOX2-dependent in LPS- challenged lungs (37, 38). The LPS activating the toll-like receptor 4 (TLR4) receptor induces NOX-mediated ROS generation (39), which in turn activates pro-inflammatory signaling factors such as the TNF-α and NF-κB (40, 41). Protein interaction with C-kinase 1 (PICK1) affects pulmonary vascular glutathione synthesis by influencing the substrate-specific component xCT of the pulmonary cystine/glutamate transporter, resulting in severe oxidative stress, according to an animal study on sepsis (42).

Alveolar epithelial injury

One of the key hallmarks of ARDS is alveolar epithelial injury, and the severity of epithelial cell injury is a significant factor in ARDS severity.

The early stage of lung injury, commonly known as the exudative phase of ARDS, is characterized by innate immune cell-mediated disruption of the alveolar endothelial cell barrier and accumulation of protein-rich edema fluid in the alveolar interstitium and alveolus (5). Macrophages in the alveoli generate pro-inflammatory substances, which attract neutrophils, monocytes, and macrophages, as well as activate alveolar epithelial cells and effector T cells, causing inflammation and tissue damage (43). Second, alveolar endothelial cells with enhanced permeability allow proteins and fluids to collect in the pulmonary interstitium, resulting in interstitial edema. The edema fluid is transmitted to the alveolar fluid at this moment due to the alveolar epithelium's normal tight barrier being compromised (4).

Dissociation of intercellular junctions

When endothelial cells mount a proinflammatory or procoagulant response to infection in neighboring epithelial cells, the alveolar epithelial-endothelial barrier occurs independently of endothelial cells, according to Kirsty et al. (44). Barrier injury, on the other hand, is linked to the breakdown of epithelial cells' tight junctions. The alveolar epithelium's tight junctions are critical for regulating fluid in the lung's distal space, and transmembrane tight junction proteins called Claudins play a significant role (45).

Claudins 3, 4, 5, 7, 8, 15, and 18 were all expressed in the distal lung. Claudins 3, 4, and 7 are mostly found in alveolar type II cells, whereas claudin-5 is found in nearly all alveolar epithelial cells (46). The loss of the tight junction protein claudin-4 is one of them, and it's linked to barrier destruction (44). Claudin-5 disrupts the function of the alveolar epithelial barrier by interfering with the interaction of claudin-18 with the scaffold protein ZO-1 (45).

Epithelial cell death

Lung epithelial cells are usually regarded as the lung's first line of defense, and epithelial cell death is the most prominent aspect of alveolar damage in ARDS, which can be induced directly by bacterial and viral invasion, acidic media, hyperoxia, hypoxia, and mechanical alterations (47, 48). Inflammatory macrophages can promote cell death through methods such as the production of tumor necrosis factors and similar apoptosis-inducing ligands, while neutrophil-derived mediators can induce cell death through many pathways, including the release of TNF (49, 50).

Epigenetics

The term "epigenetics" describes the regulatory systems that manage gene expression but are unrelated to changes in the DNA sequence. These changes include non-coding RNA control of transcription, DNA methylation, and histone changes (51). The confluence of genetics and environment is where epigenetic alterations, which affect gene expression in response to external stress, occur. Epigenetic control may be the key factor in the pathogenesis of sepsis, as per several recent types of research on immunology and human sepsis (52).

Epigenetics and sepsis-related immune suppression

Anti-inflammatory and pro-inflammatory symptoms are present in the early stages of sepsis, but, as the condition progresses, immunosuppression frequently predominates, which increases the risk of subsequent infection and death. Immunosuppression induced by sepsis is a complicated phenomenon. Endotoxin tolerance or the body's inability to respond to bacterial endotoxin is one of the current markers of sepsis-associated immunosuppression. Numerous *in vitro* studies support the idea that epigenetic changes are essential for the development of endotoxin tolerance. In researches by El Gazzar et al. (53, 54), it was demonstrated that the TNF promoter was methylated in monocytes in the stationary phase. The TNF promoter is quickly demethylated upon initial endotoxin exposure, inducing an immunological response. However, the TNF promoter is bound by the histone methyltransferase G9a throughout the endotoxin-tolerance phase, resulting in recurrent methylation of the TNF promoter,

which eventually renders the TNF promoter insensitive to endotoxin activation. miRNAs may also play a role in endotoxin tolerance, where miR-125b, miR-146a, miR-221, and miR-579 are involved in controlling the transcriptional expression of TNF (55, 56).

Epigenetics and ARDS

ARDS is a common complication of sepsis, and past studies have demonstrated that DNA methylation plays an important role in its pathogenesis. In an LPS-induced ARDS rat model, the level of 5-methylocytosine was increased, which confirmed the increased DNA methylation level (57). In an epigenomic analysis of lung tissues, more than 1,700 genes exhibited methylation differences (58). Of the 42 differential methylation genes associated with MAPK signaling, seven were found to be associated with ARDS (59). In their recent study, Chen et al. (60) confirmed the METTL3-mediated abnormal n6-methyladenosine mRNA expression in the septic lungs. Moreover, decreased METTL3 levels could exacerbate lung endothelial injury and inflammatory responses in sepsis-related ARDS. These findings provide a new direction in the research of whether sepsis-related ARDS can develop METTL3 as a biomarker or as a therapeutic intervention point.

Biomarkers of sepsis-related ARDS

A variety of biomarkers can be used to determine the severity of ARDS and the characteristics of each stage. The ideal biomarker would be based on the more precise pathophysiological pathways that have been investigated thus far. It must be extremely dependable, repeatable, disease-specific, and sensitive. The procedure is easy and low-cost in clinical practice, and short-term volatility must also be considered. Blood or plasma, urine, feces, bronchoalveolar lavage fluid, cerebrospinal fluid, bone marrow, and other clinical test specimens are currently used. It can reflect the damage or activation of epithelial cells, endothelial cells, or the coagulation system in the ARDS inflammatory response by detecting the change of a single biomarker in the specimen, which can aid in the diagnosis of the disease or the judgment of the curative effect in the treatment of the disease. Predict the present patient's cure rate or fatality rate.

Interleukin-6

Interleukin-6 (IL-6) was first discovered in 1986 under the name B cell-stimulating factor. T cells produce it, but it can also cause B cells to create antibodies. IL-6 has become a key inflammatory regulator since its discovery, and it is secreted by a variety of cells, the majority of which are found in inflammatory, infectious, and neoplastic disorders (61). IL-6 levels have been discovered to be elevated in critical conditions including sepsis and ARDS, and studies have demonstrated that it plays a key role in the disease's progression. Because IL-6 is required for clearing infections in the immune process and plays an active role as an anti-inflammatory or protective factor in most cases, future research can use IL-6 concentrations in the blood or lung as a biomarker to explain the current status of the disease.

Classical signaling and trans-signaling are the two basic types of IL-6 signaling. In recent years, a third transduction mechanism known as trans-presentation has been found. IL-6 forms the IL-6-IL-6R complex with membrane-bound IL-6R, which subsequently binds to gp130 to form signal transduction via the JAK-STAT pathway in traditional signaling. Only a few types of cells, most notably hepatocytes and some leukocytes such as macrophages and T-cell subsets, express IL-6R, but gp130 is expressed by all cell types. IL-6 does not bind to gp130 on its own; it must first form a complex with IL-6R. This signal transduction pathway is primarily responsible for IL-6's anti-inflammatory and antibacterial actions (62). IL-6R is cleaved off the cell surface and alternatively spliced to produce the soluble receptor sIL-6R in trans-signaling. The capacity of sIL-6R to bind to IL-6, and the resulting IL-6-sIL-6R complex to bind to gp130, can be performed on cells without IL-6R, extending the range of IL-6 on target cells and explaining IL-6's versatility. Protease disintegrin and metalloproteinase domain-containing protein 17 (ADAM-17), which is activated during inflammation or infection, is the major enzyme capable of completing this cleavage event, hence the pro-inflammatory effect is mostly mediated through trans-signaling (63). Trans-presentation is the third signal transduction mode. IL-6 is expected to be delivered to the plasma membrane after engaging with antigen-specific dendritic cells (DCs) and binding to IL-6R on DCs. Under the joint action of transforming growth factor-beta 2(TGF-2) and the IL-6-IL-6R complex on the surface of dendritic cells, it binds to gp130 on the surface of T cells and activates pathogenic Th17 cells (64).

In the course of sepsis or ARDS, IL-6 plays a significant role, and its management can have a favorable influence on the condition. The current study focuses on blocking IL-6, and IL-6R, neutralizing gp130, and interfering with JAK-STAT signaling, and it has yielded some promising results (62). The research on IL-6 as a biomarker in critical diseases, particularly ARDS and COVID-19, has made some headway. It is difficult to determine the concentration of circulating IL-6 and interpret the results. The cytokine peaks at different times in different disorders, making the sample time more restrictive. Changes in circadian rhythm, exercise, certain medicines, and immunometabolism comorbidities can all impact IL-6 levels and release in the bloodstream (65). There are further needs for sample processing, as il-6 and other cytokines are produced from blood cells over time, altering results (66). COVID-19 has swept the globe in the last 2 years, and its severe sufferers

are likely to develop ARDS. Due to the lack of a common definition, some of the data gathered in the current COVID-19 research on IL-6 may have varied results. Part of the explanation for this disparity could be the use of clinically-based IL-6 tests, which are notoriously less sensitive than currently available research-grade assays. A recent series of cytokine-focused prospective studies in critically ill COVID-19 patients found that IL-6 concentrations were significantly higher than previously reported using clinical IL-6 measurements, both in absolute terms and relative to other inflammatory airway conditions like ARDS (67–69). IL-6 was found to act as an independent predictor of 28-day mortality in sepsis patients, showing superior predictive power to procalcitonin and hypersensitive C-responsive proteins as per meta-analysis. With the combined application of IL-6 and neutrophil-lymphocyte ratio as a predictive model, the predictive power of death risk in sepsis patients was significantly improved (70). This aspect benefits clinicians for more appropriate and accurate management of patients with sepsis.

Angiopoietin 2

Davis et al. (71) found the vascular receptor tyrosine kinase Tie-2 and its ligand angiopoietin 1 (Ang-1) in the mid-1990s and then used homology screening of a cDNA library to find angiopoietin 2 (Ang-2). Tie-2 receptors can bind to both Ang-1 and Ang-2. Tie-2 receptors are activated and phosphorylated after Ang-1 binds, promoting blood vessel integrity and growth. Ang-2 functions as an antagonist of Ang-1, binding to Tie-2 receptors competitively and blocking Ang-1's actions, boosting inflammatory responses and capillary leakage. Because Ang-2 is implicated in the pathophysiology of a variety of disorders, it could be used as a therapeutic target, and certain Ang-2-targeted therapies have been demonstrated to be effective (72, 73).

ARDS is a leading cause of morbidity and mortality in patients around the world, and despite effective antibiotic treatment, pathogen-body interactions can lead to increased pulmonary endothelial cell permeability, which can lead to protein exudation and edema, and eventually life-threatening lung failure. Ang-2 is the principal cause of enhanced permeability in lung endothelial cells. Ang-2 has been validated as a biomarker for sepsis and ARDS risk assessment in several prior studies (74, 75). Despite considerable knowledge of Ang-2's expression and activity in pulmonary circulation, its significance in the development of pneumonia and ARDS is unknown. Gutbier et al. (76) confirmed that Ang-2 levels were significantly greater in ARDS patients than in healthy people in a prospective analysis of two different cohorts and that using Ang-2 as a particular biomarker could improve the CURB-65/CRB-65 grading system accuracy. International recommendations indicate the CURB-65 and CRB-65 scores as predictors of pneumonia fatality (77). It was discovered that Ang-1, Ang-2, and its receptor Tie-2 were considerably expressed in the lung tissue of patients with pneumonia after evaluating the lung tissue of the deceased patient. The Ang-1 protein is found in a variety of types, including lung parenchymal cells, endothelial cells, and epithelial cells. Ang-2 and Tie-2 proteins, on the other hand, were only found in pulmonary VE cells (76). Villar et al. (78) discovered that Ang-2 plays an essential role in ARDS prediction in septic patients in a multicenter observational study in Spanish intensive care units. In a prospective study, Ang-2 demonstrated a large independent association between severe sepsis and organ injury (79). These studies implied that Ang-2—a biomarker of endothelial dysfunction and damage—may play an important role in future studies on predicting the treatment and prognosis of sepsis.

Receptor for advanced glycation end products

RAGE (receptor for advanced glycation end-products) is an immunoglobulin superfamily multi-ligand pattern recognition receptor. It is mostly found in membrane-bound and soluble forms (sRAGE). Membrane-bound forms can identify a wide range of receptors, activate transcription factors *via* binding to receptors, and enhance pro-inflammatory factor production. The soluble form is a decoy receptor that suppresses membrane RAGE activation competitively (80). RAGE is extensively expressed in lung tissue under normal circumstances (81). By evaluating the level of RAGE in bronchoalveolar lavage fluid and serum of rats and people with acute lung damage, Uchida et al. (82) confirmed that RAGE is a biomarker of type I alveolar epithelial cell injury and a significant inflammatory mediator as early as 2006. This is critical because epithelial cell injury and inflammatory responses are both involved in the ARDS process, and RAGE is involved in both of these routes (80). Following research, it was discovered that sRAGE is linked to the severity of ARDS (83–85).

Two subtypes of sARGE, known as cRAGE and esRAGE, have been separated in recent years (for endogenous secretory RAGE). To facilitate the shedding of sARGE, inflammatory factors are amplified and result in the formation of cRAGE, which is created on the surface of the cell membrane by proteolytic cleavage at the extracellular and transmembrane boundaries (86). Less than 25% of the total circulating sRAGE is created by alternate splicing of the RAGE pre-mRNA (87, 88), and the specific process governing esRAGE production is currently unknown. Studies have shown that increased sRAGE levels during acute illness predict 90-day death in ARDS patients (89). RAGE is thought to be substantially overexpressed in the lung epithelium and that RAGE signaling may play a key role in the clinical symptoms of lung injury (90). High levels of sRAGE are also linked to potential mortality in sepsis

(91). Theoretically, respiratory virus illnesses like Covid-19, which are currently wreaking havoc worldwide, may also be connected. The next study objective may be to construct risk classification and sRAGE thresholds for sRAGE levels in clinical practice, which will help sRAGE as a biomarker to better serve the clinic.

In summary, past studies on the biomarkers of sepsis-related ARDS were mostly focused on two aspects: the biomarkers of VE injury and alveolar epithelial injury. The common ones included IL-6, Ang-2, and sRAGE. Despite the lack of any substantial evidence supporting the specificity of these biomarkers for such diseases, recent studies have hinted that the levels of these biomarkers are associated with an increased risk of sepsis-related ARDS development (92–94). In recent years, the researchers turn their attention to the genetic basis of these relationships. Recent studies suggest that genomic or transcriptome-based biomarkers may facilitate the establishment of predictive or prognostic stratification approaches for sepsis-associated ARDS and may, thereby, facilitate the development of novel therapeutic targets. For example, epigenetic variants and circulating microRNA have become potential biomarkers for the diagnosis or prognosis of sepsis-associated ARDS (95). However, they are possibly limited by various factors such as the sample size, ethnicity, and phenotypic heterogeneity. The current study did not detected any exact association of these novel biomarkers with sepsis-related ARDS (96). Nevertheless, this finding also provides a new direction for further research.

Conclusions

Sepsis-related ARDS is an inherently heterogeneous clinical syndrome. Several potential biomarkers have been investigated so far, with no single biomarker yet identified that can specifically reliably diagnose this disease. Current research indicates that biomarker combinations that respond to different aspects (such as epithelial and endothelial injury, epigenetic variation, and inflammation) are more likely to be applied in clinical settings. Some studies have suggested and tested the combination of several biomarkers to explore the relationship with sepsis-related ARDS (97–101), with some success. For example, Zhao et al. (98) validated an ARDS-mortality prediction model, including the age, surfactant protein D, and interleukin-8, which may be useful for risk assessment in clinical trial enrollment. However, none of these candidate research schemes has yet been clinically applied in such patients. It is therefore important to further study and clarify the potential of these candidate schemes.

Our knowledge of sepsis-related ARDS disorders has grown over the last two decades, and our capacity to detect and treat such patients has steadily increased, saving the lives of a huge number of patients. The death rate of sepsis-related ARDS patients, on the other hand, remains at the forefront of many diseases, and long-term consequences for surviving patients are also a serious issue. Further research in the following areas could assist enhance patient outcomes to change this predicament. Exploring strategies to reduce lung endothelial and epithelial cell damage and finding ways to promote lung endothelial and epithelial cell repair is necessary from a molecular standpoint. On the clinical side, we will actively investigate particular biomarkers closely associated with the disease so that the disease may be diagnosed early in clinical work and early treatment intervention can be carried out to prevent the disease from progressing further.

Author contributions

HG, YC, MC, and CL participated in the conception, drafting, and revision of the manuscript. All authors critically reviewed the paper. All authors have read and approved the final manuscript.

Funding

This work was supported by grants from the Hainan Province Science and Technology Special Fund (ZDKJ202004 and ZDKJ2021038), National Natural Science Foundation of China (81871611), and Finance Science and Technology Program of Sichuan Province (2022YFS0602).

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.

Publisher's note

References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *J Am Med Assoc.* (2016) 315:801–10. doi: 10.1001/jama.2016.0287

2. Park I, Kim M, Choe K, Song E, Seo H, Hwang Y, et al. Neutrophils disturb pulmonary microcirculation in sepsis-induced acute lung injury. *Eur Respir J.* (2019) 53:1800786. doi: 10.1183/13993003.00786-2018

3. Genga K, Russell J. Update of sepsis in the intensive care unit. *J Innate Immun.* (2017) 9:441–55. doi: 10.1159/000477419

4. Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, et al. Acute respiratory distress syndrome. *Nat Rev Dis Prim.* (2019) 5:18. doi: 10.1038/s41572-019-0069-0

5. Thompson BT, Chambers RC, Liu KD. Acute respiratory distress syndrome. *N Engl J Med.* (2017) 377:562–72. doi: 10.1056/NEJMra1608077

6. Hu Q, Hao C, Tang S. From sepsis to acute respiratory distress syndrome (ARDS): emerging preventive strategies based on molecular and genetic researches. *Biosci Rep.* (2020) 40:BSR20200830. doi: 10.1042/BSR20200830

7. Mehta Y, Dixit SB, Zirpe K, Sud R, Gopal PB, Koul PA, et al. Therapeutic approaches in modulating the inflammatory and immunological response in patients with sepsis, acute respiratory distress syndrome, and pancreatitis: an expert opinion review. *Cureus*. (2021) 13:e18393. doi: 10.7759/cureus.18393

8. Aggarwal NR, King LS, D'Alessio FR. Diverse macrophage populations mediate acute lung inflammation and resolution. *Am J Physiol Lung Cell Mol Physiol.* (2014) 306:L709–25. doi: 10.1152/ajplung.00341.2013

9. Ware LB, Matthay MA. The acute respiratory distress syndrome. *J Clin Invest.* (2012) 122:2731–40. doi: 10.1172/JCI60331

10. Lefrançais E, Mallavia B, Zhuo H, Calfee CS, Looney MR. Maladaptive role of neutrophil extracellular traps in pathogen-induced lung injury. *JCI Insight.* (2018) 3:e98178. doi: 10.1172/jci.insight.98178

11. Fan E, Erica KY, Fan J. Regulation of alveolar macrophage death in acute lung inflammation. *Respir Res.* (2018) 19:50. doi: 10.1186/s12931-018-0756-5

12. Rivera A, Siracusa MC, Yap GS, Gause WC. Innate cell communication kick-starts pathogen-specific immunity. *Nat Immunol.* (2016) 17:356–63. doi: 10.1038/ni.3375

13. Li Z, Scott MJ, Fan EK, Li Y, Liu J, Xiao G, et al. Tissue damage negatively regulates LPS-induced macrophage necroptosis. (2016) 23:1428–47. doi: 10.1038/cdd.2016.21

14. Yang J, Zhao Y, Zhang P, Li Y, Yang Y, Yang Y, et al. Hemorrhagic shock primes for lung vascular endothelial cell pyroptosis: role in pulmonary inflammation following LPS. *Cell Death Dis.* (2016) 7:e2363. doi: 10.1038/cddis.2016.274

15. Franklin BS, Bossaller L, De Nardo D, Ratter JM, Stutz A, Engels G, et al. The adaptor ASC has extracellular and 'prionoid' activities that propagate inflammation. *Nat Immunol.* (2014) 15:727–37. doi: 10.1038/ni.2913

16. Delaleu N, Bickel MJP. Interleukin-1 beta and interleukin-18: regulation and activity in local inflammation. *Periodontology*. (2004) 35:42–52. doi: 10.1111/j.0906-6713.2004.003569.x

17. Liu B, He R, Zhang L, Hao B, Jiang W, Wang W, et al. Inflammatory caspases drive pyroptosis in acute lung injury. *Front Pharmacol.* (2021) 12:631256. doi: 10.3389/fphar.2021.631256

18. He X, Qian Y, Li Z, Fan EK, Li Y, Wu L, et al. *T*LR4-upregulated IL-1β and IL-1RI promote alveolar macrophage pyroptosis and lung inflammation through an autocrine mechanism. *Sci Rep.* (2016) 6:31663. doi: 10.1038/srep31663

19. Frye M, Dierkes M, Küppers V, Vockel M, Tomm J, Zeuschner D, et al. Interfering with VE-PTP stabilizes endothelial junctions *in vivo via* Tie-2 in the absence of VE-cadherin. *J Exp Med.* (2015) 212:2267–87. doi: 10.1084/jem.20150718

20. Juettner VV, Kruse K, Dan A, Vu VH, Khan Y, Le J, et al. VE-PTP stabilizes VE-cadherin junctions and the endothelial barrier via a phosphatase-independent mechanism. *J Cell Biol.* (2019) 218:1725–42. doi: 10.1083/jcb.201807210

21. Giannotta M, Trani M, Dejana E. VE-cadherin and endothelial adherens junctions: active guardians of vascular integrity. *Dev Cell.* (2013) 26:441–54. doi: 10.1016/j.devcel.2013.08.020

22. Broermann A, Winderlich M, Block H, Frye M, Rossaint J, Zarbock A, et al. Dissociation of VE-PTP from VE-cadherin is required for leukocyte extravasation and for VEGF-induced vascular permeability *in vivo*. *J Exp Med.* (2011) 208:2393–401. doi: 10.1084/jem.20110525

23. Schnoor M, García Ponce A, Vadillo E, Pelayo R, Rossaint J, Zarbock A. Actin dynamics in the regulation of endothelial barrier functions and neutrophil recruitment during endotoxemia and sepsis. *Cell Mol Life Sci.* (2017) 74:1985–97. doi: 10.1007/s00018-016-2449-x

24. Jones J, Minshall R. Endothelial transcytosis in acute lung injury: emerging mechanisms and therapeutic approaches. *Front Physiol.* (2022) 13:828093. doi: 10.3389/fphys.2022.828093

25. Mahida RY, Scott A, Parekh D, Lugg ST, Hardy RS, Lavery GG, et al. Acute respiratory distress syndrome is associated with impaired alveolar macrophage efferocytosis. *Eur Respir J.* (2021) 58:2100829. doi: 10.1183/13993003.00829-2021

26. Mahida RY, Scott A, Parekh D, Lugg ST, Belchamber KB, Hardy RS, et al. In vitroassessment of alveolar macrophage dysfunction using an model of acute respiratory distress syndrome. *Front Med.* (2021) 8:737859. doi: 10.3389/fmed.2021.737859

27. Chopra M, Reuben JS, Sharma AC. Acute lung injury:apoptosis and signaling mechanisms. *Exper Biol Med.* (2009) 234:361–71. doi: 10.3181/0811-MR-318

28. Pan L, Yao DC Yu YZ, Li SJ, Chen BJ, Hu GH, et al. Necrostatin-1 protects against oleic acid-induced acute respiratory distress syndrome in rats. *Biochem Biophys Res Commun.* (2016) 478:1602–8. doi: 10.1016/j.bbrc.2016.08.163

29. Sauler M, Bazan IS, Lee PJ. Cell death in the lung: the apoptosis-necroptosis axis. *Annu Rev Physiol.* (2019) 81:375–402. doi: 10.1146/annurev-physiol-020518-114320

30. Rodrigue-Gervais IG, Labbé K, Dagenais M, Dupaul-Chicoine J, Champagne C, Morizot A, et al. Cellular inhibitor of apoptosis protein cIAP2 protects against pulmonary tissue necrosis during influenza virus infection to promote host survival. *Cell Host Microbe.* (2014) 15:23–35. doi: 10.1016/j.chom.2013.12.003

31. Mitra S, Exline M, Habyarimana F, Gavrilin MA, Baker PJ, Masters SL, et al. Microparticulate caspase 1 regulates gasdermin d and pulmonary vascular endothelial cell injury. *Am J Respir Cell Mol Biol.* (2018) 59:56–64. doi: 10.1165/rcmb.2017-0393OC

32. Joffre J, Hellman J. Oxidative stress and endothelial dysfunction in sepsis and acute inflammation. *Antioxid Redox Signal.* (2021) 35:1291–307. doi: 10.1089/ars.2021.0027

33. Ma H, Huang D, Guo L, Chen Q, Zhong W, Geng Q, et al. Strong correlation between lung ultrasound and chest computerized tomography imaging for the detection of acute lung injury/acute respiratory distress syndrome in rats. (2016) 8:1443–8. doi: 10.21037/jtd.2016.05.15

34. Ago T, Nunoi H, Ito T, Sumimoto H. Mechanism for phosphorylation-induced activation of the phagocyte NADPH oxidase protein p47(phox). Triple replacement of serines 303, 304, and 328 with aspartates disrupts the SH3 domain-mediated intramolecular interaction in p47(phox), thereby activating the oxidase. *J Biol Chem.* (1999) 274:33644–53. doi: 10.1074/jbc.274.47.33644

35. Geiszt, M. NADPH oxidases: new kids on the block. *Cardiovasc Res.* (2006) 71:289–99. doi: 10.1016/j.cardiores.2006.05.004

36. Pendyala S, Usatyuk PV, Gorshkova IA, Garcia JG, Natarajan V, Regulation of NADPH oxidase in vascular endothelium: the role of phospholipases, protein kinases, and cytoskeletal proteins. *Antioxid Redox Signal.* (2009) 11:841–60. doi: 10.1089/ars.2008.2231

37. Maitra U, Singh N, Gan L, Ringwood L, Li L. IRAK-1 contributes to lipopolysaccharide-induced reactive oxygen species generation in macrophages by inducing NOX-1 transcription and Rac1 activation and suppressing the expression of antioxidative enzymes. *J Biol Chem.* (2009) 284:35403–11. doi: 10.1074/jbc.M109.059501

38. Sato K, Kadiiska MB, Ghio AJ, Corbett J, Fann YC, Holland SM, et al. In vivo lipid-derived free radical formation by NADPH oxidase in acute lung injury induced by lipopolysaccharide: a model for ARDS. *FASEB J.* (2002) 16:1713–20. doi: 10.1096/fj.02-0331com

39. Imai Y, Kuba K, Neely GG, Yaghubian-Malhami R, Perkmann T, van Loo G, et al. Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. *Cell.* (2008). 133:235–49. doi: 10.1016/j.cell.2008.02.043

40. Wang Y, Hu H, Yin J, Shi Y, Tan J, Zheng L, et al. TLR4 participates in sympathetic hyperactivity Post-MI in the PVN by regulating NF-κB pathway and ROS production. *Redox Biol.* (2019) 24:101186. doi: 10.1016/j.redox.2019.101186

41. Zhang SS, Liu M, Liu DN, Yang YL, Du GH, Wang YH, TLR4-IN-C34 inhibits lipopolysaccharide-stimulated inflammatory responses via downregulating TLR4/MyD88/NF-κB/NLRP3 signaling pathway and reducing ROS generation in BV2 cells. *Inflammation.* (2022) 45:838–50. doi: 10.1007/s10753-021-01588-8

42. Qian M, Lou Y, Wang Y, Zhang M, Jiang Q, Mo Y, et al. PICK1 deficiency exacerbates sepsis-associated acute lung injury and impairs glutathione synthesis via reduction of xCT. *Free Radic Biol Med.* (2018) 118:23–34. doi: 10.1016/j.freeradbiomed.2018.02.028

43. Weichelt U, Cay R, Schmitz T, Strauss E, Sifringer M, Bührer C, et al. Prevention of hyperoxia-mediated pulmonary inflammation in neonatal rats by caffeine. *Eur Respir J.* (2013) 41:966–73. doi: 10.1183/09031936.00012412

44. Short KR, Kasper J, Van Der Aa S, Andeweg AC, Zaaraoui-Boutahar F, Goeijenbier M, et al. Influenza virus damages the alveolar barrier by disrupting epithelial cell tight junctions. *Eur Respir J.* (2016) 47:954–66. doi: 10.1183/13993003.01282-2015

45. Schlingmann B, Overgaard CE, Molina SA, Lynn KS, Mitchell LA, Dorsainvil White S, et al. Regulation of claudin/zonula occludens-1 complexes by hetero-claudin interactions. *Nat Commun.* (2016) 7:12276. doi: 10.1038/ncomms12276

46. Günzel D, Yu A. Claudins and the modulation of tight junction permeability. *Physiol Rev.* (2013) 93:525–69. doi: 10.1152/physrev.00019.2012

47. Burgoyne R, Fisher A, Borthwick L. The role of epithelial damage in the pulmonary immune response. *Cells.* (2021) 10:2763. doi: 10.3390/cells10102763

48. Budinger GS, Mutlu GM, Urich D, Soberanes S, Buccellato LJ, Hawkins K, et al. Epithelial cell death is an important contributor to oxidant-mediated acute lung injury. *Am J Respir Crit Care Med.* (2011) 183:1043–54. doi: 10.1164/rccm.201002-0181OC

49. Ye C, Li H, Bao M, Zhuo R, Jiang G, Wang W. Alveolar macrophage—derived exosomes modulate severity and outcome of acute lung injury. *Aging.* (2020) 12:6120–8. doi: 10.18632/aging.103010

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

51. Portela A, Esteller, M. Epigenetic modifications and human disease. *Nat Biotechnol.* (2010) 28:1057–68. doi: 10.1038/nbt.1685

52. Tollefsbol TO. *Advances in Epigenetic Technology, Vol 791.* Totowa, NJ: Humana Press (2011). p. 1–10. doi: 10.1007/978-1-61779-316-5_1

53. El Gazzar M, Liu T, Yoza BK. McCall CE. Dynamic and selective nucleosome repositioning during endotoxin tolerance. *J Biol Chem.* (2010) 285:1259–71. doi: 10.1074/jbc.M109.067330

54. El Gazzar M, Yoza BK, Chen X, Hu J, Hawkins GA, McCall CE. G9a and HP1 couple histone and DNA methylation to TNFalpha transcription silencing during endotoxin tolerance. *J Biol Chem.* (2008) 283:32198–208. doi: 10.1074/jbc.M803446200

55. El Gazzar M, McCall CE. MicroRNAs distinguish translational from transcriptional silencing during endotoxin tolerance. *J Biol Chem.* (2010) 285:20940–51. doi: 10.1074/jbc.M110.115063

56. El Gazzar M, Church A, Liu T, McCall CE. MicroRNA-146a regulates both transcription silencing and translation disruption of TNF-α during TLR4-induced gene reprogramming. *J Leukocyte Biol.* (2011) 90:509–19. doi: 10.1189/jlb.0211074

57. Shih CC, Liao MH, Hsiao TS, Hii HP, Shen CH, Chen SJ, et al. Procainamide inhibits DNA methylation and alleviates multiple organ dysfunction in rats with endotoxic shock. *PLoS ONE.* (2016) 11:e0163690. doi: 10.1371/journal.pone.0163690

58. Zhang XQ, Lv CJ, Liu XY, Hao D, Qin J, Tian HH, et al. Genome-wide analysis of DNA methylation in rat lungs with lipopolysaccharide-induced acute lung injury. *Mol Med Rep.* (2013) 7:1417–24. doi: 10.3892/mmr.2013.1405

59. Zhang R, Miao Q, Wang C, Zhao R, Li W, Haile CN, et al. Genome-wide DNA methylation analysis in alcohol dependence. *Addict Biol.* (2013) 18:392–403. doi: 10.1111/adb.12037

60. Chen Y, Wu Y, Zhu L, Chen C, Xu S, Tang D. Sepsis-induced acute respiratory distress syndrome. (2022) 13:897487. doi: 10.3389/fimmu.2022.897487

61. Garbers C, Heink S, Korn T, Rose-John S. Interleukin-6: designing specific therapeutics for a complex cytokine. *Nat Rev Drug Discov.* (2018) 17:395–412. doi: 10.1038/nrd.2018.45

62. McElvaney OJ, Curley GF, Rose-John S, McElvaney NG. Interleukin-6: obstacles to targeting a complex cytokine in critical illness. *Lancet Respir Med.* (2021) 9:643–54. doi: 10.1016/S2213-2600(21)00103-X

63. Heink S, Yogev N, Garbers C, Herwerth M, Aly L, Gasperi C, et al. Trans-presentation of IL-6 by dendritic cells is required for the priming of pathogenic T17 cells. *Nat Immunol.* (2017) 18:74–85. doi: 10.1038/ni.3632

64. Uciechowski P, Dempke W. Interleukin-6: a masterplayer in the cytokine network. *Oncology.* (2020) 98:131–7. doi: 10.1159/000505099

65. Nilsonne G, Lekander M, Åkerstedt T, Axelsson J, Ingre M. Diurnal variation of circulating interleukin-6 in humans: a meta-analysis. *PLoS ONE.* (2016) 11:e0165799. doi: 10.1371/journal.pone.0165799

66. Keustermans GC, Hoeks SB, Meerding JM, Prakken BJ, de Jager W. Cytokine assays: an assessment of the preparation and treatment of blood and tissue samples. *Methods.* (2013) 61:10–7. doi: 10.1016/j.ymeth.2013.04.005

67. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat Med.* (2020) 26:1636–43. doi: 10.1038/s41591-020-1051-9

68. Sinha P, Calfee CS, Cherian S, Brealey D, Cutler S, King C, et al. Prevalence of phenotypes of acute respiratory distress syndrome in critically ill patients with COVID-19: a prospective observational study. *Lancet Respir Med.* (2020) 8:1209–18. doi: 10.1016/S2213-2600(20)30366-0

69. Remy KE, Mazer M, Striker DA, Ellebedy AH, Walton AH, Unsinger J, et al. Severe immunosuppression and not a cytokine storm characterizes COVID-19 infections. *JCI Insight.* (2020) 5. doi: 10.1172/jci.insight.140329

70. Liu S, Wang X, She F, Zhang W, Liu H, Zhao X, Effects of neutrophil-to-lymphocyte ratio combined with interleukin-6 in predicting 28-day mortality in patients with sepsis. *Front Immunol.* (2021). 12:639735. doi: 10.3389/fimmu.2021.639735

71. Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL. Jain V, et al. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. *Cell.* (1996) 87:1161–9. doi: 10.1016/S0092-8674(00)81812-7

72. Lefere S, Van de Velde F, Hoorens A, Raevens S, Van Campenhout S, Vandierendonck A, et al. Angiopoietin-2 promotes pathological angiogenesis and is a therapeutic target in murine nonalcoholic fatty liver disease. *Hepatology.* (2019) 69:1087–104. doi: 10.1002/hep.30294

73. Monk BJ, Poveda A, Vergote I, Raspagliesi F, Fujiwara K, Bae DS, et al. Anti-angiopoietin therapy with trebananib for recurrent ovarian cancer (TRINOVA-1): a randomised, multicentre, double-blind, placebo-controlled phase 3 trial. *Lancet Oncol.* (2014) 15:799–808. doi: 10.1016/S1470-2045(14)70244-X

74. Calfee CS, Gallagher D, Abbott J, Thompson BT, Matthay MA, NHLBI ARDS. Network, plasma angiopoietin-2 in clinical acute lung injury: prognostic and pathogenetic significance. *Crit Care Med.* (2012) 40:1731–7. doi: 10.1097/CCM.0b013e3182451c87

75. Agrawal A, Matthay MA, Kangelaris KN, Stein J, Chu JC, Imp BM, et al. Plasma angiopoietin-2 predicts the onset of acute lung injury in critically ill patients. *Am J Respir Crit Care Med.* (2013) 187:736–42. doi: 10.1164/rccm.201208-1460OC

76. Gutbier B, Neuhauß AK, Reppe K, Ehrler C, Santel A, Kaufmann J, et al. Prognostic and pathogenic role of angiopoietin-1 and−2 in pneumonia. *Am J Respir Crit Care Med.* (2018) 198:220–31. doi: 10.1164/rccm.201708-1733OC

77. Ewig. Management-based risk prediction in community-acquired pneumonia by scores and biomarkers. *Eur Respir J.* (2013) 41:974–84. doi: 10.1183/09031936.00104412

78. Villar J, Herrán-Monge R, González-Higueras E, Prieto-González M, Ambrós A, Rodríguez-Pérez A, et al. Clinical and biological markers for predicting ARDS and outcome in septic patients. *Sci Rep.* (2021) 11:22702. doi: 10.1038/s41598-021-02100-w

79. Yu WK, McNeil JB, Wickersham NE, Shaver CM, Bastarache JA, Ware LB, Angiopoietin-2 outperforms other endothelial biomarkers associated with severe acute kidney injury in patients with severe sepsis and respiratory failure. *Crit Care.* (2021) 25:48. doi: 10.1186/s13054-021-03474-z

80. Guo WA, Knight PR, Raghavendran K. The receptor for advanced glycation end products and acute lung injury/acute respiratory distress syndrome. *Intens Care Med.* (2012) 38:1588–98. doi: 10.1007/s00134-012-2624-y

81. Fehrenbach H, Kasper M, Tschernig T, Shearman MS, Schuh D, Müller M. Receptor for advanced glycation endproducts (RAGE) exhibits highly differential cellular and subcellular localisation in rat and human lung. *Cell Mol Biol (Noisy-le-grand).* (1998) 44:1147–57.

82. Uchida T, Shirasawa M, Ware LB, Kojima K, Hata Y, Makita K, et al. Receptor for advanced glycation end-products is a marker of type I cell injury in acute lung injury. *Am J Respir Crit Care Med.* (2006) 173:1008–15. doi: 10.1164/rccm.200509-1477OC

83. Zhang H, Tasaka S, Shiraishi Y, Fukunaga K, Yamada W, Seki H, et al. Role of soluble receptor for advanced glycation end products on endotoxin-induced lung injury. *Am J Respir Crit Care Med.* (2008) 178:356–62. doi: 10.1164/rccm.200707-1069OC

84. Su X, Looney MR, Gupta N, Matthay MA. Receptor for advanced glycation end-products (RAGE) is an indicator of direct lung injury in models

of experimental lung injury. *Am J Physiol Lung Cell Mol Physiol.* (2009) 297:L1–5. doi: 10.1152/ajplung.90546.2008

85. Jabaudon M, Blondonnet R, Roszyk L, Bouvier D, Audard J, Clairefond G, et al. Soluble receptor for advanced glycation end-products predicts impaired alveolar fluid clearance in acute respiratory distress syndrome. *Am J Respir Crit Care Med.* (2015) 192:191–9. doi: 10.1164/rccm.201501-0020OC

86. Zhang L, Bukulin M, Kojro E, Roth A, Metz VV, Fahrenholz F, et al. Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases. *J Biol Chem.* (2008) 283:35507–16. doi: 10.1074/jbc.M806948200

87. Hudson BI, Carter AM, Harja E, Kalea AZ, Arriero M, Yang H, et al. Identification, classification, and expression of RAGE gene splice variants. *FASEB J.* (2008) 22:1572–80. doi: 10.1096/fj.07-9909com

88. Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, Abedin MJ. Li H, et al. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochem J.* (2003) 370:1097–109. doi: 10.1042/bj20021371

89. Jabaudon M, Blondonnet R, Pereira B, Cartin-Ceba R, Lichtenstern C. Mauri T, et al. Plasma sRAGE is independently associated with increased mortality in ARDS: a meta-analysis of individual patient data. *Intensive Care Med.* (2018) 44:1388–99. doi: 10.1007/s00134-018-5327-1

90. Erusalimsky, J. The use of the soluble receptor for advanced glycation-end products (sRAGE) as a potential biomarker of disease risk and adverse outcomes. *Redox Biol.* (2021) 42:101958. doi: 10.1016/j.redox.2021.101958

91. Brodska H, Malickova K, Valenta J, Fabio A, Drabek T. Soluble receptor for advanced glycation end products predicts 28-day mortality in critically ill patients with sepsis. *Scand J Clin Lab Invest.* (2013) 73:650–60. doi: 10.3109/00365513.2013.849357

92. Van Der Zee P, Rietdijk W, Somhorst P, Endeman H, Gommers D. A systematic review of biomarkers multivariately associated with acute respiratory distress syndrome development and mortality. *Crit Care.* (2020) 24:243. doi: 10.1186/s13054-020-02913-7

93. Jones TK, Feng R, Kerchberger VE, Reilly JP, Anderson BJ, Shashaty MG, et al. Plasma sRAGE acts as a genetically regulated causal intermediate in sepsis-associated acute respiratory distress syndrome. *Am J Respir Crit Care Med.* (2020) 201:47–56. doi: 10.1164/rccm.201810-2033OC

94. Reilly JP, Wang F, Jones TK, Palakshappa JA, Anderson BJ, Shashaty MG, et al. Plasma angiopoietin-2 as a potential causal marker in sepsis-associated ARDS development: evidence from Mendelian randomization and mediation analysis. *Intens Care Med.* (2018) 44:1849–58. doi: 10.1007/s00134-018-5328-0

95. Zheng F, Pan Y, Yang Y, Zeng C, Fang X, Shu Q, et al. Novel biomarkers for acute respiratory distress syndrome: genetics, epigenetics and transcriptomics. *Biomark Med.* (2022) 16:217–31. doi: 10.2217/bmm-2021-0749

96. Beitler JR, Thompson BT, Baron RM, Bastarache JA, Denlinger LC, Esserman L, et al. Advancing precision medicine for acute respiratory distress syndrome. *Lancet Respir Med.* (2022) 10:107–20. doi: 10.1016/S2213-2600(21)00157-0

97. Calfee CS, Ware LB, Glidden DV, Eisner MD, Parsons PE, Thompson BT, et al. Use of risk reclassification with multiple biomarkers improves mortality prediction in acute lung injury. *Crit. Care Med.* (2011) 39:711–7. doi: 10.1097/CCM.0b013e318207ec3c

98. Zhao Z, Wickersham N, Kangelaris KN, May AK, Bernard GR, Matthay MA, et al. External validation of a biomarker and clinical prediction model for hospital mortality in acute respiratory distress syndrome. *Intens Care.* (2017) 43:1123–31. doi: 10.1007/s00134-017-4854-5

99. Chen C, Shi L, Li Y, Wang X, Yang S. Disease-specific dynamic biomarkers selected by integrating inflammatory mediators with clinical informatics in ARDS patients with severe pneumonia. *Cell Biol Toxicol.* (2016) 32:169–84. doi: 10.1007/s10565-016-9322-4

100. Ding XF, Li JB, Liang HY, Wang ZY, Jiao TT, Liu Z, et al. Predictive model for acute respiratory distress syndrome events in ICU patients in China using machine learning algorithms: a secondary analysis of a cohort study. *J Transl Med.* (2019) 17:326. doi: 10.1186/s12967-019-2075-0

101. Sinha P, Delucchi KL, McAuley DF, O'Kane CM, Matthay MA, Calfee CS, et al. Development and validation of parsimonious algorithms to classify acute respiratory distress syndrome phenotypes: a secondary analysis of randomised controlled trials. *Lancet Respir Med.* (2020) 8:247–57. doi: 10.1016/S2213-2600(19)30369-8

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 23 December 2022
DOI 10.3389/fmed.2022.1102550

OPEN ACCESS

EDITED BY
Shuibang Wang,
National Institutes of Health (NIH),
United States

REVIEWED BY
Salina Gairhe,
National Institutes of Health (NIH),
United States
Mariaenrica Tinè,
University of Padua, Italy

*CORRESPONDENCE
Lingyun Zhou
✉ 352505504@qq.com
Xinyi Zhang
✉ zhangxinyi80@163.com

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 19 November 2022
ACCEPTED 12 December 2022
PUBLISHED 23 December 2022

CITATION
Xu J, Xiong Y, Xu Z, Xing H, Zhou L and Zhang X (2022) From targeted therapy to a novel way: Immunogenic cell death in lung cancer. *Front. Med.* 9:1102550. doi: 10.3389/fmed.2022.1102550

From targeted therapy to a novel way: Immunogenic cell death in lung cancer

Jiawei Xu[1,2†], Yiyi Xiong[2†], Zhou Xu[2], Hongquan Xing[1,2], Lingyun Zhou[3*] and Xinyi Zhang[1,2*]

[1]Department of Respiratory Diseases, The Second Affiliated Hospital of Nanchang University, Nanchang, China, [2]The Second Clinical Medical College of Nanchang University, Nanchang, China, [3]International Education College, Jiangxi University of Chinese Medicine, Nanchang, China

Lung cancer (LC) is one of the most incident malignancies and a leading cause of cancer mortality worldwide. Common tumorigenic drivers of LC mainly include genetic alterations of EGFR, ALK, KRAS, BRAF, ROS1, and MET. Small inhibitory molecules and antibodies selectively targeting these alterations or/and their downstream signaling pathways have been approved for treatment of LC. Unfortunately, following initial positive responses to these targeted therapies, a large number of patients show dismal prognosis due to the occurrence of resistance mechanisms, such as novel mutations of these genes and activation of alternative signaling pathways. Over the past decade, it has become clear that there is no possible cure for LC unless potent antitumor immune responses are induced by therapeutic intervention. Immunogenic cell death (ICD) is a newly emerged concept, a form of regulated cell death that is sufficient to activate adaptive immune responses against tumor cells. It transforms dying cancer cells into a therapeutic vaccine and stimulates long-lasting protective antitumor immunity. In this review, we discuss the key targetable genetic aberrations and the underlying mechanism of ICD in LC. Various agents inducing ICD are summarized and the possibility of harnessing ICD in LC immunotherapy is further explored.

KEYWORDS

immunogenic cell death, lung cancer, immunotherapy, damage-associated molecular patterns, genetic alteration

1 Introduction

Over decades, lung cancer (LC) has remained one of the most frequently diagnosed cancers and ranks as the leading cause of cancer-related death in human globally (1). The 5-year survival rate is disappointing: only 19% of patients have survived overall, and most of them have suffered from a high risk of cancer relapse (2, 3). In the past decade, significant progress has been made in the treatment of LC. Researchers found several driver gene mutations of LC and deeply investigated molecular targeted therapy, which obviously improves the landscape of non-small cell lung carcinoma (NSCLC)

treatment (4). Genetic alterations, such as mutations of epidermal growth factor receptor (EGFR), KRAS, MET, and rearrangements of ALK and ROS1, are the main contributors to LC tumorigenesis and progression (5), which dysregulate proliferation, apoptosis, migration, and invasion of cancer cells through various downstream signaling pathways. Targeting these abnormal genes or/and their downstream signaling has been used for treatment of LC. However, owing to the high heterogeneity of LC and acquired resistance to treatment, therapeutic efficacy of the targeted therapy is not guaranteed (6, 7).

Immunogenic cell death (ICD) is a novel form of regulated cell death which can evoke an adaptive immune response against cancer cells (8). Dying cancer cells can secrete damage-associated molecular patterns (DAMPs), mainly including high mobility group box 1 (HMGB1), calreticulin (CRT), adenosine triphosphate (ATP) and Type I interferon (Type I IFN). Recognized by the pattern recognition receptors (PRRs), the DAMPs enhance function of the antigen-presenting cells (APCs), activate T cells, increase the immunogenicity of tumor cells and ultimately trigger ICD (9). ICD can be triggered by many kinds of anti-cancer therapies, including chemotherapy, radiation, targeted drugs, photodynamic therapy (PDT) and immune checkpoints inhibitors (ICIs) (10–12). A shared characteristic of these various ICD is their ability to provoke endoplasmic reticulum (ER) stress and reactive oxygen species (ROS) generation. By restoring the immunogenicity of poor ICD triggers and stimulating DAMPs secretion, (ER) stress and ROS are believed to be indispensable for ICD (13, 14). Therefore, harnessing ICD to maintain the efficacy of anti-tumor therapies is crucial and challenging for LC treatment (15).

2 Genetic alterations affecting signaling pathways in lung cancer

Mutations of EGFR, KRAS, BRAF, and MET, and rearrangements of ALK and ROS1 have aroused great interest in recent years. Plenty of studies have pointed out the significance of targeting tumor driver genes in cancer therapies nowadays due to their key roles in promoting cancer survival, proliferation and cell-cycle progression through modulating downstream signaling pathways in lung cancer (**Figure 1**).

2.1 EGFR mutations

For Asian patients with lung cancer, EGFR mutant NSCLC is the most prevalent subtype (16). Over the past decades, more and more studies have showed that EGFR mutation is a common driver of tumorigenesis, and lung cancer is no exception (17, 18). EGFR mutations include in-frame mutations or point mutations and insertions, which typically occurs in exon18-21, encoding a portion of the EGFR kinase domain. In-frame deletions in exon 19 and the L858R point mutation in exon 21 account for nearly 90% of EGFR mutations and confer high sensitivity to clinical target therapies (19). These mutations confer higher sensitivity to clinical target therapies due to increased affinity of TKIs to the ATP-binding pocket of mutant EGFR compared to its wild-type. However, insertion mutation and T790M point mutation in exon 20 are often resistant to TKI (20). EGFR mutations are responsible for activation of constitutive ligand-independent receptor and regulation of downstream signaling pathways, promoting cancer proliferation and cell survival (21). Regulated downstream signaling pathways include activation of RAS/RAF/MEK/ERK, phospholipase C (PLCγ) and phosphoinositide 3-kinase (PI3K)-AKT, but inhibition the p38/MAPK and JNK/STAT pathway (22) (**Figure 2**). Oncogenic alterations may result in EGFR overexpression as well, which eventually increases the cancer incidence risk *via* regulating related signaling pathway (23).

Mutant EGFR could inhibit apoptosis *via* inhibiting BH3 -domain proteins, such as pro-apoptotic BIM and BMF (24). Trever et al. proposed that the FAS/NF-κB pathway, which promotes tumor growth, could rescue EGFR mutant lung cancer

Abbreviations: TME, the tumor microenvironment; ICD, immunogenic cell death; NSCLC, non-small cell lung carcinoma; LUAD, lung adenocarcinoma; SqCC, lung squamous cell carcinoma; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitors; IL-6, interleukin-6; DAMPs, damage-associated molecular patterns; HGMB1, high mobility group protein B1; CRT, calreticulin; ATP, adenosine triphosphate; Type I IFN, Type I interferon; PRRs, pattern recognition receptors; APCs, antigen-presenting cells; ER, endoplasmic reticulum; ROS, reactive oxygen species; PDT, photodynamic therapy; ICIs, immune checkpoints inhibitors; PI3K, phosphatidylinositol 3-kinase; BAD, Bcl-xL/Bcl-2 associated death promoter; RTK, receptor tyrosine kinases; ROR1, orphan receptor 1; SEMA7A, semaphorin 7A; PHPT1, phosphohistidine phosphatase 1; FBXO32, F-box protein 32; BTC, betacellulin; STAT3, signal transducer and activator of transcription 3; Grp94, glucose-regulated protein 94; YB-1, Y-box Binding protein; HGF, hepatic growth factor; TNF-α, tumor necrosis factor alpha; EMT, epithelial-to-mesenchymal transition; MVP, major vault protein; STAT3, signal transducer and activator of transcription; HGF, hepatocyte growth factor; Hh, hedgehog; Ral, Ras-like; RalGEF, Ras-like 2 guanine nucleotide exchange factor; PRC1, protein required for cytokinesis 1; BRAF, V-raf murine sarcoma viral oncogene homolog B; V600E, valine to glutamate substitution at codon 600; RhoGEFs, Rho guanine nucleotide exchange factors; TNFR, tumor necrosis factor receptor; TRAF, tumor necrosis factor receptor–associated factors; TINCR, terminal differentiation-induced non-coding RNA; RTKs, receptor tyrosine kinases; NPM, nucleophosmin; EML4, echinoderm microtubule associated protein like 4; PD-L1, programmed cell death-ligand 1; PD-1, programmed cell death-1; SLC34A2, solute carrier family 34 Member 2; NF-κB, nuclear factor-kappa B; GSDMD, gasdermin D; DCs, dendritic cells; TLR4, toll-like receptor 4; RAGE, receptor for advanced glycation end products; RAC1, Rac family small GTPase 1; CGAS, cyclic GMP-AMP synthase; STING1, signal transducer stimulator of IFN response cGAMP interactor 1; CXCL10, CX-C motif chemokine ligand 10; IL-1β, interleukin-1 beta; UPR, unfolded protein response; eIF2α, eukaryotic translation initiation factor 2α; PKR, protein kinase RNA; PERK, Protein Kinase RNA-activated-like ER Kinase; IRE1, inositol-requiring transmembrane kinase/endonuclease; ATE6, activating transcription factor 6; IL-12, interleukin-12; EnaV, enapotamab vedotin; AF, auranofin; TrxR, thioredoxin reductase 1; KRASmut, KRAS-mutant; NSq-NSCLC, non-squamous NSCLC; RT, radiation therapy; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; IDO, indoleamine 2.3-dioxygenase.

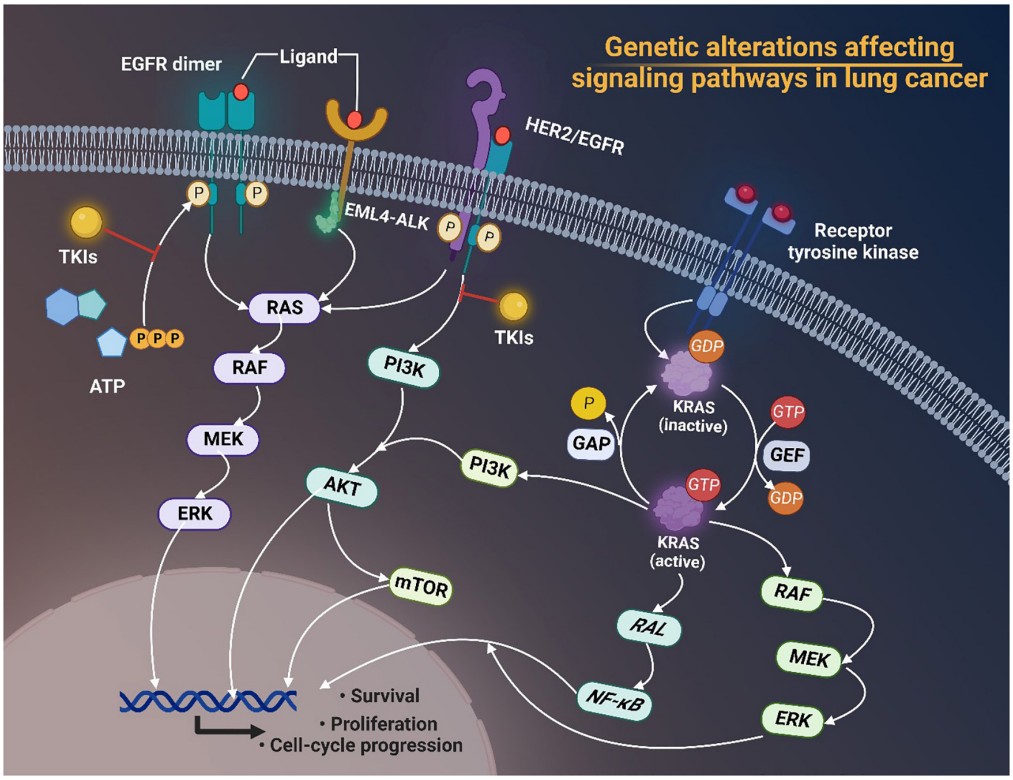

FIGURE 1
Genetic alterations of EGFR, ALK, and HER2 affecting signaling pathways in lung cancer. Tyrosine kinase inhibitors (TKIs) target receptor tyrosine kinases and prevent phosphorylation of the TK domain receptor of EGFR, thus inhibiting the activation of downstream signaling pathways such as the RAS/RAF/MEK/ERK pathway and the phosphatidylinositol 3-kinase (PI3K)-AKT pathway, thereby interfering with cell proliferation, differentiation, migration and survival. Activated KRAS proteins principally activate the downstream PI3K-AKT-mTOR signaling pathway that regulates cell proliferation and the RAS-RAF-MEK-ERK signaling pathway that regulates cell growth. Tumorigenic alterations contribute to EGFR overexpression, which ultimately increases the risk of cancer development by regulating related signaling pathways. EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitors; EML4-ALK, echinoderm microtubule associated protein like 4-activin-like kinase; HER2, human epidermal growth factor receptor 2; ATP, adenosine triphosphate; MEK, MAP kinase-ERK kinase; ERK, extracellular regulated protein kinases; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; GAP, growth-associated protein; GDP, guanosine diphosphate; GTP, guanosine triphosphate; GEF, Granule, Effervescent; RAL, Ras-like; NF-κB, nuclear factor-kappa B; RAF, rheumatoid arthritis factor; ERK, extracellular regulated protein kinases.

cells from EGFR inhibition (25). Karachaliou et al. found that knockdown of Orphan receptor 1 (ROR1) could inhibit the growth of NCI-H1975 cells [harboring EGFR L858R and T790M mutations (24)] *via* the ROR1/MEK/ERK signaling pathway, indicating the potential of ROR1 as therapeutic target for EGFR positive lung adenocarcinoma (LUAD) (26). The study of Kinehara et al. proposed that the GPI-anchored protein semaphorin 7A (SEMA7A) is overexpressed *via* induction of mTOR signaling in LUAD. Mutant EGFR lung cancer could upregulate SEMA7A/ITBG1 axis, which normally activates ERK signaling and leads to apoptosis resistance (27). Zhang et al. reported that phosphohistidine phosphatase 1 (PHPT1), often overexpressed and caused poor survival in lung cancer patients, could activate ERK/MAPK pathway targeting F-box protein 32 (FBXO32) as E3 ubiquitin ligase in EGFR mutant lung cancer (28). Previous studies have revealed that betacellulin (BTC) could binds to members of the ErbB family and mediating cancer development. Chava et al. firstly proposed that BTC could suppress apoptosis and promote cancer growth in EGFR-mutant LUAD in a MAP kinase-dependent way (29). The F-box protein FBXL2, a potential therapeutic target for EGFR mutant LC, could suppress EGFR-driven NSCLC cell growth. Niu et al. showed that glucose-regulated protein 94 (Grp94) protects the stability of EGFR *via* blockage of FBXL2, thereby promoting EGFR mutant cell proliferation and anti-apoptosis (30).

Epidermal growth factor receptor-positive lung cancer could confer potent invasive ability. Tsai et al.'s study showed that EGFR-L858R mutant LUAD could activate CXCL12-CXCR4 axis to enhance metastasis (31). Feng et al. reached a similar conclusion in their study that EGFR 19 exon deletion can promote the expression of MMP-2 and MMP-9 by enhancing the CXCR4/CXCL12 signaling pathway, leading to higher proliferation, migration, and invasion abilities (32). Li et al.'s study found that the EGFR-mutant NSCLC related brain

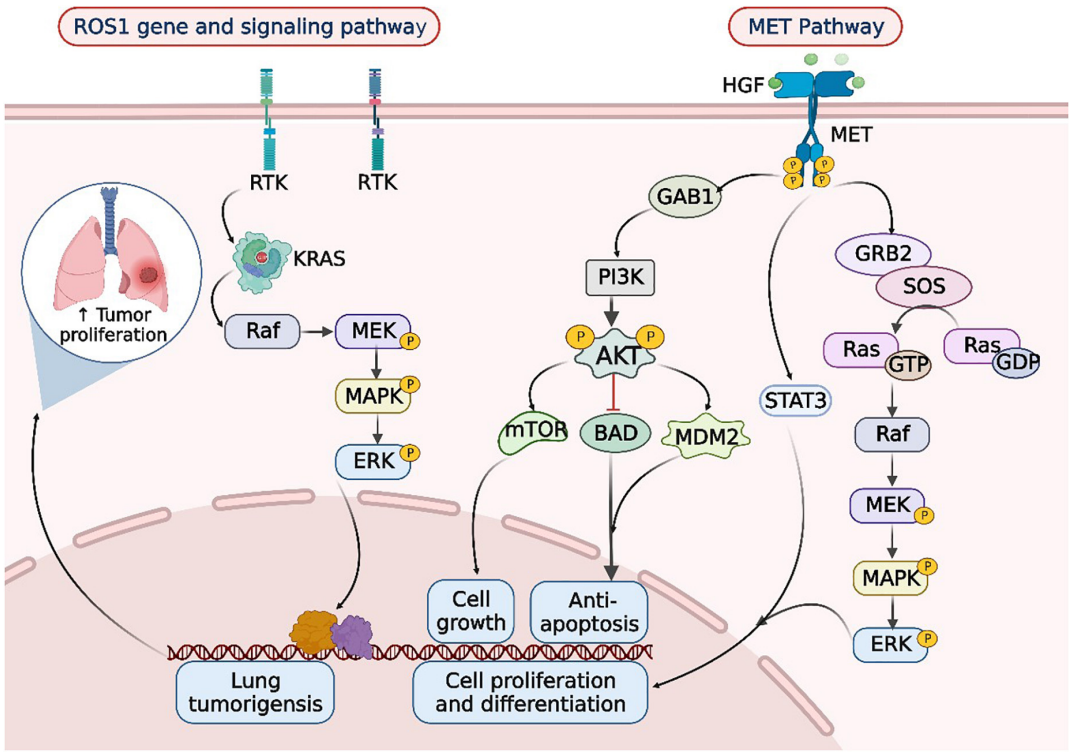

FIGURE 2
ROS1 rearrangements and MET mutations in the occurrence and development of lung cancer. When lung cancer is developed, the tyrosine kinase receptor (RTK) encoded by the ROS1 gene synergizes with oncogenic drivers such as KRAS to enhance MAPK-ERK signaling. Upon MET activation, PI3K associates with GAB1 and activates AKT/protein kinase. AKT inactivates the pro-apoptotic protein BCL-2 cell death antagonist (BAD) and triggers the E3 ubiquitin-protein ligase MDM2, thereby inhibiting apoptosis and promoting cell survival. In addition, AKT activates the mammalian target of rapamycin (mTOR) protein, promoting protein synthesis and cell growth, MET activation signals through the RAS-MAPK pathway as well. The nucleotide exchange protein Son of Sevenless (SOS) activates RAS upon binding to GRB2, which leads to activation of the v-Raf murine sarcoma viral oncogene homolog B1 (RAF) kinase, followed by stimulation of MAPK effector kinase (MEK) and resulting in MAPK activation. MAPK phosphorylates ERK, the ultimate effector of the cascade. The RAS-MAPK pathway is responsible for cell proliferation, cell motility, and cell cycle progression. Moreover, MET could relay signals to the activator of the transcription 3 (STAT3) pathway. STAT3 directly binds to MET, enabling STAT3 phosphorylation, which regulates cell transformation and invasion. RTK, receptor tyrosine kinases; Raf, rheumatoid arthritis factor; MEK, MAP kinase-ERK kinase; ERK, extracellular regulated protein kinases; HGF, hepatic growth factor; MET, mesenchymal to epithelial transition factor; GAB1, Grb2-associated binders 1; GAB2, Grb2-associated binders 2; PI3K, phosphoinositide-3 kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; BAD, Bcl-xL/Bcl-2 associated death promoter; MDM2, murine double minute 2; SOS, son of sevenless; GTP, guanosine triphosphate; GDP, guanosine diphosphate; STAT3, signal transducer and activator of transcription 3.

metastasis is associated with downregulation of WNT5A by E2F1 *via* ERK1/2 pathway (33). A recent study elucidated that Y-box Binding protein (YB-1), an important drug sensitivity modulator, could activate AKT signaling and epithelial-to-mesenchymal transition (EMT) *via* targeting major vault protein (MVP), especially in EGFR mutant LAUD (34).

Moreover, the crosstalk of EGFR with the tumor microenvironment (TME) could affect the immunity to cancer. High level of IL-6 is a biomarker in lung cancer patients, Cao et al. revealed that mutant EGFR could upregulate IL-6 *via* gp130/JAK signaling pathway targeting signal transducer and activator of transcription (STAT) 3, a known oncogenic protein (35). Patients with EGFR activation showed upregulated CD73/adenosine *via* EGFR-ERK signaling pathway, which contributes to the immune-inert environment for EGFR-mutant NSCLC (36–39). Chen et al. reported that activated EGFR NSCLC cells enhance ILT4 expression, which suppresses T cell proliferation and immunity and thereby leads to immune escape (40).

2.2 KRAS mutations

RAS is the most frequent mutant oncogene in cancer (41, 42). Among all kinds of isoforms, KRAS, which belongs to GFPase superfamily, accounts for 86% RAS-mutants (43). Research demonstrated that 20–40% of LUAD and 30% NSCLC have been observed with KRAS mutation, which is more prevalent in smokers (44). KRAS mutation could destruct the activation of GTPase, which would lead to the accumulation of KRAS under GTP binding condition and thus cause the activation of basic downstream pathways related with cellular

life events, including MAPK, PI3K, and Ras-like (Ral) 2 guanine nucleotide exchange factor (RalGEF) and so on (45, 46).

KRAS mutations act as strong drivers for tumorigenesis by modulating multiple signaling pathways. It has been demonstrated that mutant RAS gene, could activate Raf–MEK–ERK phosphorylation cascade to enhance tumorigenesis (47). SIRT1, an oncogene or tumor suppressor, was found decreased by KRAS in a PI3K and MEK dependent way, which contributes to lung carcinogenesis (48). RASSF1A is believed to be a weak suppressor of human tumors. Transgenic mice with RASSF1A-defective background demonstrated that the loss of RASSF1A apparently enhances the RAS-driven lung cancer (49). EGFR palmitoylation has been shown to inhibit EGFR activity and alter downstream signaling in the KRAS mutant lung cancer. Blocking EGFR palmitoylation decreased PI3K signaling, negatively regulating lung carcinogenesis (50, 51). The ERBB/EGFR signaling pathway is also dysregulated in lung cancer. The activation of KRAS induces the phosphorylation of iRhom2, which induces excessive shedding of ERBB ligand and tumorigenesis (52). Escaping from oncogene surveillance is a vital part in tumorigenesis. RUNX3, which serves as a mediator of multiple tumor suppressor pathways, is inactivated in KRAS mutant lung cancer. KRAS-activated cells could develop into ADCs when Runx3-mediated tumor suppress signaling pathways are abrogated (53).

Accumulating evidence suggests that inflammation is an essential factor for tumor promotion (54). The JAK-STAT pathway is considered as a central player for inflammation mediated tumorigenesis and targeting this pathway in KRAS-driven LC has been proposed. KRAS mutant LC secrete pro-inflammatory cytokines which activate JAK1 and JAK2, thereby improving cell survival. Besides, deletion of STAT3 could enhance KRAS-driven lung cancer development (55). Previous studies have found that the abnormal activation of STAT3 in the development of KRAS mutant lung cancer, which will be attenuated under anti-IL-6 therapy, suggesting a tight association between IL-6/STAT3 signaling and inflammation in KRAS-activated tumorigenesis (56, 57). A study established a murine model and confirmed that this route is gender-specific, deletion of epithelial STAT3 in KRAS mutant LC female mice will decrease tumorigenesis, while the outcome is completely opposite in male mice (58). Later, their team showed that NF-κB is activated in KRAS-driven mouse model of LUAD (59). Bassères et al.'s work indicated that NF-κB is significant in KRAS-driven tumorigenesis, as the deficiency of p65/RelA profoundly impairs KRAS-driven lung tumorigenesis. Besides, inhibition of IKKβ expression suppressed NF-κB expression in KRAS-driven lung cells (60).

More and more studies have explained the underlying mechanism from various perspectives including proliferation, invasion and EMT. By upregulating DUSP6, a negative regulator of p-ERK, KRAS mutant lung cancer retrained the ERK1/2 mediated toxicity and promote cell proliferation (61). Wang et al. demonstrated that mutant KRAS could enhance the Cathepsin L/CUX1 axis, thereby promoting lung cancer invasion and migration (62). Hsu et al. firstly reported Yes-associated protein (YAP) in LUAD, their study stressed that YAP promote the brain metastasis of NSCLC cell lines H2030-BrM3 (KRASG12C mutation), especially by, targeting the downstream genes CTGF and CYR61 (63).

2.3 BRAF mutations

V-raf murine sarcoma viral oncogene homolog B (BRAF), which plays a vital part in cell proliferation, differentiation, and growth through mediating the MAPK pathway, belongs to the RAF family of serine/threonine protein kinase (64). Over 40 missense mutations have been discovered in human, while the most common BRAF mutation occurs in exon 15 is a thymidine to adenosine transversion at the level of T1799A, leading to the valine to glutamate substitution at codon 600 (V600E), which contribute to approximately half of the BRAF-mutant NSCLC (65, 66). This alteration could result in the activation of B-RAF kinase and constitutive MAPK/ERK cascade signal transduction, which leads to 500 folds BRAF activity compared with WT (67).

Mutant BRAF plays a positive key player in the tumorigenesis (68). Li et al. reported that ARHGEF19, one of Rho guanine nucleotide exchange factors (RhoGEFs), could interact with BRAF and promote MEK1/2 phosphorylation during the NSCLC formation (69). Tumor necrosis factor receptor (TNFR)–associated factors (TRAF) is a kinases modulator of TNFR family. Wang et al. firstly proposed the relationship between BRAF and TRAF1. The study explained that overexpressed TRAF1 could regulate BRAF/MAPK/ERK axis to promote NSCLC cells' viability (70). C-RAF, which could promote adenoma initiation and growth, belongs to RAF family as well. Zanucco et al. reported that elimination of BRAF in oncogenic C-RAF expressed alveolar epithelial type II cells inactivates MAPK signal and lung Tumor growth (71). Upregulated terminal differentiation-induced non-coding RNA (TINCR) is associated with poor survival in NSCLC patients, Zhu et al. pointed out that TINCR could target BRAF and mediate downstream MAPK pathway to promote NSCLC tumorigenesis (72).

Abundant evidence indicates that inactivation of BRAF could cause cancer cell apoptosis, thus demonstrating the necessity of mutant BRAF in tumor cells (73). Lin et al. comprehensively evaluated the molecular determinants of BRAF mutant in lung cancer. Their study summarized that inhibition of MEK/ERK signaling targeting p61VE could suppress the cell escape from BRAFV600E oncogenic inhibitions in NSCLC. Besides, they found the MAPK pathway could mediate EGFR signaling and alleviate the dependence on BRAFV600E (74). Kotani et al. analyzed the role of MAPK

signaling in BRAFV600E mutant lung cancer. The results showed that EGFR signaling could govern MEK/ERK pathway more strongly, however, the situation is not the same in BRAFV600E mutant lung cancer, which induce the receptor tyrosine kinases (RTKs) resistance problem (75). However, a previous study claimed that although BRAFV600E could initiate some benign tumors, lung cancer is seldomly induced. Trejo et al. suggested that co-mutation of PIK3CAH1047R and BRAFV600E could promote lung cancer progression, including transformation of tumor phenotype into malignant and accelerate tumor growth rate (76). The team of Mcmahon also demonstrated similar conclusion. Their research confirmed that the co-mutant BRAFV600E and PIK3CAH1047R in alveolar type 2 pneumocytes accelerate cell dedifferentiation (77). Tumor suppressor STK11 (LKB1) gene is frequently deficient in lung cancer. A study revealed that Lkb1 loss could promote tumorigenesis in BRAFV600E induced LUAD (78).

2.4 ALK rearrangements

ALK gene locates on the short arm of chromosome 2 (2p23), and belongs to the insulin receptor superfamily (79). As a tyrosine kinase receptor, ALK generally expresses in the brain and spinal cord during embryo genesis and dominantly decreased following maturation (79). The high correlation between ALK and tumorigenesis was firstly identified in 1994 as a fusion partner of nucleophosmin (NPM) in anaplastic large-cell lymphoma (80). Further research revealed underlying mechanism of NSCLC genesis with ALK translocation, as well (81, 82). ALK rearrangement presented potent oncogenic drivers in approximately 5–6% of NSCLC patients population characterized with young age, barely smoking and adenocarcinoma histology (83, 84).

The most common fusion partner of ALK is EML4 (echinoderm microtubule associated protein like 4) (85). Heat shock protein 90 (Hsp90) is a novel cancer therapy target owning to its positive role in controlling oncogenic signaling proteins. Normant et al. proposed that inhibition of Hsp90 could decrease EML4-ALK thereby inducing tumor regression in ALK-driven NSCLC (86). The crosstalk between Programmed cell death-ligand 1 (PD-L1) and its receptor programmed cell death-1 (PD-1) conferred profound strength in immunotherapy. A study examined the role of ALK rearrangement in affecting PD-L1 expression, their result showed that EML4-ALK fusion could decrease the PD-L1 expression through suppression of PI3K–AKT or MEK–ERK signaling pathway, which contributes to the immune escape in NSCLC (87). Shen et al. reported that EML4-ALK G1202R mutation could increase the invasion and migration ability of A549 cells. Besides, their research proved the EML4-ALK G1202R mutation could lead to EMT phenotype transformation in NSCLC cells by activating STAT3/Slug pathway (88).

2.5 ROS1 rearrangements

The ROS1 gene, which is located on chromosome 6 (6q22.1), is a widely known proto-oncogenic gene that belongs to the sevenless subfamily of tyrosine kinase insulin receptor. ROS1 rearrangements were primarily described in glioblastoma, the close correlation with NSCLC was discovered in 2012 (89). The ROS1 tyrosine kinase has been discovered to play a vital role in plenty of intracellular signaling pathways (90).

The (c-ros oncogene1) ROS1 rearrangements, which drive malignant transformation of NSCLC, affect approximately 0.7–1.7% NSCLC patients (91). The fusion partners include CD74, SLC34A2, FIG, TPM3, SLC12A2, CCDC6, and SDC4, while CD74-ROS1 fusion is the most prevalent phenotype in NSCLC (92, 93). Gou et al. demonstrated that CD74-ROS1 mutation could lead to EMT and enhance the NSCLC invasion and migration ability by upregulating Twist1 (94). Chromosomal rearrangement of the Solute Carrier Family 34 Member 2 (SLC34A2)-ROS1 fusion accounts for more than 14% of all ROS1 fusion in NSCLCs. Cai et al.'s study revealed that BA/F3 fusion NSCLC cells (harboring SLC34A2-ROS1) could activate ROS1-SHP2 signaling to elevate PD-L1 expression and mediate immunogenicity (95). Interestingly, another study supplemented that ROS1-fusion positive NSCLC cells could target MEK/ERK signaling pathway to upregulate PD-L1 expression significantly (96).

2.6 MET mutations

The proto-oncogene MET, located on chromosome 7q31, is one of the tyrosine kinase receptors. HGF is a common ligand of MET, upon their binding, MET will be dimerized and auto-phosphorylate, thus leading to the activation of activity of intracellular tyrosine kinase. Activation of MET could lead to the modulation of multiple downstream signaling pathways including RAS/RAF/ERK/MAPKA, PI3K/AKT/mTOR, Wnt/β-catenin, STAT and so on, which play vital roles in regulating tumor growth, progression and migration (97). Aberrant activation of MET signaling pathway may contribute to the tumorigenesis process of lung cancer.

About 3–5% of NSCLC patients have MET mutations, most of which are adenocarcinoma. Up to date, various mutations of MET have been identified, including MET amplification, MET point mutations, exon 14 skipping mutation, fusions and overexpression, all of which are oncogenic in lung cancer (98). The skipping of MET exon 14 mutation occurs in 3% LUAD and 13–22% sarcomatoid lung cancer (99). Studies revealed that the exon 14 skipping could lead to the lack of Y1003-Cbl, a ligand mediating c-MET degradation and ubiquitination, which subsequently prolong the activation of c-MET, downstream proliferation, and tumorigenesis (100). MET amplification is another major subtype of MET mutations which occur mainly

in TKI-resistance lung cancer. Because single amplification of MET rarely contributes significantly to cancer development, co-mutant of MET and other cancer drivers, such as EGFR, appear more commonly (101, 102). A study indicated that only high amplification level of MET could display an oncogenic effect (103). However, there's still a lack of research to fully elucidate the mechanism of MET-driven tumor development.

Recently, more and more target therapies are focusing on mutant genes to improve the prognosis for lung cancer. However, disappointedly, clinical outcomes are not positive as expected. Firstly, the development of drug resistance is another unavoidable theme in lung cancer. Jackman et al. primarily defined acquired resistance when patients receive target therapy over 6 months but the disease progression keeps evolving (104). Accumulating evidence has elucidated that, although the acquired resistance mechanism varies, the main reasons are from three aspects: mutant gene modification, phenotypic transformation and alternative signal pathway activation (105). EGFR TKIs are the most commonly used target drugs in EGFR mutant lung cancer patients. Previous studies have revealed that the EGFR T790M mutation, MET amplification, epithelial-to-mesenchymal transition (EMT), activation of the NF-κB pathway, and so on are common foundations of later developed EGFR TKIs resistance (105–108). These various factors could induce signaling pathway cross talk in lung cancer. Hepatocyte growth factor (HGF) could induce acquired resistance to TKIs by restoring the PI3K/Akt signaling pathway *via* phosphorylation of MET in EGFR mutant NSCLC (109). Activation of Hedgehog (Hh) pathway is a common feature of TKIs resistance. A study fully illustrated the role of Hh signaling in EGFR mutant lung cancer, their result showed that, through the induction of mesenchymal properties, Hh could mediate the resistance of EGFR inhibitors (110). Several clinical studies reported acquired resistance to targeted drugs, such as Osimertinib, a MET tyrosine kinase inhibitor (111). The advance in K-RAS targeted medicine faced huge challenge (112). On one hand, clinical trial of K-RAS targeting drugs revealed disappointing results. It was found that K-ras could activate an alternative pathway *via* geranylation with resistant farnesyl transferase inhibitors (113). Blumenschein et al. reported that the downstream MEK signaling pathway inhibitor failed to achieve significant positive effect on improving the survival of lung cancer patients (114). ALK- independent resistance occurs as well. Activating bypass signaling pathways compromising KIT amplification, MAPK, MET amplification, EGFR and BRAF V600E leads to the ALK TKIs resistance (115–117). Additionally, more drugs targeting other mutant genes are under research, which is not warranted to have a certain therapeutic effect. What's more, the narrow therapeutic window is worrying. For example, the second-generation EGFR TKI afatinib displayed an apparent adverse effect in clinical trials (118). Besides, recurrence or metastasis of lung cancer occurs frequently. Previous research has elucidated that brain relapse, bone relapse, and relapse of other organs can seriously affect the prognosis process of lung cancer (119, 120).

Taken together, gene alteration is a common and significant phenomenon in tumorigenesis progression. Slight changes in these central cancer-driven genes can lead to dysregulate in downstream signal pathways, which dramatically affect lung cancer from multiple perspectives (**Figure 2**). However, the therapeutic effect is not always as positive as considered, clinical feedback suggested that the developed drug resistance is uncontrollable (5). Appropriate drug concentration is still in preclinical trials and prognostic recurrence/metastasis is beyond expectation. Therefore, a novel way of overcoming existing dilemma is urgently needed.

3 ICD in lung cancer cells

Immunogenic cell death is a novel type of cell death which primes an adaptive immune response (**Figure 3**). Its main feature is that dying cells can secrete DAMPs, including HMGB1, CRT, ATP, and Type I IFN, which can enhance the antigen presentation capability of APCs, activate T cells, and enhance the immunogenicity of tumor cells, ultimately trigger the arise of ICD (9). In addition, induction of ER stress and ROS accumulation are indispensable components for ICD which increase the DAMPs (13, 14).

Apoptosis is one of the most researched forms of ICD. Apoptosis, mediated by the activity of caspases, is regulated by both endogenous and exogenous factors at the same time, and both of them rely on the activation of caspase-3 and caspase-7 (121). Yet, it has also emerged that non-apoptotic cell death can also be immunogenic, such as necrosis and pyroptosis. Necroptosis can be initiated firstly after activating the extended tumor necrosis factor alpha (TNF-α) receptor family on the surface of the cell, and transmitted in virtue of the serine/threonine kinases, RIP1 or RIP3 interact with receptors. Then dead cells release immunogenic DAMPs to greatly activate both innate and adaptive immune systems. Pyroptosis is a lytic pro-inflammatory modality of regulated cell death (RCD), which leads to the formation of plasma membrane pores with the help of members of the gasdermin protein family, particularly gasdermin D (GSDMD) (122). In addition, the anticancer immune response can be also triggered by autophagy, while autophagy was found to be related to the resistance of cancer cells to anti-cancer therapy (12).

3.1 Extra-cellular DAMP release

One of the main features of ICD is the release of molecular signals, which are usually called "DAMPs" (14). DAMPs can recognize specific receptors and attract adaptive immune cells like neutrophils, macrophages and dendritic cells (DCs). Then

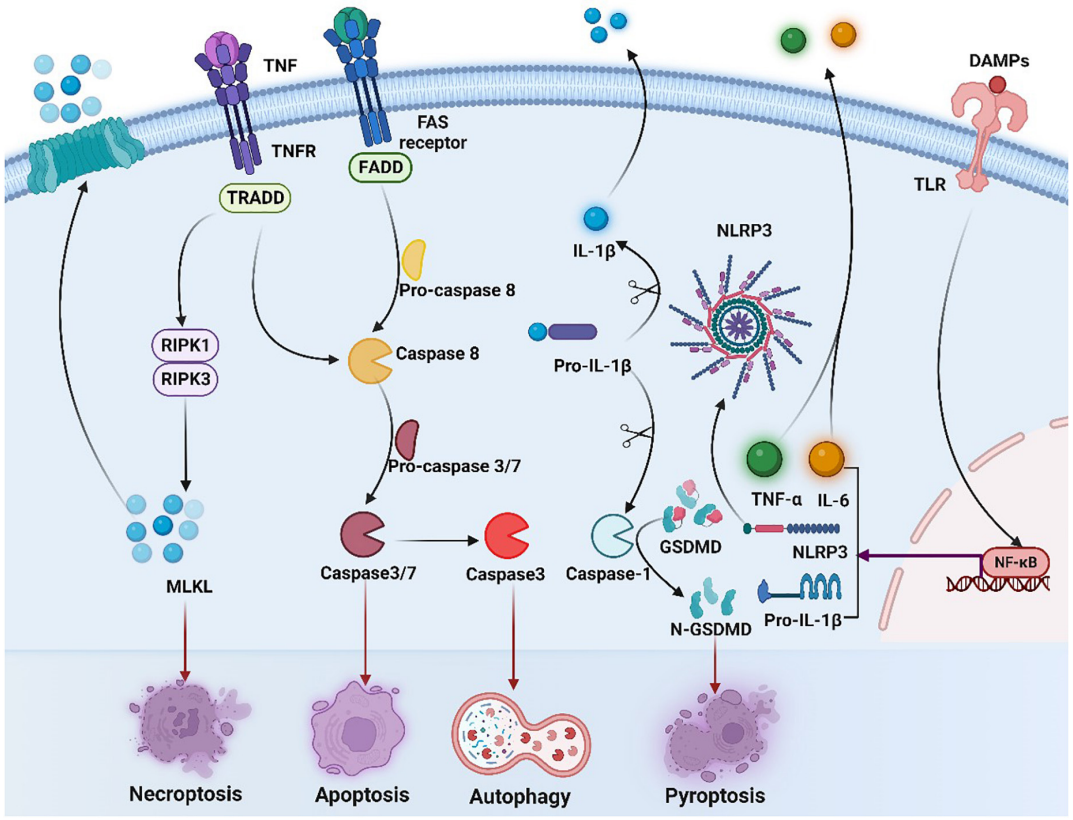

FIGURE 3
Overview of necroptosis, apoptosis, and pyroptosis signaling pathways. Binding of tumor necrosis factor (TNF) to its receptor (TNFR) activates RIPK3 *via* RIPK1, leading to the formation of necrosome, which activates mixed-lineage kinase-like (MLKL), contributing to necrosis and membrane permeation. Activation of caspase-8 induces exogenous apoptosis by triggering caspase-3 and caspase-7 activation. Meanwhile, caspase-3 induces autophagy. The occurrence of ICD in tumor cells is accompanied by the production of a series of signaling molecules in which released DAMPs can bind to pattern recognition receptors such as toll-like receptors (TLRs) on the surface of DC cells, activating NF-κB and inducing expression of NLRP3 and IL-1β/IL-18 precursors. NLRP3 recognizes various DAMPs and becomes oligomerized, contributing to activation of caspase-1 and production of mature IL-1β/IL-18. Activated caspase-1 cleaves gasdermin D (GSDMD) and releases the n-terminal (GSDMDNT) pore-forming fragments, thereby resulting in membrane permeation and pyroptosis. DAMPs, damage-associated molecular patterns; TLR, toll-like receptors; NF-κB, nuclear factor-kappa B; IL-6, interleukin-6; TNF, tumor necrosis factor, TNFR, tumor necrosis factor receptor; TNF-α, tumor necrosis factor alpha; NLRP3, NOD-like receptor thermal protein domain associated protein 3; IL-1β, interleukin-1 beta; GSDMD, gasdermin D; FADD, Fas-associating protein with a novel death domain; TRADD, TNF receptor 1 associated *via* death domain; RIPK1, receptor interacting serine/threonine kinase 1; RIPK3, receptor interacting serine/threonine kinase; MLKL, mixed lineage kinase domain-like protein.

DAMPs promote activation and maturation of these immune cells, including dead cell removal, antigen uptake, processing, and presentation, and cytokine production (123). Some of the most widely researched ICD-linked DAMPs include HMGB1, CRT, ATP, and Type I IFN (124).

High mobility group box 1, which can trigger strongly inflammatory response when released from nucleus of dead cells, is an abundant nuclear non-histone chromatin-binding protein (125). HMGB1 binds to several receptors, such as Toll-like receptor 4 (TLR4), a type of receptor for advanced glycation end products (RAGE) to activate MAPKs and NF-κB in DCs, which are widely expressed in lungs (126–129). After CRT exposure, dead lung cancer cells will secrete HMGB1, which has a dual effect depending on whether it is extracellular or intracellular (14). Extracellular HMGB1 can facilitate the processing and presentation of antigens by DCs (128, 130), while intracellular HMGB1 can promote cancer cell growth and invasion, and resist therapy (129). Studies found that high level of HMGB1 is associated with poor prognosis in NSCLC (131). Moreover, Łagiedo et al. discovered that the levels of HMGB1 in NSCLC patients' serum had a significant positive correlation with the size of the tumor (132).

Adenosine triphosphate is dependent on autophagy to be released from dying cancer cells in virtue of the active exocytosis of ATP-containing vesicles through pannexin channels (13, 133, 134). After being secreted out of cells and binding to purinergic receptor P2Y2 on the target cells, ATP will send a "find-me" signal to DCs and macrophages to promote DC maturation and

macrophage expansion (135, 136). Moreover, ATP can mediate immune stimulation by activating the NLRP3 inflammasome and the subsequent secretion of interleukin 1 beta (IL-1β) (127, 137).

Calreticulin, a soluble protein in ER lumen, is exposed on the cell surface at a premortem stage and confers an "eat me" signal (122). After that, CRT interacts with the CD91 receptor in phagocytes to effectively engulf dead cells, thus providing abundant antigenic substance (13, 138). CRT also induces increased expression of endothelial cell adhesion molecules to promote infiltration of specific lymphocytes in the TME (139). Research has found that high CRT levels were shown to be in association with eIF2α phosphorylation in biopsies from NSCLC patients, which is independently relevant to better prognosis in NSCLC (140). Besides, in the treatment of lung cancer, CRT plays a similar role as HMGB1 so that it can be used to assess the extent of ICD induced by the treatment (141).

Type I IFNs, which can be driven by RNA or DNA species, are actively synthesized and activate other downstream genes including genes coding for chemokines to favor an immune response (142, 143). In RNA species, the receptor is endosomal TLR3, whereas the latter setting mainly works through cytosolic cyclic GMP-AMP synthase (CGAS) and its signal transducer stimulator of IFN response cGAMP interactor 1 (STING1) (144–146). Moreover, type I IFN can trigger macrophages to secrete pro-inflammatory mediators and inhibit the immunosuppressive functions of regulatory T cells (147, 148). Apart from these direct immunostimulatory functions, type I IFN can also elicit the synthesis of the CX-C motif chemokine ligand 10 (CXCL10) by tumor cells in ICD *via* an autocrine signaling loop (149).

3.2 ER stress

Immunogenic cell death can be divided into two modes according to its induction mechanism (13). Instead of inducing ROS and ER stress directly, type I ICD is stimulated by indirect signals. Quite the opposite, type II ICD targets the ER, inducing ER stress and immunogenic cell death (150). The process of ER stress activation is termed as unfolded protein response (UPR), featured with phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) by Protein Kinase RNA-activated (PKR)-like ER Kinase (PERK) (151, 152). Several studies have shown that ER stress is the core of the occurrence of ICD (153). Moderate ER stress may be conducive to creating an immunosuppressive environment, while severe ER stress can stimulate immune response, as what happens in ICD (154). The more concentrated the ER stress is, the higher the immunogenicity of cell death is (155). ER stress is the main cellular mechanism for the cell surface exposure of CRT, which is closely linked to the phosphorylation of eIF2α. In Fucikova et al.'s study, they stated a subgroup of NSCLC associated with strong ER stress, which erupts in CRT expression and exposure (156). The high CRT driven by ER stress response has a positive prognostic value for NSCLC patients, however, its specific molecular mechanism remains to be further studied. ER stress also has an influence on levels of intracellular ATP by stimulating mitochondrial respiration, and cells can fill their bioenergy reserves in this way to restore cellular homeostasis (157).

4 Induction of ICD for lung cancer therapy

Immunogenic cell death can be caused by different types of stimulation and antitumor therapy, such as chemotherapy and radiation, some targeted drugs, oxygen-boosted PDT and ICIs. A lot of evidence shows that ICD can stimulate anticancer immune responses *in vivo*, and provide an opportunity to improve the cancer treatment and outcomes (158, 159). However, recently, only a few ICD inducers have been successfully translated into clinical practice. Here we elaborated on the role of ICD in the therapy of lung cancer.

4.1 Targeted therapy

Most anti-cancer drugs kill cancer cells in a non-immunogenic way. However, many studies demonstrated that ICD can be induced by different targeted agents. We will review these targeted drugs in detail to illustrate the important role of targeted therapy in the treatment of lung cancer (**Table 1**).

Crizotinib, a TKI used to treat NSCLC carrying activated ALK, ROS1, and MET, serves as an ICD stimulator *via* off-target effects (160). Drewry et al. have provided preclinical evidence that crizotinib can be expediently in combination with non-ICD inducing chemotherapeutics, as much with immune checkpoint blockade, to treat NSCLC in an effective way (161). One research has shown that the combination of cisplatin and high-dose crizotinib brings about an increase of PD-1 and PD-L1, and induces greatly ICD in NSCLC cells (12, 162). Hence, a sequential combination treatment including conventional chemotherapy together with crizotinib and immune checkpoint blockade may be effective for NSCLC. Besides, another ALK inhibitor ceritinib also has the function of targeting which can induce ICD in ALK-dependent NSCLC cell lines (124). For the past few years, the receptor tyrosine kinase AXL has been considered as a promising target for tumor treatment. The AXL signaling can promote a pro-survival pathway and reduce reactivation of the MAPK pathway to develop acquired resistance to EGFR TKI in NSCLC cells. And there is a positive correlation between AXL and autophagy (163, 164). Boshuizen et al. showed that an antibody–drug conjugate targeting AXL called enapotamab vedotin (EnaV) not

TABLE 1 Targeted therapies inducing immunogenic cell death (ICD) and their molecular targets in lung cancer treatment.

Drug	Molecular targets	Acquired mutations	Mechanisms	References
Crizotinib	ALK, ROS1	ALK, EGFR, KRAS, ROS1	Off-target effects	(124, 162, 210)
Ceritinib	ALK	ALK	Off-target effects	(124)
EnaV	AXL		Induce inflammatory response and the induction of a memory-like phenotype in cytotoxic T cells	(165)
Bemcentinib	AXL		Abrogate the transcription of autophagy-associated genes	(164)
BI2536	PLK1		Promote apoptosis and mitotic cell death, promote DC maturation and T-cell infiltration	(167)
Auranofin	Trx/TrxR	p53	Induce apoptosis and ferroptosis	(169)
Statins	RAS	KRAS	Induce severe ER stress	(170)
Biscoumarin OT52	STAT3	KRAS	Trigger cell cycle arrest and senescence, and multiple cellular stress mechanisms	(211)

EGFR, epidermal growth factor receptor; EnaV, enapotamab vedotin; DC, dendritic cell; Trx, thioredoxin; TrxR, thioredoxin reductase; ER, endoplasmic reticulum; STAT3, signal transducer and activator of transcription.

only has direct tumor killing, but also induces inflammation and ICD of tumor cells in melanoma and lung cancer models (165). Lotsberg et al. reported that a small molecule inhibitor bemcentinib inhibits the transcription of autophagy-associated genes, releases DAMPs and then gives rise to ICD in NSCLC cells by targeted inhibition of the AXL signaling pathway (164). Erlotinib, a kind of TKI, can inhibit tumor development by inhibiting intracellular phosphorylation of EGFR-related tyrosine kinases, which commonly used in the treatment of NSCLC and pancreatic cancer. Studies have shown that targeting AXL in ER cells induces massive autophagic vacuolation before death in erlotinib-resistant cancer cells and triggers ICD (164). Hence, we suggest that ICD induction may have an unexpected effect on AXL-targeted NSCLC with drug-resistant EGFR mutations. Besides, PLK1 is a member of polo-like kinase family associated with cell division. PLK1 is overexpressed in NSCLC and it often predicts a poor prognosis (166). A selective PLK1 inhibitor BI2536 can act as an ICD inducer to cause apoptosis and alter the tumor immune microenvironment by promoting DC maturation and increasing T-cell infiltration (167).

In addition, several drugs widely used for other diseases have been found to have the ability to induce ICD in lung cancer cells. Auranofin (AF), a thioredoxin reductase 1 (TrxR) inhibitor, is known as an antirheumatic drug (168). TrxR is considered as a potential target in NSCLC on account of its high expression in NSCLC patients. A recent study indicated that AF can initiate release of DAMPs and DC maturation, then trigger apoptotic and ferroptotic cell death by targeting TrxR and launching ICD. It may provide new ideas for the treatment of NSCLC (169). Moreover, statins are one of the most frequently used drugs to treat hyperlipidemia. Statins can stimulate CD8 + T cells and provoke severe ER stress by inhibiting RAS prenylation in KRAS-mutant (KRASmut) lung tumor models, thereby leading to the ICD effects (170). Besides, coumarin is a kind of natural compounds with anti-inflammatory and anti-cancer function. Lee et al. found that biscoumarin OT52 strongly inhibited the proliferation of KRASmut NSCLC cells *via* ICD pathways (171). Mechanistically, biscoumarin OT52 suppresses STAT3 transactivation and expression of its target genes. Altogether, these drugs may become novel candidates in the future for more effective treatment of lung cancer. However, the specific signaling pathway in ICD inducing and other mechanisms still need to be explored in-depth.

4.2 Chemotherapy and radiation therapy

Chemotherapeutic drugs are considered to kill cancer cells selectively *via* direct cytotoxicity (12). A main mechanism of immunity stimulation by chemotherapy involves the induction of ICD (121). Unfortunately, only a little part of anti-cancer drugs can effectively trigger ICD (172). Some kinds of chemotherapeutic agents have been tried to modulate activity of DCs, such as cyclophosphamide, doxorubicin, oxaliplatin and anthracyclines, which can make tumor antigens be vaccines to the immune system and induce ICD, consequently provoking robust adaptive immune response (173–175). Chemotherapy with a combination regimen of oxaliplatin with cyclophosphamide is approved in clinical practice for lung cancer. Previous studies found that in a lung mouse cancer model, this regimen can foster CD8+ T cell infiltration and increase TLR4+ DCs in tumor tissues, and further increase tumor sensitivity to immune checkpoint therapy (176). But the research by Fileswasser et al. has shown that oxaliplatin does not induce ICD in NSCLC cells (4). Pemetrexed, a multi-targeting antifolate antagonist which is established as the main chemotherapy drug for the first-line treatment of advanced non-squamous NSCLC (NSq-NSCLC)

and mesothelioma, has also been shown to induce ICD and to increase of immune-regulatory genes (177, 178). Liu et al. found that crizotinib, a kind of drugs used to treat NSCLC patients which carries activated ALK/ROS1, is an efficient ICD stimulator *via* off-target effects (162). Wang et al.'s results show that trametinib also has the ability to induce ICD by sensitizing lung cancer cells to endoplasmic reticulum stress and triggering the release of DAMPs, and can be effective in treating KRAS-mutant LUAD when used in combination with interleukin-12 (IL-12) (179). A study by Gao et al. showed that, DOC, a kind of tubulin stabilizer belonging to the taxane family, can induce DAMPs and significantly upregulated release of HMGB1 in human NSCLC cell line (180). In addition, the results of Furuwaka et al. suggest that osimertinib induced NSCLC tumor cell death may lead to exposure and release of CRT to induce ICD, and then improve the anti-tumor immunity (181).

As a topical treatment approach, radiation therapy (RT) is widely used in clinical cancer treatment. RT can induce ICD, which promotes DCs activation and the presentation of tumor antigen to prime CD8+ T cells (182). The CD8+ T cells then enter the unirradiated tumor area and attack cancer cells (14, 183). RT and many traditional chemotherapeutic agents give rise to DNA damage and multiform cell death ultimately (184). In various preclinical settings, similar to chemotherapy, induction of ICD by RT has been shown associated with increased sensitivity to immune checkpoint blockade (144, 185), and many clinical trials have proven that (186). In the same way, ablative RT can induce necroptosis in NSCLC and mediate HMGB1-driven immunological response (187).

4.3 PDT

Photodynamic therapy is able to kill cancer cells by manipulating photosensitizers and generating reactive ROS, which triggers ER stress and induces the anti-tumor immunity to eliminate residual or metastatic tumors effectively and selectively (155, 171, 188). After accumulating selectively in the tumor area, the photosensitizer (PS) is activated by illumination with visible light of appropriate wavelength, and then illuminated by red light (690 nm), which can induce local ICD at the tumor sites and strong anti-tumor immunity (10, 189). In recent years, the concept of PDT has been actively pursued. The binding of near-infrared PS to antibodies or nanocarriers improves the efficiency of PDT (190). One typical PS shown to induce ICD is hypericin, which is an anthraquinone derivative of natural origin with specific ER localization (155, 171). The other promising non-porphyrin PS is benzophenazine, OR141, which also has specific location in the ER (191). OR141 induces cell death mainly *via* the mammalian target of rapamycin signaling pathway and by inhibition of proteasomal deubiquitinases, leading to ER stress (192). One study reveals that in a prophylactic tumor vaccination model using PDT-treated TC1 lung cancer cells, redaporfin acts as an ICD inducer that can trigger eIF2a phosphorylation, DAMPs release and inhibit tumor growth (193). ICD can also be induced by PDT based on 8-methoxypsoralen (8- MOP) (194). But it is worth noting that it doesn't need oxygen but intercalates into DNA and forms cross-links with one or two DNA strands under UVA irradiation (195). Furthermore, it has been shown that photofrin-based PDT of Lewis lung carcinoma cells induced release of HSPs, and surface exposure of CRT *in vitro* and *in vivo* in an hour after PDT, as well as an increase of HMGB1 (196). These data also indicate that photofrin is a potential inducer of ICD.

4.4 ICIs

The antitumor effect of ICIs works by interfering with immune tolerance (178). The most clinically common immune checkpoints include: PD-1/PD-L1, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), indoleamine 2.3-dioxygenase (IDO), and CD47 (197, 198). ICIs have established a new model of lung cancer treatment and improved patients' survival benefits (199, 200). It also has revolutionized the prognosis of multiple lung cancers, especially NSCLC, which have a high sensitivity to the immunotherapy against PD-1 (201). The combination therapy of platinum, PEM and ICIs has been proposed as a standard first-line treatment for advanced LUAD (202, 203). Also, numerous studies have shown platinum-based combination chemotherapy and combination ICIs, like PD-1 or for its ligand PD-L1, can markedly prolong survival in patients with stage III unresectable NSCLC (204–207). In clinical application, the combination of ICIs and chemotherapy can improve the efficacy of anti-tumor therapy, this may be because chemotherapy drugs increase tumor sensitivity to ICIs (208). Pemetrexed and ICIs targeting PD-1/PD-L1 are applied widely for the treatment of advanced NSq-NSCLC (178). Moreover, lurbinectedin is a kind of DNA-binding inhibitors of transcription, which is efficient at inducing ICD (209). The combination therapy of lurbinectedin and ICIs targeting PD-1/PD-L1 is supposed to be a salvage therapy for relapsed SCLC be over the years (172).

5 Conclusions and perspectives

Worldwide, lung cancer is one of the most common cancers and the leading cause of cancer-related deaths. For decades, researchers are exploring the pathogenesis of lung cancer and trying to find more effective treatments, such as by finding oncogenic driver gene mutations to improve targeted therapy. However, it is disappointing that clinical results have not been as positive as we expected. In recent years, ICD was noticed for evoking adaptive immune response of cancer cells. ICD

can be induced by a variety of anticancer therapies, including chemotherapy, radiotherapy, targeted drugs, PDT and ICIs, etc. And it is becoming increasingly evident that ICD may offer a new idea in the anti-cancer therapeutic approaches in the future, especially for lung cancer.

In summary, it is a breakthrough to harness ICD to elevate the immunogenicity of tumor cells to maintain the efficacy of anti-tumor therapies for lung cancer. ICD induction is a promising area to explore and the mechanism of function and regulatory networks of ICD deserve further investigation. Finally, due to the limitations of current study, there are still many unanswered questions, such as whether ICD is associated with ferroptosis or cuproptosis, whether ICD is associated with anti-angiogenic drugs, and so on.

Author contributions

XZ: conceptualization and funding acquisition. JX and YX: writing – original draft preparation. ZX and HX: adapt the text and figures. XZ and LZ: writing – review and editing and project administration. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the Domestic Cooperation Project of the Science and Technology Department of Jiangxi Province (No. 20212BDH81021 to XZ), Project of the Science and Technology Department of Jiangxi Province (No. 20203BBGL73148 to XZ), and Project of Health Commission of Jiangxi Province (No. 202210038 to XZ).

Acknowledgments

The graphical abstracts were created with BioRender software (www.biorender.com). We sincerely appreciated the guidance from our and every member in our team.

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.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L, Jemal A. 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

2. Rudin C, Brambilla E, Faivre-Finn C, Sage J. Small-cell lung cancer. *Nat Rev Dis Primers.* (2021) 7:3. doi: 10.1038/s41572-020-00235-0

3. Rami-Porta R, Bolejack V, Giroux D, Chansky K, Crowley J, Asamura H, et al. The IASLC lung cancer staging project: the new database to inform the eighth edition of the TNM classification of lung cancer. *J Thorac Oncol.* (2014) 9:1618–24. doi: 10.1097/JTO.0000000000000334

4. Flieswasser T, Van Loenhout J, Freire Boullosa L, Van den Eynde A, De Waele J, Van Audenaerde J, et al. Clinically relevant chemotherapeutics have the ability to induce immunogenic cell death in non-small cell lung cancer. *Cells.* (2020) 9:1474. doi: 10.3390/cells9061474

5. Tan A, Tan D. Targeted therapies for lung cancer patients with oncogenic driver molecular alterations. *J Clin Oncol.* (2022) 40:611–25. doi: 10.1200/JCO.21.01626

6. Duma N, Santana-Davila R, Molina J. Non-small cell lung cancer: epidemiology, screening, diagnosis, and treatment. *Mayo Clin Proc.* (2019) 94:1623–40. doi: 10.1016/j.mayocp.2019.01.013

7. Ruiz-Cordero R, Devine W. Targeted therapy and checkpoint immunotherapy in lung cancer. *Surg Pathol Clin.* (2020) 13:17–33. doi: 10.1016/j.path.2019.11.002

8. Galluzzi L, Vitale I, Aaronson S, Abrams J, Adam D, Agostinis P, et al. Molecular mechanisms of cell death: recommendations of the nomenclature committee on cell death 2018. *Cell Death Differ.* (2018) 25:486–541. doi: 10.1038/s41418-018-0102-y

9. Garg A, Galluzzi L, Apetoh L, Baert T, Birge R, Bravo-San Pedro J, et al. Molecular and translational classifications of DAMPs in immunogenic cell death. *Front Immunol.* (2015) 6:588. doi: 10.3389/fimmu.2015.00588

10. Alzeibak R, Mishchenko T, Shilyagina N, Balalaeva I, Vedunova M, Krysko D. Targeting immunogenic cancer cell death by photodynamic therapy: past, present and future. *J Immunother Cancer.* (2021) 9:e001926. doi: 10.1136/jitc-2020-001926

11. Duan X, Chan C, Lin W. Nanoparticle-mediated immunogenic cell death enables and potentiates cancer immunotherapy. *Angew Chem Int Ed Engl.* (2019) 58:670–80. doi: 10.1002/anie.201804882

12. Fucikova J, Kepp O, Kasikova L, Petroni G, Yamazaki T, Liu P, et al. Detection of immunogenic cell death and its relevance for cancer therapy. *Cell Death Dis.* (2020) 11:1013. doi: 10.1038/s41419-020-03221-2

13. Krysko D, Garg A, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P. Immunogenic cell death and DAMPs in cancer therapy. *Nat Rev Cancer.* (2012) 12:860–75. doi: 10.1038/nrc3380

14. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. *Nat Rev Immunol.* (2017) 17:97–111. doi: 10.1038/nri.2016.107

15. Passiglia F, Commendatore O, Vitali M, Conca R. Immunotherapy in non-small-cell lung cancer: a bridge between research and clinical practice. *Future Oncol.* (2018) 14:41–60. doi: 10.2217/fon-2018-0098

16. To K, Fong W, Cho W. Immunotherapy in treating EGFR-mutant lung cancer: current challenges and new strategies. *Front Oncol.* (2021) 11:635007. doi: 10.3389/fonc.2021.635007

17. Yarden Y, Pines G. The ERBB network: at last, cancer therapy meets systems biology. *Nat Rev Cancer.* (2012) 12:553–63. doi: 10.1038/nrc3309

18. Cho J, Chen L, Sangji N, Okabe T, Yonesaka K, Francis J, et al. Cetuximab response of lung cancer-derived EGF receptor mutants is associated with asymmetric dimerization. *Cancer Res.* (2013) 73:6770–9. doi: 10.1158/0008-5472.CAN-13-1145

19. Kobayashi Y, Mitsudomi T. Not all epidermal growth factor receptor mutations in lung cancer are created equal: perspectives for individualized treatment strategy. *Cancer Sci.* (2016) 107:1179–86. doi: 10.1111/cas.12996

20. Pacini L, Jenks A, Vyse S, Wilding C, Arthur A, Huang P. Tackling drug resistance in EGFR exon 20 insertion mutant lung cancer. *Pharmacogenomics Pers Med.* (2021) 14:301–17. doi: 10.2147/PGPM.S242045

21. Lynch T, Bell D, Sordella R, Gurubhagavatula S, Okimoto R, Brannigan B, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med.* (2004) 350:2129–39. doi: 10.1056/NEJMoa040938

22. Takeuchi K, Ito F. EGF receptor in relation to tumor development: molecular basis of responsiveness of cancer cells to EGFR-targeting tyrosine kinase inhibitors. *FEBS J.* (2010) 277:316–26. doi: 10.1111/j.1742-4658.2009.07450.x

23. Roskoski R Jr. The ErbB/HER family of protein-tyrosine kinases and cancer. *Pharmacol Res.* (2014) 79:34–74. doi: 10.1016/j.phrs.2013.11.002

24. Rothenberg S, Concannon K, Cullen S, Boulay G, Turke A, Faber A, et al. Inhibition of mutant EGFR in lung cancer cells triggers SOX2-FOXO6-dependent survival pathways. *Elife.* (2015) 4:e06132. doi: 10.7554/eLife.06132

25. Bivona T, Hieronymus H, Parker J, Chang K, Taron M, Rosell R, et al. FAS and NF-κB signalling modulate dependence of lung cancers on mutant EGFR. *Nature.* (2011) 471:523–6. doi: 10.1038/nature09870

26. Karachaliou N, Gimenez-Capitan A, Drozdowskyj A, Viteri S, Moran T, Carcereny E, et al. ROR1 as a novel therapeutic target for EGFR-mutant non-small-cell lung cancer patients with the EGFR T790M mutation. *Transl Lung Cancer Res.* (2014) 3:122–30.

27. Kinehara Y, Nagatomo I, Koyama S, Ito D, Nojima S, Kurebayashi R, et al. Semaphorin 7A promotes EGFR-TKI resistance in EGFR mutant lung adenocarcinoma cells. *JCI Insight.* (2018) 3:e123093. doi: 10.1172/jci.insight.123093

28. Zhang N, Liao Y, Lv W, Zhu S, Qiu Y, Chen N, et al. FBXO32 targets PHPT1 for ubiquitination to regulate the growth of EGFR mutant lung cancer. *Cell Oncol.* (2022) 45:293–307. doi: 10.1007/s13402-022-00669-6

29. Chava S, Bugide S, Zhang X, Gupta R, Wajapeyee N. Betacellulin promotes tumor development and EGFR mutant lung cancer growth by stimulating the EGFR pathway and suppressing apoptosis. *iScience.* (2022) 25:104211. doi: 10.1016/j.isci.2022.104211

30. Niu M, Xu J, Liu Y, Li Y, He T, Ding L, et al. FBXL2 counteracts Grp94 to destabilize EGFR and inhibit EGFR-driven NSCLC growth. *Nat Commun.* (2021) 12:5919. doi: 10.1038/s41467-021-26222-x

31. Tsai M, Chang T, Wu S, Yang H, Hsu Y, Yang P, et al. EGFR-L858R mutant enhances lung adenocarcinoma cell invasive ability and promotes malignant pleural effusion formation through activation of the CXCL12-CXCR4 pathway. *Sci Rep.* (2015) 5:13574. doi: 10.1038/srep13574

32. Feng J, Wei X, Li C, Guo M, Peng M, Song Q, et al. [Mechanism of EGFR over-expression and mutations leading to biological characteristics changes of human lung adenocarcinoma cells through CXCR4/CXCL12 signaling pathway]. *Zhongguo Fei Ai Za Zhi.* (2018) 21:503–12.

33. Li H, Tong F, Meng R, Peng L, Wang J, Zhang R, et al. E2F1-mediated repression of WNT5A expression promotes brain metastasis dependent on the ERK1/2 pathway in EGFR-mutant non-small cell lung cancer. *Cell Mol Life Sci.* (2021) 78:2877–91. doi: 10.1007/s00018-020-03678-6

34. Lou L, Wang J, Lv F, Wang G, Li Y, Xing L, et al. Y-box binding protein 1 (YB-1) promotes gefitinib resistance in lung adenocarcinoma cells by activating AKT signaling and epithelial-mesenchymal transition through targeting major vault protein (MVP). *Cell Oncol.* (2021) 44:109–33. doi: 10.1007/s13402-020-00556-y

35. Cao W, Liu Y, Zhang R, Zhang B, Wang T, Zhu X, et al. Homoharringtonine induces apoptosis and inhibits STAT3 via IL-6/JAK1/STAT3 signal pathway in gefitinib-resistant lung cancer cells. *Sci Rep.* (2015) 5:8477. doi: 10.1038/srep08477

36. Han Y, Lee T, He Y, Raman R, Irizarry A, Martin M, et al. The regulation of CD73 in non-small cell lung cancer. *Eur J Cancer.* (2022) 170:91–102. doi: 10.1016/j.ejca.2022.04.025

37. Griesing S, Liao B, Yang J. CD73 Is regulated by the EGFR-ERK signaling pathway in non-small cell lung cancer. *Anticancer Res.* (2021) 41:1231–42. doi: 10.21873/anticanres.14880

38. Le X, Negrao M, Reuben A, Federico L, Diao L, McGrail D, et al. Characterization of the immune landscape of EGFR-mutant NSCLC identifies CD73/adenosine pathway as a potential therapeutic target. *J Thorac Oncol.* (2021) 16:583–600. doi: 10.1016/j.jtho.2020.12.010

39. Passarelli A, Aieta M, Sgambato A, Gridelli C. Targeting immunometabolism mediated by CD73 pathway in EGFR-mutated non-small cell lung cancer: a new hope for overcoming immune resistance. *Front Immunol.* (2020) 11:1479. doi: 10.3389/fimmu.2020.01479

40. Chen X, Gao A, Zhang F, Yang Z, Wang S, Fang Y, et al. ILT4 inhibition prevents TAM- and dysfunctional T cell-mediated immunosuppression and enhances the efficacy of anti-PD-L1 therapy in NSCLC with EGFR activation. *Theranostics.* (2021) 11:3392–416. doi: 10.7150/thno.52435

41. Bos J. ras oncogenes in human cancer: a review. *Cancer Res.* (1989) 49:4682–9.

42. Murugan A, Grieco M, Tsuchida N. RAS mutations in human cancers: roles in precision medicine. *Semin Cancer Biol.* (2019) 59:23–35. doi: 10.1016/j.semcancer.2019.06.007

43. Cox A, Fesik S, Kimmelman A, Luo J, Der C. Drugging the undruggable RAS: mission possible?. *Nat Rev Drug Discov.* (2014) 13:828–51. doi: 10.1038/nrd4389

44. Ricciuti B, Leonardi G, Metro G, Grignani F, Paglialunga L, Bellezza G, et al. Targeting the KRAS variant for treatment of non-small cell lung cancer: potential therapeutic applications. *Expert Rev Respir Med.* (2016) 10:53–68. doi: 10.1586/17476348.2016.1115349

45. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. *Nat Rev Cancer.* (2003) 3:459–65. doi: 10.1038/nrc1097

46. Ostrem J, Peters U, Sos M, Wells J, Shokat K. MK-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature.* (2013) 503:548–51. doi: 10.1038/nature12796

47. Kanno E, Kawasaki O, Takahashi K, Takano K, Endo T. DA-Raf, a dominant-negative antagonist of the Ras-ERK pathway, is a putative tumor suppressor. *Exp Cell Res.* (2018) 362:111–20. doi: 10.1016/j.yexcr.2017.11.008

48. Costa-Machado L, Martín-Hernández R, Sanchez-Luengo M, Hess K, Vales-Villamarin C, Barradas M, et al. Sirt1 protects from K-Ras-driven lung carcinogenesis. *EMBO Rep.* (2018) 19:e43879. doi: 10.15252/embr.201643879

49. Schmidt M, Hobbing K, Donninger H, Clark G. RASSF1A deficiency enhances RAS-driven lung tumorigenesis. *Cancer Res.* (2018) 78:2614–23. doi: 10.1158/0008-5472.CAN-17-2466

50. Kharbanda A, Walter D, Gudiel A, Schek N, Feldser D, Witze E. Blocking EGFR palmitoylation suppresses PI3K signaling and mutant KRAS lung tumorigenesis. *Sci Signal.* (2020) 13:eaax2364. doi: 10.1126/scisignal.aax2364

51. Kadry Y, Lee J, Witze E. Regulation of EGFR signalling by palmitoylation and its role in tumorigenesis. *Open Biol.* (2021) 11:210033. doi: 10.1098/rsob.210033

52. Sieber B, Lu F, Stribbling S, Grieve A, Ryan A, Freeman M. iRhom2 regulates ERBB signalling to promote KRAS-driven tumour growth of lung cancer cells. *J Cell Sci.* (2022) 135:jcs259949. doi: 10.1242/jcs.259949

53. Lee Y, Lee J, Song S, Kim D, Lee J, Chi X, et al. K-ras-activated cells can develop into lung tumors when runx3-mediated tumor suppressor pathways are abrogated. *Mol Cells.* (2020) 43:889–97.

54. Hanahan D, Coussens L. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell.* (2012) 21:309–22. doi: 10.1016/j.ccr.2012.02.022

55. Mohrherr J, Haber M, Breitenecker K, Aigner P, Moritsch S, Voronin V, et al. JAK-STAT inhibition impairs K-RAS-driven lung adenocarcinoma progression. *Int J Cancer.* (2019) 145:3376–88. doi: 10.1002/ijc.32624

56. Caetano M, Zhang H, Cumpian A, Gong L, Unver N, Ostrin E, et al. IL6 blockade reprograms the lung tumor microenvironment to limit the development and progression of K-ras-mutant lung cancer. *Cancer Res.* (2016) 76:3189–99. doi: 10.1158/0008-5472.CAN-15-2840

57. Clowers M, Moghaddam S. Cell type-specific roles of STAT3 signaling in the pathogenesis and progression of K-ras mutant lung adenocarcinoma. *Cancers.* (2022) 14:1785. doi: 10.3390/cancers14071785

58. Caetano M, Hassane M, Van H, Bugarin E, Cumpian A, McDowell C, et al. Sex specific function of epithelial STAT3 signaling in pathogenesis of K-ras mutant lung cancer. *Nat Commun.* (2018) 9:4589. doi: 10.1038/s41467-018-07042-y

59. Moghaddam S, Li H, Cho S, Dishop M, Wistuba I, Ji L, et al. Promotion of lung carcinogenesis by chronic obstructive pulmonary disease-like airway inflammation in a K-ras-induced mouse model. *Am J Respir Cell Mol Biol.* (2009) 40:443–53. doi: 10.1165/rcmb.2008-0198OC

60. Bassères D, Ebbs A, Cogswell P, Baldwin AS. IKK is a therapeutic target in KRAS-Induced lung cancer with disrupted p53 activity. *Genes Cancer.* (2014) 5:41–55. doi: 10.18632/genesandcancer.5

61. Unni A, Harbourne B, Oh M, Wild S, Ferrarone J, Lockwood W, et al. Hyperactivation of ERK by multiple mechanisms is toxic to RTK-RAS mutation-driven lung adenocarcinoma cells. *Elife.* (2018) 7:e33718. doi: 10.7554/eLife.33718

62. Wang L, Zhao Y, Xiong Y, Wang W, Fei Y, Tan C, et al. K-ras mutation promotes ionizing radiation-induced invasion and migration of lung cancer in part via the Cathepsin L/CUX1 pathway. *Exp Cell Res.* (2018) 362:424–35. doi: 10.1016/j.yexcr.2017.12.006

63. Hsu P, Miao J, Huang Z, Yang Y, Xu Z, You J, et al. Inhibition of yes-associated protein suppresses brain metastasis of human lung adenocarcinoma in a murine model. *J Cell Mol Med.* (2018) 22:3073–85. doi: 10.1111/jcmm.13582

64. Leicht D, Balan V, Kaplun A, Singh-Gupta V, Kaplun L, Dobson M, et al. Raf kinases: function, regulation and role in human cancer. *Biochim Biophys Acta.* (2007) 1773:1196–212. doi: 10.1016/j.bbamcr.2007.05.001

65. Wan P, Garnett M, Roe S, Lee S, Niculescu-Duvaz D, Good V, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell.* (2004) 116:855–67. doi: 10.1016/S0092-8674(04)00215-6

66. Pratilas C, Hanrahan A, Halilovic E, Persaud Y, Soh J, Chitale D, et al. Genetic predictors of MEK dependence in non-small cell lung cancer. *Cancer Res.* (2008) 68:9375–83. doi: 10.1158/0008-5472.CAN-08-2223

67. Chambard J, Lefloch R, Pouysségur J, Lenormand P. ERK implication in cell cycle regulation. *Biochim Biophys Acta.* (2007) 1773:1299–310. doi: 10.1016/j.bbamcr.2006.11.010

68. Hall R, Kudchadkar RR. BRAF mutations: signaling, epidemiology, and clinical experience in multiple malignancies. *Cancer Control.* (2014) 21:221–30. doi: 10.1177/107327481402100307

69. Li Y, Ye Z, Chen S, Pan Z, Zhou Q, Li Y, et al. ARHGEF19 interacts with BRAF to activate MAPK signaling during the tumorigenesis of non-small cell lung cancer. *Int J Cancer.* (2018) 142:1379–91. doi: 10.1002/ijc.31169

70. Wang Q, Gao G, Zhang T, Yao K, Chen H, Park M, et al. TRAF1 is critical for regulating the BRAF/MEK/ERK pathway in non-small cell lung carcinogenesis. *Cancer Res.* (2018) 78:3982–94. doi: 10.1158/0008-5472.CAN-18-0429

71. Zanucco E, El-Nikhely N, Götz R, Weidmann K, Pfeiffer V, Savai R, et al. Elimination of B-RAF in oncogenic C-RAF-expressing alveolar epithelial type II cells reduces MAPK signal intensity and lung tumor growth. *J Biol Chem.* (2014) 289:26804–16. doi: 10.1074/jbc.M114.558999

72. Zhu Z, He JK. TINCR facilitates non-small cell lung cancer progression through BRAF-activated MAPK pathway. *Biochem Biophys Res Commun.* (2018) 497:971–7. doi: 10.1016/j.bbrc.2018.02.059

73. Rustgi AK. BRAF: a driver of the serrated pathway in colon cancer. *Cancer Cell.* (2013) 24:1–2. doi: 10.1016/j.ccr.2013.06.008

74. Lin L, Asthana S, Chan E, Bandyopadhyay S, Martins M, Olivas V, et al. Mapping the molecular determinants of BRAF oncogene dependence in human lung cancer. *Proc Natl Acad Sci USA.* (2014) 111:E748–57. doi: 10.1073/pnas.1320956111

75. Kotani H, Adachi Y, Kitai H, Tomida S, Bando H, Faber A, et al. Distinct dependencies on receptor tyrosine kinases in the regulation of MAPK signaling between BRAF V600E and non-V600E mutant lung cancers. *Oncogene.* (2018) 37:1775–87. doi: 10.1038/s41388-017-0035-9

76. Trejo C, Green S, Marsh V, Collisson E, Iezza G, Phillips W, et al. Mutationally activated PIK3CA(H1047R) cooperates with BRAF(V600E) to promote lung cancer progression. *Cancer Res.* (2013) 73:6448–61. doi: 10.1158/0008-5472.CAN-13-0681

77. van Veen J, Scherzer M, Boshuizen J, Chu M, Liu A, Landman A, et al. Mutationally-activated PI3'-kinase-α promotes de-differentiation of lung tumors initiated by the BRAF(V600E) oncoprotein kinase. *Elife.* (2019) 8:e43668. doi: 10.7554/eLife.43668

78. González-Sánchez E, Martín-Caballero J, Flores J, Hernández-Losa J, Cortés J, Mares R, et al. Lkb1 loss promotes tumor progression of BRAF(V600E)-induced lung adenomas. *PLoS One.* (2013) 8:e66933. doi: 10.1371/journal.pone.0066933

79. Duyster J, Bai R, Morris S. Translocations involving anaplastic lymphoma kinase (ALK). *Oncogene.* (2001) 20:5623–37. doi: 10.1038/sj.onc.1204594

80. Morris S, Kirstein M, Valentine M, Dittmer K, Shapiro D, Saltman D, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science.* (1994) 263:1281–4. doi: 10.1126/science.8122112

81. Toyokawa G, Seto T. ALK inhibitors: what is the best way to treat patients with ALK+ non-small-cell lung cancer?. *Clin Lung Cancer.* (2014) 15:313–9. doi: 10.1016/j.cllc.2014.05.001

82. Shaw A, Kim D, Nakagawa K, Seto T, Crinó L, Ahn M, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med.* (2013) 368:2385–94. doi: 10.1056/NEJMoa1214886

83. Devarakonda S, Morgensztern D, Govindan R. Genomic alterations in lung adenocarcinoma. *Lancet Oncol.* (2015) 16:e342–51. doi: 10.1016/S1470-2045(15)00077-7

84. Shaw A, Yeap B, Mino-Kenudson M, Digumarthy S, Costa D, Heist R, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. *J Clin Oncol.* (2009) 27:4247–53. doi: 10.1200/JCO.2009.22.6993

85. Tsao A, Scagliotti G, Bunn P Jr., Carbone D, Warren G, Bai C, et al. Scientific advances in lung cancer 2015. *J Thorac Oncol.* (2016) 11:613–38. doi: 10.1016/j.jtho.2016.03.012

86. Normant E, Paez G, West K, Lim A, Slocum K, Tunkey C, et al. The Hsp90 inhibitor IPI-504 rapidly lowers EML4-ALK levels and induces tumor regression in ALK-driven NSCLC models. *Oncogene.* (2011) 30:2581–6. doi: 10.1038/onc.2010.625

87. Ota K, Azuma K, Kawahara A, Hattori S, Iwama E, Tanizaki J, et al. Induction of PD-L1 expression by the EML4-ALK oncoprotein and downstream signaling pathways in non-small cell lung cancer. *Clin Cancer Res.* (2015) 21:4014–21. doi: 10.1158/1078-0432.CCR-15-0016

88. Shen J, Meng Y, Wang K, Gao M, Du J, Wang J, et al. EML4-ALK G1202R mutation induces EMT and confers resistance to ceritinib in NSCLC cells via activation of STAT3/Slug signaling. *Cell Signal.* (2022) 92:110264. doi: 10.1016/j.cellsig.2022.110264

89. Davies K, Le A, Theodoro M, Skokan M, Aisner D, Berge E, et al. Identifying and targeting ROS1 gene fusions in non-small cell lung cancer. *Clin Cancer Res.* (2012) 18:4570–9. doi: 10.1158/1078-0432.CCR-12-0550

90. Alrifai D, Forster M, Janes S. Emerging resistance pathways in lung cancer: what has ROS-1 taught us? *Transl Lung Cancer Res.* (2018) 7(Suppl. 1):S9–12. doi: 10.21037/tlcr.2017.11.13

91. Bergethon K, Shaw A, Ou S, Katayama R, Lovly C, McDonald N, et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol.* (2012) 30:863–70. doi: 10.1200/JCO.2011.35.6345

92. Yoshida A, Tsuta K, Wakai S, Arai Y, Asamura H, Shibata T, et al. Immunohistochemical detection of ROS1 is useful for identifying ROS1 rearrangements in lung cancers. *Mod Pathol.* (2014) 27:711–20. doi: 10.1038/modpathol.2013.192

93. Rodríguez-Antolín C, Rosas-Alonso R, Cruz P, Higuera O, Sánchez-Cabrero D, Esteban-Rodríguez I, et al. Novel SLC12A2-ROS1 fusion in non-small cell lung cancer with a significant response to crizotinib: the importance of choosing the appropriate next-generation sequencing assay. *Oncologist.* (2021) 26:e908–12. doi: 10.1002/onco.13745

94. Gou W, Zhou X, Liu Z, Wang L, Shen J, Xu X, et al. CD74-ROS1 G2032R mutation transcriptionally up-regulates Twist1 in non-small cell lung cancer cells leading to increased migration, invasion, and resistance to crizotinib. *Cancer Lett.* (2018) 422:19–28. doi: 10.1016/j.canlet.2018.02.032

95. Cai L, Duan J, Qian L, Wang Z, Wang S, Li S, et al. ROS1 fusion mediates immunogenicity by upregulation of PD-L1 after the activation of ROS1-SHP2 signaling pathway in non-small cell lung cancer. *Front Immunol.* (2020) 11:527750. doi: 10.3389/fimmu.2020.527750

96. Liu Z, Zhao K, Wei S, Liu C, Zhou J, Gou Q, et al. ROS1-fusion protein induces PD-L1 expression via MEK-ERK activation in non-small cell lung cancer. *Oncoimmunology.* (2020) 9:1758003. doi: 10.1080/2162402X.2020.1758003

97. Blaquier J, Recondo G. Non-small-cell lung cancer: how to manage MET exon 14 skipping mutant disease. *Drugs Context.* (2022) 11:2022–2–2. doi: 10.7573/dic.2022-2-2

98. Terlecka P, Krawczyk P, Grenda A, Milanowski J. MET gene dysregulation as a promising therapeutic target in lung cancer-a review. *J Pers Med.* (2021) 11:1370. doi: 10.3390/jpm11121370

99. Tong J, Yeung S, Chan A, Chung L, Chau S, Lung R, et al. MET amplification and exon 14 splice site mutation define unique molecular subgroups of non-small cell lung carcinoma with poor prognosis. *Clin Cancer Res.* (2016) 22:3048–56. doi: 10.1158/1078-0432.CCR-15-2061

100. Frampton G, Ali S, Rosenzweig M, Chmielecki J, Lu X, Bauer T, et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. *Cancer Discov.* (2015) 5:850–9. doi: 10.1158/1538-7445.AM2015-1118

101. Rosell R, Chaib I, Santarpia M. Targeting MET amplification in EGFR-mutant non-small-cell lung cancer. *Lancet Respir Med.* (2020) 8:1068–70. doi: 10.1016/S2213-2600(20)30171-5

102. Song Y, Li G, Ju K, Ran W, Zhao H, Liu X, et al. Mesenchymal-epithelial transition exon 14 skipping mutation and amplification in 5,008 patients with lung cancer. *Front Oncol.* (2021) 11:755031. doi: 10.3389/fonc.2021.755031

103. Noonan S, Berry L, Lu X, Gao D, Barón A, Chesnut P, et al. Identifying the appropriate FISH criteria for defining MET copy number-driven lung adenocarcinoma through oncogene overlap analysis. *J Thorac Oncol.* (2016) 11:1293–304. doi: 10.1016/j.jtho.2016.04.033

104. Jackman D, Pao W, Riely G, Engelman J, Kris M, Jänne P, et al. Clinical definition of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. *J Clin Oncol.* (2010) 28:357–60. doi: 10.1200/JCO.2009.24.7049

105. Wu S, Shih J. Management of acquired resistance to EGFR TKI-targeted therapy in advanced non-small cell lung cancer. *Mol Cancer.* (2018) 17:38. doi: 10.1186/s12943-018-0777-1

106. Lin Y, Higashisaka K, Shintani T, Maki A, Hanamuro S, Haga Y, et al. Progesterone receptor membrane component 1 leads to erlotinib resistance, initiating crosstalk of Wnt/β-catenin and NF-κB pathways, in lung adenocarcinoma cells. *Sci Rep.* (2020) 10:4748. doi: 10.1038/s41598-020-61727-3

107. Frederick B, Helfrich B, Coldren C, Zheng D, Chan D, Bunn P Jr., et al. Epithelial to mesenchymal transition predicts gefitinib resistance in cell lines of head and neck squamous cell carcinoma and non-small cell lung carcinoma. *Mol Cancer Ther.* (2007) 6:1683–91. doi: 10.1158/1535-7163.MCT-07-0138

108. Zhong W, Zhou Q, Wu Y. The resistance mechanisms and treatment strategies for EGFR-mutant advanced non-small-cell lung cancer. *Oncotarget.* (2017) 8:71358–70. doi: 10.18632/oncotarget.20311

109. Yano S, Wang W, Li Q, Matsumoto K, Sakurama H, Nakamura T, et al. Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. *Cancer Res.* (2008) 68:9479–87. doi: 10.1158/0008-5472.CAN-08-1643

110. Della Corte C, Malapelle U, Vigliar E, Pepe F, Troncone G, Ciaramella V, et al. Efficacy of continuous EGFR-inhibition and role of Hedgehog in EGFR acquired resistance in human lung cancer cells with activating mutation of EGFR. *Oncotarget.* (2017) 8:23020–32. doi: 10.18632/oncotarget.15479

111. F Smit E, Dooms C, Raskin J, Nadal E, Tho L, Le X. INSIGHT 2: a phase II study of tepotinib plus osimertinib in MET-amplified NSCLC and first-line osimertinib resistance. *Future Oncol.* (2022) 18:1039–54. doi: 10.2217/fon-2021-1406

112. Adderley H, Blackhall F, Lindsay C. KRAS-mutant non-small cell lung cancer: converging small molecules and immune checkpoint inhibition. *EBioMedicine.* (2019) 41:711–6. doi: 10.1016/j.ebiom.2019.02.049

113. Adjei A, Mauer A, Bruzek L, Marks R, Hillman S, Geyer S, et al. Phase II study of the farnesyl transferase inhibitor R115777 in patients with advanced non-small-cell lung cancer. *J Clin Oncol.* (2003) 21:1760–6. doi: 10.1200/JCO.2003.09.075

114. Blumenschein G Jr., Smit E, Planchard D, Kim D, Cadranel J, De Pas T, et al. A randomized phase II study of the MEK1/MEK2 inhibitor trametinib (GSK1120212) compared with docetaxel in KRAS-mutant advanced non-small-cell lung cancer (NSCLC)†. *Ann Oncol.* (2015) 26:894–901. doi: 10.1093/annonc/mdv072

115. Crystal A, Shaw A, Sequist L, Friboulet L, Niederst M, Lockerman E, et al. Patient-derived models of acquired resistance can identify effective drug combinations for cancer. *Science.* (2014) 346:1480–6. doi: 10.1126/science.1254721

116. Shi R, Filho S, Li M, Fares A, Weiss J, Pham N, et al. BRAF V600E mutation and MET amplification as resistance pathways of the second-generation anaplastic lymphoma kinase (ALK) inhibitor alectinib in lung cancer. *Lung Cancer.* (2020) 146:78–85. doi: 10.1016/j.lungcan.2020.05.018

117. Katayama R, Shaw A, Khan T, Mino-Kenudson M, Solomon B, Halmos B, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung Cancers. *Sci Transl Med.* (2012) 4:120ra17. doi: 10.1126/scitranslmed.3003316

118. Park K, Tan E, O'Byrne K, Zhang L, Boyer M, Mok T, et al. Afatinib versus gefitinib as first-line treatment of patients with EGFR mutation-positive non-small-cell lung cancer (LUX-Lung 7): a phase 2B, open-label, randomised controlled trial. *Lancet Oncol.* (2016) 17:577–89. doi: 10.1016/S1470-2045(16)30033-X

119. Biswas A, Han S, Tai Y, Ma W, Coker C, Quinn S, et al. Targeting S100A9-ALDH1A1-retinoic acid signaling to suppress brain relapse in EGFR-mutant lung cancer. *Cancer Discov.* (2022) 12:1002–21. doi: 10.1158/2159-8290.CD-21-0910

120. Wu Y, Herbst R, Mann H, Rukazenkov Y, Marotti M, Tsuboi M. ADAURA: phase III, double-blind, randomized study of osimertinib versus placebo in EGFR mutation-positive early-stage NSCLC after complete surgical resection. *Clin Lung Cancer.* (2018) 19:e533–6. doi: 10.1016/j.cllc.2018.04.004

121. Galluzzi L, Vitale I, Warren S, Adjemian S, Agostinis P, Martinez A, et al. Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. *J Immunother Cancer.* (2020) 8:e000337. doi: 10.1136/jitc-2019-000337corr1

122. Ahmed A, Tait S. Targeting immunogenic cell death in cancer. *Mol Oncol.* (2020) 14:2994–3006. doi: 10.1002/1878-0261.12851

123. Ruan H, Leibowitz B, Zhang L, Yu J. Immunogenic cell death in colon cancer prevention and therapy. *Mol Carcinog.* (2020) 59:783–93. doi: 10.1002/mc.23183

124. Petrazzuolo A, Perez-Lanzon M, Liu P, Maiuri M, Kroemer G. Crizotinib and ceritinib trigger immunogenic cell death via on-target effects. *Oncoimmunology.* (2021) 10:1973197. doi: 10.1080/2162402X.2021.1973197

125. Scaffidi P, Misteli T, Bianchi M. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature.* (2002) 418:191–5. doi: 10.1038/nature00858

126. Fucikova J, Truxova I, Hensler M, Becht E, Kasikova L, Moserova I, et al. Calreticulin exposure by malignant blasts correlates with robust anticancer immunity and improved clinical outcome in AML patients. *Blood.* (2016) 128:3113–24. doi: 10.1182/blood-2016-08-731737

127. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, Ortiz C, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. *Nat Med.* (2009) 15:1170–8. doi: 10.1038/nm.2028

128. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med.* (2007) 13:1050–9. doi: 10.1038/nm1622

129. Wu X, Chen Y, Gong C, Pei D. The role of high-mobility group protein box 1 in lung cancer. *J Cell Biochem.* (2018) 119:6354–65. doi: 10.1002/jcb.26837

130. Wu L, Yang L. The function and mechanism of HMGB1 in lung cancer and its potential therapeutic implications. *Oncol Lett.* (2018) 15:6799–805.

131. Solari J, Filippi-Chiela E, Pilar E, Nunes V, Gonzalez E, Figueiro F, et al. Damage-associated molecular patterns (DAMPs) related to immunogenic cell death are differentially triggered by clinically relevant chemotherapeutics in lung adenocarcinoma cells. *BMC Cancer.* (2020) 20:474. doi: 10.1186/s12885-020-06964-5

132. Lagiedo M, Sikora J, Kaczmarek M. Damage-associated molecular patterns in the course of lung cancer–A review. *Scand J Immunol.* (2015) 82:95–101. doi: 10.1111/sji.12308

133. Anderson C, Macleod K. Autophagy and cancer cell metabolism. *Int Rev Cell Mol Biol.* (2019) 347:145–90. doi: 10.1016/bs.ircmb.2019.06.002

134. Follo C, Cheng Y, Richards W, Bueno R, Broaddus V. Autophagy facilitates the release of immunogenic signals following chemotherapy in 3D models of mesothelioma. *Mol Carcinog.* (2019) 58:1754–69. doi: 10.1002/mc.23050

135. Kronlage M, Song J, Sorokin L, Isfort K, Schwerdtle T, Leipziger J, et al. Autocrine purinergic receptor signaling is essential for macrophage chemotaxis. *Sci Signal.* (2010) 3:ra55. doi: 10.1126/scisignal.2000588

136. Kroemer G, Marino G, Levine B. Autophagy and the integrated stress response. *Mol Cell.* (2010) 40:280–93. doi: 10.1016/j.molcel.2010.09.023

137. Swanson K, Deng M, Ting J. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat Rev Immunol.* (2019) 19:477–89. doi: 10.1038/s41577-019-0165-0

138. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol.* (2013) 31:51–72. doi: 10.1146/annurev-immunol-032712-100008

139. Wang H, Lee H, Guo J, Chen S, Liao Z, Huang K, et al. Calreticulin promotes tumor lymphocyte infiltration and enhances the antitumor effects of immunotherapy by up-regulating the endothelial expression of adhesion molecules. *Int J Cancer.* (2012) 130:2892–902. doi: 10.1002/ijc.26339

140. Thul P, Lindskog C. The human protein atlas: a spatial map of the human proteome. *Protein Sci.* (2018) 27:233–44. doi: 10.1002/pro.3307

141. Inoue H, Tsutsumi H, Tanaka K, Iwama E, Shiraishi Y, Hirayama A, et al. Increased plasma levels of damage-associated molecular patterns during systemic anticancer therapy in patients with advanced lung cancer. *Transl Lung Cancer Res.* (2021) 10:2475–86. doi: 10.21037/tlcr-21-92

142. Rodriguez-Ruiz M, Buque A, Hensler M, Chen J, Bloy N, Petroni G, et al. Apoptotic caspases inhibit abscopal responses to radiation and identify a new prognostic biomarker for breast cancer patients. *Oncoimmunology.* (2019) 8:e1655964. doi: 10.1080/2162402X.2019.1655964

143. Khodarev N. Intracellular RNA sensing in mammalian cells: role in stress response and cancer therapies. *Int Rev Cell Mol Biol.* (2019) 344:31–89. doi: 10.1016/bs.ircmb.2018.08.005

144. Yamazaki T, Kirchmair A, Sato A, Buque A, Rybstein M, Petroni G, et al. Mitochondrial DNA drives abscopal responses to radiation that are inhibited by autophagy. *Nat Immunol.* (2020) 21:1160–71. doi: 10.1038/s41590-020-0751-0

145. Hopfner K, Hornung V. Molecular mechanisms and cellular functions of cGAS-STING signalling. *Nat Rev Mol Cell Biol.* (2020) 21:501–21. doi: 10.1038/s41580-020-0244-x

146. McLaughlin M, Patin E, Pedersen M, Wilkins A, Dillon M, Melcher A, et al. Inflammatory microenvironment remodelling by tumour cells after radiotherapy. *Nat Rev Cancer.* (2020) 20:203–17. doi: 10.1038/s41568-020-0246-1

147. Muller E, Christopoulos P, Halder S, Lunde A, Beraki K, Speth M, et al. Toll-like receptor ligands and interferon-gamma synergize for induction of antitumor M1 macrophages. *Front Immunol.* (2017) 8:1383. doi: 10.3389/fimmu.2017.01383

148. Gangaplara A, Martens C, Dahlstrom E, Metidji A, Gokhale A, Glass D, et al. Type I interferon signaling attenuates regulatory T cell function in viral infection and in the tumor microenvironment. *PLoS Pathog.* (2018) 14:e1006985. doi: 10.1371/journal.ppat.1006985

149. Sistigu A, Yamazaki T, Vacchelli E, Chaba K, Enot D, Adam J, et al. Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. *Nat Med.* (2014) 20:1301–9. doi: 10.1038/nm.3708

150. Yang H, Lundback P, Ottosson L, Erlandsson-Harris H, Venereau E, Bianchi M, et al. Redox modification of cysteine residues regulates the cytokine activity of high mobility group box-1 (HMGB1). *Mol Med.* (2012) 18:250–9. doi: 10.2119/molmed.2011.00389

151. Rufo N, Garg A, Agostinis P. The unfolded protein response in immunogenic cell death and cancer immunotherapy. *Trends Cancer.* (2017) 3:643–58. doi: 10.1016/j.trecan.2017.07.002

152. Tabas I, Ron D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nat Cell Biol.* (2011) 13:184–90. doi: 10.1038/ncb0311-184

153. So J. Roles of endoplasmic reticulum stress in immune responses. *Mol Cells.* (2018) 41:705–16.

154. Cubillos-Ruiz J, Mohamed E, Rodriguez P. Unfolding anti-tumor immunity: ER stress responses sculpt tolerogenic myeloid cells in cancer. *J Immunother Cancer.* (2017) 5:5. doi: 10.1186/s40425-016-0203-4

155. Garg A, Krysko D, Verfaillie T, Kaczmarek A, Ferreira G, Marysael T, et al. A novel pathway combining calreticulin exposure and ATP secretion in immunogenic cancer cell death. *EMBO J.* (2012) 31:1062–79. doi: 10.1038/emboj.2011.497

156. Fucikova J, Becht E, Iribarren K, Goc J, Remark R, Damotte D, et al. Calreticulin expression in human non-small cell lung cancers correlates with increased accumulation of antitumor immune cells and favorable prognosis. *Cancer Res.* (2016) 76:1746–56. doi: 10.1158/0008-5472.CAN-15-1142

157. Bravo R, Gutierrez T, Paredes F, Gatica D, Rodriguez A, Pedrozo Z, et al. Endoplasmic reticulum: ER stress regulates mitochondrial bioenergetics. *Int J Biochem Cell Biol.* (2012) 44:16–20. doi: 10.1016/j.biocel.2011.10.012

158. Deutsch E, Chargari C, Galluzzi L, Kroemer G. Optimising efficacy and reducing toxicity of anticancer radioimmunotherapy. *Lancet Oncol.* (2019) 20:e452–63. doi: 10.1016/S1470-2045(19)30171-8

159. Galluzzi L, Humeau J, Buque A, Zitvogel L, Kroemer G. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. *Nat Rev Clin Oncol.* (2020) 17:725–41. doi: 10.1038/s41571-020-0413-z

160. Garrido G, Rabasa A, Sanchez B, Lopez M, Blanco R, Lopez A, et al. Induction of immunogenic apoptosis by blockade of epidermal growth factor receptor activation with a specific antibody. *J Immunol.* (2011) 187:4954–66. doi: 10.4049/jimmunol.1003477

161. Drewry D, Wells C, Andrews D, Angell R, Al-Ali H, Axtman A, et al. Progress towards a public chemogenomic set for protein kinases and a call for contributions. *PLoS One.* (2017) 12:e0181585. doi: 10.1371/journal.pone.0181585

162. Liu P, Zhao L, Pol J, Levesque S, Petrazzuolo A, Pfirschke C, et al. Crizotinib-induced immunogenic cell death in non-small cell lung cancer. *Nature Commun.* (2019) 10:1486. doi: 10.1038/s41467-019-09838-y

163. Muller J, Krijgsman O, Tsoi J, Robert L, Hugo W, Song C, et al. Low MITF/AXL ratio predicts early resistance to multiple targeted drugs in melanoma. *Nat Commun.* (2014) 5:5712. doi: 10.1038/ncomms6712

164. Lotsberg M, Wnuk-Lipinska K, Terry S, Tan T, Lu N, Trachsel-Moncho L, et al. AXL targeting abrogates autophagic flux and induces immunogenic cell death in drug-resistant cancer cells. *J Thorac Oncol.* (2020) 15:973–99. doi: 10.1016/j.jtho.2020.01.015

165. Boshuizen J, Pencheva N, Krijgsman O, Altimari D, Castro P, de Bruijn B, et al. Cooperative targeting of immunotherapy-resistant melanoma and lung cancer by an AXL-targeting antibody-drug conjugate and immune checkpoint blockade. *Cancer Res.* (2021) 81:1775–87. doi: 10.1158/0008-5472.CAN-20-0434

166. Gheghiani L, Wang L, Zhang Y, Moore X, Zhang J, Smith S, et al. PLK1 induces chromosomal instability and overrides cell-cycle checkpoints to drive tumorigenesis. *Cancer Res.* (2021) 81:1293–307. doi: 10.1158/0008-5472.CAN-20-1377

167. Zhou J, Yang Q, Lu L, Tuo Z, Shou Z, Cheng J. PLK1 Inhibition induces immunogenic cell death and enhances immunity against NSCLC. *Int J Med Sci.* (2021) 18:3516–25. doi: 10.7150/ijms.60135

168. Saei A, Gullberg H, Sabatier P, Beusch C, Johansson K, Lundgren B, et al. Comprehensive chemical proteomics for target deconvolution of the redox active drug auranofin. *Redox Biol.* (2020) 32:101491. doi: 10.1016/j.redox.2020.101491

169. Freire Boullosa L, Van Loenhout J, Flieswasser T, De Waele J, Hermans C, Lambrechts H, et al. Auranofin reveals therapeutic anticancer potential by triggering distinct molecular cell death mechanisms and innate immunity in mutant p53 non-small cell lung cancer. *Redox Biol.* (2021) 42:101949. doi: 10.1016/j.redox.2021.101949

170. Nam G, Kwon M, Jung H, Ko E, Kim S, Choi Y, et al. Statin-mediated inhibition of RAS prenylation activates ER stress to enhance the immunogenicity of KRAS mutant cancer. *J Immunother Cancer.* (2021) 9:e002474. doi: 10.1136/jitc-2021-002474

171. Garg A, Krysko D, Vandenabeele P, Agostinis P. Hypericin-based photodynamic therapy induces surface exposure of damage-associated molecular patterns like HSP70 and calreticulin. *Cancer Immunol Immunother.* (2012) 61:215–21. doi: 10.1007/s00262-011-1184-2

172. Kepp O, Zitvogel L, Kroemer G. Lurbinectedin: an FDA-approved inducer of immunogenic cell death for the treatment of small-cell lung cancer. *Oncoimmunology.* (2020) 9:1795995. doi: 10.1080/2162402X.2020.1795995

173. Bezu L, Gomes-de-Silva L, Dewitte H, Breckpot K, Fucikova J, Spisek R, et al. Combinatorial strategies for the induction of immunogenic cell death. *Front Immunol.* (2015) 6:187. doi: 10.3389/fimmu.2015.00187

174. Rodriguez-Ruiz M, Vitale I, Harrington K, Melero I, Galluzzi L. Immunological impact of cell death signaling driven by radiation on the tumor microenvironment. *Nat Immunol.* (2020) 21:120–34. doi: 10.1038/s41590-019-0561-4

175. Krombach J, Hennel R, Brix N, Orth M, Schoetz U, Ernst A, et al. Priming anti-tumor immunity by radiotherapy: dying tumor cell-derived DAMPs trigger endothelial cell activation and recruitment of myeloid cells. *Oncoimmunology.* (2019) 8:e1523097. doi: 10.1080/2162402X.2018.1523097

176. Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, et al. Immunogenic chemotherapy sensitizes tumors to checkpoint blockade therapy. *Immunity.* (2016) 44:343–54. doi: 10.1016/j.immuni.2015.11.024

177. Mathew M, Enzler T, Shu C, Rizvi N. Combining chemotherapy with PD-1 blockade in NSCLC. *Pharmacol Ther.* (2018) 186:130–7. doi: 10.1016/j.pharmthera.2018.01.003

178. Sumiyoshi I, Okabe T, Togo S, Takagi H, Motomura H, Ochi Y, et al. High lymphocyte population-related predictive factors for a long-term response in non-small cell lung cancer patients treated with pemetrexed: a retrospective observational study. *J Transl Med.* (2021) 19:92. doi: 10.1186/s12967-021-02761-1

179. Wang D, Cong J, Fu B, Zheng X, Sun R, Tian Z, et al. Immunogenic chemotherapy effectively inhibits KRAS-Driven lung cancer. *Cancer Lett.* (2020) 492:31–43. doi: 10.1016/j.canlet.2020.07.043

180. Gao Q, Wang S, Chen X, Cheng S, Zhang Z, Li F, et al. Cancer-cell-secreted CXCL11 promoted CD8(+) T cells infiltration through docetaxel-induced-release of HMGB1 in NSCLC. *J Immunother Cancer.* (2019) 7:42. doi: 10.1186/s40425-019-0511-6

181. Furukawa R, Inoue H, Yoneshima Y, Tsutsumi H, Iwama E, Ikematsu Y, et al. Cytotoxic chemotherapeutic agents and the EGFR-TKI osimertinib induce calreticulin exposure in non-small cell lung cancer. *Lung Cancer.* (2021) 155:144–50. doi: 10.1016/j.lungcan.2021.03.018

182. Wang Y, Shen N, Wang Y, Li M, Zhang W, Fan L, et al. Cisplatin nanoparticles boost abscopal effect of radiation plus anti-PD1 therapy. *Biomater Sci.* (2021) 9:3019–27. doi: 10.1039/D1BM00112D

183. Ma S, Song W, Xu Y, Si X, Lv S, Zhang Y, et al. Rationally designed polymer conjugate for tumor-specific amplification of oxidative stress and boosting

antitumor immunity. *Nano Lett.* (2020) 20:2514–21. doi: 10.1021/acs.nanolett.9b05265

184. Strasser A, Cory S, Adams J. Deciphering the rules of programmed cell death to improve therapy of cancer and other diseases. *EMBO J.* (2011) 30:3667–83. doi: 10.1038/emboj.2011.307

185. Akamatsu H, Teraoka S, Hayashi H, Fujimoto D, Hayata A, Haratani K, et al. Pembrolizumab plus amrubicin in patients with relapsed SCLC: multi-institutional, single-arm phase 2 study. *JTO Clin Res Rep.* (2021) 2:100184. doi: 10.1016/j.jtocrr.2021.100184

186. Altorki N, McGraw T, Borczuk A, Saxena A, Port J, Stiles B, et al. Neoadjuvant durvalumab with or without stereotactic body radiotherapy in patients with early-stage non-small-cell lung cancer: a single-centre, randomised phase 2 trial. *Lancet Oncol.* (2021) 22:824–35. doi: 10.1016/S1470-2045(21)00149-2

187. Sprooten J, De Wijngaert P, Vanmeerbeerk I, Martin S, Vangheluwe P, Schlenner S, et al. Necroptosis in immuno-oncology and cancer immunotherapy. *Cells.* (2020) 9:1823. doi: 10.3390/cells9081823

188. Chen C, Kono H, Golenbock D, Reed G, Akira S, Rock K. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat Med.* (2007) 13:851–6. doi: 10.1038/nm1603

189. Qi J, Jin F, Xu X, Du Y. Combination cancer immunotherapy of nanoparticle-based immunogenic cell death inducers and immune checkpoint inhibitors. *Int J Nanomed.* (2021) 16:1435–56. doi: 10.2147/IJN.S285999

190. Josefsen L, Boyle R. Unique diagnostic and therapeutic roles of porphyrins and phthalocyanines in photodynamic therapy, imaging and theranostics. *Theranostics.* (2012) 2:916–66. doi: 10.7150/thno.4571

191. Pinto A, Mace Y, Drouet F, Bony E, Boidot R, Draoui N, et al. A new ER-specific photosensitizer unravels (1)O2-driven protein oxidation and inhibition of deubiquitinases as a generic mechanism for cancer PDT. *Oncogene.* (2016) 35:3976–85. doi: 10.1038/onc.2015.474

192. Doix B, Trempolec N, Riant O, Feron O. Low photosensitizer dose and early radiotherapy enhance antitumor immune response of photodynamic therapy-based dendritic cell vaccination. *Front Oncol.* (2019) 9:811. doi: 10.3389/fonc.2019.00811

193. Gomes-da-Silva L, Zhao L, Bezu L, Zhou H, Sauvat A, Liu P, et al. Photodynamic therapy with redaporfin targets the endoplasmic reticulum and Golgi apparatus. *EMBO J.* (2018) 37:e98354. doi: 10.15252/embj.201798354

194. Tatsuno K, Yamazaki T, Hanlon D, Han P, Robinson E, Sobolev O, et al. Extracorporeal photochemotherapy induces bona fide immunogenic cell death. *Cell Death Dis.* (2019) 10:578. doi: 10.1038/s41419-019-1819-3

195. Ventura A, Vassall A, Robinson E, Filler R, Hanlon D, Meeth K, et al. Extracorporeal photochemotherapy drives monocyte-to-dendritic cell maturation to induce anticancer immunity. *Cancer Res.* (2018) 78:4045–58. doi: 10.1158/0008-5472.CAN-18-0171

196. Korbelik M, Zhang W, Merchant S. Involvement of damage-associated molecular patterns in tumor response to photodynamic therapy: surface expression of calreticulin and high-mobility group box-1 release. *Cancer Immunol Immunother.* (2011) 60:1431–7. doi: 10.1007/s00262-011-1047-x

197. Kurtulus S, Madi A, Escobar G, Klapholz M, Nyman J, Christian E, et al. Checkpoint blockade immunotherapy induces dynamic changes in PD-1(-)CD8(+) tumor-infiltrating T cells. *Immunity.* (2019) 50:181–94.e6. doi: 10.1016/j.immuni.2018.11.014

198. Xiao Y, Freeman G. The microsatellite instable subset of colorectal cancer is a particularly good candidate for checkpoint blockade immunotherapy. *Cancer Discov.* (2015) 5:16–8. doi: 10.1158/2159-8290.CD-14-1397

199. Ferrara R, Mezquita L, Besse B. Progress in the management of advanced thoracic malignancies in 2017. *J Thorac Oncol.* (2018) 13:301–22. doi: 10.1016/j.jtho.2018.01.002

200. Doroshow D, Herbst R. Treatment of advanced non-small cell lung cancer in 2018. *JAMA Oncol.* (2018) 4:569–70. doi: 10.1001/jamaoncol.2017.5190

201. Chabanon R, Muirhead G, Krastev D, Adam J, Morel D, Garrido M, et al. PARP inhibition enhances tumor cell-intrinsic immunity in ERCC1-deficient non-small cell lung cancer. *J Clin Invest.* (2019) 129:1211–28. doi: 10.1172/JCI123319

202. Belani C, Wu Y, Chen Y, Kim J, Yang S, Zhang L, et al. Efficacy and safety of pemetrexed maintenance therapy versus best supportive care in patients from East Asia with advanced, nonsquamous non-small cell lung cancer: an exploratory subgroup analysis of a global, randomized, phase 3 clinical trial. *J Thorac Oncol.* (2012) 7:567–73. doi: 10.1097/JTO.0b013e31823d4f9d

203. Gridelli C, Casaluce F. Frontline immunotherapy for NSCLC: alone or not alone?. *Nat Rev Clin Oncol.* (2018) 15:593–4. doi: 10.1038/s41571-018-0070-7

204. Gandhi L, Rodriguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med.* (2018) 378:2078–92. doi: 10.1056/NEJMoa1801005

205. Socinski M, Jotte R, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *N Engl J Med.* (2018) 378:2288–301. doi: 10.1056/NEJMoa1716948

206. West H, McCleod M, Hussein M, Morabito A, Rittmeyer A, Conter H, et al. Atezolizumab in combination with carboplatin plus nab-paclitaxel chemotherapy compared with chemotherapy alone as first-line treatment for metastatic non-squamous non-small-cell lung cancer (IMpower130): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol.* (2019) 20:924–37. doi: 10.1016/S1470-2045(19)30167-6

207. Martinez P, Peters S, Stammers T, Soria J. Immunotherapy for the first-line treatment of patients with metastatic non-small cell lung cancer. *Clin Cancer Res.* (2019) 25:2691–8. doi: 10.1158/1078-0432.CCR-18-3904

208. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. *Cancer Cell.* (2015) 28:690–714. doi: 10.1016/j.ccell.2015.10.012

209. Santamaria Nunez G, Robles C, Giraudon C, Martinez-Leal J, Compe E, Coin F, et al. Lurbinectedin specifically triggers the degradation of phosphorylated RNA polymerase II and the formation of DNA breaks in cancer cells. *Mol Cancer Ther.* (2016) 15:2399–412. doi: 10.1158/1535-7163.MCT-16-0172

210. Nagano T, Tachihara M, Nishimura Y. Molecular mechanisms and targeted therapies including immunotherapy for non-small cell lung cancer. *Curr Cancer Drug Targets.* (2019) 19:595–630. doi: 10.2174/1568009619666181210114559

211. Lee J, Talhi O, Jang D, Cerella C, Gaigneaux A, Kim K, et al. Cytostatic hydroxycoumarin OT52 induces ER/Golgi stress and STAT3 inhibition triggering non-canonical cell death and synergy with BH3 mimetics in lung cancer. *Cancer Lett.* (2018) 416:94–108. doi: 10.1016/j.canlet.2017.12.007

frontiers | Frontiers in Medicine

TYPE Systematic Review
PUBLISHED 06 January 2023
DOI 10.3389/fmed.2022.1052540

OPEN ACCESS

EDITED BY
Bruno Guedes Baldi,
University of São Paulo, Brazil

REVIEWED BY
Jorge Boczkowski,
Université Paris-Est Créteil Val de Marne, France
Chunbin Zou,
University of Pittsburgh, United States

*CORRESPONDENCE
Wantao Wang
✉ wangwt0906@shutcm.edu.cn
Suhua Song
✉ sdjnssh@163.com

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 24 September 2022
ACCEPTED 08 December 2022
PUBLISHED 06 January 2023

CITATION
Meng D, Zhu C, Jia R, Li Z, Wang W and Song S (2023) The molecular mechanism of ferroptosis and its role in COPD. *Front. Med.* 9:1052540. doi: 10.3389/fmed.2022.1052540

The molecular mechanism of ferroptosis and its role in COPD

Dandan Meng[1], Chengfeng Zhu[1], Ruixue Jia[1], Zongxin Li[2], Wantao Wang[3]* and Suhua Song[1]*

[1]Department of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China, [2]Department of Second Department of Haematology, Jinan Haematology Hospital, Jinan, China, [3]Department of Basic Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China

Ferroptosis, a new type of cell death, is mainly characterized by intracellular iron accumulation and lipid peroxidation. The complex regulatory network of iron metabolism, lipid metabolism, amino acid metabolism, p53-related signaling, and Nrf2-related signaling factors is involved in the entire process of ferroptosis. It has been reported that ferroptosis is involved in the pathogenesis of neurological diseases, cancer, and ischemia–reperfusion injury. Recent studies found that ferroptosis is closely related to the pathogenesis of COPD, which, to some extent, indicates that ferroptosis is a potential therapeutic target for COPD. This article mainly discusses the related mechanisms of ferroptosis, including metabolic regulation and signaling pathway regulation, with special attention to its role in the pathogenesis of COPD, aiming to provide safe and effective therapeutic targets for chronic airway inflammatory diseases.

KEYWORDS

ferroptosis, COPD, inflammation, iron, lipid peroxidation

In 2012, Dixon (1) first proposed a new type of iron-dependent programmed cell death named ferroptosis. As a new type of cell death, ferroptosis is mainly characterized by intracellular iron accumulation and lipid peroxidation. Ferroptosis differs from other forms of cell death such as apoptosis and necrosis in terms of morphology, biochemical characteristics, and genetics (2, 3). The morphological aspects of ferroptosis are mainly characterized by mitochondrial contraction and increased density of mitochondrial membranes with a decrease or disappearance of mitochondrial cristae and disintegration of the outer membrane (1). The biochemical features of ferroptosis are as follows: accumulation of ROS and iron ions, decreased cysteine uptake and GSH synthesis, activation of the mitogen-activated protein kinase system, and release of arachidonic acid (4). Iron metabolism, lipid metabolism, amino acid metabolism, p53-related signaling factors, and Nrf2-related signaling factors are involved in the entire process of regulating ferroptosis (5). Ferroptosis, a new programmed cell death mode, has been confirmed to be closely related to tumors, central nervous system diseases, arteriosclerosis, acute kidney injury, diabetes, and ischemia–reperfusion injury (6). Recent studies found that ferroptosis is also associated with the onset of chronic obstructive pulmonary disease (COPD) and has the potential to become a new therapeutic target. COPD is a common condition that can be prevented and treated, and it is characterized by persistent respiratory symptoms and airflow restriction; in addition, COPD is caused by airway and alveolar abnormalities due to heavy exposure to harmful particles or gases,

and it is affected by host factors such as abnormal pulmonary development (7). COPD has developed into a major chronic respiratory disease that seriously threatens human health. Related studies showed that the disruption of iron homeostasis is related to the pathogenesis of COPD (8). With further study, the role of ferroptosis in the pathogenesis of COPD has gradually been revealed, and appropriate intervention in ferroptosis can delay the COPD process.

1. Mechanism of ferroptosis

The mechanism of ferroptosis is shown in Figure 1.

1.1. Abnormal iron metabolism

Iron is an element necessary for lipid peroxide accumulation and ferroptosis. Excessive iron load promotes the production of ROS through the Fenton reaction and iron-binding proteins, thereby promoting the occurrence of ferroptosis (4, 9, 10). Iron intake, transport, and storage affect ferroptosis (5). Under physiological conditions, intracellular iron absorption and metabolism should always be in a dynamic and stable state. Iron uptake and export proteins play important roles in the process of iron metabolism. On the one hand, transferrin receptor 1 (TFR1) and divalent metal transporter-1 (DMT1) take up extracellular iron in cells. On the other hand, ferroportin (FPN) transfers excess intracellular iron to the outside of the cell, a process that maintains the "on" and "off" state of intracellular iron homeostasis (4, 11). Iron in the daily diet is usually absorbed by intestinal epithelial cells in the form of Fe3+ and then enters cells through the transferrin receptor (TFR) on the cell membrane after binding to transferrin. Afterward, Fe3+ in cells is reduced to Fe2+ by six-transmembrane epithelial antigens of prostate 3 (STEAP3). Then, Fe2+ is released into the cytoplasmic iron pool by divalent metal transporter 1 (DMT1) or zinc–iron regulatory protein family 8/14 (ZIP8/14) to meet its own metabolic needs. Abnormal expression or dysfunction of related proteins increases the concentration of intracellular iron ions and leads to excess iron. Excess intracellular iron can generate lipid reactive oxygen species (ROS) through the

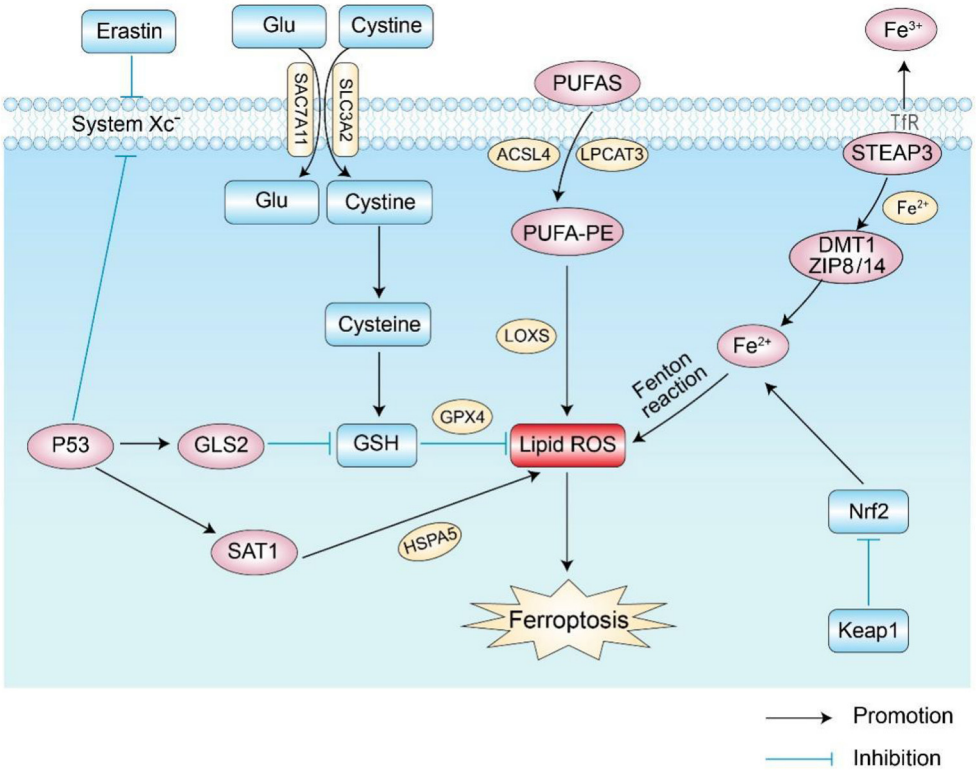

FIGURE 1
Main regulatory pathways of ferroptosis. There are two main ways of regulating ferroptosis shown in the figure: the first is the pathway of abnormal iron, amino acid, and lipid metabolism; the second involves the related signaling pathways that regulate ferroptosis, such as the P53 and Nrf2 pathways. Glu, glutamic acid; GSH, glutathione; GPX4, glutathione peroxidase 4; Nrf2, Nuclear factor erythroid 2-related factor 2; GLS2, glutaminase 2; LOXs, lipoxygenases; ROS, reactive oxygen species; DMT1, divalent metal ion transporter-1; SAT1, spermidine N1-acetyltransferase 1; TfR, transferrin receptor.

Fenton and Haber–Weiss reactions, which further accumulate and initiate lipid peroxidation (LPO) to induce ferroptosis (12).

1.2. Abnormal lipid metabolism

Lipid metabolism is critical for regulating cellular susceptibility to ferroptosis. Because plasma membrane damage caused by iron-dependent excess accumulation is an important feature during ferroptosis (13), reducing oxidative damage from lipid peroxidation is a fundamental process to inhibit ferroptosis (14–16). ROS interacts with polyunsaturated fatty acids (PUFAs) on lipid membranes to form lipid ROS, and excessive ROS accumulation in cells induces ferroptosis (17). When PUFAs exist in large amounts, they lead to more lipid peroxides and aggravate the degree of cell ferroptosis (18). Studies found that lipoxygenases (LOXs), non-heme iron-containing proteins, are involved in the formation of iron-dependent lipid ROS. When cells contain a large amount of iron ions, they catalyze PUFAs to form lipid hydroperoxides and toxic lipid-free radicals, which cause cell damage and promote ferroptosis (19). It was later found that antioxidants significantly inhibit LOXs and further inhibit the formation of lipid hydroperoxides to prevent cell ferroptosis (19). Acyl-CoA synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) are involved in helping PUFAs in cell membrane synthesis and esterification to generate PUFA-PEs, which accelerate the process of ferroptosis (20, 21). Therefore, PUFA-related biosynthetic enzymes may be potential targets for regulating ferroptosis.

1.3. Abnormal amino acid metabolism

Ferroptosis caused by abnormal amino acid metabolism is mainly related to the abnormal metabolism of GSH. GSH is a key substance in amino acid metabolism in ferroptosis and is mainly synthesized from cysteine, glutamate, and glycine (5). Extracellular cysteine and intracellular cysteine are essential for GSH biosynthesis. Cystine generates GSH through a series of enzymatic actions, and GSH is the basic substrate for the degradation of phospholipid hydrogen peroxide (PLOOH) by glutathione peroxidase 4 (GPX4). Decreased GPX4 activity leads to the accumulation of intracellular lipid peroxides, thereby inducing ferroptosis (22, 23).

Cystine/glutamate anti-transport system Xc- (System Xc-) on the cell membrane transports extracellular cystine and intracellular glutamate in a 1:1 ratio. Cells mainly acquire cystine from the extracellular space through System Xc-, where cystine is reduced to cysteine to participate in the synthesis of GSH, and erastin acts on System Xc- to inhibit the uptake of cystine by the cell membrane, thereby reducing the synthesis of GSH and further contributing to the accumulation of ROS (24). Because glutamate is a regulator of ferroptosis and is exchanged with cystine in a 1:1 ratio by System Xc-, the concentration level of glutamate affects the function of System Xc-. A previous study reported that a high concentration of extracellular glutamate further prevents the uptake of cystine by inhibiting the biological activity of System Xc-, thereby inducing ferroptosis (11, 25). Therefore, abnormal amino acid metabolism is another important mechanism in cell ferroptosis.

1.4. Pathways related to ferroptosis

The process of ferroptosis is affected by different signaling pathways. As a tumor suppressor gene for cell cycle inhibition, apoptosis, and senescence, p53 is also one of the main signaling pathways of ferroptosis in cells (26, 27). Related studies showed that p53 is involved in the ferroptosis process and is a key regulator of both the canonical and non-standard ferroptosis pathways (28–30). SLC7A11 is an important part of System Xc-, and p53 acts as a transcriptional repressor of SLC7A11, participates in the process of ferroptosis, and inhibits the acquisition of cysteine by downregulating the expression of SLC7A11 and reducing GPX activity and GSH synthesis ability; this allows ROS accumulation and induces ferroptosis (28). p53 also sensitizes ferroptosis by enhancing the expression of glutaminase 2 (GLS2) and spermidine/spermine N1-acetyl-transferase 1 (SAT1) (31, 32). In addition, p53 inhibits ferroptosis by directly inhibiting the activity of dipeptidyl peptidase 4 (DPP4) or by promoting the expression of cyclin-dependent kinase inhibitor 1A (CDKN1A/p21) (33, 34).

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key regulator of cellular antioxidant activity, and its targets play important roles in iron and lipid metabolism (35, 36). Studies found that Nrf2 further inhibits ferroptosis by increasing the expression of target genes related to iron and ROS metabolism (37). Under physiological conditions, Nrf2 expression is low, and its activity is tightly regulated by Keap1 (38). When oxidative stress occurs, Nrf2 dissociates from the Keap1 cytoplasmic inhibitor and activates the Nrf2 transcriptional gene and thus plays an antioxidant role in protecting cells from oxidative stress. Activation of Nrf2 greatly reduces iron absorption and inhibits the production of reactive oxygen species, thereby enhancing cellular antioxidant capacity (15, 39–41). Also, Nrf2 stimulates the expression of GPX4, thereby inhibiting the occurrence of ferroptosis under certain circumstances (42, 43). Thus, the regulation of the Nrf2 pathway inhibits ferroptosis.

2. Pathogenesis of COPD

2.1. Pathological mechanism of COPD

The pathogenesis of COPD is based on the response of the body to inhalation of harmful particles and gases, and

it is complex and has not been fully elucidated. Smoking is the main cause of COPD, and the pathogenesis of COPD is mostly related to inflammatory mediators, inherent immunity, oxidative stress, and protease/antiprotease imbalance (7, 44). Among many theories regarding the pathogenesis of COPD, ferroptosis is likely to be a major internal manifestation (8), and environmental interaction that induces respiratory inflammation is the main factor leading to the pathogenesis (45). Chronic airway inflammation and defects in epithelial repair remain core problems in COPD (46).

Cigarette smoke (CS) and harmful particles induce the body to produce highly reactive molecules such as ROS and reactive nitrogen species (RNS), thus inducing oxidative stress. The accumulation of neutrophils and macrophages in airways and pulmonary blood vessels causes the release of a large number of inflammatory mediators, which induces an inflammatory response and leads to lung tissue damage and protease/antiprotease imbalance, ultimately accelerating the progression of COPD (47). Oxidative stress causes the release of IL-1β and tumor necrosis factor-α (TNF-α) by regulating redox transcription factors such as nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1), thereby enhancing the inflammatory response of lungs. It is worth noting that the increase in inflammatory cells and pro-inflammatory cytokines not only maintains the chronic inflammatory response in this population but also causes systemic damage (48).

2.2. Iron-dependent oxidative stress

Oxidative stress is an important pathogenic factor in COPD, and the presence of a large amount of ROS in the inflammatory response inactivates antiproteases and leads to lung tissue damage. Excessive secretion and accumulation of neutrophils lead to the production of a large amount of reactive oxygen species (ROS). The accumulation of ROS reduces the activity of histone deacetylases (HDACs) and increases the activity of histone acetyltransferases, leading to further accumulation of neutrophils, which exacerbates oxidative stress (49).

Iron homeostasis may be disrupted in inflammatory diseases, resulting in the production of excess reactive oxygen species with deleterious effects on cells and tissues. Epithelial cells and macrophages in lung tissues produce iron metabolism-related proteins, which regulate iron homeostasis and prevent the occurrence of oxidative stress (50). Disruption of pulmonary iron homeostasis is closely related to the development of COPD (51), and oxidative stress occurs due to excess iron in the lungs caused by endogenous or exogenous factors. Studies in rats showed high levels of pulmonary oxidative stress following intravenous injection of iron-containing compounds (iron dextran and iron carboxymaltose), which are mainly manifested by increased levels of nitrotyrosine and protein carbonyl modifications (52). Lung administration of Fe2O3 nanoparticles by inhalation induces ROS generation in rat lungs (53). Chio et al. found that cigarette smoke (CS) has an important effect on iron homeostasis in the lungs. Exposure of mouse and human bronchial epithelial cells to CS increases the concentrations of iron, Ft, serum ferritin, and non-heme iron in lung cells (54).

2.3. Lipid peroxidation

Lipid peroxidation is the loss of hydrogen atoms of intracellular lipids under the action of peroxidase or free radicals, resulting in oxidation, fragmentation, and shortening of carbon chains as well as lipid-free radicals, malondialdehyde (MDA), and 4-hydroxy-2-nonenal (4-HNE) peroxidation products, which eventually oxidatively degrade lipids and damage the lipid bilayer structure of cell membranes. (55) Currently, MDA, HNE, F2-isoprostanes (F2-isoP), and 8-iso-prostaglandin F2α (8-iso-PGF2α) are the main biomarkers for evaluating lipid peroxides.

Smoking increases the content of lipid peroxidation in the body, and lipid peroxidation is closely related to the pathological progression of COPD (56). A survey of community residents in Germany showed that the level of cotinine in plasma and the amount of smoking in community residents are proportional to the level of 8-iso-PGF2α in urine, and the survey also reported that the level of 8-iso-PGF2α in smokers is significantly higher than that in non-smokers. The study further showed that the smoking index constructed based on 71 smoking-related methylation sites has a positive dose–response relationship with the log value of 8-iso-PGF2α (57). In addition, air pollution is also a major risk factor for COPD, and air pollutants lead to elevated levels of lipid peroxides in the body. In a cohort study of 97 elderly people in the United States, Zhang et al. found that the levels of carbon monoxide, nitrogen oxides, and other related pollutants and ultrafine particulate matter (PM0.18) are closely related to the elevation of the MDA oxidative stress marker, and they also reported that the effect of the component with a smaller particle size is stronger. (58) A survey of adults commuting on the highway for 3 h during the morning rush hour in Atlanta reported that exhaled nitric oxide, C-reactive protein, and MDA levels of patients with asthma and without asthma are significantly higher than baseline levels, indicating that the inhalation of harmful gases causes pulmonary inflammation and oxidative stress (59).

3. Role of ferroptosis in COPD

The role of ferroptosis in COPD is shown in Figure 2.

Ferroptosis is involved in the pathogenesis of COPD in a COPD mouse model. Murine lung epithelial cells exposed to CS exhibit unstable iron accumulation and increased lipid

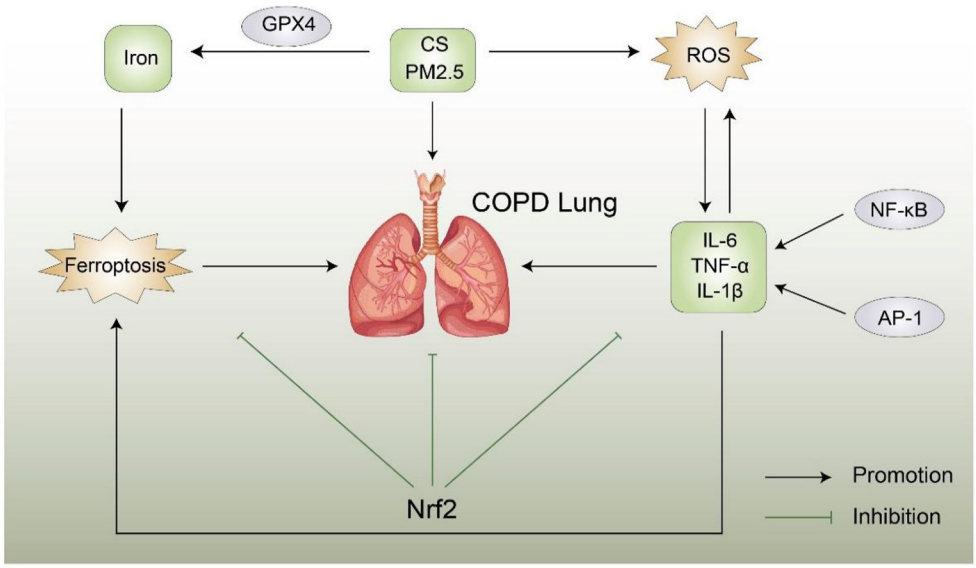

FIGURE 2
Possible relationship between ferroptosis and COPD, ROS, CS, and PM2.5 trigger COPD, resulting in an inflammatory reaction and abnormal levels of inflammatory factors. Conversely, abnormal inflammatory factors can aggravate COPD, Nrf2 can inhibit the production of reactive oxygen species and iron and the occurrence of COPD and ferroptosis. A direct link between ferroptosis and COPD remains unclear. Nrf2, Nuclear factor erythroid 2-related factor 2; CS, cigarette smoke; ROS, reactive oxygen species; AP-1, activator protein 1; NF-κB, nuclear factor of kappa B.

peroxidation accompanied by a non-apoptotic mode of cell death negatively regulated by GPX4. In addition, the mouse model further confirmed that the treatment of lung epithelial cells with deferoxamine and Fer-1 effectively reduces the lipid peroxidation induced by CSE, and inhibition of GPX4 also has the same effect (8). Therefore, related inhibitors such as deferoxamine and Fer-1 are potential approaches for the prevention of ferroptosis and COPD treatment. PM2.5 is one of the pathogenic factors of COPD. Studies found that, after inhalation of PM2.5 particles, the iron content and ROS concentration in human endothelial cells significantly increased, whereas the expression of GSH and NADPH decreased; in addition, the changes in the expression of TfR and Ft lead to an imbalance in cellular iron homeostasis, thereby inducing ferroptosis. The use of Fer-1 and deferoxamine improves GSH and nicotinamide adenine dinucleotide phosphate (NADPH) levels (60). Tang et al. found that cigarette smoke extract (CSE) aggravates the damage and death of BEAS-2B cells as well as increases the levels of IL-6 and TNF-α inflammatory factors, resulting in iron property changes. *In vivo* studies showed that CS causes lung injury in COPD rats, increases inflammatory cell infiltration, increases inflammatory cytokine secretion, and induces ferroptosis in lung tissue cells of COPD rats. *In vitro* and *in vivo* studies showed that CSE/CS increases the MDA content, increases the iron content, downregulates GPX4, decreases ferritin heavy chain levels, and upregulates transferrin receptor levels and also reported that curcumin reverses CS-induced lung inflammatory damage and epithelial cell ferroptosis (61).

Morphological aspects of ferroptosis mainly manifest as mitochondrial shrinkage, reduction or disappearance of mitochondrial cristae, and increased mitochondrial membrane density. Liu et al. found that dihydroquercetin (DHQ) inhibits CS-induced ferroptosis in the pathogenesis of COPD by activating the Nrf2-mediated pathway and attenuating CSE-induced mitochondrial morphological changes (62). *In vitro* and *in vivo* studies reported that the mRNA and protein expression levels of SLC7A11 and GPX4 are increased after DHQ treatment, and they also demonstrated that CSE-induced lipid peroxidation in HBE cells is significantly reduced after DHQ treatment. In addition, DHQ reverses CSE-induced excess MDA and ROS production, and it has also been reported that the DHQ-induced increase in SLC7A11 and GPX4 mRNA and protein levels is reversed by the use of an Nrf2-specific inhibitor (ML38.5). These findings suggest new options for the treatment of patients with COPD.

Nrf2 is a key factor in maintaining the oxidative/antioxidative balance, which can prevent the occurrence of COPD by resisting oxidative stress and lung inflammation. Recent studies reported that CpG hypermethylation in the promoter causes downregulation of Nrf2 expression in the lung tissue of patients with COPD (65). Zhang et al. found that, in HBE cells treated with CSE, the expression levels of reactive oxygen species (ROS), lipid

peroxides, and MDA are increased, and they also reported that the levels of IL-1β and IL-8 are increased but that the expression levels of GPX4 and SOD are decreased, indicating that CSE induces ferroptosis in HBE cells and increases the release of inflammatory factors (63). However, increasing the expression of Nrf2 enhances the expression of GPX4 and SOD but inhibits the expression of ferroptosis and related inflammatory factors in the supernatant. Further studies found that CS-/CSE-induced hypermethylation may lead to abnormal expression of Nrf2, and targeting methylation and ferroptosis may prevent the progression of COPD, which may be a new strategy for the treatment of COPD in the future.

Macrophages are innate immune cells that play a key role in alleviating inflammation and defending against invasion by external pathogens (66, 67). Studies found that macrophage activation is closely related to the occurrence of COPD, and the total number of macrophages is proportional to the severity of smoking and COPD (68). Using both *in vitro* and *in vivo* studies, Liu et al. found increased levels of M2 macrophages, MMP9 expression, and MMP12 expression in patients with COPD, CS-exposed mice, and THP-M cells cocultured with CSE-treated human bronchial epithelial (HBE) cells, suggesting that NCOA4 and ferroptosis are involved in the pathogenesis of COPD (64). This trend is further reversed using NCOA4 siRNA and the ferrostatin-1 ferroptosis inhibitor. Therefore, blocking NCOA4 may be a promising therapeutic strategy for COPD, which provides a new direction for COPD diagnosis and treatment research. The Mechanism of ferroptosis in COPD is shown in Table 1.

4. Ferroptosis as a potential therapeutic strategy in COPD

The imbalance of iron absorption and metabolism is an important factor affecting the progression of COPD. Therefore, correcting the local metabolism of iron is another key method for the treatment of COPD. Studies showed that iron chelators, antioxidants, iron supplementation, and dietary restriction are effective ways to treat COPD.

4.1. Iron chelators

Common iron chelators include deferoxamine (DFO), deferiprone, and deferasirox. DFO is one of the drugs that have been approved by the FDA for the treatment of iron overdose (69). In addition, a previous study found that DFO reduces the levels of inflammatory factors and reactive oxygen species *in vitro* and that it exerts an anti-inflammatory effect and reduces the inflammatory response (70). However, genetic factors are one of the important factors in the complex pathogenesis of COPD (71). Another study showed that iron regulatory protein 2 (IRP2, also known as IREB2) is increased in the lung tissue of patients with COPD, and IRP2 has been identified as a COPD susceptibility gene (72). Through COPD model mouse experiments, Cloonan et al. found that mice lacking the IRP2 gene are protected from CS-induced COPD, and they identified IRP2 as a regulator of mouse lung mitochondrial function. They further found that mice treated with a mitochondrial iron chelator (deferiprone) or fed a low-iron diet are protected from CS-induced COPD; in addition, CS-induced mucociliary clearance (MCC) impairment, lung inflammation, and lung injury in mice are attenuated (73). Therefore, these findings suggest that mitochondrial iron chelators may be a new potential approach for treating COPD.

4.2. Antioxidants

Oxidative stress is an important cause of airway inflammation, lung parenchyma destruction, and lung function

TABLE 1 Mechanism of ferroptosis in COPD.

Reagents	Model	Key mechanisms	References
DFO, Fer-1	HBEC; COPD mouse model	CS promotes the accumulation of unstable iron *via* NCOA4-mediated ferritinophagy, inducing lipid peroxidation and ferroptosis	(8)
Fer-1, DFOM	EA.hy 926; HUVECs	PM2.5 causes iron overload and oxidative stress and further induces ferroptosis.	(60)
Fer-1, DFO, CUR	BEAS-2B; COPD mouse model	CS/CSE causes cell damage and enhances inflammation, and oxidative stress induces ferroptosis	(61)
Dihydroquercetin (DHQ); ML385,	HBE cells; COPD mouse model	CSE induces cellular oxidative stress and ferroptosis	(62)
Fer-1	HBE cells; COPD mouse model	CSE induces increased levels of cellular ROS, lipid peroxides, and MDA, with IL-1β and IL-8 inducing ferroptosis and inflammatory responses, respectively	(63)
Fer-1	HBE/THP-M cells; COPD mouse model	NCOA4-induced ferroptosis promotes macrophage M2 polarization	(64)

decline in COPD, mainly induced by inhalation of air pollutants such as CS/CSE and dust. The acute exacerbation of COPD is closely related to oxidative stress and oxidant/antioxidant imbalance in the blood (74). Studies showed that H2S-activated Nrf2 pathway-mediated antioxidant effects play an important role in the progression of COPD (75). Wang et al. found that, after PM2.5 treatment of airway epithelial cells, the mitochondrial membrane density increases and characteristic changes in ferroptosis occur. Furthermore, COX2 expression is increased in patients with COPD. In the PM2.5-mediated mouse model and cell injury model, the levels of LIP ROS, total ROS, and MDA increased, and the levels of GSH, GSH Px, and GPX4 antioxidants decreased, indicating that PM2.5-mediated ferroptosis is involved in the pathogenesis of COPD (76). Further studies found that H2S inhibits lipid peroxidation-mediated ferroptosis by restoring the redox balance and regulating the Nrf2–PPAR ferritin in the phagocytosis pathway, thereby reducing PM2.5-induced emphysema and airway inflammation. These results suggest that inhibition of ferroptosis may be a potential therapeutic target for diseases with oxidative stress as the core pathogenesis, and H2S may be a potential antioxidant that blocks PM2.5 and causes COPD pathogenesis.

5. Conclusion and outlook

Ferroptosis, a new type of cell death, is involved in the pathogenesis of various diseases. Ferroptosis is a process involving abnormal metabolism of iron, amino acids, and lipids, which are involved in cell proliferation and differentiation. The metabolic process of ferroptosis is complex, and its mechanism has been preliminarily studied. Both *in vitro* and *in vivo* studies showed that ferroptosis is closely related to a variety of disease processes, and appropriate intervention in ferroptosis can treat the disease and delay the progression of related diseases. Although some progress has been made in the role of ferroptosis in lung cancer, ALI, and pulmonary fibrosis, the role of ferroptosis in COPD is not fully understood, and further clinical and experimental studies are needed. Although the existing ferroptosis inducers are effective, the therapeutic modalities for ferroptosis are still insufficient and only include iron inhibitors, iron chelators, and antioxidants. Identifying effective diagnostic markers of COPD may aid in the treatment of ferroptosis in COPD. For example, the known important regulator of iron metabolism, hepcidin, and its receptor, Fpn1, have been demonstrated to be effective diagnostic markers for COPD, and they may be promising targets for future drug development. In conclusion, with increasing studies on ferroptosis, new related regulators and their functions are constantly being explored. Considering ferroptosis as an entry point to develop COPD treatment regimens and targeted drugs has important clinical value.

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 authors.

Author contributions

DM and CZ wrote the manuscript. RJ and ZL provided language assistance. WW proofread the manuscript. SS was involved in the design and review of this study. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Shandong Province Postgraduate Education Quality Curriculum Construction Project (SDYKC20054) and the Qilu Medical School Traditional Chinese Medicine Inheritance Project (Lu Weihan [2022] No. 93).

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.

Publisher's note

References

1. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell.* (2012) 149:1060–72. doi: 10.1016/j.cell.2012.03.042

2. Zhou B, Liu J, Kang R, Klionsky DJ, Kroemer G, Tang D. Ferroptosis is a type of autophagy-dependent cell death. *Semin Cancer Biol.* (2020) 66:89–100. doi: 10.1016/j.semcancer.2019.03.002

3. Gao M, Jiang X. To eat or not to eat-the metabolic flavor of ferroptosis. *Curr Opin Cell Biol.* (2018) 51:58–64. doi: 10.1016/j.ceb.2017.11.001

4. Dixon SJ, Stockwell BR. The role of iron and reactive oxygen species in cell death. *Nat Chem Biol.* (2014) 10:9–17. doi: 10.1038/nchembio.1416

5. Stockwell BR, Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, et al. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell.* (2017) 171:273–85. doi: 10.1016/j.cell.2017.09.021

6. Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, et al. Ferroptosis: process and function. *Cell Death Differ.* (2016) 23:369–79. doi: 10.1038/cdd.2015.158

7. Global Initiative for Chronic Obstructive Lung Disease. *Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease 2022 Report* [EB/OL]. http://www.goldcopd.org.

8. Yoshida M, Minagawa S, Araya J, Sakamoto T, Hara H, Tsubouchi K, et al. Involvement of cigarette smoke-induced epithelial cell ferroptosis in COPD pathogenesis. *Nat Commun.* (2019) 10:3145. doi: 10.1038/s41467-019-10991-7

9. Kehrer JP. The Haber-Weiss reaction and mechanisms of toxicity. *Toxicology.* (2000) 149:43–50. doi: 10.1016/S0300-483X(00)00231-6

10. Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M. Targeting free radicals in oxidative stress-related human diseases. *Trends Pharmacol Sci.* (2017) 38:592–607. doi: 10.1016/j.tips.2017.04.005

11. Schweichel JU, Merker HJ. The morphology of various types of cell death in prenatal tissues. *Teratology.* (1973) 7:253–66. doi: 10.1002/tera.1420070306

12. Fischbacher A, von Sonntag C, Schmidt TC. Hydroxyl radical yields in the Fenton process under various pH, ligand concentrations and hydrogen peroxide/Fe(II) ratios. *Chemosphere.* (2017) 182:738–44. doi: 10.1016/j.chemosphere.2017.05.039

13. Ye Z, Liu W, Zhuo Q, Hu Q, Liu M, Sun Q. et al. Ferroptosis: final destination for cancer? *Cell Prolif.* (2020) 53:e12761. doi: 10.1111/cpr.12761

14. Zilka O, Shah R, Li B, Angeli JPF, Griesser M, Conrad M, et al. On the Mechanism of cytoprotection by ferrostatin-1 and liproxstatin-1 and the role of lipid peroxidation in ferroptotic cell death. *ACS Cent Sci.* (2017) 3:232–43. doi: 10.1021/acscentsci.7b00028

15. Sun X, Ou Z, Chen R, Niu X, Chen D, Kang R, et al. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. *Hepatology.* (2016) 63:173–84. doi: 10.1002/hep.28251

16. Chen L, Hambright WS, Na R, Ran Q. Ablation of the ferroptosis inhibitor glutathione peroxidase 4 in neurons results in rapid motor neuron degeneration and paralysis. *J Biol Chem.* (2015) 290:28097–106. doi: 10.1074/jbc.M115.680090

17. Florean C, Song S, Dicato M, Diederich M. Redox biology of regulated cell death in cancer: a focus on necroptosis and ferroptosis. *Free Radic Biol Med.* (2019) 134:177–89. doi: 10.1016/j.freeradbiomed.2019.01.008

18. D'Herde K, Krysko DV. Ferroptosis: oxidized pes trigger death. *Nat Chem Biol.* (2017) 13:4–5. doi: 10.1038/nchembio.2261

19. Kagan VE, Mao G, Qu F, Angeli JPF, Doll S, Croix CS, et al. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat Chem Biol.* (2017) 13:81–90. doi: 10.1038/nchembio.2238

20. Dixon SJ, Winter GE, Musavi LS, Lee ED, Snijder B, Rebsamen M, et al. Human haploid cell genetics reveals roles for lipid metabolism genes in nonapoptotic cell death. *ACS Chem Biol.* (2015) 10:1604–9. doi: 10.1021/acschembio.5b00245

21. Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, et al. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol.* (2017) 13:91–8. doi: 10.1038/nchembio.2239

22. Kim S, Kang SW, Joo J, Han SH, Shin H, Nam BY, et al. Characterization of ferroptosis in kidney tubular cell death under diabetic conditions. *Cell Death Dis.* (2021) 12:160. doi: 10.1038/s41419-021-03452-x

23. Ingold I, Berndt C, Schmitt S, Doll S, Poschmann G, Buday K, et al. Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. *Cell.* (2018) 172:409–22. doi: 10.1016/j.cell.2017.11.048

24. Liang C, Zhang X, Yang M, Dong X. Recent progress in ferroptosis inducers for cancer therapy. *Adv Mater.* (2019) 31:e1904197. doi: 10.1002/adma.201904197

25. Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT. Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. *Neuron.* (1989) 2:1547–58. doi: 10.1016/0896-6273(89)90043-3

26. Green DR, Kroemer G. Cytoplasmic functions of the tumour suppressor p53. *Nature.* (2009) 458:1127–30. doi: 10.1038/nature07986

27. Khoo KH, Verma CS, Lane DP. Drugging the p53 pathway: understanding the route to clinical efficacy. *Nat Rev Drug Discov.* (2014) 13:217–36. doi: 10.1038/nrd4236

28. Jiang L, Kon N, Li T, Wang S-J, Su T, Hibshoosh H, et al. Ferroptosis as a p53-mediated activity during tumour suppression. *Nature.* (2015) 520:57–62. doi: 10.1038/nature14344

29. Liu Y, Gu W. The complexity of p53-mediated metabolic regulation in tumor suppression. *Semin Cancer Biol.* (2021). doi: 10.1016/j.semcancer.2021.03.010

30. Liu Y, Gu W. p53 in ferroptosis regulation: the new weapon for the old guardian. *Cell Death Differ.* (2022) 29:895–910. doi: 10.1038/s41418-022-00943-y

31. Hu W, Zhang C, Wu R, Sun Y, Levine A, Feng Z. Glutaminase 2, a novel p53 target gene regulating energy metabolism and antioxidant function. *Proc Natl Acad Sci USA.* (2010) 107:7455–60. doi: 10.1073/pnas.1001006107

32. Ou Y, Wang S-J, Li D, Chu B, Gu W. Activation of SAT1 engages polyamine metabolism with p53-mediated ferroptotic responses. *Proc Natl Acad Sci U S A.* (2016) 113:E6806–12. doi: 10.1073/pnas.1607152113

33. Xie Y, Zhu S, Song X, Sun X, Fan Y, Liu J, et al. The tumor suppressor p53 limits ferroptosis by blocking DPP4 activity. *Cell Rep.* (2017) 20:1692–704. doi: 10.1016/j.celrep.2017.07.055

34. Tarangelo A, Magtanong L, Bieging-Rolett KT, Li Y, Ye J, Attardi LD, et al. p53 suppresses metabolic stress-induced ferroptosis in cancer cells. *Cell Rep.* (2018) 22:569–75. doi: 10.1016/j.celrep.2017.12.077

35. Ma Q. Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol.* (2013) 53:401–26. doi: 10.1146/annurev-pharmtox-011112-140320

36. Kerins MJ, Ooi A. The roles of NRF2 in modulating cellular iron homeostasis. *Antioxid Redox Signal.* (2018) 29:1756–73. doi: 10.1089/ars.2017.7176

37. Lei P, Bai T, Sun Y. Mechanisms of ferroptosis and relations with regulated cell death: a review. *Front Physiol.* (2019) 10:139. doi: 10.3389/fphys.2019.00139

38. Song X, Long D. Nrf2 and ferroptosis: a new research direction for neurodegenerative diseases. *Front Neurosci.* (2020) 14:267. doi: 10.3389/fnins.2020.00267

39. Sun X, Niu X, Chen R, He W, Chen D, Kang R, et al. Metallothionein-1G facilitates sorafenib resistance through inhibition of ferroptosis. *Hepatology.* (2016) 64:488–500. doi: 10.1002/hep.28574

40. Chen G, Wei J, Lyu X. Research progress of nuclear factor-erythroid 2 related factor 2 in acute lung injury. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue.* (2018) 30:270–4. doi: 10.3760/cma.j.issn.2095-4352.2018.03.016

41. Dodson M, Castro-Portuguez R, Zhang DD. NRF2 plays a critical role in mitigating lipid peroxidation and ferroptosis. *Redox Biol.* (2019) 23:101107. doi: 10.1016/j.redox.2019.101107

42. Chen D, Tavana O, Chu B, Erber L, Chen Y, Baer R, et al. NRF2 is a major target of ARF in p53-independent tumor suppression. *Mol Cell.* (2017) 68:224–32. doi: 10.1016/j.molcel.2017.09.009

43. Telorack M, Meyer M, Ingold I, et al. A glutathione-Nrf2-thioredoxin cross-talk ensures keratinocyte survival and efficient wound repair. *PLoS Genet.* (2016) 12:e1005800. doi: 10.1371/journal.pgen.1005800

44. Cho WK, Lee CG, Kim LK. COPD as a disease of immunosenescence. *Yonsei Med J.* (2019) 60:407–13. doi: 10.3349/ymj.2019.60.5.407

45. Chen X, Que C, Yao Y, Han Y, Zhang H, Li X, et al. Susceptibility of individuals with lung dysfunction to systemic inflammation associated with ambient fine particle exposure: a panel study in Beijing. *Total Environ.* (2021) 788:147760. doi: 10.1016/j.scitotenv.2021.147760

46. Bernhagen J. A new cytokine target for chronic obstructive pulmonary disease? *EBioMedicine.* (2021) 69:103479. doi: 10.1016/j.ebiom.2021.103479

47. Szulakowski P, Crowther AJL, Jiménez LA, Donaldson K, Mayer R, Leonard TB, et al. The effect of smoking on the transcriptional regulation of lung inflammation in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* (2006) 174:41–50. doi: 10.1164/rccm.200505-725OC

48. Barnes PJ. Targeting cytokines to treat asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol.* (2018) 18:454–66. doi: 10.1038/s41577-018-0006-6

49. Sahakian E, Chen J, Powers JJ, Chen X, Maharaj K, Deng SL, et al. Essential role for histone deacetylase 11 (HDAC11) in neutrophil biology. *J Leukoc Biol.* (2017) 102:475–86. doi: 10.1189/jlb.1A0415-176RRR

50. Ali MK, Kim RY, Karim R, Mayall JR, Martin KL, Shahandeh A, et al. Role of iron in the pathogenesis of respiratory disease. *Int J Biochem Cell Biol.* (2017) 88:181–95. doi: 10.1016/j.biocel.2017.05.003

51. Philippot Q, Deslée G, Adair-Kirk TL, Woods JC, Byers D, Conradi S, et al. Increased iron sequestration in alveolar macrophages in chronic obstructive pulmonary disease. *PLoS ONE.* (2014) 9:e96285. doi: 10.1371/journal.pone.0096285

52. Bailie GR, Schuler C, Leggett RE Li H, Li H-D, Patadia H, et al. Oxidative effect of several intravenous iron complexes in the rat. *Biometals.* (2013) 26:473–8. doi: 10.1007/s10534-013-9632-4

53. Sadeghi L, Yousefi BV, Espanani HR. Toxic effects of the Fe2O3 nanoparticles on the liver and lung tissue. *Bratisl Lek Listy.* (2015) 116:373–8. doi: 10.4149/BLL_2015_071

54. Ghio AJ, Hilborn ED, Stonehuerner JG, Dailey LA, Carter JD, Richards JH, et al. Particulate matter in cigarette smoke alters iron homeostasis to produce a biological effect. *Am J Respir Crit Care Med.* (2008) 178:1130–8. doi: 10.1164/rccm.200802-334OC

55. Ayala A, Muñoz MF, Argüelles S. A Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev.* (2014) 2014:360438. doi: 10.1155/2014/360438

56. Basu S, Helmersson J, Jarosinska D, Sällsten G, Mazzolai B, Barregård L. Regulatory factors of basal F(2)-isoprostane formation: population, age, gender and smoking habits in humans. *Free Radic Res.* (2009) 43:85–91. doi: 10.1080/10715760802610851

57. Gao X, Gào X, Zhang Y, Breitling LP, Schöttker B, Brenner H. Assoiations of self-reported smoking, cotinine levels and epigenetic smoking indicators with oxidative stress among older adults: a population-based study. *Eur J Epidemiol.* (2017) 32:443–56. doi: 10.1007/s10654-017-0248-9

58. Zhang X, Staimer N, Gillen DL, Tjoa T, Schauer JJ, Shafer MM, et al. Associations of oxidative stress and inflammatory biomarkers with chemically-characterized air pollutant exposures in an elderly cohort. *Environ Res.* (2016) 150:306–19. doi: 10.1016/j.envres.2016.06.019

59. Sarnat JA, Golan R, Greenwald R, Raysoni AU, Kewada P, Winquist A, et al. Exposure to traffic pollution, acute inflammation and autonomic response in a panel of car commuters. *Environ Res.* (2014) 133:66–76. doi: 10.1016/j.envres.2014.05.004

60. Wang Y, Tang M. PM25 induces ferroptosis in human endothelial cells through iron overload and redox imbalance. *Environ Pollut.* (2019) 254:112937. doi: 10.1016/j.envpol.2019.07.105

61. Tang X, Li Z, Yu Z, Li J, Zhang J, Wan N, et al. Effect of curcumin on lung epithelial injury and ferroptosis induced by cigarette smoke. *Hum Exp Toxicol.* (2021) 40:S753–62. doi: 10.1177/09603271211059497

62. Liu X, Ma Y, Luo L, Zong D, Li H, Zeng Z, et al. Dihydroquercetin suppresses cigarette smoke induced ferroptosis in the pathogenesis of chronic obstructive pulmonary disease by activating Nrf2-mediated pathway. *Phytomedicine.* (2022) 96:153894. doi: 10.1016/j.phymed.2021.153894

63. Zhang Z, Fu C, Liu J, Sai X, Qin C, Di T, et al. Hypermethylation of the Nrf2 Promoter Induces Ferroptosis by Inhibiting the Nrf2-GPX4 Axis in COPD. *Int J Chron Obstruct Pulmon Dis.* (2021) 16:3347–62. doi: 10.2147/COPD.S340113

64. Liu J, Zhang Z, Yang Y, Di T, Wu Y, Bian T. NCOA4-mediated ferroptosis in bronchial epithelial cells promotes macrophage M2 polarization in COPD emphysema. *Int J Chron Obstruct Pulmon Dis.* (2022) 17:667–81. doi: 10.2147/COPD.S354896

65. Vucic EA, Chari R, Thu KL, Wilson IM, Cotton AM, Kennett JY, et al. DNA methylation is globally disrupted and associated with expression changes in chronic obstructive pulmonary disease small airways. *Am J Respir Cell Mol Biol.* (2014) 50:912–22. doi: 10.1165/rcmb.2013-0304OC

66. Gimenes JJ, Srivastava V, ReddyVari H, et al. Rhinovirus-induces progression of lung disease in a mouse model of COPD via IL-33/ST2 signaling axis. *Clin Sci.* (2019) 133:983–96. doi: 10.1042/CS20181088

67. Wang L, Zhang Y, Zhang N, Xia J, Zhan Q, Wang C. Potential role of M2 macrophage polarization in ventilator-induced lung fibrosis. *Int Immunopharmacol.* (2019) 75:105795. doi: 10.1016/j.intimp.2019.105795

68. Feng H, Yin Y, Ren Y, Li M, Zhang D, Xu M, et al. Effect of CSE on M1/M2 polarization in alveolar and peritoneal macrophages at different concentrations and exposure *in vitro*. *In Vitro Cell Dev Biol Anim.* (2020) 56:154–64. doi: 10.1007/s11626-019-00426-4

69. Crisponi G, Nurchi VM, Lachowicz JI. Iron chelation for iron overload in thalassemia. *Met Ions Life Sci.* (2019) 19:9783110527872–009. doi: 10.1515/9783110527872-003

70. Ramezanpour M, Smith JLP, Ooi ML, Gouzos M, Psaltis AJ, Wormald PJ, et al. Deferiprone has anti-inflammatory properties and reduces fibroblast migration *in vitro*. *Sci Rep.* (2019) 9:2378. doi: 10.1038/s41598-019-38902-2

71. Siedlinski M, Tingley D, Lipman PJ, Cho MH, Litonjua AA, Sparrow D, et al. Dissecting direct and indirect genetic effects on chronic obstructive pulmonary disease (COPD) susceptibility. *Hum Genet.* (2013) 132:431–41. doi: 10.1007/s00439-012-1262-3

72. DeMeo DL, Mariani T, Bhattacharya S, Srisuma S, Lange C, Litonjua A, et al. Integration of genomic and genetic approaches implicates IREB2 as a COPD susceptibility gene. *Am J Hum Genet.* (2009) 85:493–502. doi: 10.1016/j.ajhg.2009.09.004

73. Cloonan SM, Glass K, Laucho-Contreras ME, Bhashyam AR, Cervo M, Pabón MA, et al. Mitochondrial iron chelation ameliorates cigarette smoke-induced bronchitis and emphysema in mice. *Nat Med.* (2016) 22:163–74. doi: 10.1038/nm.4021

74. Babaoglu E, Kilic H, Hezer H, Dag O, Parlak E, Senturk A, et al. Comparison of thiol/disulphide homeostasis parameters in patients with COPD, asthma and ACOS. *Eur Rev Med Pharmacol Sci.* (2016) 20:1537–43. Available online at: https://pm.yuntsg.com/searchList.html

75. Han W, Dong Z, Dimitropoulou C, Su Y. Hydrogen sulfide ameliorates tobacco smoke-induced oxidative stress and emphysema in mice. *Antioxid Redox Signal.* (2011) 15:2121–34. doi: 10.1089/ars.2010.3821

76. Wang Y, Liao S, Pan Z, Jiang S, Fan J, Yu S, et al. Hydrogen sulfide alleviates particulate matter-induced emphysema and airway inflammation by suppressing ferroptosis. *Free Radic Biol Med.* (2022) 186:1–16. doi: 10.1016/j.freeradbiomed.2022.04.014

frontiers | Frontiers in Medicine

TYPE Mini Review
PUBLISHED 19 January 2023
DOI 10.3389/fmed.2023.1124008

OPEN ACCESS

EDITED BY
Shuibang Wang,
National Institutes of Health, United States

REVIEWED BY
Stephen C. Land,
University of Dundee, United Kingdom

*CORRESPONDENCE
Elena Lesma
✉ elena.lesma@unimi.it

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 14 December 2022
ACCEPTED 06 January 2023
PUBLISHED 19 January 2023

CITATION
Bernardelli C, Caretti A and Lesma E (2023) Dysregulated lipid metabolism in lymphangioleiomyomatosis pathogenesis as a paradigm of chronic lung diseases. *Front. Med.* 10:1124008. doi: 10.3389/fmed.2023.1124008

Dysregulated lipid metabolism in lymphangioleiomyomatosis pathogenesis as a paradigm of chronic lung diseases

Clara Bernardelli[1], Anna Caretti[2] and Elena Lesma[1]*

[1]Laboratory of Pharmacology, Department of Health Sciences, Università degli Studi di Milano, Milan, Italy, [2]Laboratory of Biochemistry and Molecular Biology, Department of Health Sciences, Università degli Studi di Milano, Milan, Italy

A chronic inflammatory condition characterizes various lung diseases. Interestingly, a great contribution to inflammation is made by altered lipids metabolism, that can be caused by the deregulation of the mammalian target of rapamycin complex-1 (mTORC1) activity. There is evidence that one of mTOR downstream effectors, the sterol regulatory element-binding protein (SREBP), regulates the transcription of enzymes involved in the *de novo* fatty acid synthesis. Given its central role in cell metabolism, mTOR is involved in several biological processes. Among those, mTOR is a driver of senescence, a process that might contribute to the establishment of chronic lung disease because the characteristic irreversible inhibition of cell proliferation, associated to the acquisition of a pro-inflammatory senescence-associated secretory phenotype (SASP) supports the loss of lung parenchyma. The deregulation of mTORC1 is a hallmark of lymphangioleiomyomatosis (LAM), a rare pulmonary disease predominantly affecting women which causes cystic remodeling of the lung and progressive loss of lung function. LAM cells have senescent features and secrete SASP components, such as growth factors and pro-inflammatory molecules, like cancer cells. Using LAM as a paradigm of chronic and metastatic lung disease, here we review the published data that point out the role of dysregulated lipid metabolism in LAM pathogenesis. We will discuss lipids' role in the development and progression of the disease, to hypothesize novel LAM biomarkers and to propose the pharmacological regulation of lipids metabolism as an innovative approach for the treatment of the disease.

KEYWORDS

chronic lung diseases, inflammation, senescence-mediated inflammation, mTOR, lymphangioleiomyomatosis, lipids

Introduction

Lungs rely on a unique lipid biology that ensures respiratory function and is involved in the regulation of the immune response. The alveolar area sustains active lipid metabolism to maintain surfactant homeostasis thus ensuring optimal respiration cycle. Mostly composed of phospholipids with 5–10% of cholesterol and small amount of sphingolipids, surfactant is essential in reducing the surface tension in the alveolar walls (1). Sterol-regulatory element-binding proteins (SREBPs) regulate lung lipid biosynthesis and sterol homeostasis

at transcriptional level (2). This family of transcription factors promotes the expression of cholesterogenic and lipogenic genes involved in fat storage (3). Growing evidence shows that SREBPs are involved in numerous pathogenic processes such as endoplasmic reticulum stress and inflammation. LPS-challenged macrophages from mice with a targeted deficiency in the gene encoding SREBP-1a, failed to activate lipogenesis, secretion of IL-1β and gene encoding Nlrp1a, which is a core inflammasome component (4). Oishi et al. demonstrated that in macrophages, SREBP1 reprograms lipid metabolism to produce the anti-inflammatory polyunsaturated fatty acids thus promoting the late resolution of TLR4-induced gene activation (5). SREBP1 induction in alveolar type 2 cells of *Insig1/2*$^{\Delta/\Delta}$ mice causes neutral lipid accumulation in type II cells and in the alveoli, resulting in pulmonary inflammation and airspace remodeling (6). Transcriptome network analysis reveals that SREBP1 activation promotes lipotoxicity that in turn induces inflammation and fibrosis in the lung (7). Recent data have shown that SREBP2 integrates cholesterol metabolism with NLRP3 inflammasome activation in macrophages (8). SREBP1 is regulated by the mammalian target of rapamycin (mTOR) (9) that is a regulator of lipid and nucleotide synthesis. Once activated, SREBP1 translocates from the endoplasmic reticulum to the nucleus to promote the transcription of the enzymes responsible for glugoneogenesis and lipogenesis (10).

Lymphangioleiomyomatosis (LAM) is a rare progressive lung disease characterized by lung cystic destruction, and the progressive loss of lung function (11). LAM occurs sporadically or associated to Tuberous Sclerosis Complex (TSC) (12). Loss of *TSC* gene function causes to dysregulated mTOR signaling (13). mTOR has a central role in cell metabolism, integrating environmental signals to promote anabolic processes (14). Recently, likely because of mTOR dysregulation, several studies indicated the alteration of lipid metabolism in LAM as an interesting target to uncover novel therapeutic approaches (15).

The minireview describes the current state of knowledge of emerging role of lipids in LAM considering their contribution in inflammation and in the pathogenesis and progression of chronic lung diseases.

Lipids in pulmonary diseases

Inflammation

Lung inflammatory diseases are tightly linked with aberrant SREBP activity in respiratory epithelial cells and with dyslipidemia. SREBP2-mediated lipid metabolism promotion correlates with (COVID-19)-induced cytokine storm (16). The increased expression of SREBP-1 after respiratory virus infection (i.e., SARS-CoV), regulates the accumulation of lipid droplets, cell organelles involved in the amplification of inflammatory mediators' production (17). Dysregulation of free fatty acids, cholesterol, and ceramides homeostasis in the lungs is associated with the pathogenesis and the progression of chronic obstructive pulmonary disease (COPD). Cigarette smoke–induced alveolar accumulation of lipids is an important event that triggers inflammation in alveolar macrophages (18). Several studies have demonstrated that ceramides, the central hub of sphingolipids metabolism, are elevated in COPD and contribute to chronic inflammation (19). Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease characterized by inflammation and fibrosis. As recently reviewed by Suryadevara et al. (20), bioactive lipid mediators derived from fatty acids, glycerolipids, phospholipids, and sphingolipids exhibit pro- or anti-fibrotic/inflammatory effects in lung tissues of IPF patients. Acute Respiratory Distress Syndrome (ARDS) is a severe form of clinical acute lung injury (ALI), both sharing severe inflammatory conditions. Phospholipase A2 enzymes (sPLA2s), that generate free fatty acids and lysophospholipids from glycerophospholipids, are upregulated in different cell types in the ALI/ARDS lung compartment. Significant evidence demonstrates that in these pathological settings, PLA2s are functionally important as regulators of inflammatory signaling (21). Caused by several factors, pneumonia is the inflammation localized to the terminal airways, the alveoli, and the interstitium of the lungs. A pilot study conducted in 2017 during community-acquired pneumonia (22), reported that monounsaturated and polyunsaturated fatty acid accumulation elicits an inflammatory response during pneumonia progression. Cystic Fibrosis (CF) is an inherited recessive disease. CF is caused by mutations in the CF transmembrane conductance regulator gene, with most of the mortality given by the lung dysfunction (23), being inflammation an independent risk factor for disease progression. We previously demonstrated that CF bronchial epithelial cells accumulate the sphingolipid ceramide (24), which contributes to CF airways inflammation (25), as well as glycerophospholipids and lyso-glycerophospholipids, the latest considered pro-inflammatory molecules (26), cholesterols, cholesterol esters and triacylglycerols (27), compared to normal cells.

Senescence-mediated inflammation

Senescence is a stress-response process characterized by the irreversible arrest of the cell cycle following DNA damage. Senescent cells develop a senescence-associated secretory phenotype (SASP) to maintain their status and to communicate within their microenvironment. SASP factors, both released as soluble molecules or within extracellular vesicles (EVs), have strong biological activities on neighboring cells, including the promotion of inflammation. Indeed, the study of SASP in all *in vitro* generated models demonstrated the presence of the proinflammatory interleukin-6 (IL-6), CXC chemokine ligand 8 (IL-8), and monocyte chemoattractant protein 1 (28). Even if senescence allows the maintaining of tissue homeostasis, an irreversible proliferation arrest might limit the regenerative capacity of the tissue, and the presence of a pro-inflammatory milieu might sustain the onset of pathological condition and age-related disorders. In pulmonary diseases, accelerated or premature aging emerged to be significant. The oxidative stress caused by cigarette smoke induces senescence in alveolar epithelial cells (AEC) and endothelial cells that accumulate in COPD lungs, while in IPF the shortening of the telomeres might be responsible for the senescent status of fibroblasts and AEC (29). Of note, AEC might be driven to senescence also after the serine/threonine kinase mTOR hyperactivation (30). The constitutive activation of mTOR in the lung epithelial cells of transgenic mice results in weakness tight junctions' (TJ), that is a sign of injury in the lung epithelium. In parallel, also epithelial to mesenchymal transition (EMT) was enhanced, ultimately contributing to lung fibrosis. In this study, however, senescence was not observed, but the authors suggested that this might be due to differences in the

TABLE 1 Anti-inflammatory efficient compounds targeting lipid pathways in pulmonary diseases.

Pulmonary disease	Lipid target	Anti-inflammatory compound	References
SARS-CoV-2 infection	Leukotrienes (LTB4 and cysLT)	A922500, DGAT-1 inhibitor	(61)
	Sphingosine	Fingolimod (FTY720), sphingosine analog	(74)
ALI/ARDS	Ceramide	D609, aSMAse inhibitor	(75)
	Sphingosine	Fingolimod (FTY720), Sphingosine analog	(76, 77)
CF	Ceramide	Myriocin, SPT inhibitor	(26, 78)
		Amitriptyline, Trimipramine, Desipramine, aSMAse inhibitors	(78)
	Glucosylceramide	Miglustat, GBA2 inhibitor	(80)
PF	Eicosanoids	AK106-001616, PLA2 inhibitor	(62)
	Cholesterol	Statins, HMG-CoA reductase inhibitors	(81)
Asthma	Cholesterol	Statins, HMG-CoA reductase inhibitors	(82)
	Diacylglicerols	R59949, DGKα inhibitor	(83)
COPD	Ceramide	Myriocin, SPT inhibitor	(84)
	Cholesterol	Atorvastatin, HMG-CoA reductase inhibitors	(85, 86)
LAM	Ganglioside D3	Rapamycin	(61)
	LPCs	Rapamycin Torin1	(64)
	AdPLA2 (PLA2G16)	MAFP	(66)
	DAG	Rintanserin ± chloroquine	(67)

cysLT, cysteinyl leukotriene; LTB4, leukotriene B4; DGAT, acyl-CoA:diacylglycerol acyltransferase-1; aSMAse, acid sphingomyelinase; SPT, serine palmitoyltransferase; PF, pulmonary fibrosis; GBA2, glucosylceramidase beta 2; PLA2, phospholipase A2; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LPCs, lysophosphatidylcholines; MAFP, methyl arachidonyl fluorophosphate; AdPLA2, adipocyte phospholipase A2; DAG, diacylglycerol.

timing of mTOR activation compared to previous studies (31). Moreover, the peculiar contribution of the AEC to local inflammation might be ascribed also to the release of EVs. Interestingly, it was recently demonstrated that AEC released EVs that differs on their miRNA content, depending on the apical or basolateral origin site of their release. EVs released from the apical side of epithelial cells are enriched in miRNAs enhancing the mTOR signaling pathways, probably being responsible for the TJ vulnerability and for the EMT, which can cause lung injury (32). Of note, senescence and lipid metabolism share many regulatory proteins, among which mTOR has a crucial role (33). Senescent cells have increased lipids uptake and accumulation in lipids droplets (34) and it has recently arisen that the regulation of specific lipid species plays a critical role in senescence contributing to the chronic inflammation associated with SASP (35). For example, the prostaglandin E2 (PGE_2), which is synthesized from the arachidonic acid, is expressed at higher levels in COPD fibroblasts compared to healthy controls and is responsible for the autocrine and paracrine spreading of senescence in neighboring cells, as well as of the induction of inflammatory response through the up-regulation of its receptors EP2 and EP4 in a cyclooxygenase 2 -dependent reactive oxygen species response (36). Notably, PGE_2 is released also by epithelial cells to relax airways smooth muscle cells, indicating that the roles of lipid molecules in the crosstalk between cells in the lung environment is complex and needs further investigation (37). Additionally, the signaling lipid leukotrienes, that are SASP components, are secreted by senescent fibroblasts and contribute to worsen IPF promoting fibrosis in lung (38). Finally, lysophosphatidylcholines (LPCs) have SASP activity, being expressed at high levels in senescent fibroblasts and being capable to induce the secretion of IL-8 and IL-6 (35).

LAM and mTOR

Lymphangioleiomyomatosis (LAM) primarily affects women of childbearing age. LAM cells are smooth muscle-like cells bearing a mutation in *TSC1* or *TSC2* tumor suppressor genes that causes the hyperactivation of Rheb with an increase of mTORC1 and adenosine 5′-monophosphate activated protein kinase (AMPK) activity (39, 40). AMPK control the energy levels by promoting the catabolic processes and by inhibiting the anabolic activities. Interestingly, tuberin-null cells show the AMPK hyperactivation which correlates with the cytoplasmic localization of p27Kip1 that negatively regulates the cyclin dependent kinase 2 (41). mTORC1 or mTORC2 induce the expression and the proteolytic process of SREBP1; therefore, mTORC1 hyperactivation, through SREBP1 and acetyl-CoA carboxylase, controls the lipid accumulation (10). Moreover, the synthesis of PC is controlled by mTORC1 through SREBP1 (9). As a consequence of the *TSC* mutations, the loss of heterozygosity in *TSC1* or *TSC2*, which causes the lack of hamartin or tuberin, leads to the hyperactivation of mTOR that promotes cell growth and proliferation by stimulating anabolic metabolism with the increase of protein and lipid synthesis (42, 43). Furthermore, constitutive mTOR activation leads to an increased invasion of lymphatics and lungs where LAM cells and wild type stromal cells form cysts with the consequent pneumothorax and progressive loss of pulmonary function (44).

The proliferation of LAM cells in the lung parenchyma is associated with diffuse cystic lesions and their infiltration into the lymphatic walls causes lymphatic abnormalities leading to damage or obstruction of lymphatic vessels (45). Lymphangiogenesis plays a pathogenetic role whereby LAM cells invade and spread through

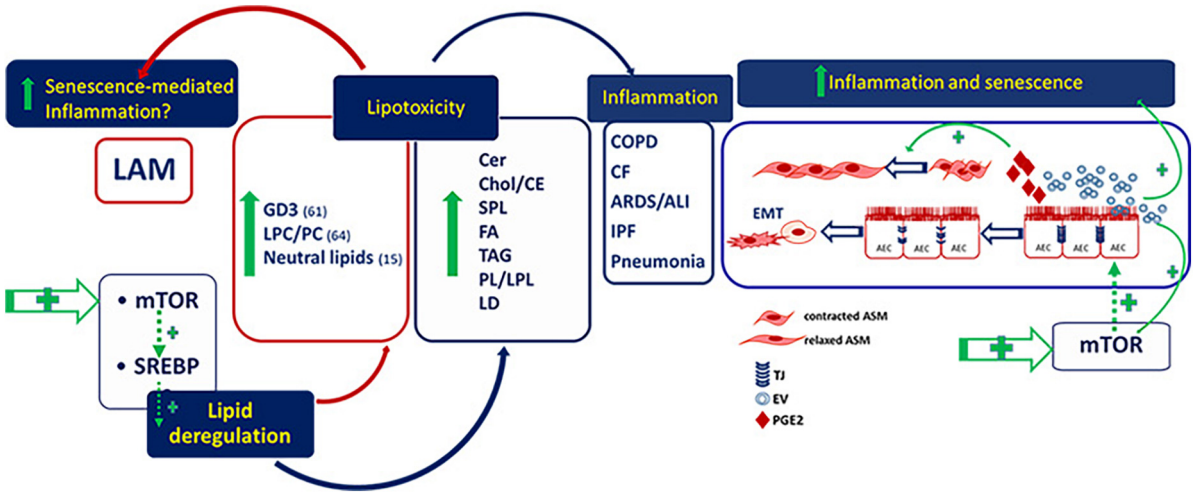

FIGURE 1
Deregulated lipids in lymphangioleiomyomatosis (LAM) and pulmonary diseases. GD3, ganglioside D3; LPC/PC, lysophosphatidylcholine/phosphatidylcholine; Cer, ceramide; Chol/CE, cholesterol/cholesterol esthers; SPL, sphingolipids; FA, fatty acids; TAG, riacylglycerols; PL/LPL, phospholipids/lysophospholipids; LD, lipid droplets; AEC, alveolar epithelial cells; ASM, airway smooth muscle.

the lymphatic, a process that is related to a high levels of vascular endothelial growth factor (VEGF)-D (46). As well, nearby and into the LAM lesions, the increased expression of VEGF-A stimulates angiogenesis that also might be a mechanism by which LAM cells invade the circulation (47). Clinical manifestations of LAM include progressive dyspnea, cough, chest pain, recurrent pneumothoraces, and chylous complications including chylothorax, chyloptysis, and chylous ascites (13). Angiomyolipomas, benign tumors composed of LAM cells, smooth muscle cells, adipose tissue, and vessels, can often occur in LAM patients.

The discovery of the genetic basis of LAM cells led to consider the inhibition of mTOR hyperactivation as a therapeutic approach. Rapamycin indeed inhibits LAM cell proliferation resulting in the stabilization of the lung function, in the reduction of lymphatic abnormalities, and of the angiomyolipoma volumes, with no efficacy in the regression of the existing pulmonary lesions. So far, the treatment with rapamycin is the only approved for LAM (48).

After being classified as an interstitial lung disease, LAM has been recently reconsidered as a low-grade, destructive, metastasizing neoplasm for several features as the invasive LAM cell properties, the recurrency in donor allografts of patients who have undergone lung transplantation, and the metabolic reprogramming. However, LAM differs from other neoplasms for the bilateral and symmetrical disruption of the lung without a clear primary tumor site and dominant mass lesions (49).

We recently demonstrated that LAM/TSC cells, derived by chylous thorax of a LAM/TSC patient, have senescent features dependent from mTOR hyperactivation and the capability to induce senescence in neighboring cells (50). As demonstrated in other respiratory disease such as IPF, senescence might drive the progressive loss of parenchymal structure in LAM which ultimately causes the impairment of lung function (51). It has been demonstrated that LAM cells secrete molecules known to be SASP components comprised metalloproteinases (52), proinflammatory cytokines IL-6 (53) and IL-8 (50), kathepsin K (54), and VEGF-D (39) reinforcing the hypothesis of a LAM cell communication with the microenvironment and a SASP modulation of the remodeling of the lung parenchyma. Interestingly, IL-8, a potent neutrophil chemotactic factor that triggers chemotaxis and neutrophil activation through a phosphorylation cascade in the inflammatory response (55), has been demonstrated to be involved in the pathogenesis and progression of lung diseases such as ARDS and SARS CoV-2, suggesting the possibility to use IL-8 as a biomarker or as a therapeutic target (56). In LAM, besides its role in senescence, IL-8 might reinforce the senescence/inflammatory milieu driving the progression of the disease.

Furthermore, mTOR role in LAM senescence can be supported by the senolitic effect of rapamycin and the relapse following the suspension of rapamycin treatment caused by an irreversible senescent state, called geroconversion, sustained by mTOR hyperactivation (57).

Lipids as novel potential therapeutic targets and biomarkers

Pulmonary diseases

In view of the deep interplay between dysregulated lipid metabolism and chronic inflammatory condition that characterizes several lung diseases, restoring lipid homeostasis could represent a novel therapeutic approach. Herein, there are examples of preclinical studies focusing on the potential anti-inflammatory efficacy of compounds targeting lipid pathway in pulmonary diseases (**Table 1**). Treatment with A922500, a pharmacological inhibitor of acyl-CoA:diacylglycerol acyltransferase-1, inhibits lipid droplets biogenesis triggered by SARS-CoV-2 infection in A549 human epithelial cells and in primary human monocytes. Thereon, the synthesis of pro-inflammatory lipid production and cytokinesis is downregulated (58). Interestingly, we were able to also reduce glycerol- and cholesterol-based lipids, to promote fatty acids oxidation, to reduce inflammation in *in vitro* model of CF by reducing ceramide accumulation with Myriocin, the inhibitor of the rate-limiting step in sphingolipid biosynthesis (27). Finally, in an *in vivo*

model, the bleomycin-induced lung fibrosis and inflammation in rats was attenuated by AK106-001616 that inhibits the cytosolic PLA2 responsible for the generation of pro-inflammatory eicosanoids (59).

LAM

Lymphangioleiomyomatosis (LAM) cells have a metabolic signature of increased fatty acid uptake and synthesis, a characteristic in common with cancer cells (60), making altered lipid species in LAM both disease relevant biomarkers and potential therapeutic targets.

For instance, in the perspective of a LAM immunotherapy, it was demonstrated that LAM lung sections of both patients and $Tsc2^{-/-}$ mice have a strong positivity to the ganglioside D3 (GD3), whose expression is a characteristic that LAM cells maintain also after multiple passages *in vitro* (61). Interestingly, LAM patients have lower anti-GD3 antibodies in their serum compared to healthy controls and the *in vitro* treatment of LAM cells with commercial anti-GD3 antibodies plus human complement induce death in the 12–42% of the population. This might suggest an approach similar to melanoma, in which the targeting of GD3 by antibodies reduces tumor growth through the activation of the natural killer T cells (62).

Even if GD3 expression in LAM cells and lipids accumulation in the renal angiomyolipomas of LAM patients can be considered a consequence of mTOR deregulation (63), emerging researches demonstrate the existence of uncovered TSC1/2-dependent-mTORC1-independent regulation of lipids metabolism that may contribute to the LAM pathogenesis and progression.

In the first systematic study of the LAM lipidome, high levels of four LPCs (C16:0, C18:0, C18:1, and C20:4) were found in the plasma of women with LAM compared with healthy controls, suggesting novel potential biomarkers (64). LPCs are bioactive lipids generated by the activity of phospholipase A (PLA) on the precursor PC (9) and preclinical models of lung cancer showed that those molecules might have a direct role in tumor angiogenesis (65), a condition that, together with lymphangiogenesis, is characteristic also in pulmonary and extrapulmonary LAM (49). Interestingly, the increase of LPCs were also observed in $Tsc2^{-/-}$ mouse embryonic fibroblasts (MEFs), consistently with the hypothesis that tuberin loss might enhance several phospholipid and neutral lipid species. The treatment with the mTOR inhibitors rapamycin or torin1, or the down-regulation of SREBP1 does not suppress LPCs, while the down-regulation of specific PLAs decreases selectively the proliferation of $Tsc2^{-/-}$ MEFs compared with $Tsc2^{+/+}$ MEFs (58). Among PLAs, the accumulation of the PLA2G16 isoform was observed in the lung nodules and in the renal angiomyolipomas of LAM patients, and it was demonstrated that the presence of tuberin negatively regulates PLA2G16 overexpression both *in vitro* and *in vivo*, affecting also the production of prostaglandins, that are critical mediators of chronic inflammation and cancer progression. In the same study, $Tsc2^{-/-}$ MEFs treated with rapamycin or torin1 did not change the expression of PLA2G16, while the PLA inhibitor methyl arachidonyl fluorophosphonate (MAFP) reduced the growth and induced apoptosis on tuberin-deficient cells derived from LAM patient. Interestingly, the tuberin induced-expression in these cells prevents the effects of MAFP treatment (66).

Since mTORC1 hyperactivation causes the reduction of autophagy and induces a metabolic reprogramming of tuberin-deficient cells, a novel therapeutic approach was proposed in a recent study which demonstrates that $Tsc2^{-/-}$ MEFs are sensitive to the treatment with rintaserin in combination with chloroquine, while the impact of those two drugs on $Tsc2^{+/+}$ MEFs is minimal (67). Rintaserin is an inhibitor of the diacylglycerol kinase alpha (DGKA), that induces the formation of the phosphatidic acid from diacylglycerol, ultimately regulating the homeostasis of cell membranes (68). $Tsc2^{-/-}$ MEFs increased the expression and the activity of DGKA, with a consequent 5-fold higher accumulation of phosphatidic acid compared to $Tsc2^{+/+}$ MEFs and a higher capability to uptake nutrients from the extracellular space through macropinocytosis. The treatment with rintaserin blocks macropinocytosis and leads to the accumulation of diacylglycerol, reprogramming the phospholipid metabolism by reducing the storage of lipids in droplets and enhancing the synthesis of phospholipids (67). Interestingly, high levels of DGKA, DGKD, DGKQ, and DGKZ were found in the angiomyolipomas of TSC patients, that often develop LAM as pulmonary manifestation (69). *In vivo*, the injection of tuberin-deficient cells with the downregulation of DGKA does not cause the enlargement of lung alveoli observed in preclinical models of LAM (67, 70).

Taken together, these studies indicate that targeting of key metabolic pathways in lipids synthesis might be novel therapeutic approaches for LAM.

Moreover, lipids can be relevant biomarkers to understand LAM pathogenesis and progression. Indeed, the first evaluation of the LAM serum metabolome showed that there are differences in the metabolic profile when LAM patients were stratified according to menopausal status, lung function, disease burden and disease activity (15). These metabolic abnormalities involved almost exclusively sphingolipids, phospholipids and acylcarnitine fatty acids, reflecting metabolic processes downstream of mTOR. Remarkably, the observation that FEV_1 loss, used as measure of airflow limitation, was related to sphingolipid and acylcarnitine fatty acid metabolism, might reflect the extent of lung parenchyma disruption, resulting in the loss of elastic recoil. In the same way, the airflow obstruction in patients with COPD is associated with glycerophospholipids and sphingolipids metabolites, suggesting a common mechanism involving mTOR hyperactivation (71). Moreover, uncovered changes in glycerophospholipids were observed in patients with sporadic LAM compared with LAM/TSC ones after rapamycin treatment (15).

Finally, the altered lipid metabolism in LAM cells can be exploited to identify novel metabolic imaging biomarkers for the disease. Indeed, the previously reported enhancing in PC levels and neutral lipids in LAM cells (64) allowed to test the *in vivo* uptake of [^{18}F]fluorocholine (FCH) and [^{18}F]fluoroacetate (FACE) to detect tuberin-deficient cells in solid tumors and to monitor the response to rapamycin through dynamic PET (72). [^{18}F]FCH uptake, but not [^{18}F]FACE uptake in tuberin-deficient xenografts was rapamycin-sensitive and *in vitro* study on tuberin-deficient cells indicated that this difference might be due to an accumulation of these two compounds in two different cells compartment. In fact, [^{18}F]FCH is mainly incorporated into lipids, while [^{18}F]FACE enters into mitochondria and can be used as marker of mitochondrial activity, which is not suppressed by rapamycin, confirming a specific metabolic feature of tuberin-deficiency in LAM cells (73).

Conclusion

Regulation of precise lipid species plays a critical role in senescence and senescence-mediated inflammation associated to chronic pulmonary diseases. Aberrant activity of SREBP and its upstream regulator, mTOR, is highly involved in lipotoxicity that induces inflammation and fibrosis in several lung diseases including LAM (**Figure 1**). The evidence of an altered lipid metabolism in tuberin-null cells, e.g., involving LPCs and GD3, and the demonstration of metabolic abnormalities in LAM patient serum, mainly LPCs, sphingolipids, phospholipids and acylcarnitine fatty acids, provides the need to promote the comprehension of lipid dysmetabolism in LAM as novel biomarkers for a metabolic signature for stratifying LAM patients and to suggest useful therapeutic targets, also to associate to mTOR inhibitor rapamycin, for the treatment of LAM.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Acknowledgments

The authors acknowledge support from the University of Milan through the APC initiative.

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.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Agudelo C, Samaha G, Garcia-Arcos I. Alveolar lipids in pulmonary disease. A review. *Lipids Health Dis.* (2020) 19:122. doi: 10.1186/s12944-020-01278-8

2. Horton J, Goldstein J, Brown M. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest.* (2002) 109:1125–31. doi: 10.1172/JCI0215593

3. Walker A, Yang F, Jiang K, Ji J, Watts J, Purushotham A, et al. Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. *Genes Dev.* (2010) 24:1403–17. doi: 10.1101/gad.1901210

4. Im S, Yousef L, Blaschitz C, Liu J, Edwards R, Young S, et al. Linking lipid metabolism to the innate immune response in macrophages through sterol regulatory element binding protein-1a. *Cell Metab.* (2011) 13:540–9. doi: 10.1016/j.cmet.2011.04.001

5. Oishi Y, Spann N, Link V, Muse ED, Strid T, Edillor C, et al. SREBP1 contributes to resolution of pro-inflammatory TLR4 signaling by reprogramming fatty acid metabolism. *Cell Metab.* (2017) 25:412–27. doi: 10.1016/j.cmet.2016.11.009

6. Plantier L, Besnard V, Xu Y, Ikegami M, Wert S, Hunt A, et al. Activation of sterol-response element-binding proteins (SREBP) in alveolar type II cells enhances lipogenesis causing pulmonary lipotoxicity. *J Biol Chem.* (2012) 287:10099–114. doi: 10.1074/jbc.M111.303669

7. Shichino S, Ueha S, Hashimoto S, Otsuji M, Abe J, Tsukui T, et al. Transcriptome network analysis identifies protective role of the LXR/SREBP-1c axis in murine pulmonary fibrosis. *JCI Insight.* (2019) 4:e122163. doi: 10.1172/jci.insight.122163

8. Guo C, Chi Z, Jiang D, Xu T, Yu W, Wang Z, et al. Cholesterol homeostatic regulator SCAP-SREBP2 integrates NLRP3 inflammasome activation and cholesterol biosynthetic signaling in macrophages. *Immunity.* (2018) 49:842.e–56.e. doi: 10.1016/j.immuni.2018.08.021

9. Quinn W, Wan M, Shewale S, Gelfer R, Rader D, Birnbaum M, et al. mTORC1 stimulates phosphatidylcholine synthesis to promote triglyceride secretion. *J Clin Invest.* (2017) 127:4207–15. doi: 10.1172/JCI96036

10. Yi J, Zhu J, Wu J, Thompson C, Jiang X. Oncogenic activation of PI3K-AKT-mTOR signaling suppresses ferroptosis via SREBP-mediated lipogenesis. *Proc Natl Acad Sci.* (2020) 117:31189–97. doi: 10.1073/pnas.2017152117

11. Meraj R, Wikenheiser-Brokamp K, Young L, McCormack F. Lymphangioleiomyomatosis: new concepts in pathogenesis, diagnosis, and treatment. *Semin Respir Crit Care Med.* (2012) 33:486–97. doi: 10.1055/s-0032-1325159

12. Volpi A, Sala G, Lesma E, Labriola F, Righetti M, Alfano R, et al. Tuberous sclerosis complex: new insights into clinical and therapeutic approach. *J Nephrol.* (2019) 32:355–63. doi: 10.1007/s40620-018-0547-6

13. McCarthy C, Gupta N, Johnson S, Yu J, McCormack F. Lymphangioleiomyomatosis: pathogenesis, clinical features, diagnosis, and management. *Lancet Respir Med.* (2021) 9:1313–27. doi: 10.1016/S2213-2600(21)00228-9

14. Kennedy B, Lamming D. The mechanistic target of rapamycin: the grand conductor of metabolism and aging. *Cell Metab.* (2016) 23:990–1003. doi: 10.1016/j.cmet.2016.05.009

15. Bottolo L, Miller S, Johnson S. Sphingolipid, fatty acid and phospholipid metabolites are associated with disease severity and mTOR inhibition in lymphangioleiomyomatosis. *Thorax.* (2020) 75:679–88. doi: 10.1136/thoraxjnl-2019-214241

16. Sohrabi Y, Reinecke H, Godfrey R. Altered cholesterol and lipid synthesis mediates hyperinflammation in COVID-19. *Trends Endocrinol Metab.* (2021) 32:132–4. doi: 10.1016/j.tem.2021.01.001

17. Yuan S, Chu H, Chan J, Ye Z, Wen L, Yan B, et al. SREBP-dependent lipidomic reprogramming as a broad-spectrum antiviral target. *Nat Commun.* (2019) 10:120. doi: 10.1038/s41467-018-08015-x

18. Lugg S, Scott A, Parekh D, Naidu B, Thickett D. Cigarette smoke exposure and alveolar macrophages: mechanisms for lung disease. *Thorax.* (2022) 77:94–101. doi: 10.1136/thoraxjnl-2020-216296

19. Chakinala R, Khatri A, Gupta K, Koike K, Epelbaum O. Sphingolipids in COPD. *Eur Respir Rev.* (2019) 28:190047.

20. Suryadevara V, Ramchandran R, Kamp D, Natarajan V. Lipid mediators regulate pulmonary fibrosis: potential mechanisms and signaling pathways. *Int J Mol Sci.* (2020) 21:4257. doi: 10.3390/ijms21124257

21. Letsiou E, Htwe Y, Dudek S. Secretory phospholipase A2 enzymes in acute lung injury. *Cell Biochem Biophys.* (2021) 79:609–17. doi: 10.1007/s12013-021-01003-x

22. Zheng Y, Ning P, Luo Q, He Y, Yu X, Liu X, et al. Inflammatory responses relate to distinct bronchoalveolar lavage lipidome in community-acquired pneumonia patients: a pilot study. *Respir Res.* (2019) 20:82. doi: 10.1186/s12931-019-1028-8

23. Elborn J. Cystic fibrosis. *Lancet.* (2016) 388:2519–31. doi: 10.1016/S0140-6736(16)00576-6

24. Zulueta A, Peli V, Dei Cas M, Colombo M, Paroni R, Falleni M, et al. Inflammatory role of extracellular sphingolipids in cystic fibrosis. *Int J Biochem Cell Biol.* (2019) 116:105622. doi: 10.1016/j.biocel.2019.105622

25. Teichgräber V, Ulrich M, Endlich N, Riethmüller J, Wilker B, de Oliveira–Munding C, et al. Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. *Nat Med.* (2008) 14:382–91. doi: 10.1038/nm1748

26. Chiurchiù V, Leuti A, Maccarrone M. Bioactive lipids and chronic inflammation: managing the fire within. *Front Immunol.* (2018) 9:38. doi: 10.3389/fimmu.2018.00038

27. Mingione A, Dei Cas M, Bonezzi F, Caretti A, Piccoli M, Anastasia L, et al. Inhibition of sphingolipid synthesis as a phenotype-modifying therapy in cystic fibrosis. *Cell Physiol Biochem.* (2020) 54:110–25. doi: 10.33594/000000208

28. di Micco R, Krizhanovsky V, Baker D, d'Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. *Nat Rev Mol Cell Biol.* (2021) 22:75–95. doi: 10.1038/s41580-020-00314-w

29. Barnes P, Baker J, Donnelly L. Cellular senescence as a mechanism and target in chronic lung diseases. *Am J Respir Crit Care Med.* (2019) 200:556–64. doi: 10.1164/rccm.201810-1975TR

30. Summer R, Shaghaghi H, Schriner D, Roque W, Sales D, Cuevas-Mora K, et al. Activation of the mTORC1/PGC-1 axis promotes mitochondrial biogenesis and induces cellular senescence in the lung epithelium. *Am J Physiol Lung Cell Mol Physiol.* (2019) 316:L1049–60. doi: 10.1152/ajplung.00244.2018

31. Saito M, Mitani A, Ishimori T, Miyashita N, Isago H, Mikami Y, et al. Active mTOR in lung epithelium promotes epithelial–mesenchymal transition and enhances lung fibrosis. *Am J Respir Cell Mol Biol.* (2020) 62:699–708. doi: 10.1165/rcmb.2019-0255OC

32. Schindler V, Alhamdan F, Preußer C, Hintz L, Alashkar Alhamwe B, Nist A, et al. Side-directed release of differential extracellular vesicle-associated microRNA profiles from bronchial epithelial cells of healthy and asthmatic subjects. *Biomedicines.* (2022) 10:622. doi: 10.3390/biomedicines10030622

33. Iglesias-Bartolome R, Patel V, Cotrim A, Leelahavanichkul K, Molinolo A, Mitchell J, et al. mTOR inhibition prevents epithelial stem cell senescence and protects from radiation-induced mucositis. *Cell Stem Cell.* (2012) 11:401–14. doi: 10.1016/j.stem.2012.06.007

34. Flor A, Wolfgeher D, Wu D, Kron SJ. A signature of enhanced lipid metabolism, lipid peroxidation and aldehyde stress in therapy-induced senescence. *Cell Death Discov.* (2017) 3:17075. doi: 10.1038/cddiscovery.2017.75

35. Hamsanathan S, Gurkar A. Lipids as regulators of cellular senescence. *Front Physiol.* (2022) 13:796850. doi: 10.3389/fphys.2022.796850

36. Dagouassat M, Gagliolo J, Chrusciel S, Bourin M, Duprez C, Caramelle P, et al. The cyclooxygenase-2–prostaglandin E 2 pathway maintains senescence of chronic obstructive pulmonary disease fibroblasts. *Am J Respir Crit Care Med.* (2013) 187:703–14. doi: 10.1164/rccm.201208-1361OC

37. Ruan Y, Zhou W, Chan H. Regulation of smooth muscle contraction by the epithelium: role of prostaglandins. *Physiology.* (2011) 26:156–70. doi: 10.1152/physiol.00036.2010

38. Wiley C, Brumwell A, Davis S, Jackson J, Valdovinos A, Calhoun C, et al. Secretion of leukotrienes by senescent lung fibroblasts promotes pulmonary fibrosis. *JCI Insight.* (2019) 4:e130056. doi: 10.1172/jci.insight.130056

39. Johnson S, Taveira-DaSilva A, Moss J. Lymphangioleiomyomatosis. *Clin Chest Med.* (2016) 37:389–403. doi: 10.1016/j.ccm.2016.04.002

40. Short J, Houston K, Dere R, Cai S, Kim J, Johnson C, et al. AMP-Activated protein kinase signaling results in cytoplasmic sequestration of p27. *Cancer Res.* (2008) 68:6496–506. doi: 10.1158/0008-5472.CAN-07-5756

41. Lacher M, Pincheira R, Zhu Z, Camoretti-Mercado B, Matli M, Warren R, et al. Rheb activates AMPK and reduces p27Kip1 levels in Tsc2-null cells via mTORC1-independent mechanisms: implications for cell proliferation and tumorigenesis. *Oncogene.* (2010) 29:6543–56. doi: 10.1038/onc.2010.393

42. Ben-Sahra I, Hoxhaj G, Ricoult S, Asara J, Manning B. mTORC1 induces purine synthesis through control of the mitochondrial tetrahydrofolate cycle. *Science.* (2016) 351:728–33. doi: 10.1126/science.aad0489

43. Ricoult S, Yecies J, Ben-Sahra I, Manning B. Oncogenic PI3K and K-Ras stimulate de novo lipid synthesis through mTORC1 and SREBP. *Oncogene.* (2016) 35:1250–60. doi: 10.1038/onc.2015.179

44. Clements D, Dongre A, Krymskaya V, Johnson S. Wild type mesenchymal cells contribute to the lung pathology of lymphangioleiomyomatosis. *PLoS One.* (2015) 10:e0126025. doi: 10.1371/journal.pone.0126025

45. Kumasaka T, Seyama K, Mitani K, Sato T, Souma S, Kondo T, et al. Lymphangiogenesis in lymphangioleiomyomatosis. *Am J Surg Pathol.* (2004) 28:1007–16. doi: 10.1097/01.pas.0000126859.70814.6d

46. Terraneo S, Lesma E, Ancona S, Imeri G, Palumbo G, Torre O, et al. Exploring the role of matrix metalloproteinases as biomarkers in sporadic lymphangioleiomyomatosis and tuberous sclerosis complex. A pilot study. *Front Med.* (2021) 8:605909. doi: 10.3389/fmed.2021.605909

47. Dodd K, Yang J, Shen M, Sampson J, Tee A. mTORC1 drives HIF-1α and VEGF-A signalling via multiple mechanisms involving 4E-BP1, S6K1 and STAT3. *Oncogene.* (2015) 34:2239–50. doi: 10.1038/onc.2014.164

48. Yao J, Taveira-DaSilva A, Jones A, Julien-Williams P, Stylianou M, Moss J. sustained effects of sirolimus on lung function and cystic lung lesions in lymphangioleiomyomatosis. *Am J Respir Crit Care Med.* (2014) 190:1273–82. doi: 10.1164/rccm.201405-0918OC

49. McCormack F, Travis W, Colby T, Henske E, Moss J. Lymphangioleiomyomatosis. *Am J Respir Crit Care Med.* (2012) 186:1210–2. doi: 10.1164/rccm.201205-0848OE

50. Bernardelli C, Ancona S, Lazzari M, Lettieri A, Selvaggio P, Massa V, et al. LAM Cells as potential drivers of senescence in lymphangioleiomyomatosis microenvironment. *Int J Mol Sci.* (2022) 23:7040. doi: 10.3390/ijms23137040

51. Yao C, Guan X, Carraro G, Parimon T, Liu X, Huang G, et al. Senescence of alveolar type 2 cells drives progressive pulmonary fibrosis. *Am J Respir Crit Care Med.* (2021) 203:707–17. doi: 10.1164/rccm.202004-1274OC

52. Ancona S, Orpianesi E, Bernardelli C, Chiaramonte E, Chiaramonte R, Terraneo S, et al. Differential modulation of matrix metalloproteinases-2 and-7 in lam/tsc cells. *Biomedicines.* (2021) 9:1760. doi: 10.3390/biomedicines9121760

53. Wang J, Filippakis H, Hougard T, Du H, Ye C, Liu H, et al. Interleukin-6 mediates PSAT1 expression and serine metabolism in TSC2-deficient cells. *Proc Natl Acad Sci.* (2021) 118:e2101268118. doi: 10.1073/pnas.2101268118

54. Dongre A, Clements D, Fisher A, Johnson S. Cathepsin K in lymphangioleiomyomatosis. *Am J Pathol.* (2017) 187:1750–62. doi: 10.1016/j.ajpath.2017.04.014

55. Ortiz-Montero P, Londoño-Vallejo A, Vernot J. Senescence-associated IL-6 and IL-8 cytokines induce a self- and cross-reinforced senescence/inflammatory milieu strengthening tumorigenic capabilities in the MCF-7 breast cancer cell line. *Cell Commun Signal.* (2017) 15:17. doi: 10.1186/s12964-017-0172-3

56. Cesta M, Zippoli M, Marsiglia C, Gavioli E, Mantelli F, Allegretti M, et al. The role of interleukin-8 in lung inflammation and injury: implications for the management of COVID-19 and hyperinflammatory acute respiratory distress syndrome. *Front Pharmacol.* (2022) 12:808797. doi: 10.3389/fphar.2021.808797

57. Walters H, Cox L. mTORC inhibitors as broad-spectrum therapeutics for age-related diseases. *Int J Mol Sci.* (2018) 19:2325. doi: 10.3390/ijms19082325

58. Dias S, Soares V, Ferreira A, Sacramento C, Fintelman-Rodrigues N, Temerozo J, et al. Lipid droplets fuel SARS-CoV-2 replication and production of inflammatory mediators. *PLoS Pathog.* (2020) 16:e1009127. doi: 10.1371/journal.ppat.1009127

59. Shimizu H, Ito A, Sakurada K, Nakamura J, Tanaka K, Komatsu M, et al. AK106-001616, a potent and selective inhibitor of cytosolic phospholipase A 2: in vivo efficacy for inflammation, neuropathic pain, and pulmonary fibrosis. *J Pharmacol Exp Ther.* (2019) 369:511–22. doi: 10.1124/jpet.118.255034

60. Mossmann D, Park S, Hall M. mTOR signalling and cellular metabolism are mutual determinants in cancer. *Nat Rev Cancer.* (2018) 18:744–57. doi: 10.1038/s41568-018-0074-8

61. Gilbert E, Eby J, Hammer A, Klarquist J, Christensen D, Barfuss A, et al. Positioning ganglioside D3 as an immunotherapeutic target in lymphangioleiomyomatosis. *Am J Pathol.* (2013) 183:226–34. doi: 10.1016/j.ajpath.2013.04.002

62. Park J, Wu D, Prendes M, Lu S, Ragupathi G, Schrantz N, et al. Fine specificity of natural killer T cells against GD3 ganglioside and identification of GM3 as an inhibitory natural killer T-cell ligand. *Immunology.* (2008) 123:145–55. doi: 10.1111/j.1365-2567.2007.02760.x

63. Zhang H, Huang J, Düvel K, Boback B, Wu S, Squillace R, et al. Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. *PLoS One.* (2009) 4:e6189. doi: 10.1371/journal.pone.0006189

64. Priolo C, Ricoult S, Khabibullin D, Filippakis H, Yu J, Manning B, et al. Tuberous sclerosis complex 2 loss increases lysophosphatidylcholine synthesis in lymphangioleiomyomatosis. *Am J Respir Cell Mol Biol.* (2015) 53:33–41. doi: 10.1165/rcmb.2014-0379RC

65. Linkous A, Yazlovitskaya E, Hallahan D. Cytosolic phospholipase A2 and lysophospholipids in tumor angiogenesis. *JNCI J Natl Cancer Inst.* (2010) 102:1398–412. doi: 10.1093/jnci/djq290

66. Li C, Zhang E, Sun Y, Lee P, Zhan Y, Guo Y, et al. Rapamycin-insensitive up-regulation of adipocyte phospholipase A2 in tuberous sclerosis and lymphangioleiomyomatosis. *PLoS One.* (2014) 9:e104809. doi: 10.1371/journal.pone.0104809

67. Kovalenko A, Sanin A, Kosmas K, Zhang L, Wang J, Akl E, et al. Therapeutic targeting of DGKA-mediated macropinocytosis leads to phospholipid reprogramming in tuberous sclerosis complex. *Cancer Res.* (2021) 81:2086–100. doi: 10.1158/0008-5472.CAN-20-2218

68. Boroda S, Niccum M, Raje V, Purow B, Harris T. Dual activities of ritanserin and R59022 as DGKα inhibitors and serotonin receptor antagonists. *Biochem Pharmacol.* (2017) 123:29–39. doi: 10.1016/j.bcp.2016.10.011

69. Martin K, Zhou W, Bowman M, Shih J, Au K, Dittenhafer-Reed K, et al. The genomic landscape of tuberous sclerosis complex. *Nat Commun.* (2017) 8:15816. doi: 10.1038/ncomms15816

70. Bernardelli C, Chiaramonte E, Ancona S, Sirchia S, Cerri A, Lesma E. Primary TSC2-/meth cells induce follicular neogenesis in an innovative TSC mouse model. *Int J Mol Sci.* (2022) 23:9713. doi: 10.3390/ijms23179713

71. Bowler R, Jacobson S, Cruickshank C, Hughes G, Siska C, Ory D, et al. Plasma sphingolipids associated with chronic obstructive pulmonary disease phenotypes. *Am J Respir Crit Care Med.* (2015) 191:275–84. doi: 10.1164/rccm.201410-1771OC

72. Verwer E, Kavanagh T, Mischler W, Feng Y, Takahashi K, Wang S, et al. [18F]Fluorocholine and [18F]Fluoroacetate PET as imaging biomarkers to assess phosphatidylcholine and mitochondrial metabolism in preclinical models of TSC and LAM. *Clin Cancer Res.* (2018) 24:5925–38. doi: 10.1158/1078-0432.CCR-17-3693

73. Parkhitko A, Priolo C, Coloff J, Yun J, Wu J, Mizumura K, et al. Autophagy-dependent metabolic reprogramming sensitizes TSC2-deficient cells to the antimetabolite 6-aminonicotinamide. *Mol Cancer Res.* (2014) 12:48–57. doi: 10.1158/1541-7786.MCR-13-0258-T

74. Tasat D, Yakisich J. Rationale for the use of sphingosine analogues in COVID-19 patients. *Clin Med.* (2021) 21:e84–7. doi: 10.7861/clinmed.2020-0309

75. Pandolfi R, Barreira B, Moreno E, Lara-Acedo V, Morales-Cano D, Martínez-Ramas A, et al. Role of acid sphingomyelinase and IL-6 as mediators of endotoxin-induced pulmonary vascular dysfunction. *Thorax.* (2017) 72:460–71. doi: 10.1136/thoraxjnl-2015-208067

76. Huang Z, Liu H, Zhang X, Wen G, Zhu C, Zhao Y, et al. Transcriptomic analysis of lung tissues after hUC-MSCs and FTY720 treatment of lipopolysaccharide-induced acute lung injury in mouse models. *Int Immunopharmacol.* (2018) 63:26–34. doi: 10.1016/j.intimp.2018.06.036

77. Zhang Z, Li W, Heng Z, Zheng J, Li P, Yuan X, et al. Combination therapy of human umbilical cord mesenchymal stem cells and FTY720 attenuates acute lung injury induced by lipopolysaccharide in a murine model. *Oncotarget.* (2017) 8:77407–14. doi: 10.18632/oncotarget.20491

78. Caretti A, Bragonzi A, Facchini M, de Fino I, Riva C, Gasco P, et al. Anti-inflammatory action of lipid nanocarrier-delivered myriocin: therapeutic potential in cystic fibrosis. *Biochim Biophys Acta Gen Subj.* (2014) 1840:586–94. doi: 10.1016/j.bbagen.2013.10.018

79. Becker K, Riethmüller J, Lüth A, Döring G, Kleuser B, Gulbins E. Acid sphingomyelinase inhibitors normalize pulmonary ceramide and inflammation in cystic fibrosis. *Am J Respir Cell Mol Biol.* (2010) 42:716–24. doi: 10.1165/rcmb.2009-0174OC

80. Dechecchi M, Nicolis E, Mazzi P, Cioffi F, Bezzerri V, Lampronti I, et al. Modulators of sphingolipid metabolism reduce lung inflammation. *Am J Respir Cell Mol Biol.* (2011) 45:825–33. doi: 10.1165/rcmb.2010-0457OC

81. Kou L, Kou P, Luo G, Wei S. Progress of statin therapy in the treatment of idiopathic pulmonary fibrosis. *Oxid Med Cell Longev.* (2022) 2022:1–18. doi: 10.1155/2022/6197219

82. Han W, Li J, Tang H, Sun L. Treatment of obese asthma in a mouse model by simvastatin is associated with improving dyslipidemia and decreasing leptin level. *Biochem Biophys Res Commun.* (2017) 484:396–402. doi: 10.1016/j.bbrc.2017.01.135

83. Kambayashi T, Deshpande D. The role of diacylglycerol kinases in allergic airway disease. *Curr Opin Pharmacol.* (2020) 51:50–8. doi: 10.1016/j.coph.2020.07.008

84. Karandashova S, Kummarapurugu A, Zheng S, Chalfant C, Voynow J. Neutrophil elastase increases airway ceramide levels via upregulation of serine palmitoyltransferase. *Am J Physiol Lung Cell Mol Physiol.* (2018) 314:L206–14. doi: 10.1152/ajplung.00322.2017

85. Mroz R, Lisowski P, Tycinska A, Bierla J, Trzeciak P, Minarowski L, et al. Anti-inflammatory effects of atorvastatin treatment in chronic obstructive pulmonary disease. A controlled pilot study. *J Physiol Pharmacol.* (2015) 66:111–28.

86. Blamoun A, Batty G, DeBari V, Rashid A, Sheikh M, Khan M. Statins may reduce episodes of exacerbation and the requirement for intubation in patients with COPD: evidence from a retrospective cohort study. *Int J Clin Pract.* (2008) 62:1373–8. doi: 10.1111/j.1742-1241.2008.01731.x

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 14 March 2023
DOI 10.3389/fmed.2023.1110977

OPEN ACCESS

EDITED BY
Shuibang Wang,
National Institutes of Health (NIH), United States

REVIEWED BY
Gunnar N. Hillerdal,
Karolinska University Hospital, Sweden
Dong Xie,
Tongji University, China

*CORRESPONDENCE
Catherine Quantin
✉ catherine.quantin@chu-dijon.fr

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 29 November 2022
ACCEPTED 27 February 2023
PUBLISHED 14 March 2023

CITATION
Bernard A, Cottenet J, Pages P-B and Quantin C (2023) Is there variation between hospitals within each region in postoperative mortality for lung cancer surgery in France? A nationwide study from 2013 to 2020. *Front. Med.* 10:1110977. doi: 10.3389/fmed.2023.1110977

Is there variation between hospitals within each region in postoperative mortality for lung cancer surgery in France? A nationwide study from 2013 to 2020

Alain Bernard[1], Jonathan Cottenet[2], Pierre-Benoit Pages[1] and Catherine Quantin[2,3]*

[1]Department of Thoracic and Cardiovascular Surgery, Dijon University Hospital, Dijon, France, [2]Service de Biostatistiques et d'Information Médicale (DIM), CHU Dijon Bourgogne, INSERM, Université de Bourgogne, CIC 1432, Module Épidémiologie Clinique, Dijon, France, [3]Université Paris-Saclay, Université de Versailles Saint-Quentin-en-Yvelines (UVSQ), Inserm, Centre de recherche en Epidémiologie et Santé des Populations (CESP), Villejuif, France

Introduction: The practice of thoracic surgery for lung cancer is subject to authorization in France. We evaluated the performance of hospitals using 30-day post-operative mortality as a quality indicator, estimating its distribution within each region and measuring its variability between regions.

Material and methods: All data for patients who underwent pulmonary resection for lung cancer in France (2013–2020) were collected from the national hospital administrative database. Thirty-day mortality was defined as any patient who died in hospital (including transferred patients) within the first 30 days after the operation and those who died later during the initial hospitalization. The Standardized Mortality ratio (SMR) was the smoothed, adjusted, hospital-specific mortality rate divided by the expected mortality. To describe the variation in hospital mortality between hospitals in each region, we used different commonly used indicators of variation such as coefficients of variation (CV), interquartile interval or range (IQR), extreme ratio, and systematic component of variance (SCV).

Results: In 2013–2020, 87,232 patients underwent lung resection for cancer in France. The number of deaths was 2,537, a rate of 2.91%. The median SMR of 199 hospitals was 0.99 with an IQR of 0.86 to 1.18 and a CV of 0.25. Among the regions that had the most hospitals performing lung resections for cancer, the extreme ratio was >2, which means that the maximum value is twice as high as the minimum value. The SCV between hospitals was >10 for two of these regions, which is considered indicative of very high variation. For the other regions (with few hospitals performing lung resections for cancer), the variation between hospitals was lower. Globally, the variability between regions concerning the SMR was moderate, 6% of the variance was due to differences across regions. On the contrary, the hospital volume was significantly related to the SMR ($p = 0.003$) with a negative linear trend, whatever the region.

Conclusion: This work shows significant differences in the practices of the various hospitals within regions. However, overall, the variability in the 30-day mortality rate between regions was moderate. Our findings raises questions regarding the regionalization of major surgical procedures in France.

KEYWORDS

lung cancer surgery, standardized mortality rate, variation, region, quality of care

Introduction

The practice of lung cancer surgery in France has been the subject of several publications using the national hospital (PMSI) medico-administrative database (1–3). Although the number of hospitals performing this surgery was reduced following the implementation of the French National Cancer Plan (4), the number still remains high. The corollary of this situation is that many surgical teams have relatively low surgical activity (1, 2). The number of lung resections performed per year, called hospital volume, is one of the indicators influencing post-operative mortality (2, 5). Post-operative mortality is one of the quality indicators of lung cancer surgery, as recently demonstrated by Fernandez et al. (6).

Thoracic surgery for lung cancer (LC) is a surgical act that is subject to authorization in France, and this authorization is granted to hospitals by the regional authorities (4). There is yet to be an evaluation of the quality of care of hospitals in different regions in France for this type of surgery.

Our work consisted of evaluating the performance of hospitals using one quality indicator, 30-day post-operative mortality, and then estimating the distribution of this indicator within each region. Our secondary objective was to measure the variability of this indicator from one region to another.

Materials and methods

Data source and study population

All data for patients who underwent pulmonary resection for LC in France from January 2013 to December 2020 were collected from the national hospital administrative database. This database, called PMSI for "Programme de Médicalisation des Systèmes d'Information," was inspired by the US Medicare system. The reliability and validity of PMSI data have already been assessed (7). Routinely collected medical information includes the principal diagnosis, secondary diagnoses and procedures performed. Diagnoses identified during the hospital stay are coded according to the International Classification of Diseases, tenth revision (ICD-10) (8). We selected patients for whom a diagnosis of primary lung cancer was coded as the principal discharge diagnosis (all codes C34). Procedures are coded according to the CCAM (Classification Commune des Actes Médicaux). For all patients, LC was confirmed by pathology analyses according to the 2004 World Health Organization classification of LC (7). Surgery-related variables included the surgical approach [thoracotomy, video assisted thoracic surgery (VATS) or robot-assisted surgery], the type of resection (limited resection, lobectomy, bi-lobectomy and pneumonectomy), bronchioplasty, and the extent of the pulmonary resection (to the chest wall, the left atrium, the carina, the diaphragm, and the superior vena cava).

Patient consent was not required. Ethics approval for use of this database was obtained from the French National Commission for Data protection (*Commission Nationale de l'Informatique et des Libertés*: No 1576793), and this study adhered to the tenets of the Declaration of Helsinksi.

Patient characteristics

Patient age and sex were included as baseline demographic characteristics. From the national administrative database, we included the following comorbidities: pulmonary disease (chronic bronchitis, emphysema), heart disease (coronary artery disease, cardiac arrhythmia, congestive heart failure, valvular heart disease, pulmonary artery hypertension, pulmonary embolism), peripheral vascular disease, liver disease, cerebrovascular events, neurological diseases (hemiplegia or paraplegia), renal disease, hematologic disease (leukemia, lymphoma), metabolic disease, anemia, other therapies (preoperative chemotherapy, steroids), and infectious disease. We also calculated the modified Charlson Comorbidity Index (CCI) as a marker of comorbidity (9).

Region and hospital characteristics

Metropolitan France comprises 13 regions: Auvergne Rhones-Alpes (ARA), Bourgogne Franche-Comté (BFC), Bretagne (BRE), Centre Val-de-Loire (CVL), Corse (COR), Grand-Est (GE), Hauts-de-France (HdF), L'ile de France (IdF), Normandie (NOR), Nouvelle Aquitaine (NA), Occitanie (OC), Pays de Loire (PdL) and Provence Alpes Côte d'Azur (PACA). For each hospital within a region, we determined the number of times each type of pulmonary resection was performed from January 1, 2013, to December 31, 2020. Hospital volume was defined as the median number of procedures performed per year. For the purpose of the analysis, the hospital volume was represented as a continuous variable that was transformed into a logarithm.

Outcome measurements

Thirty-day mortality for a patient was defined as the occurrence of death in hospital (including transferred patients) either within the first 30-days after the operation or later on during the initial hospitalization. In other words, in the calculation of 30-day in-hospital mortality, we included deaths that occurred during the surgical stay, as well as any death that occurred during a subsequent hospitalization within 30-days of admission for the initial surgery.

Statistical analysis

To obtain a reliable measure of hospital quality, we used the hierarchical logistic regression model with "shrinkage" estimators. The adjusted Standardized Mortality ratio (SMR) was determined as the smoothed, adjusted, hospital-specific mortality rate divided by the expected mortality. The expected mortality was estimated from a fixed-effects component of hierarchical logistic model regression (10), using comorbidities, age, sex, modified CCI score, and the type of pulmonary resection (the approach and extent of resection) as adjustment factors. The hierarchical logistic regression model was developed using BUGS

software (Bayesian Inference Using Gibbs Sampling, version 0.60; MRC Biostatistics Unit, Cambridge, United Kingdom). The credible 95% probability interval (PI) for each provider was then estimated.

In addition, we constructed funnel plots to determine outliers for 30-day mortality according to Spiegelhalter's methodology (11).

We used several methods to describe between-hospital differences in patient characteristics, the different procedures and the SMR of the hospitals within each region. Two groups of statistics of variation are commonly used: those that describe the distribution of rates, such as coefficients of variation (CV), interquartile, interval or range (IQR), extreme ratio, and those that use differences between expected and observed cases, such as the systematic component of variation (SCV) (12). The CV is the ratio of the standard deviation to the mean and measures how the data spreads around the average. The higher the coefficient of variation, the greater the level of dispersion around the mean. If the coefficient of variation is >1, it shows relatively high variability in the data sets. The IQR defines the interval or the range between the 1st and the 3rd quartile of the distribution, and measures how the data spreads around the average. The wider the range, the greater the level of dispersion around the mean. The extreme ratio corresponds to the ratio between the maximum and minimum values. An extreme ratio >2 implies that the maximum value is twice as high as the minimum value. Finally, SCV is the variation arising from the differences between the independent variable. A SCV >10 can be considered indicative of very high variation.

To estimate the potential influence of hospital volume on the SMR, we also studied the relationship between hospital volume and SMR by calculating a linear trend.

To estimate the variability of the SMR between regions, we used the intraclass correlation coefficient (ICC), which was calculated by fitting a multilevel regression model with a fixed coefficient for hospital volume and random intercept for the region.

The calculations for the hierarchical and multilevel logistics regression models were carried out using STATA 14 software (StataCorp, College Station, Tex), and for the full Bayesian analysis we used the R2jags module of R software (http://www.r-project.org).

Results

From 2013 to 2020, 87,232 patients underwent lung resection for cancer in France. The number of deaths was 2,537, resulting in a death rate of 2.91%. The variations in patient characteristics between the 199 hospitals in the 13 French regions is reported in Table 1. Regarding demographics, the median rate of female patients was 0.33, with an IQR of [0.28–0.37], and the median age was 65 years with IQR of [64–66 years] (Table 1). The pneumonectomy and bilobectomy rates varied highly across hospitals, resulting in a CV of 1.7 and 2.09, respectively (Table 1). Hospitals in the different regions had a VATS or robot-assisted surgery rate ranging from 0.075 to 0.47 with a CV of 0.89 (Table 1). The median hospital volume was 37, with an IQR of 9 to 87 procedures per year and a CV of 1.49. Figure 1 illustrates the high variability in hospital volume across the regions.

TABLE 1 Between hospital variation in the characteristics of patients undergoing lung cancer surgery in France, January 2013 to December 2020.

	Mean	Median	IQR	cv
Gender				
Female	0.31	0.33	0.28–0.37	0.53
Age (years)	65	65	64–66	0.09
Comorbidities				
Pulmonary disease	0.36	0.31	0.2–0.435	0.68
Heart disease	0.18	0.15	0.085–0.22	0.97
Peripheral vascular disease	0.087	0.07	0.02–0.13	1.18
Liver disease	0.007	0.01	0–0.01	1.47
Neurological disease	0.05	0.03	0.007–0.06	1.9
Metabolic disease	0.11	0.12	0.07–0.14	0.85
Renal disease	0.02	0.02	0–0.03	0.92
Anemia	0.15	0.10	0.045–0.19	1.22
Hematological disease	0.05	0.024	0.006–0.055	2.22
Infectious disease	0.005	0.0025	0–0.007	1.54
Other treatment	0.09	0.044	0.006–0.13	1.51
Modified CCI score				
1	0.098	0.07	0.017–0.143	1.32
2	0.113	0.085	0.05–0.13	1.28
≥3	0.39	0.38	0.24–0.5	0.62
Type of pulmonary resection				
Lobectomy	0.65	0.73	0.62–0.78	0.4
Bilobectomy	0.04	0.03	0.01–0.04	2.09
Pneumonectomy	0.075	0.06	0.03–0.086	1.7
Surgery approach				
VATS or Robot-assisted	0.31	0.24	0.075–0.47	0.89
Extended resection	0.13	0.06	0.014–0.13	1.52
30-day mortality rate	0.06	0.03	0.014–0.04	2.57

Cv, coefficient of variation (=standard deviation/mean); IQR, interquartile interval (1^{st} quartile – 3^{rd} quartile); CCI, Charlson Comorbidity Index. For the line regarding female gender, we observed that the average rate of women among the 199 hospitals was 31%, with a median of 33%. The IQR means that 50% of rate of women among these hospitals lie between 28 and 37%. Finally, the standard deviation of the rate of women is 53% the size of the mean (CV = 0.53).

Standardized mortality rate

In order to calculate the SMR, the model used to obtain the expected 30-day mortality included 16 comorbidities, age, sex, modified CCI score, and the type of pulmonary resection (the approach and extent of resection). The reliability of 30-day mortality was 0.43 with 95% confidence interval (CI) of 0.32 to 0.53. The median SMR of 199 hospitals was 0.99 with an IQR of 0.86 to 1.18 and a CV of 0.25.

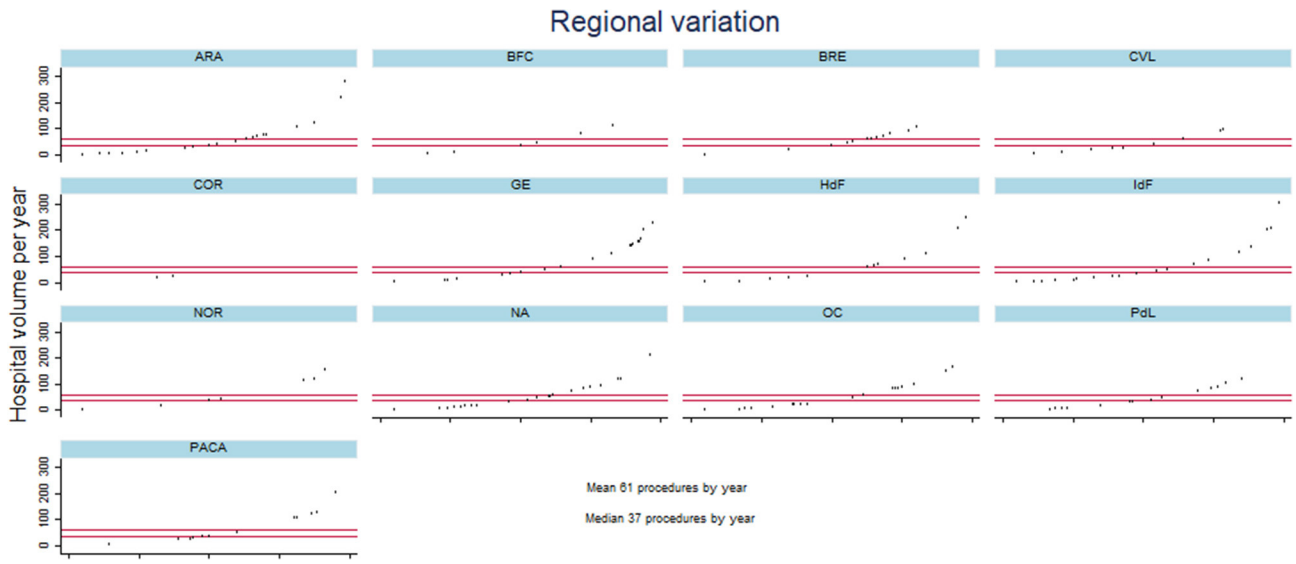

FIGURE 1
Description of hospital volume per year for the different hospitals in each French region from January 2013 to December 2020. Metropolitan France comprises 13 regions: ARA, Auvergne Rhones-Alpes; BFC, Bourgogne Franche-Comté; BRE, Bretagne; CVL, Centre Val-de-Loire; COR, Corse; GE, Grand-Est; HdF, Hauts-de-France; IdF, L'ile de France; NOR, Normandie; NA, Nouvelle Aquitaine; OC, Occitanie; PdL, Pays de Loire; and PACA, Provence Alpes Côte d'Azur. The X axis of each figure represents the different hospitals per region and the Y axis the hospital volume.

Identification of quality-of-care outliers

The funnel plot for the SMR describing hospital performance is displayed in Figure 2. It shows that out of 199 hospitals, 13 lie below the lower limit of the central 95% region, indicating performance that was better than expected. The thirteen hospitals that lie above the upper limit performed significantly worse than expected.

Regional variation

Out of the 13 regions, four regions (COR, BFC, CVL and NOR) had less than ten hospitals performing lung resections for cancer, five regions (HdF, BRE, PdL, PACA and OC) had between 10 and 20 hospitals, and four regions (GE, NA, IdF and ARA) had between twenty and thirty hospitals.

The variation in the SMR between hospitals in different regions is reported in Table 2, which shows the following measures: mean, CV, extreme ratio, interquartile ratio and SCV. Amongst the regions with the highest number of hospitals performing lung resections for cancer, the extreme ratio (i.e., the ratio of the highest SMR to the lowest SMR) was >2. On the other hand, the SCV was >10 in four regions (ARA, IdF, NOR, and PdL), which can be considered indicative of very high variation in these regions. For the OC region, the SCV was 4.9, while the extreme ratio was 4.45. Some regions with few hospitals, such as BRE, CVL, NOR and PdL had a high variability with an extreme ratio around 2. For the other regions, the variation between hospitals was smaller.

Figure 3 shows the relationship between the hospital volume (i.e., the median number of procedures performed per year) and SMR. The hospital volume was significantly related to the SMR ($p = 0.003$) with a negative linear trend, whatever the region (Figure 3). However, we noted a difference in the slope of the linear trend of the SMR between regions. The variability between regions was globally moderate with an ICC of 0.06 (95% CI: 0.014–0.23), indicating that 6% of the SMR variance is due to differences across regions. This is consistent with the value of IQRs observed from one region to another, seeing as the intervals overlap (Table 2). Statistically, this means that the SMRs are not different from one region to another.

Discussion

To the best of our knowledge, this is the first study of this type to be carried out in France. The literature on the study of regional variations in surgical practice in hospitals is fairly poor (13–15). Our work underscores the fact that there is currently a large number of French hospitals performing resections for lung cancer. Surgical practice is dispersed and in-hospital mortality varies considerably from one hospital to another. The mean SMR was similar across regions, but with great variability between hospitals within regions. Nevertheless, the variability was more marked for certain regions, particularly those in which many hospitals perform the surgery.

Our work reveals that there is variability in the practices of the different hospitals as well as in the different regions. There are still many low-volume hospitals in France despite the required authorization. One of the characteristics of French health care is the dispersed supply of care, which is also seen in other European countries (16–19). However, the number of hospitals per region practicing this type of surgery is not in line with the needs of the population in regions such as in Auvergne Rhône-Alpes or Nouvelle Aquitaine.

To our knowledge, the variation in hospital comorbidities within regions has not yet been described in the literature. Our work reveals a considerable variation in the rate of certain

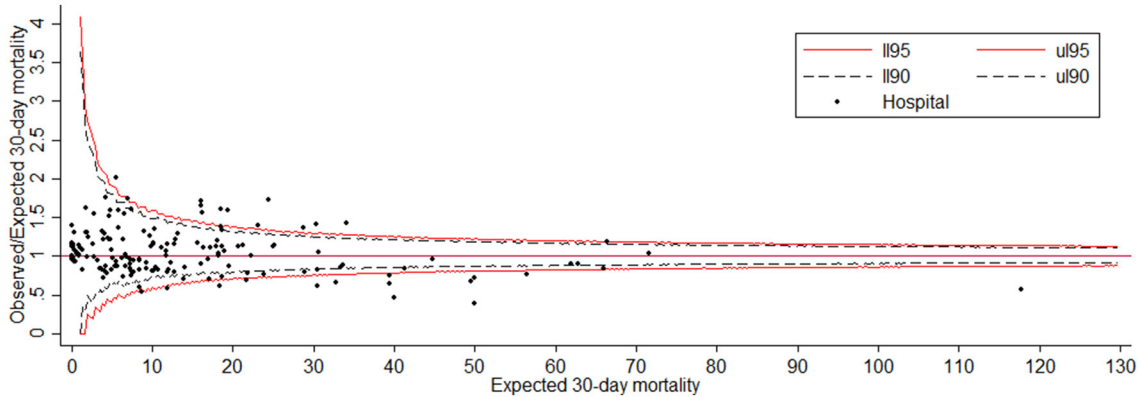

FIGURE 2
Funnel plot for the 30-day mortality with adjusted Observed/Expected ratio (SMR). The red line corresponds to the 95% control limit and the dash black line corresponds to the 90% control limit.

TABLE 2 Between hospital variation in adjusted standardized mortality rates in each region of France.

Region	Hospitals (*n*)	cv	Extreme ratio	Interquartile ratio	IQR	SCV
Auvergne Rhone-Alpes	27	0.178	2.55	1.235	0.81–1.00	12.23
Bourgogne Franche-Comté	6	0.094	1.33	1.04	0.96–1.00	7.00
Bretagne	14	0.183	1.99	1.27	0.91–1.16	7.5
Centre-Val de Loire	9	0.223	2.41	1.18	0.96–1.13	1.85
Corse	2	0.453	1.94	1.94	0.82–1.60	1.13
Grand-Est	20	0.265	2.73	1.36	0.82–1.12	0.73
Hauts de France	13	0.190	1.80	1.35	0.75–1.01	1.18
Ile de France	26	0.330	3.86	1.42	0.82–1.17	18
Normandie	9	0.195	1.91	1.18	0.86–1.02	17
Nouvelle Aquitaine	23	0.244	2.83	1.55	0.83–1.29	2.52
Occitanie	19	0.270	4.45	1.3	0.94–1.21	4.90
Pays de Loire	15	0.275	2.44	1.62	0.96–1.55	11
Provence Alpes Cote d'Azur	16	0.220	2.00	1.33	0.99–1.32	2.80

cv, coefficient of variation (=standard deviation/mean), if greater than 1, it shows relatively high variability in the data sets; Extreme ratio (=Maximum/Minimum), greater than 2 implies that the maximum value is twice as high as the minimum value; Interquartile ratio, 75th percentile rate divided by the 25th percentile; IQR, interquartile interval (1st quartile – 3rd quartile); SCV, systematic component of variance, greater than 10 can be considered indicative of very high variation. For the line regarding Auvergne Rhone-Alpes, we observed that the standard deviation of SMR is 17.8% the size of the mean (CV = 0.178). Among the 27 hospitals, the maximum value of the SMR is more than twice as high as the minimum value of the SMR (extreme ratio = 2.55). The IQR means that 50% of the SMR among these hospitals lie between 0.81 and 1.00, knowing that the ratio between the 3rd quartile and the 1st quartile is 1.235. Finally, the SCV is 12.23.

comorbidities, such as peripheral vascular disease, liver disease, anemia and hematological disease. The coefficient of variation was 1.18, 1.47, 1.22, and 2.22, respectively. It is possible that the patients managed in the different hospitals are different in terms of comorbidities or that the coding for the associated PMSI diagnoses is not consistent from one hospital to another. Because of our data source, it is not possible to know which of these hypotheses is most likely to explain the differences observed.

The type of lung resection also varied from one hospital to another, particularly bilobectomies and pneumonectomies, and we cannot compare our results with the literature because lung cancer surgery has not been published using the same methodology as ours. Pneumonectomies are complex procedures more often performed by specialized teams in high volume units, which may explain the variability observed between hospitals.

The median 30-day mortality rate was 3%, which appears to be higher than in some European countries, which is consistent with a recent systematic review (20) comparing mortality following lung cancer resection in France with other European countries. The systematic review showed that France has a higher rate than some other European countries, including England, Denmark, Holland or Finland. Our work confirms the great variability in post-operative mortality between hospitals, as shown by the interquartile values and the coefficient of variation. It is a complex issue to understand why France (20) has a higher rate of post-operative mortality than other European countries.

The fact that volume of hospital activity is significantly related to excess mortality (21–25) has been demonstrated many times in the literature. Countries in which fewer centers offer thoracic surgery, such as in the UK, have a lower overall post-operative

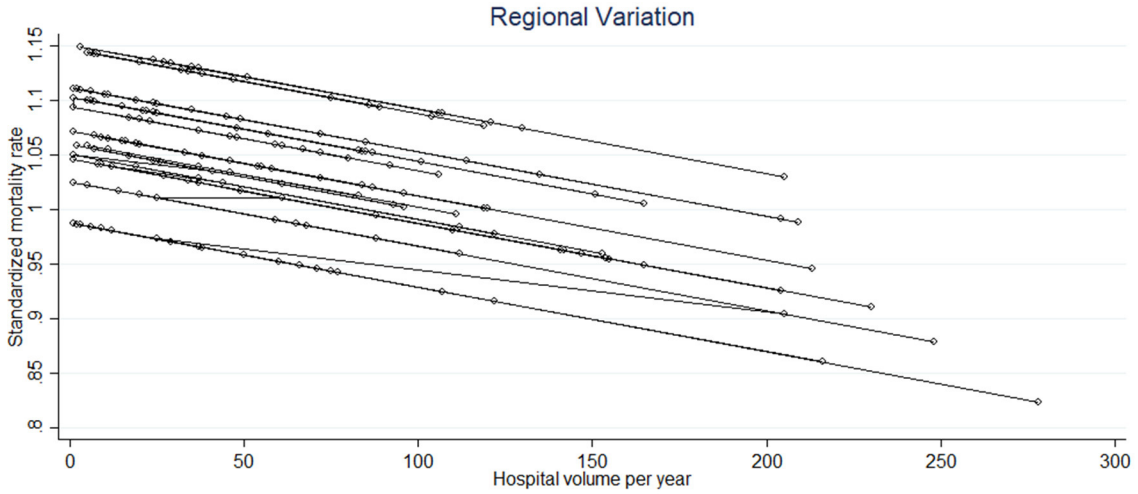

FIGURE 3
The significant linear relationship between the standardized mortality rate and the hospital volume of the different hospitals in each region (each circle corresponds to a hospital and each line to a region).

mortality rate than France. On average, their centers also have a higher volume of activity than French centers (16–19), and an interesting English study showed that high-volume centers had better survival after lung cancer surgery (23). The literature unanimously illustrates that high-volume centers have better results in terms of mortality, confirmed by our study, whatever the French region. Obviously, hospital volume is not the only component of quality of care that can have a role in hospital mortality, and we know that the nurse to patient staffing ratio or the processes in the different units could have an important role. For example, in a study by Aiken et al. (26) in nine European countries, the authors showed that an increase of one patient per nurse is associated with a 7% increase in the risk of a surgical patient dying within 30-days of admission. Further studies are needed to assess these additional factors, but we unfortunately do not have access to such data in the PMSI database.

Our work provides an estimation of the variability in post-operative mortality between regions, a subject for which the literature is scarce (27). We have shown that the difference in 30-day mortality between regions is ultimately moderate, and the most striking variability was observed between hospitals within regions. Our findings raise questions regarding current regionalization policies for major surgical procedures such as lung cancer surgery. Some countries have moved toward the centralization of complex surgeries in selected hospitals in an attempt to group together the technical skills needed to improve quality of care (28, 29). This idea seems attractive, especially if we want to be able to measure hospital performance reliably. Compared with other countries, France offers dispersed care for major interventions like thoracic surgery or pancreatic surgery, which could be one of the reasons that the death rate is higher in France than in other countries (20).

The differences in the 30-day mortality of hospitals in France could also be explained by the lack of quality measurement, in contrast to certain European countries (16–19). European surgical teams that undertake regular assessments of their practice are informed of their level of performance and can thus implement actions to improve their results.

We recognize that our study has some limitations. In particular, the choice of mortality as the key outcome is a limitation because we did not assess the overall quality of care but only one indicator, the 30-day mortality rate, which is only one of several potential indicators. Other indicators, for instance post-operative complications after lung resection, such as pneumopathy or atelectasis, which are good quality indicators, cannot be obtained through the PMSI. We only had access to in-hospital mortality data, but it is nevertheless a relevant indicator for this type of surgery. Another limitation concerns the TNM stage, which is not recorded in the PMSI, but is a factor that can influence mortality. The final limitation concerns the risk of underestimating certain comorbidities because of the quality of the coding. Indeed, coding practices may vary among institutions since the people who perform the coding of diagnoses can be clinicians or information system technicians. Nevertheless, the quality of coding is checked in a standardized way by medical information professionals in each hospital in order to correct diagnoses (internal quality assessment), and the level of re-cording of co-morbidities has increased significantly in recent years, following its impact on the tariff of hospital stays. Because of the impact on hospital budget allocation, it has been shown that hospital claims data are becoming more accurate. In addition, a national external quality assessment program has been implemented to verify the quality of discharge abstracts in each hospital.

In conclusion, this work shows significant differences in the practices of the various hospitals within each French region. However, between regions, there were only moderate variations in the 30-day mortality rate. These results should encourage surgical teams in France to implement quality of care measures based on the analysis of outcomes. Finally, our findings call into question the current policies relative to the regionalization of major surgical procedures in France.

Data availability statement

The datasets presented in this article are not readily available because the use of these data by our department was approved by the National Committee for data protection. We are not allowed to transmit these data. PMSI data are available for researchers who meet the criteria for access to these French confidential data (this access is submitted to the approval of the National Committee for data protection) from the national agency for the management of hospitalization (ATIH-Agence technique de l'information sur l'hospitalisation). Requests to access the datasets should be directed to Agence technique de l'information sur l'hospitalisation 117 boulevard Marius Vivier Merle 69329 Lyon Cedex 03.

Author contributions

AB was involved in the conception and design of the study, in charge of the analysis, involved in the interpretation, wrote the first draft, and approved the final version. JC was responsible for the data collection, accessed and verified the data, involved in the interpretation, critically reviewed the first draft, and approved the final version. P-BP was involved in the conception and design of the study, involved in the interpretation, critically reviewed the first draft, and approved the final version. CQ was the coordinator of the study, responsible for the data collection, accessed and verified the data, involved in the interpretation, and critically reviewed the first draft. All authors approved the final version.

Acknowledgments

The authors thank Harbajan Chadha-Boreham for reviewing the English and Gwenaëlle Periard for her help with the layout and management of this article.

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.

Publisher's note

References

1. Pagès PB, Cottenet J, Mariet AS, Bernard A, Quantin C. In-hospital mortality following lung cancer resection: nationwide administrative database. *Eur Respir J.* (2016) 47:1809–17. doi: 10.1183/13993003.00052-2016

2. Bernard A, Cottenet J, Mariet AS, Quantin C, Pagès PB. Is an activity volume threshold really realistic for lung cancer resection? *J Thorac Dis.* (2018) 10:5685–94. doi: 10.21037/jtd.2018.09.77

3. Bernard A, Pagès PB, Mariet AS, Pforr A, Madelaine L, Cottenet J, et al. Evaluation de la pratique du cancer bronchique en France à partir de la base nationale du PMSI. evaluation of surgical practice in the treatment of lung cancer in france from the pmsi national database. *Revue des Mal Respir.* (2018) 36:31–8. doi: 10.1016/j.rmr.2018.01.014

4. Ministère de l'Enseignement Supérieur et de la Recherche, Ministère de la Santé et des Sports. *Plan Cancer 2009– 2013.* Available online at: http://www.e-cancer.fr/Plan-cancer/Les-Plans-cancer-de-2003-a-2013/Le-Plancancer-2009-2013 (accessed March 2016).

5. Kozower BD, Stukenborg GJ. The relationship between hospital lung cancer resection volume and patient mortality risk. *Ann Surg.* (2011) 254:1032–7. doi: 10.1097/SLA.0b013e31821d4bdd

6. Fernandez FG, Kosinski AS, Burfeind W, Park B, DeCamp MM, Seder C, et al. STS lung cancer resection risk model: higher quality data and superior outcomes. *Ann Thorac Surg.* (2016) 102:370–7. doi: 10.1016/j.athoracsur.2016.02.098

7. World Health Organization. *International Statistical Classification of Diseases and Related Health Problems 10th Revision.* Available online at: http://apps.who.int/classifications/icd10/browse/2016/en (accessed March 1, 2016).

8. Iezzoni LI. Assessing quality using administrative data. *Ann Intern Med.* (1997) 127:666–74. doi: 10.7326/0003-4819-127-8_Part_2-199710151-00048

9. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. *J Clin Epidemiol.* (1994) 47:1245–51. doi: 10.1016/0895-4356(94)90129-5

10. Normand SLT, Shahian DM. Statistical and clinical aspects of hospitals outcomes profiling. *Stat Sci.* (2007) 22:206–26. doi: 10.1214/088342307000000096

11. Spiegelhalter D. Funnel plots for comparing institutional performance. *Statist Med.* (2005) 24:1185–202. doi: 10.1002/sim.1970

12. Ibáñez B, Librero J, Bernal-Delgado E, Peiró S, López-Valcarcel BG, Martínez N, et al. Is there much variation in variation? revisiting statistics of small area variation in health services research. *BMC Health Serv Res.* (2009) 9:60. doi: 10.1186/1472-6963-9-60

13. Wouters MW, Siesling SS, Jansen-Landheer ML, et al. variation in treatment and outcome in patients with nonsmall cell lung cancer by region, hospital type and volume in the Netherlands. *Eur J Surg Oncol.* (2010) 36:S83–92. doi: 10.1016/j.ejso.2010.06.020

14. Zettervall SL, Soden PA, Buck DB, Cronenwett JL, Goodney PP, Eslami MH, et al. Significant regional variation exists in morbidity and mortality after repair of abdominal aortic aneurysm. *J Vasc Surg.* (2017) 65:1305–12. doi: 10.1016/j.jvs.2016.08.110

15. Gurney JK, McLeod M, Stanley J, Robson B, Campbell D, Dennett E, et al. Regional variation in post-operative mortality in New Zealand. *ANZ J Surg.* (2022) 92:1015–25. doi: 10.1111/ans.17510

16. Jakobsen E, Green A, Oesterlind K, Rasmussen TR, Iachina M, Palshof T. Nationwide quality improvement in lung cancer care: the role of the danish lung cancer group and registry. *J Thorac Oncol.* (2013) 8:1238–47. doi: 10.1097/JTO.0b013e3182a4070f

17. Beck N, Hoeijmakers F, van der Willik EM, Heineman DJ, Braun J, Tollenaar RAEM, et al. National comparison of hospital performances in lung cancer surgery: the role of case mix adjustment. *Ann Thorac Surg.* (2018) 106:412–20. doi: 10.1016/j.athoracsur.2018.02.074

18. Helminena O, Valoa J, Andersenb H, Lautamäkia A, Vuohelainena V, Sihvoa E. Real-world guideline-based treatment of lung cancer improves short- and long-term outcomes and resection rate: a population-based study. *Lung Cancer.* (2020) 140:1–7. doi: 10.1016/j.lungcan.2019.12.002

19. Royal College of Physicians. *Lung Cancer Clinical Outcomes Publication 2018 (for surgical operations performed in 2016).* London: RCP (2018).

20. Bernard AA, Riviere A, Cottenet J, Madeleine L, Quantin C, Pages PB. Comparison of mortality of lung cancer resections in France to other European countries. *Rev Mal Respir.* (2022) 39:669–75. doi: 10.1016/j.rmr.2022.08.002

21. Lien YC, Huang MT, Lin HC. Association between surgeon and hospital volume and in-hospital fatalities after lung cancer resections: the experience of an Asian contry. *Ann Thorac Surg.* (2007) 83:1837–43. doi: 10.1016/j.athoracsur.2006.12.008

22. Sioris T, Sihvo E, Sankila R, Salo J. effect of surgical volume and hospital type on outcome in non-small cell lung cancer surgery: a finnish population-based study. *Lung Cancer.* (2008) 59:119–25. doi: 10.1016/j.lungcan.2007.07.020

23. Lüchtenborg M, Riaz SP, Coupland VH, Lim E, Jakobsen E, Krasnik M, et al. High procedure volume is strongly associated with improved survival after lung cancer surgery. *J Clin Oncol.* (2013) 31:3141–6. doi: 10.1200/JCO.2013.49.0219

24. Ross JS, Normand S-LT, Wang Y, Ko DT, Chen J, Drye EE, et al. Hospital volume and 30-day mortality for three common medical conditions. *N Engl J Med.* (2010) 362:1110–8. doi: 10.1056/NEJMsa0907130

25. von Meyenfeldt EM, Gooiker GA, Van Gijn W, Post PN, van de Velde CJH, Tollenaar RAEM, et al. The relationship between volume or surgeon speciality and outcome in the surgical treatment of lung cancer. a systematic review and meta-analysis. *J Thorac Oncol.* (2012) 7:1170–8. doi: 10.1097/JTO.0b013e318257cc45

26. Aiken LH, Sloane DM, Bruyneel L, Van den Heede K, Griffiths P, Busse R, et al. Nurse staffing and education and hospital mortality in nine European countries: a retrospective observational study. *Lancet.* (2014) 383:1824–30. doi: 10.1016/S0140-6736(13)62631-8

27. Birkmeyer JD, Reames BN, McCulloch P, Carr AJ, Campbell WB, Wennberg JE, et al. Understanding regional variation in the use of surgery health public. *Lancet.* (2013) 382:1121–9. doi: 10.1016/S0140-6736(13)61215-5

28. Bendzsak AM, Baxter NN, Darling GE, Austin PC, Urbach DR. Regionalization and outcome of lung cancer surgery in Ontario Canada. *J Clin Oncol.* (2017) 35:2772–80. doi: 10.1200/JCO.2016.69.8076

29. Gooiker GA, van der Geest LGM, Wouters MWJM, Vonk M, Karsten TM, Tollenaar RAEM, et al. Quality improvement of pancreatic surgery by centralization in the Western part of the Netherlands. *Ann Surg Oncol.* (2011) 18:1821–9. doi: 10.1245/s10434-010-1511-4

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 04 April 2023
DOI 10.3389/fmed.2023.1142836

OPEN ACCESS

EDITED BY
Bruno Guedes Baldi,
University of São Paulo,
Brazil

REVIEWED BY
Kazuya Hosokawa,
Kyushu University,
Japan
Michael Ramsay,
Baylor University Medical Center,
United States

*CORRESPONDENCE
Yuichi Tamura
✉ tamura.u1@gmail.com

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 12 January 2023
ACCEPTED 28 February 2023
PUBLISHED 04 April 2023

CITATION
Tamura Y, Tamura Y, Taniguchi Y and Atsukawa M (2023) Current clinical understanding and effectiveness of portopulmonary hypertension treatment. *Front. Med.* 10:1142836. doi: 10.3389/fmed.2023.1142836

Current clinical understanding and effectiveness of portopulmonary hypertension treatment

Yuichi Tamura[1,2]*, Yudai Tamura[1,2], Yu Taniguchi[3] and Masanori Atsukawa[4]

[1]Pulmonary Hypertension Center, International University of Health and Welfare Mita Hospital, Tokyo, Japan, [2]Department of Cardiology, International University of Health and Welfare School of Medicine, Narita, Japan, [3]Division of Cardiovascular Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan, [4]Division of Gastroenterology and Hepatology, Nippon Medical School, Tokyo, Japan

Portopulmonary hypertension (PoPH) is a rare subtype of Group 1 pulmonary arterial hypertension (PAH) with a poor prognosis. According to the most up-to-date definition, PoPH is characterized by a mean pulmonary arterial pressure (PAP) of >20mmHg at rest, a pulmonary artery wedge pressure of ≤15mmHg, and a pulmonary vascular resistance (PVR) of >2 Wood units with portal hypertension. Like PAH, PoPH is underpinned by an imbalance in vasoactive substances. Therefore, current guidelines recommend PAH-specific therapies for PoPH treatment; however, descriptions of the actual treatment approaches are inconsistent. Given the small patient population, PoPH is often studied in combination with idiopathic PAH; however, recent evidence suggests important differences between PoPH and idiopathic PAH in terms of hemodynamic parameters, treatment approaches, survival, socioeconomic status, and healthcare utilization. Therefore, large, multi-center registry studies are needed to examine PoPH in isolation while obtaining statistically meaningful results. PoPH has conventionally been excluded from clinical drug trials because of concerns over hepatotoxicity. Nevertheless, newer-generation endothelin receptor antagonists have shown great promise in the treatment of PoPH, reducing PVR, PAP, and World Health Organization functional class without causing hepatotoxicity. The role of liver transplantation as a treatment option for PoPH has also been controversial; however, recent evidence shows that this procedure may be beneficial in this patient population. In the future, given the shortage of liver donors, predictors of a favorable response to liver transplantation should be determined to select the most eligible patients. Collectively, advances in these three areas could help to standardize PoPH treatment in the clinic.

KEYWORDS

portopulmonary hypertension, treatment, endothelin receptor antagonist, liver transplantation, pulmonary arterial hypertension, screening

1. Introduction

Pulmonary hypertension (PH) is the overarching term used to describe a complex group of conditions that are characterized by loss and obstructive remodeling of the pulmonary vascular bed, leading to an increase in pulmonary vascular resistance (PVR) and pulmonary arterial pressure (PAP). These changes in PVR and PAP cause strain on the right side of the heart, and if this persists for a prolonged period, right-sided heart failure and functional decline can occur (1, 2).

In the World Health Organization (WHO) clinical classification (3), portopulmonary hypertension (PoPH) is positioned as a subtype of Group 1 pulmonary artery hypertension (PAH), and registry data suggest that PoPH accounts for 5–16% of cases of PAH (4–9). PoPH develops in 1.1–6.3% of patients with portal hypertension (10–13), and although most cases in this patient population are related to cirrhosis, non-cirrhotic causes of portal hypertension leading to PoPH have also been noted, including portal vein thrombosis, granulomatous disease, autoimmune diseases, drug reactions, infections (such as hepatitis C), and congenital abnormalities (such as congenital portosystemic shunt) (14–17). The prevalence of PoPH in liver transplant patients is 5–10% (18, 19), and among those with advanced liver disease, such as patients undergoing liver transplantation, women have a higher risk of developing PoPH than men; however, liver disease severity does not appear to be directly related to the risk of PoPH (11).

In terms of the diagnosis of portal hypertension, patients can be diagnosed by the presence of clinical signs, such as ascites, varices, or both, as well as splenomegaly, portal vein dilation, portal vein occlusion, collateral vessel formation, and declining platelet counts (20). Imaging studies, such as Doppler ultrasonography, computed tomography, and magnetic resonance imaging, as well as blood tests, are used to diagnose portal hypertension and determine the presence of the abovementioned features (20). Portal venous pressure is a product of the portal blood flow volume and the resistance to flow; however, direct measurement of portal pressure is not routine due to its invasive nature. A less invasive and indirect measure is the hepatic venous pressure gradient (HVPG), which is considered the best surrogate indicator of portal hypertension in patients with cirrhosis (21). In healthy individuals, the HVPG is 2–5 mmHg, while an HVPG of ≥6 mmHg constitutes portal hypertension and an HVPG of ≥10 mmHg constitutes clinically significant portal hypertension (20). The HVPG is calculated by subtracting the wedged hepatic venous pressure from the free hepatic venous pressure, which are determined by fluoroscopy (20).

In terms of the diagnosis of PH, many patients present as outpatients with symptoms, such as dyspnea, fatigue, or syncope. Others come to the attention of the clinician during screening evaluations, and some present acutely as inpatients (22). Once PH is suspected, echocardiography is commonly used to assess the tricuspid regurgitant velocity, pulmonary artery systolic pressure, and right ventricular wall thickness and function, and right heart catheterization may also be performed (23). Other tests may involve a clinical history and examination, complete pulmonary function testing, thoracic computed tomography, chest radiography, and nocturnal plethysmography to evaluate sleep-disordered breathing (23). The previous right heart catheterization criteria for the diagnosis of PoPH were a mean PAP of ≥25 mmHg at rest, a pulmonary artery wedge pressure of ≤15 mmHg, and a PVR of >3 Wood units with portal hypertension (9). However, the latest definition specifies a mean PAP of >20 mmHg (24). Certain et al. (25) also recently proposed a lower cut-off PVR value of 2 Wood units based on its benefit in achieving an early diagnosis. The cut-off value for pulmonary artery wedge pressure remains at ≤15 mmHg (24). For PoPH specifically, serological analysis for markers of liver failure will also be performed (23).

The pathogenesis of PoPH has been reviewed in detail previously (17) (Figure 1) (26–36). Briefly, cirrhosis causes an increase in intrahepatic resistance and an increased portal pressure gradient, which leads to portosystemic collateralization through the reperfusion/dilation of existing vessels and the generation of new vessels (37). At the molecular level, portosystemic shunting causes blood containing vasoactive substances to bypass the liver, thus evading hepatic metabolism. This reduction in peripheral vascular resistance, combined with indirect vasodilation *via* intestinal vasoactive substances that bypass the liver and reach the systemic circulation, culminates in a hyperdynamic state (37). Endothelin-1 and interleukin-6 are among the substances that are thought to increase in PoPH (38, 39), and this imbalance in vasoactive substances (such as endothelin-1) and pro-inflammatory cytokines (such as interleukin-6) in the pulmonary vasculature leads to net vasoconstriction and an increase in PVR. Thus, one commonly accepted pathogenic mechanism of PoPH is an imbalance in vasoactive substances in the pulmonary circulation in patients with cirrhosis (40, 41). Like PoPH, PAH also results in an imbalance in vasoactive substances and circulating factors; therefore, European Society of Cardiology and European Respiratory Society guidelines (42) recommend that PoPH treatment should follow that of PAH.

The 5-year survival rate of untreated patients with PoPH is as low as 14.2% (24); however, despite its poor prognosis, the rarity of this condition means that descriptions of the clinical features and treatment approaches for PoPH are scarce and inconsistent (43). A number of studies examining the treatment approaches to PoPH have been published in recent years (Table 1), many of which have not yet been reviewed. In this review, we aim to provide an up-to-date analysis of recent literature to establish the current clinical understanding and effectiveness of PoPH treatment. We will briefly discuss the reasons for the limited knowledge of PoPH; consider the controversies around studying PoPH in combination with idiopathic PAH; discuss treatment trends, including the potential of newer-generation endothelin receptor antagonists; and consider evidence for the usefulness of liver transplantation in PoPH patients. We will also provide our expert opinion on how this knowledge could be used to design future clinical trials to deepen the understanding of PoPH and standardize treatment in the clinic.

2. Reasons for the limited knowledge of PoPH

Specific knowledge of PoPH is limited, and it is relatively understudied compared with other subtypes of Group 1 PAH. There are several possible reasons for this. First, PoPH has conventionally been excluded from drug trials because of concerns about hepatotoxicity (50). For example, in a previous study, bosentan (an endothelin receptor antagonist) led to an elevation in transaminases in approximately 10% of patients with Group 1 PAH without previous liver disease (51). Second, the low incidence of PoPH means that patients with this disease are often studied in combination with patients having idiopathic PAH to ensure sufficient sample sizes. Idiopathic PAH is defined as PAH of unknown cause under the WHO functional classification (3). Thus, the disease etiologies are highly

Abbreviations: PAH, pulmonary arterial hypertension; PAP, pulmonary arterial pressure; PH, pulmonary hypertension; PoPH, portopulmonary hypertension; PVR, pulmonary vascular resistance; WHO, World Health Organization.

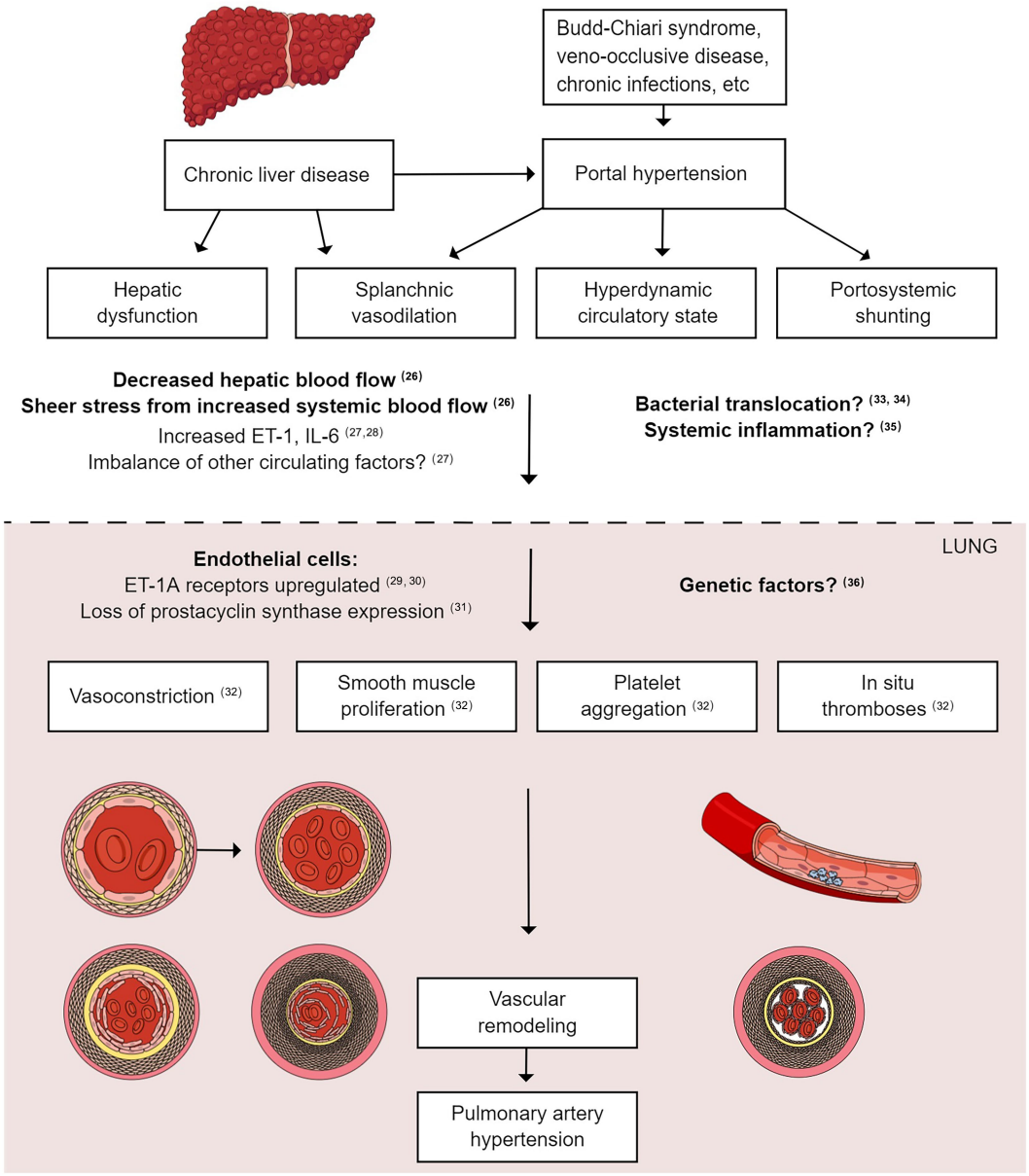

FIGURE 1
Pathophysiology of portopulmonary hypertension. Liver cirrhosis increases intrahepatic resistance and increases the portal pressure gradient, leading to portal hypertension. The reperfusion/dilation of existing vessels causes portosystemic collateralization. Vasoactive substances bypass the liver and evade hepatic metabolism due to portosystemic shunting, leading to a hyperdynamic state. Endothelin-1 and interleukin-6 are among the circulating substances that reach the pulmonary vasculature, leading to net vasoconstriction and an increase in PVR. Smooth muscle cell proliferation, platelet aggregation, and *in situ* thrombosis also occur, leading to vascular remodeling and PAH. PAH, pulmonary arterial hypertension; PVR, pulmonary vascular resistance.

variable, and the mechanisms that underpin disease development in specific patients may vary substantially. Multiple causes of PoPH have also been reported (14–17); however, case numbers are small, which further adds to the complexity of studying this condition in large enough numbers to obtain statistically meaningful results. Therefore, grouping PoPH patients with idiopathic PAH patients to study the effects of drug treatment means that the observations may not necessarily be an accurate reflection of the PoPH population. Third, PoPH has a lower prevalence than other complications, such as hepatic encephalopathy and ascites, in patients with portal hypertension. Therefore, the diagnosis of PoPH might be less of a priority for hepatologists who must diagnose and treat various complications in patients with liver cirrhosis.

3. Review of recent literature

3.1. Controversy in studying PoPH collectively with idiopathic PAH

Given the low incidence of PoPH, it is often studied in combination with idiopathic PAH when examining the effects of drug

TABLE 1 Summary of recently published studies.

Publication	Study design	Population	Intervention/ Exposure	Comparator	Outcomes
Sitbon et al. (44)	Multi-center, randomized controlled phase 4 trial	PoPH and Child–Pugh class A/B ($n = 85$)	Intervention: Macitentan (10 mg) for a 12 week double-blind period, followed by a 12 week open-label period	Placebo for a 12 week double-blind period, followed by a 12 week open-label period	35% reduction in PVR with macitentan; 84% (macitentan) and 79% (placebo) of patients experienced adverse events; 21% (macitentan) and 14% (placebo) experienced serious adverse events; most frequent adverse event was edema (macitentan: 26% vs. placebo: 5%); no hepatic safety concerns
Preston et al. (45)	Multi-center, open-label, phase 3 trial	PoPH and Child–Pugh class A/B ($n = 31$)	Intervention: Ambrisentan for 24 weeks, followed by long-term extension for 24–28 weeks	Without ambrisentan (and treatment-naïve) at baseline	Significant reduction in PVR (7.1 ± 5 vs. 3.8 ± 1.8 Wood units); no change in 6-MWD; RAP, mPAP, and CI improved; PCWP unchanged; significant improvement in WHO functional class; most common drug-related adverse events were edema (38.7%) and headache (22.5%)
Savale et al. (46)	Prospective cohort study	PoPH ($n = 637$)	Exposure: Monotherapy with PDE-5i, ERA, or a prostacyclin analog with or without liver transplantation	Dual therapy or triple therapy with or without liver transplantation	Patients treated with dual therapy had a significantly greater median change in PVR than those treated with monotherapy; in the overall cohort, survival from PoPH was better in those who underwent liver transplantation than in those who did not (92, 83, and 81% at 1, 3, and 5 years, respectively, vs. 84, 69, and 51%); in survivors of liver transplantation, PAH therapy was simplified from combination to monotherapy in 16% and discontinued in 22%
DuBrock et al. (47)	Cross-sectional study	PoPH ($n = 57$) vs. I/H-PAH ($n = 344$)	Exposure: PoPH	I/H-PAH	Patients with PoPH had similar WHO functional class, 6-MWD, and mPAP and a higher CI than patients with I/H-PAH; fewer PoPH patients received combination therapy (46.4% vs. 62.2%) and ERAs (28.6% vs. 55.1%) at enrollment, but treatment was similar between PoPH and I-PAH at follow-up; patients with PoPH had more ED visits and hospitalizations in the 6 months preceding enrollment
Salvador et al. (48)	Registry study	PoPH ($n = 237$) vs. I/H-PAH ($n = 678$)	Exposure: PoPH	I/H-PAH	Patients with PoPH were predominantly male, older, had a better WHO functional class, and had better hemodynamics; heart failure biomarkers were worse in PoPH patients; age- and sex-adjusted 5 year survival rate from diagnosis was 49.3% for PoPH and 68.7% for I/H-PAH; PAH- and liver-related causes accounted for 30.2 and 24.7% of deaths, respectively, in PoPH patients; PoPH patients less frequently received PAH-specific therapy, but first-line treatment with PAH-specific therapy was associated with better survival; 3.4% of patients underwent liver transplantation
Takahashi et al. (16)	Retrospective cohort study	PoPH ($n = 82$) vs. I/H-PAH ($n = 1,112$)	Exposure: PoPH	I/H-PAH	Patients with PoPH had higher CO and CI values and lower PVR; fewer PoPH patients received combination therapy; overall and disease-specific survival were similar between PoPH and I/H-PAH
Atsukawa et al. (49)	Retrospective database study	PoPH ($n = 386$)	N/A	N/A	Treatment preferences in PoPH patients: loop diuretics (70.2%), pulmonary vasodilator monotherapy or combination therapy (37.0%), prostacyclin (prostaglandin I_2) monotherapy (8.8%), ERA + nitric oxide combination therapy (7.0%)
Tamura et al. (9)	Retrospective database study	PoPH ($n = 62$)	Intervention: Combination therapy (≥2 PAH-specific drugs)	Monotherapy	Mean PAP, PVR, and CI were significantly improved with combination therapy

CI, cardiac index; CO, cardiac output; ED, emergency department; ERA, endothelin receptor antagonist; I/H-PAH, idiopathic/heritable pulmonary arterial hypertension; mPAP, mean pulmonary arterial pressure; PAH, pulmonary arterial hypertension; PCWP, pulmonary capillary wedge pressure; PDE-5i, phosphodiesterase type-5 inhibitor; PoPH, portopulmonary hypertension; PVR, pulmonary vascular resistance; RAP, right atrial pressure; WHO, World Health Organization; 6-MWD, 6 min walk distance.

therapy. However, differences have been identified between these two patient populations, which suggests that they should be studied independently wherever possible (Table 1). For example, Takahashi et al. (16) extracted data on patients with PoPH from the National Research Project on Intractable Disease in Japan and compared them with data on patients with idiopathic PAH. Patients with PoPH had a higher cardiac output, higher cardiac index, lower PVR, and better 6 min walk distance than patients with idiopathic PAH. In another recent study, DuBrock et al. (47) studied health disparities and treatment approaches between PoPH and idiopathic PAH as part of the Pulmonary Hypertension Association Registry. Dissimilar to Takahashi et al. (16), the authors found that patients with PoPH had a similar 6 min walk distance to patients with idiopathic PAH, as well as a similar WHO functional class and mean PAP. However, similar to Takahashi et al. (16), they identified a higher cardiac index in patients with PoPH than in patients with idiopathic PAH.

In the study by Takahashi et al. (16), although treatments were similar between patients with PoPH and those with idiopathic PAH, the use of prostaglandin I_2 and endothelin receptor antagonists was lower, and the use of phosphodiesterase type 5 inhibitors was higher in patients with PoPH than in patients with idiopathic PAH. Similarly, in DuBrock et al.'s study (47), fewer PoPH patients than idiopathic PAH patients underwent treatment with endothelin receptor antagonists, including macitentan (28.6% vs. 55.1%, respectively), at enrollment. Moreover, fewer PoPH patients than idiopathic PAH patients were treated with combination therapy (46.4% vs. 62.2%, respectively) at enrollment. However, treatment was similar between PoPH and idiopathic PAH at follow-up. Interestingly, patients with PoPH had more emergency department visits and hospitalizations in the 6 months before enrollment than patients with idiopathic PAH, which could suggest that the addition of endothelin receptor antagonists at follow-up (50% at follow-up vs. 28.6% at enrollment) was effective in reducing the rate of hospitalizations and emergency department visits. This observation corroborates the findings of recent studies by Sitbon et al. (44) and Preston et al. (45), which also demonstrated the beneficial effects of the endothelin receptor antagonists macitentan and ambrisentan, respectively, in patients with PoPH. Initial phosphodiesterase type 5 inhibitor monotherapy was initiated for most PoPH patients with preserved cardiac output and a lower PVR, and a second pulmonary vasodilator (endothelin receptor antagonist) was added sequentially if the improvement in mean PAP was not sufficient. Prior to the clinical trials on macitentan and ambrisentan, endothelin receptor antagonists were not often used as first-line agents in patients with PoPH because of their hepatotoxicity. Therefore, phosphodiesterase type 5 inhibitors were selected first, followed by endothelin receptor antagonists.

Overall, DuBrock et al. (47) showed that patients with PoPH had a worse socioeconomic status, were less likely to be treated with combination therapy at enrollment, and had increased healthcare utilization than patients with idiopathic PAH. However, the study noted that the sample size was too small to detect racial/ethnic differences and differences in survival between patients with PoPH and those with idiopathic PAH.

Adding to the differences between PoPH patients and idiopathic PAH patients identified by DuBrock et al. (47), the Spanish Registry of PAH (48) showed that patients with PoPH were predominantly male and had a better functional class and better hemodynamics than patients with idiopathic PAH. Similar to DuBrock et al.'s study (47), patients with PoPH were less likely to receive PAH-targeted therapy, which was associated with greater mortality. Moreover, first-line PAH monotherapy was associated with better survival. The Spanish Registry of PAH (48) also identified a significant difference in survival between PoPH and idiopathic PAH, reporting age- and sex-adjusted 5-year survival rates of 49.3 and 68.7%, respectively.

Taken together, this recent evidence illustrates important differences between the PoPH and idiopathic PAH populations, including differences in hemodynamics at diagnosis and differences in the therapeutic response to monotherapy, emphasizing the need for large-scale, multi-center trials to enable the PoPH population to be studied in isolation.

3.2. Potential of newer-generation endothelin receptor antagonists and treatment trends in PoPH

Although PoPH has conventionally been studied in combination with idiopathic PAH, Sitbon et al. (44) conducted the first randomized controlled trial of PAH therapy in a specific PoPH patient population. The trial adopted a prospective, multi-center, phase 4 study design, comparing the effects of macitentan with placebo in patients with PoPH without severe hepatic impairment. At baseline, 63.5% of patients were undergoing background PAH therapy. Preston et al. (45) conducted another prospective, multi-center, open-label trial in which patients were treated with ambrisentan for 24 weeks, followed by a long-term extension of 24–28 weeks. However, unlike Sitbon et al.'s study (44), patients were treatment-naïve. Importantly, in the study of Sitbon et al. (44), PVR was reduced by 35% in the macitentan group compared with the placebo group, with no hepatic safety concerns. A similar observation was made in the study of Preston et al. (45), in which ambrisentan was associated with a reduction in PVR.

Despite their effects on reducing PVR without hepatic safety concerns, macitentan (44) and ambrisentan (45) had no effect on 6 min walk distance. Moreover, macitentan (44) had no effect on mean right atrial pressure, while ambrisentan improved right atrial pressure as well as mean PAP and cardiac index. However, pulmonary capillary wedge pressure remained unchanged (45). Macitentan did not reduce WHO functional class (44); however, ambrisentan led to a significant improvement in WHO functional class (45). However, direct and simple comparisons of efficacy between these drugs may not be appropriate because the study design (open-label or double-blind) and sample size differed between these studies. For example, the clinical trial on ambrisentan included treatment-naïve patients, while more than half of the patients (64%) in the clinical trial on macitentan were already undergoing other treatments. This could explain the differences in the results between the two trials.

Given their ability to reduce mean PAP and WHO functional class (ambrisentan) and PVR (macitentan and ambrisentan), which are the defining features of PoPH, the endothelin receptor antagonists macitentan and ambrisentan illustrate great promise as therapeutic options for PoPH without causing hepatotoxicity (44, 45, 52), which is a fundamental reason why patients with PoPH have conventionally been excluded from clinical drug trials. However, it should not be disregarded that despite showing promising effects overall, macitentan and ambrisentan have been associated with adverse side effects, such as hypersensitivity, alveolitis, PAH worsening, anemia, peripheral edema, and headache (44, 45), which should be monitored in future trials.

Two recent studies on the current trends in PoPH therapy have been published in Japan. A recent database study by Atsukawa et al. (49) showed that of 386 Japanese patients with PoPH, the combined proportion of patients treated with pulmonary vasodilator monotherapy or combination therapy was 37.0% within 90 days (less than half of patients). Prostacyclin (prostaglandin I_2) was used in 8.8% of patients within 90 days, and combination therapy with endothelin receptor antagonists plus nitric oxide was used in 7.05% of patients; thus, the use of vasodilators in patients with PoPH remains low.

The low proportion of patients treated with vasodilator therapy (49) is surprising given the beneficial effects demonstrated with these agents. For example, in the Japan Pulmonary Hypertension Registry,

Tamura et al. (9) evaluated current treatment patterns and clinical events, as well as changes in hemodynamic and clinical parameters associated with PAH-specific therapy. The results showed that mean PAP, PVR, and cardiac index were significantly improved in the combination therapy group (defined as treatment with ≥2 PAH-specific drugs administered simultaneously during the follow-up period), although the improvement was not significant in the monotherapy group. There were no significant differences in mortality, PH worsening, PAH-specific drug discontinuation due to side effects, or WHO functional class improvement between the monotherapy and combination therapy groups.

Taken together, this new evidence suggests that although vasodilator therapy, and endothelin receptor antagonists in particular, has shown great promise for the treatment of patients with PoPH, its use remains limited. As a limitation of retrospective observational studies, potential bias between groups could be inevitable. Randomized controlled trials examining the use of monotherapy and combination therapy for PoPH should be conducted to validate these findings and to take a step toward treatment standardization in patients with PoPH.

3.3. The role of liver transplantation in patients with PoPH

A previous review by Thomas et al. (17) emphasized the controversy surrounding the role of liver transplantation in patients with PoPH; however, recent studies have reported the beneficial effects of this treatment approach. For example, in Savale et al.'s study (46), the effects of PAH-specific therapies were examined in a large cohort of patients with PoPH from the French Pulmonary Hypertension Registry. In total, 637 patients were analyzed, 57% of whom had mild cirrhosis. PAH-specific therapy was used in 74% of patients, and survival from PoPH was significantly better in the subgroup that underwent liver transplantation. In support of these findings, Deroo et al. (53) performed a meta-analysis in which pulmonary hemodynamics and survival were examined in patients with PoPH treated with vasodilators, liver transplantation, or both. They revealed that the risk of death in patients treated with vasodilators was significantly higher than in patients who underwent vasodilator therapy combined with liver transplantation. Furthermore, in a pooled analysis of the clinical outcomes of patients from all three Mayo Clinic liver transplantation centers, 50 out of 228 patients underwent liver transplantation and showed significant hemodynamic improvement after PAH-specific therapy, with 21 patients even able to discontinue PAH-specific therapy after liver transplantation (54).

Identifying the beneficial effects of PAH-specific therapy when used in combination with liver transplantation to treat patients with PoPH is important because liver transplantation is not without its complications. For example, on reperfusion of the liver graft, pronounced systemic hemodynamic changes, such as an increase in cardiac output, are often observed (55), which can exacerbate PH and cause potential right-sided heart failure with liver graft congestion and reverse flow in the hepatic veins (56). This condition is extremely difficult to treat with existing drugs, such as milrinone, nitric oxide, and norepinephrine. For example, milrinone increases myocardial contractility, reduces systemic afterload, and reduces PVR; however, its use is limited because it can cause systemic vasodilation and resultant hypotension (57). Therefore, the use of more effective treatments preoperatively, such as macitentan or ambrisentan, which can be used alongside liver transplantation (both preoperatively and continued postoperatively as needed) provides more options to manage such patients.

Despite the controversy around the role of liver transplantation in patients with PoPH, recent studies have demonstrated clear benefits regarding survival and the ability to subsequently discontinue PAH-specific therapy. However, given the shortage of liver donors, this approach is not feasible for every patient with PoPH. Therefore, further studies are needed to identify patients with PoPH that may benefit most from liver transplantation. Jose et al. (58) suggested that PVR predicts mortality and transplantation failure in patients with PoPH; however, the exact predictors of a favorable response to liver transplantation are still unknown and should be clarified in the future.

4. Future perspectives

Studies examining PoPH treatment have conventionally grouped PoPH patients with idiopathic PAH patients because of the low incidence of PoPH. However, several recent studies have demonstrated differences between idiopathic PAH and PoPH in terms of hemodynamic parameters, treatment approaches, and survival, as well as socioeconomic status and healthcare utilization. Thus, grouping PoPH and idiopathic PAH may not be the best approach to pharmacotherapy studies. Instead, further multi-center trials and registry studies, such as the recent Japan Pulmonary Hypertension Registry (9), should be encouraged to ensure sufficient sample sizes to study PoPH in isolation and to obtain more specific results in this patient population. Despite both reports being conducted in Japan, Atsukawa et al. (49) reported from the hepatologist's point of view that PAH-specific drug use is limited, while Tamura et al. (9) reported that the combination of PAH-specific drugs was useful for PoPH. Collaboration between physicians specializing in hepatology and PH may help to bridge the gap in their treatment strategies. This collaboration would drive larger studies on the use of PAH-specific therapies in patients with PoPH.

Promisingly, the potential of newer-generation endothelin receptor antagonists, such as macitentan and ambrisentan, has been demonstrated recently in patients with PoPH, without hepatic safety concerns (44, 45), which is a fundamental reason why PoPH has conventionally been excluded from clinical trials. Moreover, PAH-specific monotherapy and combination therapy have demonstrated promise by leading to significant improvements in key hemodynamic parameters, including mean PAP, PVR, and cardiac index. In the future, we hope to perform a clinical trial to examine the efficacy of endothelin receptor antagonist-based combination therapy.

In addition to PAH-specific drug therapy, liver transplantation has demonstrated beneficial effects in patients with PoPH, including improved survival and lower mortality (46, 53). Moreover, some patients were able to discontinue PAH-specific drug therapy after liver transplantation (54). However, a shortage of liver donors limits the feasibility of implementing this treatment strategy in all PoPH

patients; thus, it is important to ascertain the predictors of a favorable response to liver transplantation to identify suitable candidates. It would be clinically meaningful to examine the appropriateness of early intervention for PoPH, even in patients with mild disease, as this could improve survival during and after the liver transplantation waiting period.

Regardless of the indication for liver transplantation, patients with PoPH should undergo drug therapy with PAH-specific agents. Some patients with PoPH have advanced cirrhosis, while others do not. For those that do, it would be meaningful to evaluate the impact of PAH medication on survival to liver transplantation and transplant outcomes. In patients with mild cirrhosis, treatment evaluation of PH itself should be implemented, as in other types of PAH. Furthermore, as more is learned about this disease, stratification of the background liver disease status should be examined.

5. Conclusion

In summary, PoPH is classified as a subtype of Group 1 PAH that is primarily seen in patients with decompensated liver cirrhosis resulting from liver disease. PoPH has a poor prognosis; however, the rarity of PoPH limits the study of this disease in isolation. PoPH is often studied with idiopathic PAH; however, recent studies suggest important differences between PoPH and idiopathic PAH. Further large-sample, multi-center trials with sufficient sample sizes are required to generate statistically meaningful results in the PoPH population. In recent clinical trials, newer-generation endothelin receptor antagonists have shown beneficial effects in the treatment of PoPH without causing hepatotoxicity. Moreover, evidence suggests that liver transplantation is beneficial in patients with PoPH, with some patients being able to discontinue PAH-specific therapy. However, the predictors of a favorable response to liver transplantation are unknown and should be examined in future studies. Collectively, future advances in these treatment strategies could help to standardize the management of patients with PoPH in the clinic.

Author contributions

YudT, YuiT, YT, and MA wrote and edited the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by the Japan Agency for Medical Research and Development (AMED).

Acknowledgments

The authors would like to thank Emily Woodhouse, PhD, of Edanz Pharma for providing medical writing services, which were funded by the Japan Agency for Medical Research and Development (AMED) under Grant no. JP21ek0109567.

Conflict of interest

YuiT has received remuneration from Janssen Pharmaceuticals and Daiichi Sankyo, as well as research funds from Mochida. YT has received research grants from Janssen Pharmaceuticals and Nippon Shinyaku. MA has received remuneration from Janssen Pharmaceuticals.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger JR, Nicolls MR, et al. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *Eur Respir J*. (2019) 53:1801887. doi: 10.1183/13993003.01887-2018

2. Simonneau G, Robbins IM, Beghetti M, Channick RN, Delcroix M, Denton CP, et al. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol*. (2009) 54:S43–54. doi: 10.1016/j.jacc.2009.04.012

3. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J*. (2019) 53:1801913. doi: 10.1183/13993003.01913-2018

4. Benza RL, Miller DP, Gomberg-Maitland M, Frantz RP, Foreman AJ, Coffrey CS, et al. Predicting survival in pulmonary arterial hypertension: insights from the registry to evaluate early and long-term pulmonary arterial hypertension disease management (REVEAL). *Circulation*. (2010) 122:164–72. doi: 10.1161/CIRCULATIONAHA.109.898122

5. Benza RL, Miller DP, Barst RJ, Badesch DB, Frost AE, McGoon MD. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL registry. *Chest*. (2012) 142:448–56. doi: 10.1378/chest.11-1460

6. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Pulmonary arterial hypertension in France: results from a national registry. *Am J Respir Crit Care Med*. (2006) 173:1023–30. doi: 10.1164/rccm.200510-1668OC

7. Jansa P, Jarkovsky J, Al-Hiti H, Popelova J, Ambroz D, Zatocil T, et al. Epidemiology and long-term survival of pulmonary arterial hypertension in the Czech Republic: a retrospective analysis of a nationwide registry. *BMC Pulm Med*. (2014) 14:45. doi: 10.1186/1471-2466-14-45

8. Krowka MJ, Miller DP, Barst RJ, Taichman D, Dweik RA, Badesch DB, et al. Portopulmonary hypertension: a report from the US-based REVEAL registry. *Chest*. (2012) 141:906–15. doi: 10.1378/chest.11-0160

9. Tamura Y, Tamura Y, Taniguchi Y, Tsujino I, Inami T, Matsubara H, et al. Clinical management and outcomes of patients with portopulmonary hypertension enrolled in the Japanese multicenter registry. *Circ Rep*. (2022) 4:542–9. doi: 10.1253/circrep.CR-22-0098

10. Atsukawa M, Tsubota A, Hatano M, Kondo C, Shioda K, Ohno H, et al. Prevalence and characteristics of portopulmonary hypertension in cirrhotic patients who underwent both hepatic vein and pulmonary artery catheterization. *Hepatol Res*. (2020) 50:1244–54. doi: 10.1111/hepr.13560

11. Kawut SM, Krowka MJ, Trotter JF, Roberts KE, Benza RL, Badesch DB, et al. Clinical risk factors for portopulmonary hypertension. *Hepatology*. (2008) 48:196–203. doi: 10.1002/hep.22275

12. Krowka MJ, Swanson KL, Frantz RP, McGoon MD, Wiesner RH. Portopulmonary hypertension: results from a 10-year screening algorithm. *Hepatology*. (2006) 44:1502–10. doi: 10.1002/hep.21431

13. Mancuso L, Scordato F, Pieri M, Valerio E, Mancuso A. Management of portopulmonary hypertension: new perspectives. *World J Gastroenterol*. (2013) 19:8252–7. doi: 10.3748/wjg.v19.i45.8252

14. Schouten JNL, Garcia-Pagan JC, Vaila DC, Janssen HLA. Idiopathic noncirrhotic portal hypertension. *Hepatology*. (2011) 54:1071–81. doi: 10.1002/hep.24422

15. Sithamparanathan S, Nair A, Thirugnanasothy L, Coghlan JG, Condliffe R, Dimopoulos K, et al. Survival in portopulmonary hypertension: outcomes of the United Kingdom National Pulmonary Arterial Hypertension Registry. *J Heart Lung Transplant*. (2017) 36:770–9. doi: 10.1016/j.healun.2016.12.014

16. Takahashi Y, Yamamoto K, Sakao S, Takeuchi T, Suda R, Tanabe N, et al. The clinical characteristics, treatment, and survival of portopulmonary hypertension in Japan. *BMC Pulm Med*. (2021) 21:89. doi: 10.1186/s12890-021-01452-3

17. Thomas C, Glinskii V, de Jesus PV, Sahay S. Portopulmonary hypertension: from bench to bedside. *Front Med*. (2020) 7:569413. doi: 10.3389/fmed.2020.569413

18. Li J, Zhuang Q, Zhang X, Zheng Y, Qiao Z, Zhang J, et al. Prevalence and prognosis of portopulmonary hypertension in 223 liver transplant recipients. *Can Respir J*. (2018) 2018:9629570. doi: 10.1155/2018/9629570

19. Rodríguez-Roisin R, Krowka MJ, Hervé PH, Fallon MB. ERS task force pulmonary-hepatic vascular disorders (PHD) scientific committee. Pulmonary–hepatic vascular disorders (PHD). *Eur Respir J*. (2004) 24:861–80. doi: 10.1183/09031936.04.00010904

20. Koh C, Heller T. Approach to the diagnosis of portal hypertension. *Clin Liver Dis (Hoboken)*. (2012) 1:133–5. doi: 10.1002/cld.78

21. Castera L, Pinzani M, Bosch J. Non invasive evaluation of portal hypertension using transient elastography. *J Hepatol*. (2012) 56:696–703. doi: 10.1016/j.jhep.2011.07.005

22. Rubin LJ, Hopkins W. Clinical features and diagnosis of pulmonary hypertension of unclear etiology in adults (2022). Available from: https://www.uptodate.com/contents/clinical-features-and-diagnosis-of-pulmonary-hypertension-of-unclear-etiology-in-adults (Accessed February 10, 2023).

23. Maron BA, Galiè N. Pulmonary arterial hypertension diagnosis, treatment, and clinical management in the contemporary era. *JAMA Cardiol*. (2016) 1:1056–65. doi: 10.1001/jamacardio.2016.4471

24. Galiè N, McLaughlin VV, Rubin LJ, Simonneau G. An overview of the 6th world symposium on pulmonary hypertension. *Eur Respir J*. (2019) 53:1802148. doi: 10.1183/13993003.02148-2018

25. Certain MC, Baron A, Turpin M, Ebstein N, Boucly A, Beurnier A, et al. Outcomes of cirrhotic patients with pre-capillary pulmonary hypertension and pulmonary vascular resistance between 2 and 3 wood units. *Eur Respir J*. (2022) 60:2200107. doi: 10.1183/13993003.00107-2022

26. Lejealle C, Paradis V, Bruno O, de Raucourt E, Francoz C, Soubrane O, et al. Evidence for an association between intrahepatic vascular changes and the development of hepatopulmonary syndrome. *Chest*. (2019) 155:123–36. doi: 10.1016/j.chest.2018.09.017

27. Pellicelli AM, Barbaro G, Puoti C, Guarascio P, Lusi EA, Bellis L, et al. Plasma cytokines and portopulmonary hypertension in patients with cirrhosis waiting for orthotopic liver transplantation. *Angiology*. (2010) 61:802–6. doi: 10.1177/0003319710369101

28. Benjaminov FS, Prentice M, Sniderman KW, Siu S, Liu P, Wong F. Portopulmonary hypertension in decompensated cirrhosis with refractory ascites. *Gut*. (2003) 52:1355–62. doi: 10.1136/gut.52.9.1355

29. Yokomori H, Oda M, Ogi M, Kamegaya Y, Tsukada N, Nakamura M, et al. Enhanced expression of endothelin receptor subtypes in cirrhotic rat liver. *Liver*. (2001) 21:114–22. doi: 10.1034/j.1600-0676.2001.021002114.x

30. Angus PW. Role of endothelin in systemic and portal resistance in cirrhosis. *Gut*. (2006) 55:1230–2. doi: 10.1136/gut.2005.088633

31. Tuder RM, Cool CD, Geraci MW, Wang J, Abman SH, Wright L, et al. Prostacyclin synthase expression is decreased in lungs from patients with severe pulmonary hypertension. *Am J Respir Crit Care Med*. (1999) 159:1925–32. doi: 10.1164/ajrccm.159.6.9804054

32. Krowka MJ, Edwards WD. A spectrum of pulmonary vascular pathology in portopulmonary hypertension. *Liver Transpl*. (2000) 6:241–2. doi: 10.1002/lt.500060209

33. Sztrymf B, Libert JM, Mougeot C, Lebrec D, Mazmanian M, Humbert M, et al. Cirrhotic rats with bacterial translocation have higher incidence and severity of hepatopulmonary syndrome. *J Gastroenterol Hepatol*. (2005) 20:1538–44. doi: 10.1111/j.1440-1746.2005.03914.x

34. Fouts DE, Torralba M, Nelson KE, Brenner DA, Schnabl B. Bacterial translocation and changes in the intestinal microbiome in mouse models of liver disease. *J Hepatol*. (2012) 56:1283–92. doi: 10.1016/j.jhep.2012.01.019

35. Zhong Y, Yu PB. Decoding the link between inflammation and pulmonary arterial hypertension. *Circulation*. (2022) 146:1023–5. doi: 10.1161/CIRCULATIONAHA.122.059949

36. Austin ED, Loyd JE. The genetics of pulmonary arterial hypertension. *Circ Res*. (2014) 115:189–202. doi: 10.1161/CIRCRESAHA.115.303404

37. Bolognesi M, Di Pascoli M, Verardo A, Gatta A. Splanchnic vasodilation and hyperdynamic circulatory syndrome in cirrhosis. *World J Gastroenterol*. (2014) 20:2555–63. doi: 10.3748/wjg.v20.i10.2555

38. Benjaminov FS, Prentice M, Sniderman KW, Siu S, Liu P, Wong F. Portopulmonary hypertension in decompensated cirrhosis with refractory ascited. *Gut*. (2003) 52:1355–62. doi: 10.1136/gut.52.9.1355

39. Pellicelli AM, Barbaro G, Puoti C, Guarascio P, Lusi EA, Bellis L, et al. Plasma cytokines and portopulmonary hypertension in patients with cirrhosis waiting for orthopedic liver transplantation. *Angiology*. (2010) 61:802–6. doi: 10.1177/0003319710369101

40. Porres-Aguilar M, Altamirano JT, Torre-Delgadillo A, Charlton MR, Duarte-Rojo A. Portopulmonary hypertension and hepatopulmonary syndrome: a clinician-oriented overview. *Eur Respir Rev*. (2012) 21:223–33. doi: 10.1183/09059180.00007211

41. Shenoda B, Boselli J. Vascular syndromes in liver cirrhosis. *Clin J Gastroenterol*. (2019) 12:387–97. doi: 10.1007/s12328-019-00956-0

42. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension: the joint task force for the diagnosis and treatment of pulmonary hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J*. (2016) 37:67–119. doi: 10.1093/eurheartj/ehv317

43. Shao Y, Yin X, Qin T, Zhang R, Zhang Y, Wen X. Prevalence and associated factors of portopulmonary hypertension in patients with portal hypertension: a case-control study. *Biomed Res Int*. (2021) 2021:5595614. doi: 10.1155/2021/5595614

44. Sitbon O, Bosch J, Cottreel E, Csonka D, de Groote P, Hoeper MM, et al. Macitentan for the treatment of portopulmonary hypertension (PORTICO): a multicentre, randomised, double-blind, placebo-controlled, phase 4 trial. *Lancet Respir Med*. (2019) 7:594–604. doi: 10.1016/S2213-2600(19)30091-8

45. Preston IR, Burger CD, Bartolome S, Safdar Z, Krowka M, Sood N, et al. Ambrisentan in portopulmonary hypertension: a multicenter, open-label trial. *J Heart Lung Transplant*. (2020) 39:464–72. doi: 10.1016/j.healun.2019.12.008

46. Savale L, Guimas M, Ebstein N, Fertin M, Jevnikar M, Renard S, et al. Portopulmonary hypertension in the current era of pulmonary hypertension management. *J Hepatol*. (2020) 73:130–9. doi: 10.1016/j.jhep.2020.02.021

47. DuBrock HM, Burger CD, Bartolome SD, Feldman JP, Dunbar Ivy D, Rosenzweig EB, et al. Health disparities and treatment approaches in portopulmonary hypertension and idiopathic pulmonary arterial hypertension: an analysis of the Pulmonary Hypertension Association registry. *Pulm Circ*. (2021) 11:20458940211020913. doi: 10.1177/20458940211020913

48. Salvador ML, Loaiza CAQ, Padial LR, Barberá JA, López-Meseguer M, López-Reyes R, et al. Portopulmonary hypertension: prognosis and management in the current treatment era – results from the REHAP registry. *Intern Med J*. (2021) 51:355–65. doi: 10.1111/imj.14751

49. Atsukawa M, Takano M, Omura J. Treatment pattern and clinical outcomes in portopulmonary hypertension: a database study in Japan. *J Gastroenterol Hepatol*. (2022) 6:763–73. doi: 10.1002/jgh3.12820

50. Burger CD, DuBrock HM, Cartin-Ceba R, Moss JE, Shapiro BP, Frantz RP. Topic-based, recent literature review on pulmonary hypertension. *Mayo Clin Proc*. (2021) 96:3109–21. doi: 10.1016/j.mayocp.2021.05.026

51. Rubin LJ, Badesch DB, Barst RJ, Galiè N, Black CM, Keogh A, et al. Bosentan therapy for pulmonary arterial hypertension. *N Engl J Med*. (2002) 346:896–903. doi: 10.1056/NEJMoa012212

52. Enderby CY, Burger C. Medical treatment update on pulmonary arterial hypertension. *Ther Adv Chronic Dis*. (2015) 6:264–72. doi: 10.1177/2040622315590757

53. Deroo R, Trépo E, Holvoet T, De Pauw M, Geerts A, Verhelst X, et al. Vasomodulators and liver transplantation for portopulmonary hypertension: evidence from a systematic review and meta-analysis. *Hepatology*. (2020) 72:1701–16. doi: 10.1002/hep.31164

54. Cartin-Ceba R, Burger C, Swanson K, Vargas H, Aqel B, Keaveny AP, et al. Clinical outcomes after liver transplantation in patients with portopulmonary hypertension. *Transplantation*. (2021) 105:2283–90. doi: 10.1097/TP.0000000000003490

55. Kwon H-M, Hwang G-S. Cardiovascular dysfunction and liver transplantation. *Korean J Anesthesiol*. (2018) 71:85–91. doi: 10.4097/kjae.2018.71.2.85

56. Bozbas SS, Bozbas H. Portopulmonary hypertension in liver transplant candidates. *World J Gastroenterol*. (2016) 22:2024–9. doi: 10.3748/wjg.v22.i6.2024

57. Botha P, Parry G, Dark JH, Macgowan GA. Acute hemodynamic effects of intravenous sildenafil citrate in congestive heart failure: comparison of phosphodiesterase type-3 and -5 inhibition. *J Heart Lung Transplant*. (2009) 28:676–82. doi: 10.1016/j.healun.2009.04.013

58. Jose A, Shah SA, Anwar N, Jones CR, Sherman KE, Elwing JM. Pulmonary vascular resistance predicts mortality and graft failure in transplantation patients with portopulmonary hypertension. *Liver Transpl*. (2021) 27:1811–23. doi: 10.1002/lt.26091

frontiers | Frontiers in Medicine

TYPE Systematic Review
PUBLISHED 05 April 2023
DOI 10.3389/fmed.2023.1121036

OPEN ACCESS

EDITED BY
Shuibang Wang,
National Institutes of Health (NIH),
United States

REVIEWED BY
Diego Arauna,
University of Talca, Chile
S. Gokul,
Yerala Dental College, India

*CORRESPONDENCE
Zhong-guang Sun
✉ sunzhongguang@outlook.com
Xiao-wen Wang
✉ Wangxiaowen2003@126.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 11 December 2022
ACCEPTED 20 March 2023
PUBLISHED 05 April 2023

CITATION
Li T-t, Wang H-y, Zhang H, Zhang P-p, Zhang M-c, Feng H-y, Duan X-y, Liu W-b, Wang X-w and Sun Z-g (2023) Effect of breathing exercises on oxidative stress biomarkers in humans: A systematic review and meta-analysis.
Front. Med. 10:1121036.
doi: 10.3389/fmed.2023.1121036

Effect of breathing exercises on oxidative stress biomarkers in humans: A systematic review and meta-analysis

Ting-ting Li[1†], Hong-ying Wang[1†], Hui Zhang[1], Ping-ping Zhang[1], Ming-chen Zhang[1], Hai-yang Feng[1], Xiao-yong Duan[2], Wen-bo Liu[3,4], Xiao-wen Wang[1,5*] and Zhong-guang Sun[1*]

[1]School of Rehabilitation Medicine, Weifang Medical University, Weifang, China, [2]Weifang People's Hospital and Brain Hospital, Weifang, China, [3]School of First Clinical Medical, Weifang Medical University, Weifang, China, [4]Weifang People's Hospital, Weifang, China, [5]Affiliated Hospital of Weifang Medical University, Weifang, China

Background: Breathing exercises improve oxidative stress in healthy young adults and patients with diabetes, hypertension, and chronic obstructive pulmonary disease. Furthermore, the mechanism of respiratory intervention is controversial. Therefore, in this meta-analysis, we aimed to systematically evaluate the effects of breathing exercises on oxidative stress biomarkers in humans and provide evidence for the clinical application of breathing exercises.

Methods: The Embase, PubMed, Cochrane Library, Web of Science, CNKI, and WANFANG databases were searched for studies about the effects of breathing exercises on human oxidative stress levels, with no restraints regarding time, race, or language. The experimental group included various breathing exercises, and the outcome index included malondialdehyde, superoxide dismutase, and glutathione, nitric oxide, vitamin C, or total antioxidant capacity levels from a randomized controlled trial. Data were extracted by more than two authors and reviewed by one author.

Results: Ten studies were included from five countries. Data from patients with no disease, chronic obstructive pulmonary disease, hypertension, or diabetes were included. Participants who performed breathing exercises had greater changes in the included biomarkers than those who did not, suggesting that these biomarkers can be used to evaluate oxidative stress after respiratory interventions.

Conclusion: Breathing exercises increased SOD and GSH activities and decreased MDA content.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022337119, identifier CRD42022337119.

KEYWORDS

breathing exercises, oxidative stress, malondialdehyde, superoxide dismutase, glutathione, nitric oxide

1. Introduction

Oxidative stress (OS) is the imbalance between the production of the reactive oxygen species (ROS) by oxidation and the clearance of antioxidants in organisms (1, 2), which leads to the accumulation of free radicals, vascular endothelial lipid peroxidation, and inflammatory reactions in the body (3–6). ROS consist of O_2 and oxygen-containing molecules that have not been completely reduced (7). ROS are mainly produced in mitochondria and released into the mitochondrial matrix, intermembrane space, and cytoplasm (8–10). A small amount of ROS promotes cell proliferation, although a large quantities accumulated ROS negatively affects cell functions, resulting in lipid peroxidation that produces malondialdehyde (MDA) and, ultimately, cell death (4). Superoxide dismutase (SOD), which is widely distributed in the body, plays an important role in protecting the body from injury by dismutating the superoxide ions in internal and external environments and is an important free radical scavenger in the body (11). Reducing glutathione (GSH) combines with superoxide and free radicals to resist ROS damage to thiol groups, protecting the cell membrane that contains sulfhydryl proteins and sulfhydryl enzymes and preventing free radical damage to important organs (12). Nitric oxide (NO) bioavailability–which is mediated by ROS *via* endothelial NO synthase (eNOS) activation–leads to chronic inflammatory responses and cardiovascular injury (13).

When the body is exposed to harmful stimuli, the oxidant-antioxidant imbalance leads to the imbalance of musculin breakdown and synthesis, ultimately resulting in varying degrees of skeletal muscle atrophy and thereby leading to decreased muscle strength, which affects exercise endurance and quality of life (14). *Via* the attenuation of the mitochondrial oxidative respiratory chain and cell death, inflammatory cells activate and accumulate a large number of free oxygen radicals to promote the lipid generation of MDA, aggravating the damage of OS (15–17). Changes in the OS index are related to harmful stimulation, aging, and disease progression (18–20). The diagnosis of a disease or chronic inflammation is associated with changes in OS markers and, often, with an increase in antioxidant biomarker levels and a decrease in oxidative stress biomarker levels as the disease ameliorates (21–28). OS is measured by analyzing the composition of urine, sputum, blood, and exhaled gas (29–31). The lipid peroxidation product MDA, antioxidant enzyme SOD, and non-enzyme GSH are the most commonly used indexes (2, 32).

Moderate-intensity training improves OS while high-intensity training does not reverse OS and muscle damage during aerobic exercises (33–37). Breathing exercises, a moderate-intensity training technique used in pulmonary rehabilitation, have been widely used in clinical practice (38). By prolonging the breathing time, slowing the breathing rate, and reducing the alveolar-arterial oxygen gradient, breathing exercises enhance the strength and endurance of the respiratory muscles to increase pulmonary ventilation and improve the efficiency of gas exchange (39–44). These exercises effectively relieve the body of hypoxia and improve endurance, maintaining the overall health of the body.

Breathing exercises improve OS in healthy young adults and patients with diabetes, hypertension, and COPD (12, 45–53). However, the sample sizes of the previous studies were small, and the impact of respiratory interventions on patients without primary lung diseases is also controversial (54, 55). Therefore, in this meta-analysis, we aimed to systematically evaluate the effects of breathing exercises on OS biomarkers in humans to provide evidence for the clinical application of breathing exercises.

2. Methods

The meta-analysis was registered with PROSPERO (registration number: CRD42022337119).

2.1. Data sources and eligibility criteria

The Embase, PubMed, Cochrane Library, Web of Science, CNKI, and WANFANG databases were searched for randomized controlled trials regarding the effects of breathing exercises. There were no restraints in terms of time, race, or language. The keywords "breathing exercise" and "oxidative stress" were used to link the corresponding free words for the advanced retrieval of studies. In two Chinese databases, Chinese vocabulary retrieval was used to obtain Chinese studies (**Table 1**).

2.2. Inclusion criteria

Randomized controlled trials in which the intervention was breathing training, such as slow and fast deep breathing, a simple prototype respiratory muscle trainer, diaphragmatic breathing exercises, pranayama of yoga, straight leg raising breathing, inspiratory muscle training, or time-efficient inspiratory muscle strength training and the outcome indexes of OS were MDA, SOD, GSH, NO, vitamin C, or total antioxidant capacity (TAC) levels were included in this meta-analysis. The participants of the included studies were healthy individuals or patients with COPD, hypertension, or diabetes.

Studies that were not randomized controlled trials, including abstracts of conference papers, and those with missing data or data that could not be extracted or obtained from the author were excluded from the meta-analysis. Studies that used non-blood outcome indicators such as urine, sputum, and exhaled gas and those that did not include OS outcome indexes were also excluded. If the clinical randomized controlled trial design was determined as not rigorous, or the Jadad score was <3, the study was not included in the meta-analysis. Moreover, studies in which the breathing exercises were not included in the experimental group were excluded from the meta-analysis.

2.3. Study selection and data collection processes

Data extraction, including the title, author, year, country, the population in each group, intervention methods, intervention time, and outcome index data, was performed by two authors. Mean ± standard deviation values were directly extracted, while the data included in the figures were extracted using error bar graphs with error lines. Disputes were resolved by the examiner, and a consensus was achieved through discussion.

TABLE 1 Electronic database search strategies.

Electronic database	Search stragety	
	Mesh	Text word
Embase/pubmed/ cochrane/web of science	Breathing exercises	Exercise, breathing; respiratory muscle training; muscle training, respiratory; training, respiratory muscle
	Oxidative stress	Oxidative stresses; stress, oxidative; antioxidative stress; antioxidative stresses; stress, antioxidative; anti-oxidative stress; anti oxidative stress; anti-oxidative stresses; stress, anti-oxidative; oxidative damage; damage, oxidative; oxidative damages; oxidative stress injury; injury, oxidative stress; oxidative stress injuries; stress injury, oxidative; oxidative injury; injury, oxidative; oxidative injuries; oxidative cleavage; cleavage, oxidative; oxidative cleavages; oxidative dna damage; dna damage, oxidative; damage, oxidative dna; oxidative dna damages; dna oxidative damage; dna oxidative damages; damage, dna oxidative; oxidative damage, dna; oxidative and nitrosative stress; oxidative nitrative stress; nitrative stress, oxidative; oxidative nitrative stresses; stress, oxidative nitrative; nitro-oxidative stress; nitro oxidative stress; nitro-oxidative stresses; stress, nitro-oxidative; stresses, and nitro-oxidative
CNKI	[Subject: breathing exercise (accurate)] OR [Subject: breathing rehabilitation (accurate)] OR [Subject: inspiratory muscle training (accurate)] AND [Subject: oxidative stress (accurate)] OR [Subject: oxidation-antioxidation (accurate)] OR [Subject: oxidation reduction state (accurate)]	
WANFANG	[Subject: (breathing exercise) or Subject: (breathing rehabilitation) or Subject: (inspiratory muscle training)] and [Subject: (oxidative stress) or Subject: (oxidation-antioxidation) or Subject: (oxidation reduction state)]	

2.4. Quality evaluation

Quality assessment was independently performed by two authors using the Cochrane risk-of-bias tool to assess the literature grade based on the low risk criteria for A, B, and C. In cases of disagreement, the authors reevaluated their judgment and reached an agreement through discussion.

2.5. Statistical analysis

The extracted data were meta-analyzed using RevMan 5.4 and Stata 16.0 software. Continuous variables are presented as weighted mean differences (MD) or e mean differences, and the 95% CI was determined. Bias risk maps and forest maps were created. When I^2 was 50% or less, the fixed-effects model was used. When I^2 was more than 50%, the heterogeneity was considered to be large, and a sensitivity analysis was performed to reduce the heterogeneity. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Description of studies

A total of 408 articles were identified in the database search. Of these, 32 duplicate articles were excluded and 356 were excluded based on the title and abstract. A total of 20 articles were read in full, and 10 articles were included in this meta-analysis. The references of the included studies were searched for additional studies, but none met the inclusion criteria (**Figure 1**).

The 10 studies included in this meta-analysis were conducted in five different countries and included 519 participants, including 267 in the treatment group and 252 in the control group (**Table 2**). Breathing exercises included slow and fast deep breathing (52), a simple prototype respiratory muscle trainer (51), diaphragmatic breathing exercises (47), pranayama of yoga (45, 46, 48, 49), straight leg raising breathing (50), inspiratory muscle training (13), and time-efficient inspiratory muscle strength training (53). The shortest intervention time was 20 days (53), and the longest was 3 months (46, 47, 50). The earliest study was published in 2002 and revealed the changes in MDA and SOD levels in healthy, young individuals after pranayama yoga breathing interventions (45). Two studies were conducted in Thailand (51, 52), one in South Korea (48), five in India (45–47, 49, 50), one in the United States (14), and one in China (53). The most recent studies (13, 53) included inspiratory muscle training exercises. Patients with COPD (51–53), diabetes (46, 47, 49), and hypertension (13, 50) were included in some studies, and two studies included healthy, young adults (45, 48). The main indexes were MDA (45–48, 50–53), SOD (45–50, 53), GSH (46, 47, 49–53), and NO levels (13, 48, 51, 52). TAC (51, 52) and catalase (CAT) (48), vitamin C (46, 47, 50), and F2-isoprostane levels (48) were also measured in some studies (**Table 2**).

3.2. Risk of bias in included studies

Among the 10 included articles, three were grade A and seven were grade B. Studies that did not explicitly refer to randomized trials and allocation concealment were included in the unclear category. One study (47) did not use randomness and was considered to have a high risk of bias for the generation of random sequences. Another study (51) regarding the application of a simple prototype respiratory muscle trainer for breathing exercises did not include a complete explanation of the experimental design (**Figures 2**, **3**).

3.3. Meta-analysis

3.3.1. MDA

The MDA level was used as the main OS index in eight studies including 443 participants. MDA levels decreased significantly

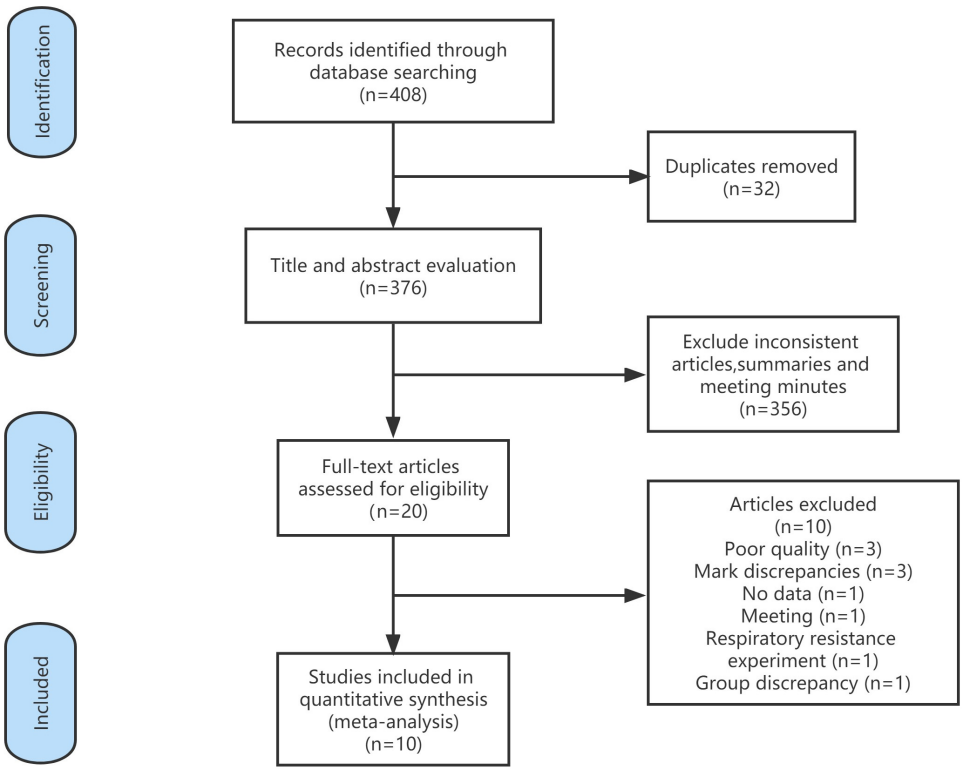

FIGURE 1
Flow diagram.

more in the experimental group than in the control group (MD = −1.31; 95% CI = −1.93 to −0.7; $P < 0.001$) (Figure 4).

3.3.2. SOD

The SOD level was used as an indicator of OS in seven studies including 433 participants. I^2 was greater than 50%. The increase in SOD levels was significantly greater in the experimental group than in the control group (MD = 1.55; 95% CI = 0.53–2.57; $P < 0.001$). However, after removing one study (48) from the sensitivity analysis, there was no significant difference in the levels of SOD between the groups (MD = 0.73; 95% CI = −0.03–1.50; $P = 0.001$) (Figure 5).

3.3.3. GSH

Glutathione levels were recorded in seven studies including 383 participants. One study (53) may have included bias as the heterogeneity reduced after its removal (I^2 = 76.7%). The GSH level of the experimental group was significantly higher than that of the control group (MD = 0.83; 95% CI = 0.28–1.38; $P < 0.001$) (Figure 6).

3.3.4. NO

Four studies including 111 participants reported NO level. The NO levels were not significantly different between the groups (MD = 2.55; 95% CI = –0.53 to 5.64; $P < 0.001$) (Figure 7).

3.3.5. Vitamin C

A total of three studies involving 220 patients discussed the changes in vitamin C levels in the human body. Vitamin C concentration did not change significantly (MD = 0.48; 95% CI = −0.08 to 1.04; $P = 0.025$) (Figure 8).

3.3.6. TAC

Total antioxidant capacity levels were measured in 50 people in two studies. The difference was not statistically significant (MD = 0.18; 95% CI = –0.37 to 0.74; $P = 0.596$) (Figure 9).

4. Discussion

Breathing exercises have been a method of rehabilitation of patients with respiratory system diseases for decades (56). Respiratory muscle training has been proven to improve lung function in healthy participants and in patients with COPD, hypertension, diabetes, and chronic kidney disease (57), as it increases exercise endurance and the overall quality of life. OS, as an indicator to measure the survival and mortality of human aging and later disease stages (26, 58–60), is a hotspot of current research, although few studies regarding the impact of breathing exercises on OS have been conducted. The role of breathing exercises in aging or disease progression can be investigated as the effect of respiratory interventions on OS indexes.

In this study, MDA, SOD, and GSH levels, the main outcome indexes of breathing exercises for OS, were investigated. Breathing exercise increased SOD and GSH activities and decreased MDA content. SOD is converted into hydrogen peroxide by catalyzing oxygen reduction, which is then converted to water by GSH. It can effectively remove oxygen free radicals, protect

TABLE 2 Characteristics of included studies.

References	Country	Population and number (treatment/control group)	Intervention of treatment group	Intervention time	Oxidative stress biomarkers
Leelarungrayub et al. (52)	Thailand	Chronic obstructive pulmonary disease 30 (15/15)	Fast deep breathing techniques	1 month; twice daily, morning and evening	MDA, GSH, NO, TAC
Leelarungrayub et al. (51)	Thailand	Chronic obstructive pulmonary disease 20 (10/10)	A simple prototype respiratory muscle trainer	6 weeks; 1–5 daily sessions of 15–30 min	MDA, GSH, NO, TAC
Lim and Cheong (48)	Korea	Young healthy People 25 (12/13)	voluntary regulation of breath (pranayama)	12 weeks; 1 day a week for 90 min	MDA, SOD, GSH, NO, CAT
Pati et al. (50)	India	Grade-1 Hypertension 57 (28/29)	Breathing practices, Straight leg raising breathing, Pranayama	3 months; 6 days in a week for 1 h	MDA, SOD, GSH, Vitamin C
Hegde et al. (47)	India	Type 2 diabetes 123 (60/63)	Diaphragmatic breathing exercise	3 months; twice daily for 15–20 min	MDA, SOD, GSH, Vitamin C
Mahapure et al. (49)	India	Diabetics 40 (30/10)	Pranayama (breathing exercises)	6 weeks; 6 days in a week for 1 h	SOD
Bhattacharya et al. (45)	India	Young healthy males 60 (30/30)	Pranayama (breathing exercises)	10 weeks; daily for 30 min	MDA, SOD
Hegde et al. (46)	India	Type 2 diabetes 40 (20/20)	Pranayama (breathing exercises)	3 months; 6 days in a week for 75–90 min	MDA, SOD, GSH, Vitamin C
Craighead et al. (13)	USA	Midlife/Older adults with above-normal blood pressure 36 (18/18)	Time-efficient inspiratory muscle strength training	6 weeks; 6 days per week	NO, TAS
Xu et al. (53)	China	Acute exacerbation of chronic obstructive pulmonary disease 88 (44/44)	Inspiratory muscle training	20 days; twice daily for 30 min	MDA, SOD, GSH

MDA, malonaldehyde; SOD, superoxide dismutase; GSH, glutathione; NO, nitric oxide; TAC, total antioxidant capacity; CAT, catalase; TAS, total antioxidant status.

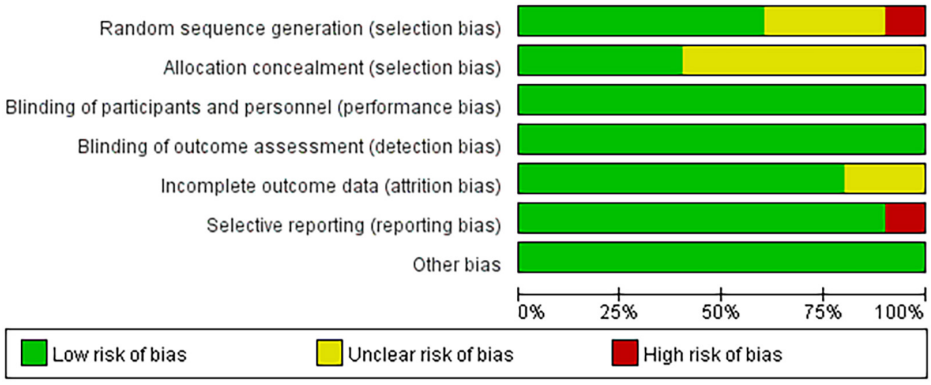

FIGURE 2
Risk of bias graph.

endothelial cells from damage, relax vascular smooth muscle by increasing NO release, thus achieving the purpose of lowering OS (48). Participants who performed breathing exercises had greater changes in these biomarkers than those who did not, suggesting that these biomarkers can be used to evaluate OS after respiratory interventions.

Malondialdehyde is an oxidation product. MDA levels decreased significantly more in participants who performed breathing exercises than in those who did not (45, 47, 48, 50, 51, 53). This may be due to the fact that MDA is the end product of lipid peroxidation (32), a reaction caused by oxygen free radicals by attacking polyunsaturated fatty acids of biofilms. MDA participates in various physiological and pathological processes in the body (4). Leelarungrayub et al. (52) and Hegde et al. (46) reported a significant decrease in MDA levels in the experimental and control groups but no statistical differences between the

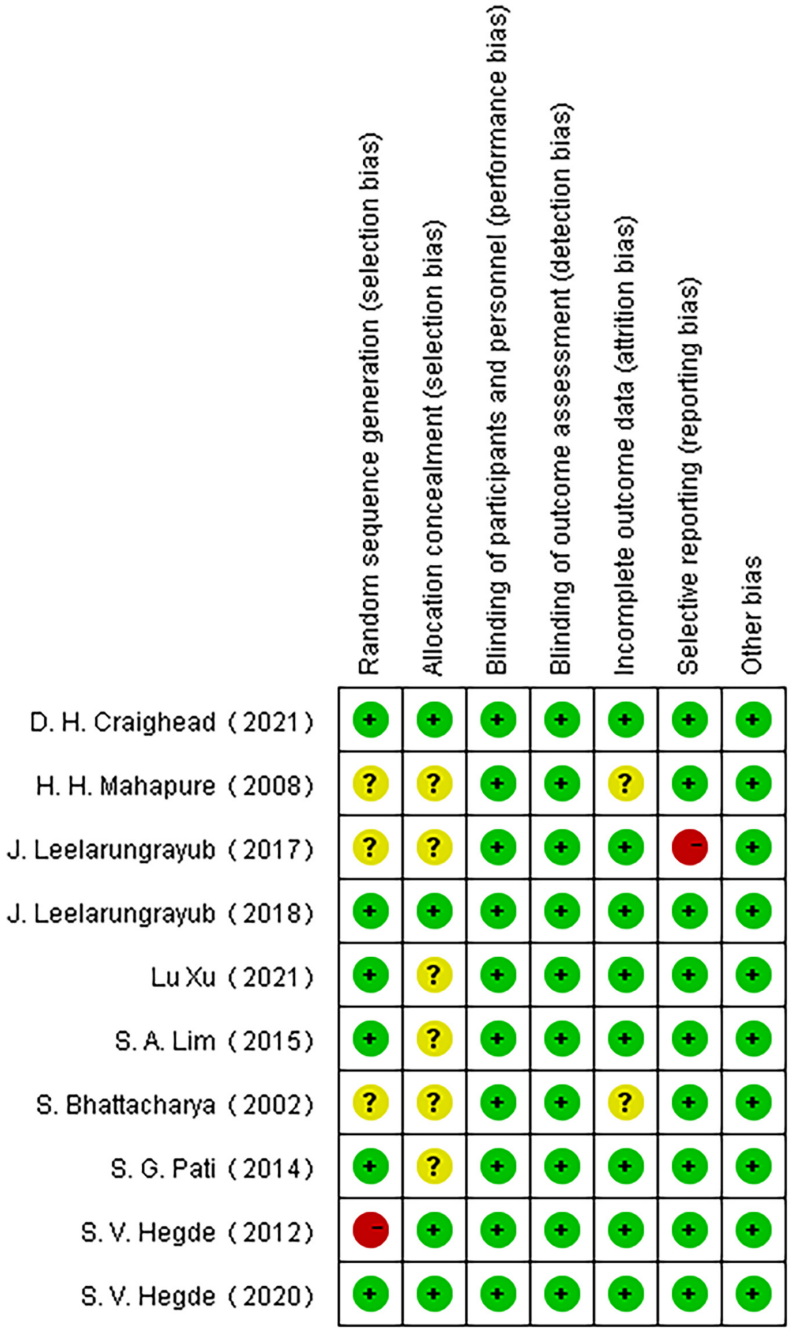

FIGURE 3
Risk of bias summary.

groups. In two studies (35, 54), non-aerobic exercises as part of the slow deep breathing method and yoga required a certain intensity of training, and both improved the body's antioxidant capacity and reduced the MDA content compared with those in the control groups (35), accounting for the lack of significant differences between the groups. These results are similar to those of another study (54) that compared breathing exercises to aerobic exercises in patients undergoing hemodialysis.

In two studies (45, 47), SOD levels decreased in patients with diabetes who performed breathing exercises. This decrease may be due to the adverse effects of long-term, low-intensity training on the body's antioxidant capacity (61). Chronically increased blood glucose levels increase the content of glycation end products in the body, leading to increased oxidase activity, self-oxidation of glucose, and glycosylation of proteins, resulting in OS reactions (62–64). However, Mahapure et al. (49) reported that SOD levels increased significantly in patients with diabetes performing breathing exercises, which may be related to yoga exercises that are more intense than the breathing exercises. High-intensity exercises resulted in increased SOD levels in patients with diabetes, improving the OS state (65). After a sensitivity analysis and the exclusion of this previous study, the total effect size was not

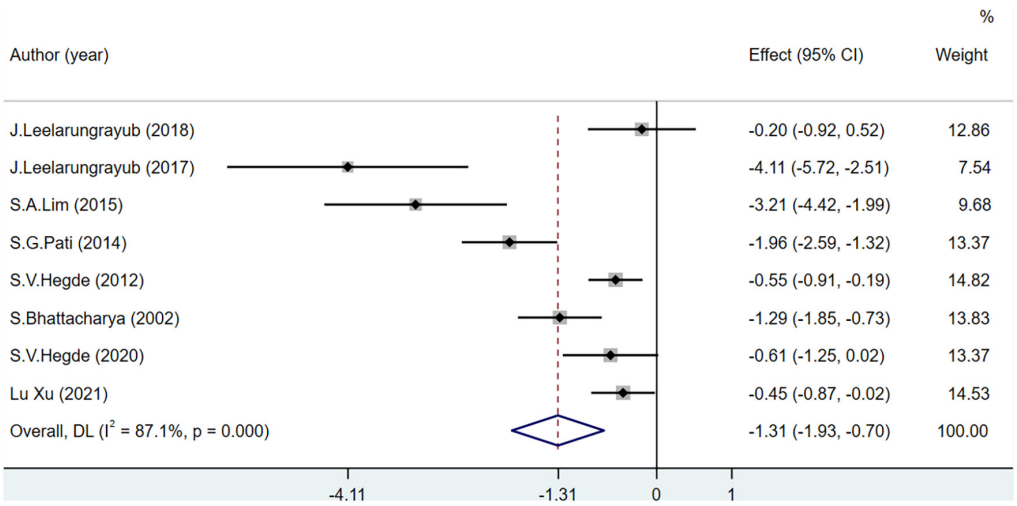

FIGURE 4
Effects of breathing exercises on malondialdehyde levels.

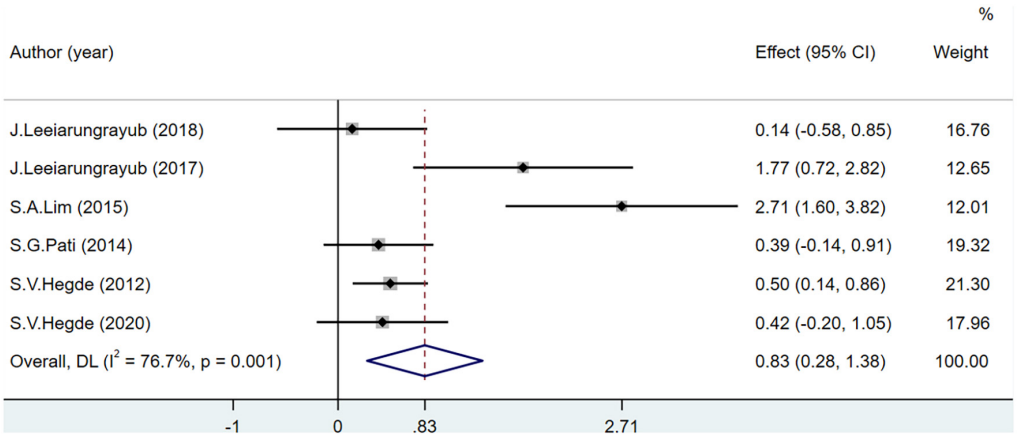

FIGURE 5
Effects of breathing exercises on superoxide dismutase levels.

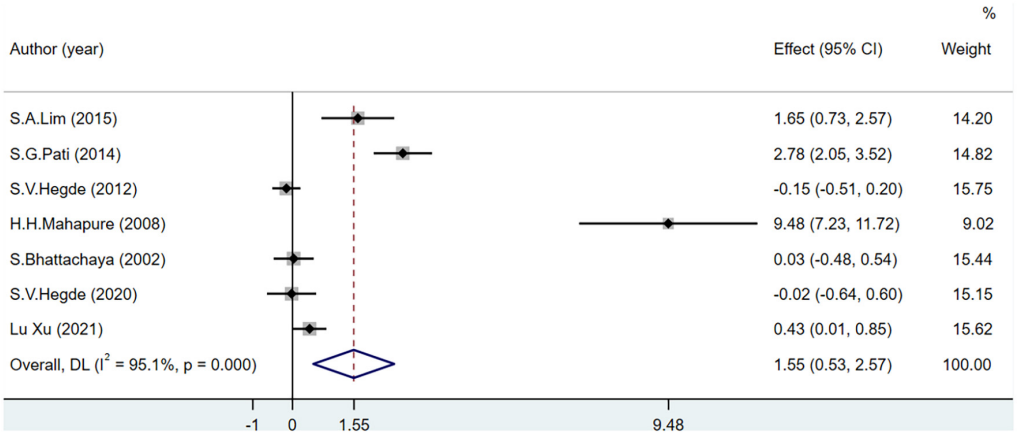

FIGURE 6
Effects of breathing exercises on glutathione levels.

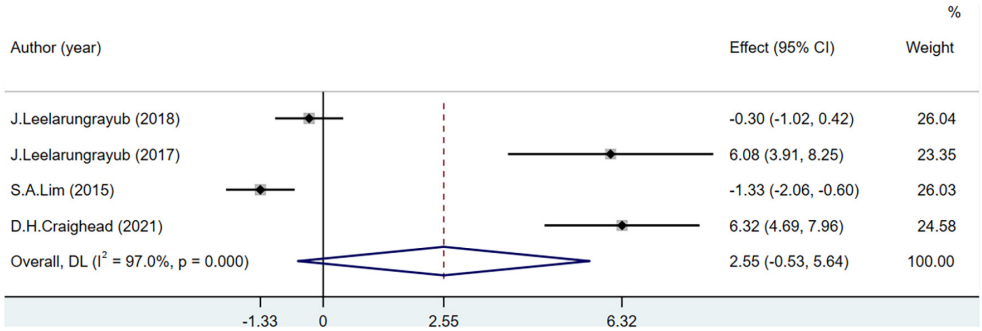

FIGURE 7
Effects of breathing exercises on nitric oxide levels.

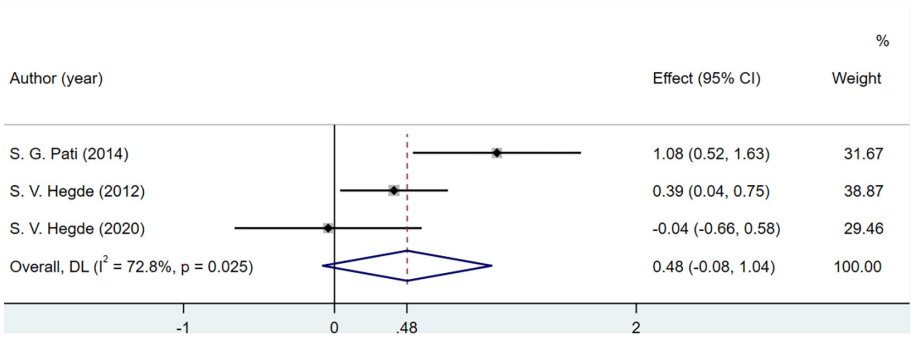

FIGURE 8
Effects of breathing exercises on vitamin C levels.

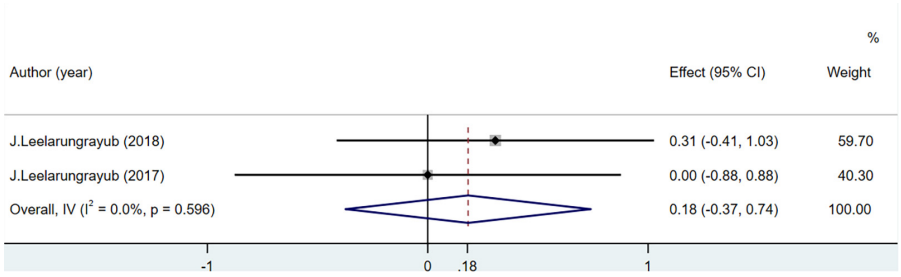

FIGURE 9
Effects of breathing exercises on total antioxidant capacity levels.

statistically significant in this meta-analysis. In healthy participants and patients with hypertension and COPD (45, 48, 50, 53), SOD dismutates the superoxide anions to generate hydrogen peroxide and oxygen during stable breathing exercises, protecting the body from damage caused by the internal and external environments and maintaining normal physiological activities (11).

The total effect size of the increase in GSH levels was significant in participants performing breathing exercises in this meta-analysis; however, Leelarungrayub et al. (51, 52) concluded that there was no significant change in GSH levels in patients with COPD. This may be due to the training method or the disease period of the population of the included studies. Another study (53) used a Drager Evi-ta-II ventilator to conduct respiratory interventions on patients with acute exacerbations of COPD and reported increased GSH levels, which improved the antioxidant capacity of the body. However, the sensitivity analysis in this study showed that there may be some bias in the previous study. In studies regarding the increase in GSH levels in patients with metabolic diseases and in healthy participants (46–48, 50), GSH improved the antioxidant defense mechanism by reducing the hydrogen peroxide dismutated from SOD into water during breathing exercises (12).

In this analysis, the variation of NO levels was the biggest difference, which may be due to the specific exercise programs in each included study. Moderate exercise increases the secretion of NO by endothelial cells, while long-term and high-intensity exercise can reduce the secretion of NO by endothelial cells (65). In patients with COPD, different respiratory interventions have resulted in conflicting data (13, 48, 51). These differences may

also be related to the stimulation degree of endothelial cells in different disease states (66). A high NO level in patients with COPD is related to the severity of the disease and airflow obstruction (67). Breathing exercises stimulate endothelial cells to reduce NO secretion, which helps to prevent deterioration due to the disease (52). In patients with hypertension, oxidation imbalance leads to the production of a large quantity of ROS (68). The bioavailability of NO is increased by the activation of eNOS with ROS, improving OS (13). Under normal conditions, no changes in OS will occur in healthy individuals, and the increase in NO levels will cause damage to the tissues and cells of the body (69). Breathing exercises do not increase NO levels in healthy individuals (48). Therefore, when NO is used as an indicator of OS in different physiological states of the body, its influence on overall OS should be further determined according to the reaction mechanism in the body.

Total antioxidant capacity and CAT, vitamin C, and F2-isoprostane levels were not included in the meta-analysis. TAC and CAT levels were not significantly different between the groups in three studies (48, 51, 52), which may be due to the low sensitivity of these indicators or because respiratory interventions did not result in significant changes. Vitamin C may not be an accurate biomarker of oxidation-antioxidant status, as it can be obtained through food and vitamins (70, 71). Of the 10 studies included in this meta-analysis, one (48) showed the serum F2-isoprostane levels in healthy individuals and reported that the experimental group had lower levels than the control group. The F2-isoprostane content in exhaled air among patients with COPD increased after breathing exercises were performed but decreased after 6 min of walking (72). This may be due to the high variability and small sample size in the previous study. Rapid or gentle breathing regulates the contraction and relaxation of skeletal muscle, which can affect the F2-isoprostane content in exhaled air (73, 74). Aerobic exercise has been reported to reduce F2-isoprostane levels in patients with circulatory system or metabolic diseases (75, 76). However, the effects and action mechanism of breathing exercises on F2-isoprostane remain unknown. Future studies evaluating the role of F2-isoprostane in the OS state are needed to confirm the reference values in patients performing breathing exercises.

In the early stages of aging and diseases, the body reacts to harmful environmental stimuli by increasing the antioxidant levels (77). However, when these levels exceed the antioxidant capacity of the body, exogenous ROS cause inflammation (78), stimulate the release of endogenous superoxide and hydrogen peroxide to produce OS, and lead to a series of pathological reactions such as mitochondrial disorders, lipid peroxidation, and apoptosis. Inflammatory and pathological reactions further promote OS, creating a vicious cycle that worsens the disease (79). Breathing exercises are steady and medium-intensity exercises (54, 74), which effectively inhibit lipid peroxidation reactions, maintain the antioxidant capacity at a high level, enhance the stability of the internal environment, and promote the removal of harmful substances (80, 81).

4.1. Study limitations

Owing to the limited number of published articles, the specific methods of respiratory interventions and disease types were not strictly distinguished in this study. The differences in conclusions regarding changes in OS indicators in the included studies may be related to different breathing exercises and disease states included in the studies.

5. Conclusion

Breathing exercises can improve the main biological indicators of OS toward the direction of antioxidation and improve the OS state by increasing the levels of antioxidants and reducing those of oxidative markers. In addition, the MDA level is the most commonly used and most sensitive indicator to evaluate the impact of breathing exercises on the OS state. However, the effect of SOD on patients with diabetes and the role of GSH in patients with COPD require further research. The reaction mechanism of NO in patients with different diseases should be considered when it is used as an indicator of OS.

Data availability statement

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

Author contributions

TTL: conceptualization, methodology, formal analysis, investigation, and writing—original draft. HYW and HZ: investigation and editing. PPZ: conceptualization and writing—review and editing. MCZ and HYF: investigation. XYD and WBL: supervision. XWW and ZGS: writing—review and editing. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Youth Project of Shandong Natural Science Foundation (grant number: ZR2022QH094) and the National Food Safety Risk Assessment Center Scientific Research Joint Research Plan Project (grant number: LH2022GG11).

Acknowledgments

We are grateful to XYD and WBL for providing language support.

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.

Publisher's note

References

1. Betteridge D. What is oxidative stress? *Metabolism.* (2000) 49 (Suppl 1):3–8. doi: 10.1016/S0026-0495(00)80077-3

2. Marrocco I, Altieri F, Peluso I. Measurement and clinical significance of biomarkers of oxidative stress in humans. *Oxid Med Cell Longev.* (2017) 2017:6501046. doi: 10.1155/2017/6501046

3. Alkadi H. A review on free radicals and antioxidants. *Infect Disord Drug Targets.* (2020) 20:16–26. doi: 10.2174/1871526518666180628124323

4. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: analytical and biological challenges. *Anal Biochem.* (2017) 524:13–30. doi: 10.1016/j.ab.2016.10.021

5. El Assar M, Angulo J, Rodríguez-Mañas L. Oxidative stress and vascular inflammation in aging. *Free Radic Biol Med.* (2013) 65:380–401. doi: 10.1016/j.freeradbiomed.2013.07.003

6. Reuter S, Gupta S, Chaturvedi M, Aggarwal B. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med.* (2010) 49:1603–16. doi: 10.1016/j.freeradbiomed.2010.09.006

7. Jakubczyk K, Dec K, Kałduńska J, Kawczuga D, Kochman J, Janda K. Reactive oxygen species - sources, functions, oxidative damage. *Pol Merkur Lekarski.* (2020) 48:124–7.

8. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol.* (2004) 55:373–99. doi: 10.1146/annurev.arplant.55.031903.141701

9. Yang S, Lian G. ROS and diseases: role in metabolism and energy supply. *Mol Cell Biochem.* (2020) 467:1–12. doi: 10.1007/s11010-019-03667-9

10. Zhang J, Wang X, Vikash V, Ye Q, Wu D, Liu Y, et al. ROS and ROS-mediated cellular signaling. *Oxid Med Cell Longev.* (2016) 2016:4350965. doi: 10.1155/2016/4350965

11. Wang Y, Branicky R, Noë A, Hekimi S. Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling. *J Cell Biol.* (2018) 217:1915–28. doi: 10.1083/jcb.201708007

12. Diaz-Vivancos P, de Simone A, Kiddle G, Foyer C. Glutathione–linking cell proliferation to oxidative stress. *Free Radic Biol Med.* (2015) 89:1154–64. doi: 10.1016/j.freeradbiomed.2015.09.023

13. Craighead D, Heinbockel T, Freeberg K, Rossman M, Jackman R, Jankowski L, et al. Time-efficient inspiratory muscle strength training lowers blood pressure and improves endothelial function, NO bioavailability, and oxidative stress in midlife/older adults With Above-normal blood pressure. *J Am Heart Assoc.* (2021) 10:e020980. doi: 10.1161/JAHA.121.020980

14. Rossi P, Marzani B, Giardina S, Negro M, Marzatico F. Human skeletal muscle aging and the oxidative system: cellular events. *Curr Aging Sci.* (2008) 1:182–91. doi: 10.2174/1874609810801030182

15. Cheresh P, Kim S, Tulasiram S, Kamp D. Oxidative stress and pulmonary fibrosis. *Biochim Biophys Acta.* (2013) 1832:1028–40. doi: 10.1016/j.bbadis.2012.11.021

16. Kudryavtseva A, Krasnov G, Dmitriev A, Alekseev B, Kardymon O, Sadritdinova A, et al. Mitochondrial dysfunction and oxidative stress in aging and cancer. *Oncotarget.* (2016) 7:44879–905. doi: 10.18632/oncotarget.9821

17. Peoples J, Saraf A, Ghazal N, Pham T, Kwong J. Mitochondrial dysfunction and oxidative stress in heart disease. *Exp Mol Med.* (2019) 51:1–13. doi: 10.1038/s12276-019-0355-7

18. Liu C, Lyass A, Larson M, Massaro J, Wang N, D'Agostino R Sr., et al. Biomarkers of oxidative stress are associated with frailty: the Framingham Offspring Study. *Age (Dordr).* (2016) 38:1. doi: 10.1007/s11357-015-9864-z

19. Saum K, Dieffenbach A, Jansen E, Schöttker B, Holleczek B, Hauer K, et al. Association between oxidative stress and frailty in an elderly German population: results from the Esther cohort study. *Gerontology.* (2015) 61:407–15. doi: 10.1159/000380881

20. Soysal P, Isik A, Carvalho A, Fernandes B, Solmi M, Schofield P, et al. Oxidative stress and frailty: a systematic review and synthesis of the best evidence. *Maturitas.* (2017) 99:66–72. doi: 10.1016/j.maturitas.2017.01.006

21. Radi E, Formichi P, Battisti C, Federico A. Apoptosis and oxidative stress in neurodegenerative diseases. *J Alzheimers Dis.* (2014) 42 (Suppl 3):S125–52. doi: 10.3233/JAD-132738

22. Bisht S, Faiq M, Tolahunase M, Dada R. Oxidative stress and male infertility. *Nat Rev Urol.* (2017) 14:470–85. doi: 10.1038/nrurol.2017.69

23. Chen Z, Zhong C. Oxidative stress in Alzheimer's disease. *Neurosci Bull.* (2014) 30:271–81. doi: 10.1007/s12264-013-1423-y

24. Kattoor A, Pothineni N, Palagiri D, Mehta J. Oxidative stress in atherosclerosis. *Curr Atheroscler Rep.* (2017) 19:42. doi: 10.1007/s11883-017-0678-6

25. Khatri N, Thakur M, Pareek V, Kumar S, Sharma S, Datusalia A. Oxidative stress: major threat in traumatic brain injury. *CNS Neurol Disord Drug Targets.* (2018) 17:689–95. doi: 10.2174/1871527317666180627120501

26. Shaw P, Werstuck G, Chen Y. Oxidative stress and aging diseases. *Oxid Med Cell Longev.* (2014) 2014:569146. doi: 10.1155/2014/569146

27. Steven S, Frenis K, Oelze M, Kalinovic S, Kuntic M, Bayo Jimenez M, et al. Vascular inflammation and oxidative stress: major triggers for cardiovascular disease. *Oxid Med Cell Longev.* (2019) 2019:7092151. doi: 10.1155/2019/7092151

28. Vona R, Gambardella L, Cittadini C, Straface E, Pietraforte D. Biomarkers of oxidative stress in metabolic syndrome and associated diseases. *Oxid Med Cell Longev.* (2019) 2019:8267234. doi: 10.1155/2019/8267234

29. Antus B. Oxidative stress markers in sputum. *Oxid Med Cell Longev.* (2016) 2016:2930434. doi: 10.1155/2016/2930434

30. Tóthová L, Kamodyová N, Èervenka T, Celec P. Salivary markers of oxidative stress in oral diseases. *Front Cell Infect Microbiol.* (2015) 5:73. doi: 10.3389/fcimb.2015.00073

31. Patsiris S, Stelios G, Papanikolaou I, Exarchos T, Vlamos P. Exhaled breath condensate: a potential tool to evaluate the relationship between inflammation and dyspnea in patients with chronic obstructive pulmonary disease. *J Lung Health Dis.* (2021) 5:3–7. doi: 10.29245/2689-999X/2021/1.1168

32. Sánchez-Rodríguez M, Mendoza-Núñez V. Oxidative stress indexes for diagnosis of health or disease in humans. *Oxid Med Cell Longev.* (2019) 2019:4128152. doi: 10.1155/2019/4128152

33. Mendoza-Núñez V, Arista-Ugalde T, Rosado-Pérez J, Ruiz-Ramos M, Santiago-Osorio E. Hypoglycemic and antioxidant effect of Tai chi exercise training in older adults with metabolic syndrome. *Clin Interv Aging.* (2018) 13:523–31. doi: 10.2147/CIA.S157584

34. Niu A. Effect of "TAI chi" exercise on antioxidant enzymes activities and immunity function in middle-aged participants. *Afr J Tradit Complement Altern Med.* (2016) 13:87–90.

35. Powers S, Radak Z, Ji L. Exercise-induced oxidative stress: past, present and future. *J Physiol.* (2016) 594:5081–92. doi: 10.1113/JP270646

36. Fiorenza M, Gunnarsson T, Ehlers T, Bangsbo J. High-intensity exercise training ameliorates aberrant expression of markers of mitochondrial turnover but not oxidative damage in skeletal muscle of men with essential hypertension. *Acta Physiol (Oxf).* (2019) 225:e13208. doi: 10.1111/apha.13208

37. Cezar M, Oliveira Junior S, Damatto R. Moderate-intensity resistance training improves oxidative stress in heart. *Arq Bras Cardiol.* (2021) 116:12–3.

38. Cornelison S, Pascual R. Pulmonary rehabilitation in the management of chronic lung disease. *Med Clin North Am.* (2019) 103:577–84. doi: 10.1016/j.mcna.2018.12.015

39. Beaumont M, Forget P, Couturaud F, Reychler G. Effects of inspiratory muscle training in COPD patients: a systematic review and meta-analysis. *Clin Respir J.* (2018) 12:2178–88. doi: 10.1111/crj.12905

40. Illi S, Held U, Frank I, Spengler C. Effect of respiratory muscle training on exercise performance in healthy individuals: a systematic review and meta-analysis. *Sports Med.* (2012) 42:707–24. doi: 10.1007/BF03262290

41. Yan H, Ouyang Y, Wang L, Luo X, Zhan Q. Effect of respiratory rehabilitation training on elderly patients with COVID-19: a protocol for systematic review and meta-analysis. *Med (Baltim).* (2020) 99:e22109. doi: 10.1097/MD.0000000000022109

42. Khandekar J, Vasavi V, Singh V, Samuel S, Sudhan S, Khandelwal B. Effect of yoga on blood pressure in prehypertension: a systematic review and meta-analysis. *Sci World J.* (2021) 2021:4039364. doi: 10.1155/2021/4039364

43. de Medeiros A, Fuzari H, Rattesa C, Brandão D, de Melo Marinho P. Inspiratory muscle training improves respiratory muscle strength, functional capacity and quality of life in patients with chronic kidney disease: a systematic review. *J Physiother.* (2017) 63:76–83. doi: 10.1016/j.jphys.2017.02.016

44. Senaratna C, Perret J, Lodge C, Lowe A, Campbell B, Matheson M, et al. Prevalence of obstructive sleep apnea in the general population: a systematic review. *Sleep Med Rev.* (2017) 34:70–81. doi: 10.1016/j.smrv.2016.07.002

45. Bhattacharya S, Pandey U, Verma N. Improvement in oxidative status with yogic breathing in young healthy males. *Indian J Physiol Pharmacol.* (2002) 46:349-354.

46. Hegde S, Adhikari P, Kotian S, Shastry R. Effects of yoga Versus sham yoga on oxidative stress, glycemic status, and anthropometry in type 2 diabetes mellitus: a single-blinded randomized pilot study. *Int J Yoga Therap.* (2020) 30:33-39. doi: 10.17761/D-18-2020-00018

47. Hegde S, Adhikari P, Subbalakshmi N, Nandini M, Rao G, D'Souza V. Diaphragmatic breathing exercise as a therapeutic intervention for control of oxidative stress in type 2 diabetes mellitus. *Complement Ther Clin Pract.* (2012) 18:151-153. doi: 10.1016/j.ctcp.2012.04.002

48. Lim S, Cheong K. Regular yoga practice improves antioxidant status, immune function, and stress hormone releases in young healthy people: a randomized, double-blind, controlled pilot study. *J Altern Complement Med.* (2015) 21:530-538. doi: 10.1089/acm.2014.0044

49. Mahapure H, Shete S, Bera T. Effect of yogic exercise on super oxide dismutase levels in diabetics. *Int J Yoga.* (2008) 1:21-26. doi: 10.4103/0973-6131.36792

50. Patil S, Dhanakshirur G, Aithala M, Naregal G, Das K. Effect of yoga on oxidative stress in elderly with grade-I hypertension: a randomized controlled study. *J Clin Diagn Res.* (2014) 8:BC04-BC07. doi: 10.7860/JCDR/2014/9498.4586

51. Leelarungrayub J, Pinkaew D, Puntumetakul R, Klaphajone J. Effects of a simple prototype respiratory muscle trainer on respiratory muscle strength, quality of life and dyspnea, and oxidative stress in COPD patients: a preliminary study. *Int J Chron Obstruct Pulmon Dis.* (2017) 12:1415-1425. doi:10.2147/COPD.S131062

52. Leelarungrayub J, Puntumetakul R, Sriboonreung T, Pothasak Y, Klaphajone J. Preliminary study. Preliminary study: comparative effects of lung volume therapy between slow and fast deep-breathing techniques on pulmonary function, respiratory muscle strength, oxidative stress, cytokines, 6-minute walking distance, and quality of life in persons with COPD. *Int J Chron Obstruct Pulmon Dis.* (2018) 13:3909-3921. doi: 10.2147/COPD.S181428

53. Xu L, Li G, Gan Y, Zhang J, Li X. Effects of external diaphragmatic pacing combined with inspiratory muscle training on arterial blood gas parameters,respiratory mechanical parameters,and oxidative stress response in patients with AECOPD. *Anhui Med Pharm J.* (2021) 25:777–81.

54. Figueiredo P, Lima M, Costa H, Martins J, Flecha O, Gonçalves P, et al. Effects of the inspiratory muscle training and aerobic training on respiratory and functional parameters, inflammatory biomarkers, redox status and quality of life in hemodialysis patients: a randomized clinical trial. *PLoS One.* (2018) 13:e0200727. doi: 10.1371/journal.pone.0200727

55. Campos N, Marizeiro D, Florêncio A, Silva Í, Meneses G, Bezerra G, et al. Effects of respiratory muscle training on endothelium and oxidative stress biomarkers in hemodialysis patients: a randomized clinical trial. *Respir Med.* (2018) 134:103–9. doi: 10.1016/j.rmed.2017.12.005

56. Pardy R, Reid W, Belman M. Respiratory muscle training. *Clin Chest Med.* (1988) 9:287–96. doi: 10.1016/S0272-5231(21)00505-0

57. Göhl O, Walker D, Walterspacher S, Langer D, Spengler C, Wanke T, et al. [Respiratory muscle training: state of the art]. *Pneumologie.* (2016) 70:37–48. doi: 10.1055/s-0041-109312

58. Mills D, Johnson M, Barnett Y, Smith W, Sharpe G. The effects of inspiratory muscle training in older adults. *Med Sci Sports Exerc.* (2015) 47:691–7. doi: 10.1249/MSS.0000000000000474

59. Nakamura T, Naguro I, Ichijo H. Iron homeostasis and iron-regulated ROS in cell death, senescence and human diseases. *Biochim Biophys Acta Gen Subj.* (2019) 1863:1398–409. doi: 10.1016/j.bbagen.2019.06.010

60. Brieger K, Schiavone S, Miller F Jr., Krause K. Reactive oxygen species: from health to disease. *Swiss Med Wkly.* (2012) 142:w13659. doi: 10.4414/smw.2012.13659

61. Woo J, Yeo N, Shin K, Lee H, Yoo J, Kang S. Antioxidant enzyme activities and DNA damage in children with type 1 diabetes mellitus after 12 weeks of exercise. *Acta Paediatr.* (2010) 99:1263–8. doi:10.1111/j.1651-2227.2010.01736.x

62. Halim M, Halim A. The effects of inflammation, aging and oxidative stress on the pathogenesis of diabetes mellitus (type 2 diabetes). *Metab Syndr.* (2019) 13:1165–72. doi: 10.1016/j.dsx.2019.01.040

63. Rehman K, Akash M. Mechanism of generation of oxidative stress and pathophysiology of type 2 diabetes mellitus: how are they interlinked? *J Cell Biochem.* (2017) 118:3577–85. doi: 10.1002/jcb.26097

64. Nowotny K, Jung T, Höhn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. *Biomolecules.* (2015) 5:194–222. doi: 10.3390/biom5010194

65. Gordon L, Morrison E, McGrowder D, Young R, Fraser Y, Zamora E, et al. Effect of exercise therapy on lipid profile and oxidative stress indicators in patients with type 2 diabetes. *BMC Complement Altern Med.* (2008) 8:21. doi: 10.1186/1472-6882-8-21

66. Wong B, Marsch E, Treps L, Baes M, Carmeliet P. Endothelial cell metabolism in health and disease: impact of hypoxia. *EMBO J.* (2017) 36:2187–203. doi: 10.15252/embj.201696150

67. ben Anes A, Fetoui H, Bchir S, ben Nasr H, Chahdoura H, Chabchoub E, et al. Increased oxidative stress and altered levels of nitric oxide and peroxynitrite in Tunisian patients with chronic obstructive pulmonary disease: correlation with disease severity and airflow obstruction. *Biol Trace Elem Res.* (2014) 161:20–31. doi: 10.1007/s12011-014-0087-4

68. Guzik T, Touyz R. Oxidative stress, inflammation, and vascular aging in hypertension. *Hypertension.* (2017) 70:660–7. doi: 10.1161/HYPERTENSIONAHA.117.07802

69. Pacher P, Beckman J, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev.* (2007) 87:315–424. doi: 10.1152/physrev.00029.2006

70. Oudemans-van Straaten H, Spoelstra-de Man A, de Waard M. Vitamin C revisited. *Crit Care.* (2014) 18:460. doi: 10.1186/s13054-014-0460-x

71. Kaźmierczak-Barańska J, Boguszewska K, Adamus-Grabicka A, Karwowski B. Two faces of vitamin C-antioxidative and pro-oxidative agent. *Nutrients.* (2020) 12:1501. doi: 10.3390/nu12051501

72. Kaminsky D, Guntupalli K, Lippmann J, Burns S, Brock M, Skelly J, et al. Effect of yoga breathing (pranayama) on exercise tolerance in patients with chronic obstructive pulmonary disease: a randomized, controlled trial. *J Altern Complement Med.* (2017) 23:696–704. doi: 10.1089/acm.2017.0102

73. Nikolaidis M, Kyparos A, Vrabas ISF. 2-isoprostane formation, measurement and interpretation: the role of exercise. *Prog Lipid Res.* (2011) 50:89–103. doi: 10.1016/j.plipres.2010.10.002

74. Briskey D, Vogel K, Johnson M, Sharpe G, Coombes J, Mills D. Inspiratory flow-resistive breathing, respiratory muscle-induced systemic oxidative stress, and diaphragm fatigue in healthy humans. *J Appl Physiol (1985).* (2020) 129:185–93. doi: 10.1152/japplphysiol.00091.2020

75. Liu G, Zhao J, Cao Y. Arterial stiffness response to regular aerobic training in hypercholesterolemia patients. *Int J Sports Med.* (2018) 39:413–8. doi: 10.1055/a-0574-3096

76. Ikizler T, Robinson-Cohen C, Ellis C, Headley S, Tuttle K, Wood R, et al. Metabolic effects of diet and exercise in patients with moderate to severe CKD: a randomized clinical trial. *J Am Soc Nephrol.* (2018) 29:250–9. doi: 10.1681/ASN.2017010020

77. Zhang P, Xu X, Li X. Cardiovascular diseases: oxidative damage and antioxidant protection. *Eur Rev Med Pharmacol Sci.* (2014) 18:3091–6.

78. Barnes P. Cellular and molecular mechanisms of asthma and COPD. *Clin Sci (Lond).* (2017) 131:1541–58. doi: 10.1042/CS20160487

79. Barnes P. Oxidative stress-based therapeutics in COPD. *Redox Biol.* (2020) 33:101544. doi: 10.1016/j.redox.2020.101544

80. Ryrsø C, Thaning P, Siebenmann C, Lundby C, Lange P, Pedersen B, et al. Effect of endurance versus resistance training on local muscle and systemic inflammation and oxidative stress in COPD. *Scand J Med Sci Sports.* (2018) 28:2339–48. doi: 10.1111/sms.13227

81. Alcazar J, Losa-Reyna J, Rodriguez-Lopez C, Navarro-Cruz R, Alfaro-Acha A, Ara I, et al. Effects of concurrent exercise training on muscle dysfunction and systemic oxidative stress in older people with COPD. *Scand J Med Sci Sports.* (2019) 29:1591–603. doi: 10.1111/sms.13494

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 20 April 2023
DOI 10.3389/fmed.2023.1146782

OPEN ACCESS

EDITED BY
Shuibang Wang,
National Institutes of Health (NIH),
United States

REVIEWED BY
Nicol Bernardinello,
University of Padua, Italy
Gaetano Rea,
Monaldi Hospital, Italy

*CORRESPONDENCE
Elzbieta Radzikowska
✉ e.radzikowska@wp.pl

RECEIVED 17 January 2023
ACCEPTED 03 April 2023
PUBLISHED 20 April 2023

CITATION
Radzikowska E and Fijolek J (2023) Update on cryptogenic organizing pneumonia.
Front. Med. 10:1146782.
doi: 10.3389/fmed.2023.1146782

Update on cryptogenic organizing pneumonia

Elzbieta Radzikowska * and Justyna Fijolek

III Department of Lung Diseases and Oncology, National Tuberculosis and Lung Diseases Research Institute, Warsaw, Poland

Cryptogenic organizing pneumonia (COP) is a form of idiopathic interstitial pneumonia that results from the pulmonary reaction to various unidentified injuries. Secondary organizing pneumonia is diagnosed when the triggering factor has been identified; it is mainly caused by infections, toxic substance exposure, drugs, connective tissue diseases, malignancies, autoimmune diseases, bone marrow, or organ transplantation, and radiotherapy. There has been an increase in the number of reports of drug-induced organizing pneumonia (OP). New biological therapies, interferon, monoclonal antibodies, anti-interleukin antibodies, and PD1/PDL-1 inhibitors may induce this specific pulmonary reaction. The classical form of COP is usually subacute and does not manifest as severe disease. Patients maintain sufficient respiratory function, and treatment with steroids is usually effective. Several specific forms of OP (e.g., the cicatricial variant or acute fibrinous type) have distinct clinical and histological features, require higher doses of immunosuppressive drugs, and have a worse prognosis. In the era of administering steroid-sparing therapies for the treatment of interstitial lung diseases, connective tissue dases, and other conditions, it is important to emphasize this type of therapy for patients with COP.

KEYWORDS

cryptogenic organizing pneumonia, secondary organizing pneumonia, acute fibrinous organizing pneumonia, cicatrices organizing pneumonia, prednisone, clarithromycin

Definition

Organizing pneumonia (OP) is a specific pulmonary reaction to a diverse range of pneumotoxic agents, both internal and external, which produce a radiologically and histologically characteristic type of inflammatory lesion that causes the distal airways to fill with an organizing fibrous exudate and inflammatory cells, in the absence of disrupted lung architecture (1–6).

According to the classification of idiopathic interstitial pneumonias proposed by the European Respiratory Society and the American Thoracic Society, OP is a separate clinical entity within this large group of diseases (1).

OP is regarded as cryptogenic organizing pneumonia (COP) when the causative factor has not been identified, and secondary organizing pneumonia (SOP) when the possible cause of disease is known (7–18) (Tables 1–3).

The disease is classified into the following forms according to the course and nature of lesions detected during histological examination: OP, acute fibrinous organizing pneumonia (AFOP), and cicatricial variant of organizing pneumonia (CIOP) (1, 5, 6, 19–25).

Focal OP is a specific form that usually progresses asymptomatically; lesion resection is sufficient treatment in many cases (3, 5, 6).

Epidemiology

The epidemiology of OP is poorly documented. The incidence of OP differs among populations; it is estimated to be 1.97–7/100000 (3, 5, 6). An Icelandic study revealed incidences of 1.10/100000 for COP and 0.87/100000 for SOP (26). The incidence of COP has been decreasing in recent years because of improvements in the diagnosis of causative factors. Approximately 3% of patients with interstitial lung disease are presumed to have a diagnosis of OP (5, 6). According to a Greek registry, the COP prevalence is approximately 5% among patients with interstitial lung disease; a similar registry in Spain indicates that approximately 10% of patients with interstitial lung disease also have COP (26, 27). The number of patients with OP described in previous reports varies from a few cases to 48 in the study by Lazor et al., 66 patients with COP in the study by Radzikowska et al., 76 patients with COP in the study by Yoo et al., and 100 patients in the study by Yilmaz et al. (14, 28–39). Recently, Zhang et al. reported a cohort of 1,346 patients with OP, among which 176 had COP and 1,170 had SOP (40). Epler et al. evaluated 2,500 specimens from patients with interstitial lung disease; 57 (2.3%) cases exhibited bronchiolitis along with an organizing inflammatory exudate in the alveolar lumen (5).

The disease is most often diagnosed during the 5th or 6th decade of life, although cases in children have been reported. Generally, the disease exhibits no ethnicity- or sex-specific bias. Although some studies revealed that greater proportions of patients were women and nonsmokers, others indicated that OP was more common in men. At the time of diagnosis, < 30% of patients were smokers, and > 55% of patients were nonsmokers (28–40).

Etiology and pathogenesis

Damage to the alveolar basement membrane and type II pneumocytes induced by various intrinsic and extrinsic factors is an important aspect of OP development. Vascular endothelial cells are mostly undamaged. This disruption of alveolar integrity results in the leakage of a fibrotic inflammatory exudate into the alveolar lumen, which subsequently expands into alveolar ducts and respiratory bronchioles (3, 5, 6, 41–43). Polymorphic inflammatory cells and fibroblasts migrate; undergo transformation into myofibroblasts; and bind to fibronectin, collagen I, procollagen III, tenascin C, and proteoglycans within the loose extracellular matrix. The architecture of the lung parenchyma remains unchanged. Epithelial cells proliferate, causing re-epithelialization of damaged fragments. The loose extracellular matrix containing collagen III fibers is susceptible to degradation by metalloproteases, gelatinase, and stromelysin. This process also involves increased activity among inflammatory cells, which secrete multiple cytokines that stimulate the release of enzymes capable of degrading the fibrillar exudate; the cytokines also stimulate apoptotic activity in fibroblasts (44, 45). Vasculogenesis is another mechanism involved in the disease process; it is mediated by vascular growth factors, fibroblast growth factors, and matrix metalloproteinases. Although the role of alveolar macrophages is not fully understood, cytokines released by these cells (e.g., platelet-derived growth factor-β and interleukin [IL]-8) are involved in disease progression (3, 46). Elevated levels of IL-6, IL-8, transforming growth factor-β, monocyte chemoattractant protein-1, IL-10, and IL-12 are present in serum and bronchoalveolar lavage fluid (BALF) samples from patients with OP. These observations indicate that many inflammatory cells are involved in the disease process (3, 47–49). Moreover, reduced levels of IL-6 and transforming growth factor-β have been associated with response to treatment and disease resolution (47, 49). During the resolution process, the number of inflammatory cells decreases, and fibrin deposits are removed. Concentric clusters of myofibroblasts, collagen I fibers, procollagen III, and fibronectin are formed. These clusters move into the interstitium, forming collagen foci, and the alveolar surface undergoes re-epithelialization. High apoptotic activity has been observed in fibromyxoid lesions during vascularization; this process is important during lesion healing (44, 45). Disruptions during the resolution of intra-alveolar exudate and collagen foci result in the scarring variant of OP. In COP, the inflammatory process is generally simultaneous, and the lung architecture is preserved. The presence of fibrotic lesions usually suggests overlap syndromes, such as OP with nonspecific interstitial pneumonia or with usual interstitial pneumonia (3, 25, 50).

Symptoms

COP usually develops in a subacute manner, such that the first pseudo-flu-like symptoms precede diagnosis by 2–3 months. These symptoms generally include a subfebrile state, cough (often dry or with minimal sputum), decreased exercise tolerance, weakness, weight loss, chest pain, and night sweats. Rarely, patients report copious sputum; even more rarely, they report hemoptysis (< 5%) or pneumothorax. Uncharacteristic symptoms can lead to a diagnostic delay of 1–5 months (28–39).

Rarely, OP progresses to a severe form with features of respiratory failure. Its main histological counterpart is AFOP (19–24), which is characterized by richly fibrinous inflammatory exudate in the alveoli, respiratory tract, and bronchioles; this exudate comprises spherical conglomerates, observed as diffuse bilateral shadows on radiographic imaging. AFOP is most often secondary to lung transplantation, allergic alveolitis, reactions to pneumotoxic agents, connective tissue diseases, and infections. AFOP has a high mortality rate, particularly in transplant patients (3, 6, 28–40).

Patients with focal OP are usually asymptomatic.

Physical examinations reveal weakened protrusions in areas of lesions, along with the sound of fine-bubble rales. No lesions are detected during physical examinations in approximately 30% of patients (3, 5, 6, 28–39).

COP patients usually do not show clubbed fingers, but this phenomenon is evident in patients with overlap syndromes such as usual interstitial pneumonia or nonspecific interstitial pneumonia; it

Abbreviations: AFOP, acute fibrinous organizing pneumonia; CAM, clarithromycin; CEP, chronic eosinophilic pneumonia; CIOP, cicatricial variant of organizing pneumonia; COP, cryptogenic organizing pneumonia; CT, computed tomography; HRCT, high resolution computed tomography; NSIP, non-specific interstitial pneumonia; OP, organizing pneumonia; PD1, programmed death receptor 1; PD-L1, programmed cell death-ligand 1; PRE, prednisone; ROP, radiotherapy induced organizing pneumonia; SOP, secondary organizing pneumonia; UIP, usual interstitial pneumonia.

may also be present in patients with underlying diseases (f.c. circulatory insufficiency, neoplastic) (3, 5, 28–39).

Laboratory tests

Laboratory tests detect increases in serum C-reactive protein concentration, erythrocyte sedimentation rate, and lymphocytosis in approximately 40% of patients.

TABLE 1 Autoimmunological diseases and other causes of SOP.

Autoimmunological diseases	Rheumatoid arthritis
	Dermatomyositis/ myositis
	Sjögren's disease
	Scleroderma
	Ankylosing spondylitis
	Behcét's disease
	Mixed cryoglobulinemia
	Periarteritis nodosa
	Systemic lupus erythematous
	Chronic thyroiditis
	Inflammatory bowel diseases
Other diseases	Acute respiratory distress syndrome (ARDS)
	Hypersensitivity pneumonitis
	Chronic eosinophilic pneumonia
	Overlap with nonspecific interstitial pneumonia (NSIP) and usual interstitial pneumonia (UIP)
	Acute exacerbation of idiopathic interstitial pneumonia (with UIP pattern)
	Cystic fibrosis
	Emphysema
	Bronchiectasis
	Choking
	Distally from a closed bronchus
	As part of a reaction around an abscess or infiltrate in the granulomatosis polyangiitis (GPA)
	Sarcoidosis
Neoplastic diseases	Tumors of the respiratory and digestive system
	Lymphoproliferative and myeloproliferative disorders
Transplantation	Bone marrow
	Parenchymal organs including lungs
Other diseases	Chronic heart or kidney failure
	Common variable immunodeficiency and other immune disorders
	Coronary by-pass grafts
Exposition	Toxic fumes (industrial gases, hydrogen sulfate)
	e-cigarette or other vaping products
	Cocaine inhalation
	Marijuana smoking

OP may precede the onset of autoimmune disease by many months or years. Thus, appropriate serological diagnostics should be conducted to assess rheumatic factor, anti-cyclic citrullinated peptide antibody, anti-nuclear antibody, anti-topoisomerase antibody (anti-SCl-70), anti-Jo-1 antibody, anti-Ro52 antibody, anti-dsDNA antibody, and other such factors (3, 5, 6, 28–39).

Radiological examination

Chest radiography

Routine chest radiography of patients with OP usually reveals bilateral opacities localized in peripheral parts of the lungs, without abnormal lung volume (Figure 1). Less frequently, diffuse lesions may be present, in the form of fine-spotted and nodules, single nodules, or mass like lesions. The changes are usually localized in the middle and lower lung fields, although they are also observed in the upper fields in one-third of patients. Lesions undergo migration in 50–75% of cases, and approximately 10% of patients exhibit spontaneous regression (3, 5, 6, 28–41, 43). The standard radiography is poorly sensitive and specific for OP.

Chest computed tomography

High-resolution computed tomography (HRCT) is the gold standard in the evaluation of OP. It reveals multifocal areas of consolidation, often with a characteristic air bronchogram. Additionally, patchy alveolar consolidations, nodules, areas of ground glass opacity, perilobular infiltrations, bronchial wall thickening, and reticular fibrous changes may be present in peripheral parts of both

TABLE 2 Infectious factors which can induce SOP.

Viral	Human immunodeficiency virus (HIV)
	Adenoviruses
	Influenza and Parainfluenza
	SARS- CoV-2
	SARS -CoV
	MERS- CoV
Bacterial	Mycoplasma sp.
	Chlamydia sp.
	Legionella pneumophila
	Streptococcus pnumoniae
	Staphylococcus aureus
	Actinomyces israeli
	Serratia sp.
	Nocardia sp.
Fungal	Aspergillus sp.
	Pneumocystis jiroveci
	Cryptococcus neoformans
	Penicyllium sp.
Protozoan	Plasmodium vivax

lungs. Thickening around areas of ground glass opacity with an "atoll" or "crazy-paving" pattern may also be present, although it is less common. Additionally, nodular lesions, pleural thickening, and rarely enlargement of hilar and mediastinal lymph nodes, are present; emphysema or pleural effusion may also be observed (28–40). Honeycomb-type lesions are not in the spectrum of pulmonary changes observed in COP but might be evident in patients who exhibit interstitial pulmonary fibrosis with a component of OP (Figures 2–6).

The micronodular form of OP is more frequently observed during the course of OP accompanying myeloproliferative diseases or infections, or after exposure to marijuana smoke (3, 28–40).

Some case series have reported higher numbers of solitary mass-like lesions mimicking lung neoplasia, whereas other case series have indicated that such lesions were present in only a few patients (Table 4) (3, 6, 40, 51, 52).

TABLE 3 Drug induced SOP.

Antibiotics, antibacterial, and antifungal chemotherapeutics	Minocycline,
	Nitrofurantoin
	Cephalosporins Amphotericin
Antiarrhythmic	Amiodarone
	Beta- blockers
	Phenytoin
	Hydralazine
	Timolol
Biological agents	Interferons
	Trastuzumab
	Rituximab
	Bortezomib
	Ceritinib
	Tocilizumab
	Etanercept
	Infliximab
	Ipilimumab
	Other new biological drugs
Kinases inhibitors	Sirolimus, Everolimus
	Anti EGFR inhibitors
	Anti ALK inhibitors
PD-1/PD-L1 inhibitors	Pembrolizumab
	Atezolizumab
	Nivolumab
Antineoplastic chemotherapeutics	Azathioprine
	Chlorambucil
	Cladribine
	Bleomycin
	Busulfan
	Mitomycin
	Methotrexate
	Doxorubicin
	Daptomycin
	Oxaliplatin
	Thalidomide
Radiotherapy	Particularly breast
Other	Statins
	Dihydroergocryptine
	Penicillamine
	Propylthiouracil
Antiepileptics	Carbamazepine

For all possible causes of OP induced by treatment see www.pneumotox.com.

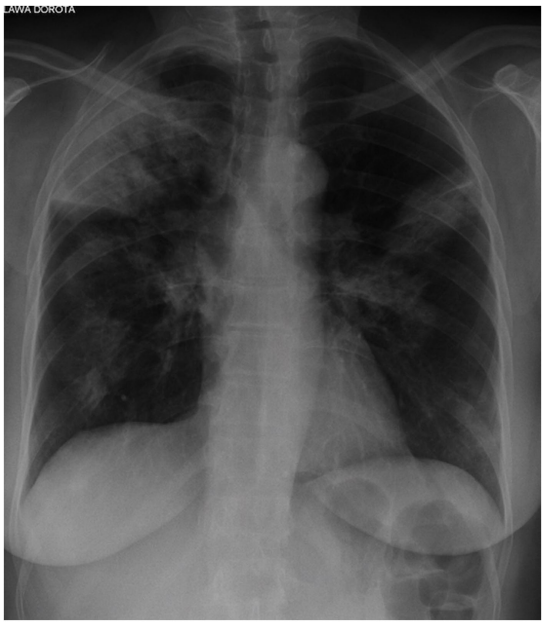

FIGURE 1
Chest X-ray of OP. Bilateral opacities localized in the peripheral parts of the both lungs.

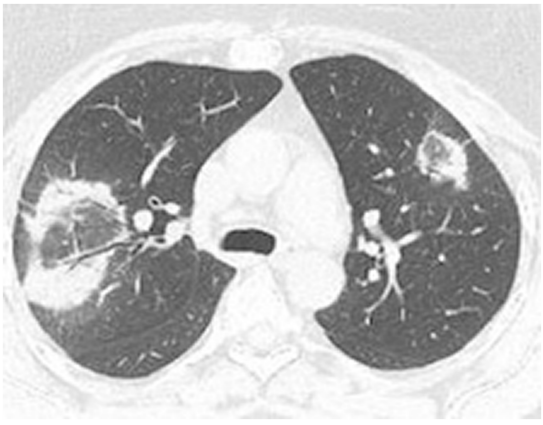

FIGURE 2
High resolution computed tomography scan of a patient with COP. Bilateral ground glass opacities with associated peripheral consolidations forming an atoll sign.

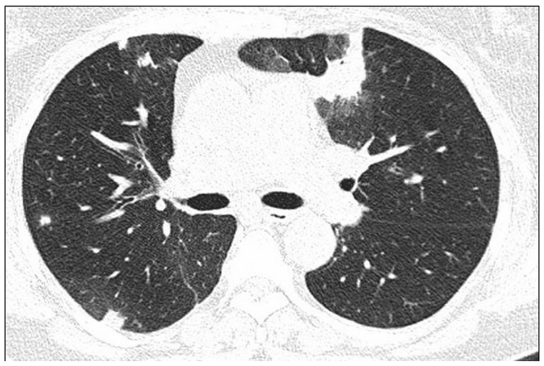

FIGURE 3
High resolution computed tomography scan of a patient with COP. Bilateral nodular consolidations.

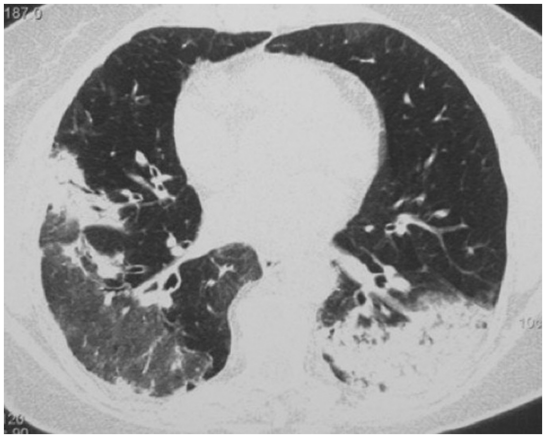

FIGURE 4
High resolution computed tomography scan of a patient with COP. Massive bilateral opacities with presence of air bronchogram and accompanying ground glass opacities on the right lung.

Bronchoscopy and bronchoalveolar lavage

Bronchoscopy and bronchoalveolar lavage are used to exclude possible causes of the observed lesions (i.e., infections, neoplasia, eosinophilic pneumonia, and alveolar hemorrhage) (53). The lymphocyte percentage in BALF is usually high (20–40%), and eosinophils and neutrophils are present (approximately 7–10%). In approximately 40% of patients, lymphocytosis with a decreased $CD4^+/CD8^+$ lymphocyte ratio is detected, but this is not a pathognomonic finding for the disease. Patients with an increased eosinophil percentage may have overlap syndrome comprising OP with chronic eosinophilic pneumonia.

Specimens obtained *via* transbronchial lung biopsy may be insufficient for diagnosis, particularly when the clinical and radiological pictures are questionable. The positive and negative predictive values of transbronchial lung biopsy were approximately 94 and 40%, respectively (54). Cryobiopsy may be an effective diagnostic route (55, 56). However, the gold standard for diagnosis is histological evaluation of specimens obtained by open lung biopsy (1, 3, 5, 6, 57–59).

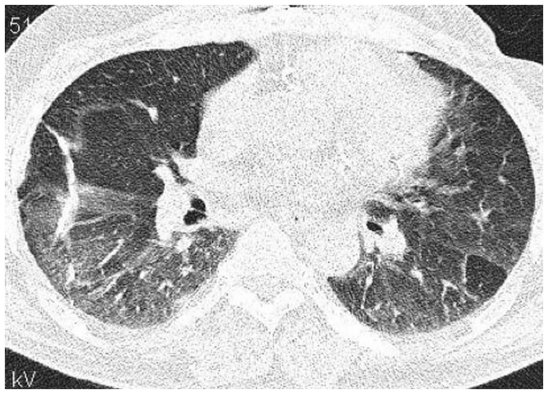

FIGURE 5
High resolution computed tomography scan of a patient with SOP in the course of dermatomyositis. In both lungs evidence of GGO with patchy distribution and parenchymal banding with "arcade" sign typical of OP. Status post lung biopsy on the right side.

Histological examination

In OP, histological examination detects a rich fibrous exudate in alveolar spaces, which expands into alveolar ducts and respiratory bronchioles; it forms characteristic polypoid lesions accompanied by a polymorphic inflammatory infiltrate composed of macrophages, plasma cells, lymphocytes, and eosinophils (Figure 7).

Some degree of inflammation is observed in the lung interstitium, but the lung architecture remains intact. The lesions usually have a unilocular appearance; typically, advanced fibrotic changes are not present at the time of diagnosis. OP involves extensive apoptotic activity in the extracellular matrix. The damaged lung is remodeled by re-epithelialization and repair of the basement membrane, along with resorption of the accompanying inflammatory exudate.

During the development of CIOP, clusters of fibrin and banded fibrous lesions are present in peribronchial spaces, sometimes accompanied by foci of dendritic ossification. This form of disease presumably results in a predisposition toward the onset of nonspecific interstitial pneumonia. Rarely, typical lesions of OP are accompanied by poorly formed granulomas (3, 5, 41).

Similarly, AFOP is detected in cases of sporadic disease with a severe and aggressive course. Histological criteria for this form are the presence of balls of organized fibrin conglomerates in the alveoli and respiratory ducts, patchy distribution in the absence of vitreous membranes, inflammatory granulomas, eosinophilic pneumonia, and bronchiolitis with abscess formation (41, 42, 57).

Pulmonary function tests

Despite the extensive radiographic changes, ventilatory parameters usually are not significantly impaired; they are within normal ranges in approximately 30% of patients. Impaired transfer rate for carbon monoxide and restrictive-type ventilatory

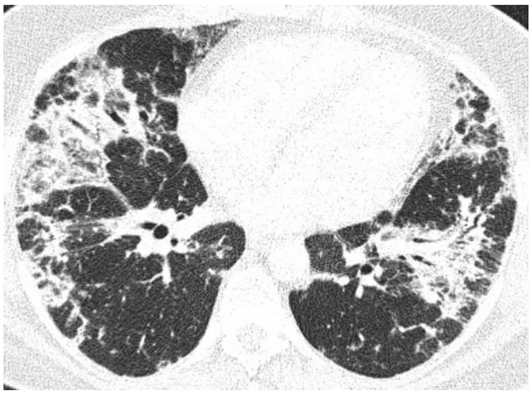

FIGURE 6
HRCT scan. Etanercept induced AFOP, in patient with rheumatic arthritis and pulmonary fibrosis. Bilateral consolidations with air bronchogram, interlobular septa thickening, and ground glass opacities predominated in the peripheral parts of both lungs.

abnormalities are most commonly detected in approximately 60–70% of patients. Features of hypoxemic respiratory failure are less frequently observed. In contrast, increased ventilatory and blood gas abnormalities are more often associated with SOP (28–38, 60).

Arterial hypoxemia at rest is uncommon (< 20% of patients), whereas arterial hypoxemia during exertion is more frequent and corresponds to impairments in diffusion capacity for carbon monoxide (3, 6, 28–40).

Diagnosis

The diagnosis of the disease is based on:

- Analysis of the clinical and radiological findings
- Exclusion of all possible causes of OP
- Histological examination of samples obtained by open lung biopsy or transbronchial lung biopsy
- Examination of lung samples obtained during transbronchial lung biopsy may be insufficient for patients with atypical clinical and radiological pictures; the OP lesions may be accompanied by other forms of interstitial pulmonary fibrosis, infections, and tumors

Diagnosis of AFOP

- Rapid clinical course leading to respiratory failure
- Radiological picture of extensive interstitial opacities
- Histological findings of richly fibrinous inflammatory exudate within the alveoli and in the respiratory tract, along with the formation of fibrin conglomerates, while excluding the presence of vitreous membranes, eosinophilia, bronchiolitis, and granulomas

Generally, a diagnosis of COP should be based on multidisciplinary consultation (1, 3, 6).

TABLE 4 Radiological findings detected in OP patients.

The most frequent	Patchy alveolar opacities
	Consolidations with and without air bronchogram
	Migration of lesions
	Subpleural localization
	Bilateral lesions
Frequent	Ground glass opacities
	Reversed halo sign
	Nodules
	Multiple masses
	Septal thickening
Rare	Lymphadenopathy
	Reticular opacities
	Centrilobular nodules
	Perilobular and linear opacities
Extremely rare	Diffuse micronodules
	Pneumothorax
	Pleural effusion

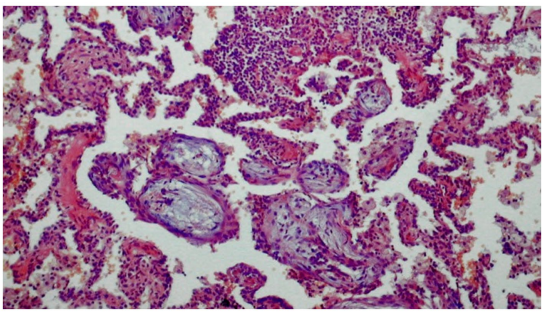

FIGURE 7
Histological image of organizing pneumonia (H-E). Alveoli filled with loose tissue forming polypoid formations extending into the respiratory tracts with a small diffuse inflammatory infiltrate in the interalveolar spaces.

Differentiation

Diagnosis requires a specific determination of possible etiology, particularly with respect to potentially reversible causes of disease, such as infections (e.g., coronavirus disease 2019), autoimmune disease, neoplastic disease, drug reactions, and toxic lung damage. Additionally, there is a need to determine whether OP is a component of another disease, including nonspecific interstitial pneumonia or usual interstitial pneumonia.

Chronic eosinophilic pneumonia may resemble OP in terms of clinical and radiological pictures. The disease more often affects women in the 5th to 6th decades of life, usually with a history of allergy, as well as sputum and > 25% eosinophils in BALF.

Opacities with air bronchogram are visualized by computed tomography, although they frequently do not exhibit migration;

these shadows can be a sign of mucosa-associated lymphoid tissue lymphoma or adenocarcinoma of the lung.

Other forms of idiopathic interstitial pneumonitis should be considered in the differential diagnosis, and the key assessment comprises evaluation of lung sections.

Nodular lesions that raise suspicion of malignancy require histological verification. Patients who have undergone radiation therapy for breast cancer are at high risk, such that 1–3% subsequently develop OP (3, 6, 59, 61–65).

Treatment

Treatment strategies for COP should be based on assessment of the patient's clinical status and the disease severity, with particular attention to respiratory sufficiency. The disease spontaneously resolves in <10% of cases.

Patients with localized COP treated by surgical resection of the lesion have a good prognosis; this is frequently sufficient treatment (40).

Steroid administration is the standard treatment for COP and SOP. Clinical and radiological improvements occur within a few days after the initiation of treatment; however, approximately 50% of patients experience recurrence, including approximately 20% who have multiple recurrences of COP. Relapses mainly occur during the first year after the initial episode. New infiltration occurs during steroid tapering (< 10 mg of prednisone) or within a few months after the completion of treatment. The factors associated with greater probability of relapse have not been fully elucidated, and different case series have yielded discordant results. However, putative factors include diagnostic and treatment delays, respiratory insufficiency, extension of pulmonary infiltrates, the presence of multifocal opacities, higher levels of inflammatory markers, and the presence of gastroesophageal reflux. Recurrence does not affect the mortality rate, and prolonged steroid treatment does not prevent recurrence. However, prolonged steroid treatment increases the likelihood of adverse events during therapy, which occur in 12–50% of patients; some events can be very severe, including pulmonary embolism, vertebral fracture, diabetes, and tuberculosis. The possibility of secondary causes of disease (e.g., autoimmune diseases and malignancy) should be considered in patients with recurrent OP. The optimal steroid dosage regimen and duration of therapy have not been established. The most common recommendation comprises the administration of 0.5–1 mg/kg per day of prednisone in gradually decreasing doses for 6–12 months. The dose and duration of treatment should be modified in accordance with the patient's clinical condition. Relapses are usually treated with lower doses of steroids (approximately 20 mg/day for 6 months) (28–40) (Table 5).

Clinically severe forms of the disease, which mainly occur in patients with SOP, have been treated with intravenous boluses of corticosteroids (500–1,000 mg methylprednisolone intravenously for 3–5 days with subsequent oral prednisone treatment at 1 mg/kg) and other immunosuppressive drugs (e.g., cyclophosphamide, azathioprine, cyclosporine A, mycophenolate mofetil, and rituximab), usually in combination with glucocorticoids (3, 6, 24, 38–40).

Generally, COP exhibits a very good response to corticosteroid treatment in patients with respiratory sufficiency, good clinical status, and no symptoms of severe disease; steroid-sparing therapies with macrolides have demonstrated promising results. There is increasing evidence regarding the efficacy of clarithromycin (CAM) treatment in COP patients without features of respiratory distress (67–75) (Table 6). The most effective dosage regimen is oral administration of CAM at 2 × 500 mg/day for 3 months. Significant improvement occurs after 1 month of treatment, rather than within a few days (e.g., the duration expected during steroid treatment). More than 80% of patients achieve complete regression of lesions after a 3-month period of treatment, and the recurrence rate is <10%. Adverse effects of this treatment are limited to allergic reactions and dyspeptic symptoms (68). Lower doses of CAM (2 × 500 mg for 1 week, 500 mg for 3 weeks, and 250 mg for 8 weeks) in combination with prednisone also led to good therapeutic effects (63% complete regression and 38% partial regression), but 80% of the patients exhibited OP recurrence. In the control group treated for 6 months with prednisone alone, the proportions of patients who achieved complete and partial remission were 81 and 14%, respectively; relapses occurred in more than half of the patients (75).

CAM was effective treatment for patients with radiotherapy-induced OP.

Similar to the first-line treatment, steroids are used as treatment for relapses. However, relapses can be treated with lower doses of glucocorticoids (< 20 mg/day) or CAM (5, 68).

Prophylactic treatment of *Pneumocystis jiroveci* infection is recommended for patients receiving steroids.

There have been no randomized controlled trials of steroid or CAM treatments for COP. The recommendations for treatment are based on individual experience and the results of uncontrolled studies. Considering the treatment duration, results, adverse events, and probability of relapse, CAM should be the first choice for treatment in patients with COP who exhibit respiratory sufficiency. Steroids should be considered for patients with a severe and aggressive disease course, particularly patients with underlying conditions (e.g., connective tissue disease, malignancy, or suspected drug reaction). Among patients who fail to respond to steroids, more aggressive treatment with cyclophosphamide, azathioprine, or rituximab is recommended.

Monitoring

Relapses of OP are common, particularly in steroid-treated patients; they most frequently occur during the period of prednisone dose reduction to approximately 5–10 mg or within 2–3 months after the completion of treatment. Therefore, follow-up examinations are recommended during the first year after completion of treatment. Additionally, particular attention is necessary for patients whose lesions have not completely resolved, and for patients in whom OP is regarded as the first symptom of a developing connective tissue disease. Factors associated with a higher risk of recurrence are diagnostic delay (> 2 months), severe disease, the presence of multifocal lesions and dilatation with stretching on radiographs, transfer factor of the lung for carbon monoxide <50%, hypoxemia <70 mmHg, and the presence of fibrin conglomerates and cicatricial lesions on histological examination (3, 28–40, 66, 68, 75).

TABLE 5 COP treatment with corticosteroids – observational retrospective studies of cohorts over than 20 patients (COP patients were extracted from presented populations).

Authors	Patients no	Initial dose of PRE	Duration of treatment (months)	Complete response rate *n* (%)	Rapid progression	Relapse *n* (%)	Adverse events	Observation period (months)
Epler et al. (5)	37	NR	3–12	24 (65%) 9 (%) partial response		8 from 24 (33%)	2 deaths from OP 2 pulmonary embolism 1 death aneurysm	Mean 4 years, range 2–10 years
King (6)	96	NR GR:1–1.5 mg/kg/d	NR GR: About 12	76 (63%)		NR 30 (37%) persistent disease	11 (12%) patients died	NR
Lohr et al. (62)	20	51 mg/d	Mean 12.7			4(13%)	5-year survival 73%	36–48
Cazzato et al. (33)	43	40 mg/d (20-120 mg/d)	6–9	NR 5 spontaneous remissions	2	15 (26%)	3 deaths	NR
Lazor et al. (28)	48	50 ± 17 mg/d	>12 68% patients were still on treatment	20 (42%)		28 (58%)	12(25%) No deaths	35 ± 31 23 median
Barroso et al. (63)	33	56 ± 12 mg/d	Non relapsed 12 ± 3; Relapsed 42 ± 10, $p < 0.01$, multiple relapsed 98 ± 48 months, $p = 0.03$	14/32 (44%)		18/32 (56%)	2 patients died (one of tuberculosis)	33 patients alive at 144 months 2 patients were on steroids in the time of analysis
Yoo et al. (14)	76 36- PRE 40- PRE+ CTX	54.6 ± 12.6 mg/d	10.5 (4.0–14.7)	35 (46%)	Rapid progression 5 (6.6%) Stabile 2 (2.6%)	14 (20%)	11 (14.5%) Disease related death	Median 38.2 (13.0–68.6)
Sveinsson et al. (64)	40	42.4 mg/d	NR	NR		8 (20%)	NR	Mean 4.7 years
Drakopanagiotakis et al. (38)	30	NR	Nr	17 (47%)		13 (43%)	2 (5.3%) 1-year mortality	NR
Onishi et al. (39)	40	0.5–0.8 mg/kg/d	1–6	25 (62%)		15 (38%)	No deaths caused by OP NR	NR
Radzikowska et al. (29)	22 (respiratory sufficient)	0.67 ± 0.24 mg/kg/d	mean of 8.59 ± 3.05	21 (100%)		12 (54.5)	8 (6.5%) patients (1 death)	67 ± 45.6
Saito et al. (66)	33	11 severe patients received 1.0 methylprednisolone iv. for 3 days	Meantime 282 ± 183 days in non-relapse group and 281 ± 174 days in relapse group	23 (70%)		10 (30%)	NR	NR
Zhou et al. (37)	73	20-200 mg/d	6–12 months	60 (68.5%)		23 (31.5%)		50.3 ± 26.8 months (range 9.3–96.4 months)
Zhang et al. (40)	53	1–2 mg/kg/d	Min. 12	12 (23%)	PR7 (13%)	35 (70%)	3 deaths	98.3% 5-year survival
Radzikowska unpublished data	32	0.73 ± 0.24 mg/kg/d	Mean 8.68 ± 4.08	32 (100%)		17 (53%) multiple relapse 15(%)	17 (53%) 2 deaths	64.3 ± 46.0 No one on steroids in the time of analysis

CTX, cyclophosphamide; GR, general recommendations; NR, not reported; PRE, prednisone; PR, partial response.

10.3389/fmed.2023.1146782

TABLE 6 COP treatment with clarithromycin – observational retrospective studies.

Author	Patients no	Treatment	Dose of CAM	Initial dose of PRE	Duration of treatment (months)	Complete response rate n (%)	Partial response>50% n (%)	Relapse *n* (%)	Adverse events
Ichikawa et al. (67)	6	Erythromycin 600 mg/d			3–4	6 (100%)		NR Observation mean 3.8 months	without
Epler et al. (5)	37	PRE		1 mg/kg/d	NR	24 (65%)	13 (26%)	33% treated with PRE	2 deaths of OP
	7	Tetracycline. erythromycin		NR	NR	3(43%)		NR	NR
	4	No treatment				2 (50%)			
Stover et al. (69)	6 (3 COP 3 ROP)	CAM	2×250 2×500		1.5–6	5 (83%)	1 (17%)	1 (17%) patient with ROP *	NR
Sveinsson et al. (64)	40	PRE	NR	Mean 42.4	NR	NR	NR	22 (55%)	1 death steroid responsive 1 death acute OP
	1	Erythromycin	NR					0	
	17	Surgery						0	
Drakopanagiotakis et al. (38)	30	PRE		NR	NR	NR	NR	38	9.4%
	3	Macrolides	NR					NR	
	9	No treatment							
Pathak et al. (71)	3 cases second line treatment 1 case first line	CAM	Two patients 2×0,5 Two patients 2×0.25	NR	5 or NER	4 (100%)		NR	NR
Radzikowska et al. (29)	40	CAM	2 ×0.5		3	35 (88%)	4 (10%)	10	1 (2.5%)
	22	PRE		Mean 0.67 ± 0.24 mg/kg/d	8.59 ± 3.05	22 (100%)	0	54	8 (36%) 1(5%) death
Petitpierre et al. (75)	16	CAM + PRE	2×0.5 for 1 week 0.5 for 3 weeks 0.25 for 8 weeks	0.75	3	10 (63%)	6 (38%)	81	0
	21 Previously published	PRE		0.75	6	17 (81%)	3 (14%)	52	0
Ciftci et al. (74)	7	CAM	2×0.5		3–9	7 (100%)		0	
Zhou et al. (37)	73	PRE		0.75	12	NR	NR	32	NR
	8	CAM		NR	4.2 ± 2.7	7 (64%)	NR	36	NR
	3	Azithromycin		NR					

CAM, clarithromycin; PRE, prednisone; COP, cryptogenic organizing pneumonia; SOP, secondary organizing pneumonia: ROP, radiotherapy induced organizing pneumonia; NR, not reported, NER -not exactly reported patient with large cell lymphoma.

10.3389/fmed.2023.1146782

Prognosis

Typical COP has a good prognosis. Spontaneous regression occurs in approximately 10% of cases, and > 75% of patients recover fully.

Poor response to treatment occurs in patients with a disease pattern that is suggestive of OP overlap with nonspecific interstitial pneumonia, patients in whom the disease is caused by exposure to pneumotoxic agents, patients with connective tissue diseases (including diseases induced by biological drugs), patients with myeloproliferative diseases, and patients who underwent lung or bone marrow transplantation. Very rarely, COP can result in death (3, 6, 28–40, 68–75).

Prevention

Because the cause of disease is not fully understood, there are no established methods for prevention.

Avoidance of infection is recommended; this includes efforts to undergo immunization against influenza, pneumonia, and coronavirus disease 2019.

Variants of organizing pneumonia

AFOP

More than 150 patients with AFOP have been described thus far, and the significance of the histological findings remains unclear (24, 76–80). This disease may be idiopathic or (more frequently) secondary, related to diffuse alveolar damage, adverse drug reaction, transplantation, and connective tissue disease. Although AFOP was first identified in patients with acute respiratory failure, the course of the disease can be acute or subacute. Confluent, bilateral, massive consolidations in basal areas of the lungs are usually observed. Patients with a fulminant clinical course and rapid progression to death exhibit radiological findings similar to diffuse alveolar damage, along with diffuse consolidations and ground glass opacities in lower parts of the lungs. In patients with a subacute clinical course, the radiological picture is more compatible with typical OP, including diffuse and focal consolidations with an air bronchogram and ground glass opacities. These patients also have better prognoses. Histologically, AFOP is characterized by the presence of organized fibrinous balls filling the alveoli, combined with type II pneumocyte hyperplasia and the absence of hyaline membrane. In the original report by Beasley et al., 17 patients with AFOP were identified among 114 cases with diffuse alveolar damage and OP (19). Relevant possible causes of the lesions were present in 11 patients; the possible cause of disease was unknown in the remaining 6 patients. The prognosis of AFOP is poor; 50% of the patients in the cohort presented by Beasley et al. died, including all patients who received mechanical ventilation. Onishi et al. identified 19 cases of idiopathic AFOP in a group of 34 patients diagnosed with AFOP. Corticosteroids were effective in 94% of the patients in the AFOP group, but relapses occurred in 76% of the patients (23). Additionally, a higher corticosteroid dose was needed during recurrence than during the initial course of disease. These patients had favorable outcomes, but two deaths from respiratory failure occurred among patients with underlying diseases. In that study, AFOP was more common than previously reported, and the patient prognosis was better than in other studies. In a review of cases and cohorts of patients with AFOP, Chen et al. reported that 33% of patients had idiopathic AFOP; underlying conditions responsible for disease were identified in the remaining patients (i.e., those patients had secondary AFOP) (24). Dyspnea (72%), nonproductive cough (71%), and fever (43%) were the most common symptoms, and they usually exhibited subacute onset (41%). Acute disease onset was identified in 27% of patients; it was nonspecific in the remaining patients. Bilateral consolidations, mainly in the lower and peripheral parts of both lungs, were present in 77% of patients. Consolidations, ground glass opacities, and nodules were evident in 54, 42, and 20% of patients, respectively. Lesions in the form of consolidations were more common in patients with idiopathic AFOP than in patients with secondary AFOP (70% vs. 47%, respectively). Rarely, pleural fluid (5%) and solitary nodules (1.3%) were detected in patients with secondary AFOP. Steroid administration was the most frequently prescribed therapy (88%); the dose and duration considerably varied. Immunosuppressive agents (e.g., cyclophosphamide, mycophenolate mofetil, tacrolimus, and azathioprine) were administered to 17 patients, most of whom exhibited secondary AFOP during the course of autoimmune disease. Drug-induced AFOP was identified in 17 (11%) patients; withdrawal of the possible causative drug and administration of corticosteroids were recommended (3, 6, 12).

Disease-related deaths were reported in 20% of idiopathic AFOP patients and 49% of secondary AFOP patients (80).

Moreover, close monitoring of patients with cryptogenic AFOP is recommended because it can reveal connective tissue disease or malignancy as the cause of the lesions.

Cicatricial variant of organizing pneumonia

CIOP is a rare histological variant of OP, which has been referred to by various names, including fibrosing OP, collagenized OP, and scarring variant of OP; these names should be consolidated to a single term (16, 25, 81–83). Yousem et al. found this variant in histological specimens from 12 of 223 patients with OP, which had been collected over a 20-year period. Additionally, 30 other patients had CIOP secondary to connective tissue diseases and malignancies. In this group, the disease was mainly present in middle-aged men; bilateral nodular or reticulonodular lesions were the most frequent manifestation. Histological examination revealed that fibromyxoid material filled distant airways and alveoli; dense eosinophilic fibrosis was evident in the center of the lesions, along with preservation of the lung architecture. In the central parts of these changes, elastic tissue was revealed by specific stains (e.g., Elastica van Gieson). Mild lymphocytic bronchiolitis and patchy alveolar septal infiltrates were present. In some cases, dendriform ossifications, fibrous pleuritis, and dust deposits were present. The lesions were persistent or progressive in >50% of patients. There has been discussion of separating this variant from classical COP (25). Woge et al. reported the presence of OP in 56 cases diagnosed *via* surgical biopsy specimens that had been collected over a period of 9 years. Thirty-two of 56 cases (57.1%) exhibited ≥10% cicatricial elements within fibromyxoid balls; cicatricial elements comprised ≥50% of OP in 9 of these cases. Intraluminal ossification was present in five of these nine cases. In 6 of these patients with major

cicatricial changes, the clinical condition was good over a median follow-up period of 47 months (83).

Recently, Zaizen et al. analyzed the histological findings of 121 patients with fibrotic interstitial pneumonia; they found that CIOP coexisted with usual interstitial pneumonia, nonspecific interstitial pneumonia, and chronic hypersensitivity pneumonia patterns. CIOP as a predominant type of lesion was present in 7 of 48 patients who exhibited any CIOP changes. CIOP was regarded as a variant of OP, as well as a histological lesion that can occur within other types of fibrotic interstitial pneumonia. Moreover, the presence of CIOP in patients with fibrotic interstitial pneumonia was associated with a low risk of acute exacerbation, slight improvement of ventilatory impairment, and better prognosis (82).

Conclusion

Cryptogenic organizing pneumonia (COP) is a result on the pulmonary reaction to various unidentified injuries. It has usually subacute course, and rarely manifests as severe disease.

The diagnosis of COP requires detailed evaluation of possible causes of secondary disease such as: infections, toxic substance exposure, drugs, connective tissue diseases, malignancies, autoimmune diseases, bone marrow or organ transplantation, and radiotherapy.

There have been no randomized controlled trials of steroid, clarithromycin, and other immunosuppressive treatments for COP. The recommendations for treatment are based on individual experience and the results of uncontrolled studies. Considering the treatment duration, results, adverse events, and probability of relapse, CAM should be the first choice for treatment in patients with COP who exhibit respiratory sufficiency. Steroids should be considered for patients with a severe and aggressive disease course, particularly patients with underlying conditions (e.g., connective tissue disease, malignancy, or suspected drug reaction). Among patients who fail to respond to steroids, more aggressive treatment with cyclophosphamide, azathioprine, or rituximab should be considered.

Author contributions

RE and FJ equally contributed to the conception and design of the work and drafted the article. All authors contributed to the article and approved the submitted version.

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.

Publisher's note

References

1. Travis WD, Costabel U, Hansell DM, King TE, Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med*. (2013) 188:733–8. doi: 10.1164/rccm.201308-1483ST

2. Epler GR. Bronchiolitis obliterans organizing pneumonia: definition and clinical features. *Chest*. (1992) 102:2S–26S. doi: 10.1378/chest.102.1.2S

3. Cordier JF. Cryptogenic organising pneumonia. *Eur Respir J*. (2006) 28:422–6. doi: 10.1183/09031936.06.00013505

4. Davison AG, Heard BE, McAllister WA, Turner-Warwick ME. Cryptogenic organizing pneumonitis. *Q J Med*. (1983) 52:382–4.

5. Epler GR, Colby TV, McLoud TC, Carrington CB, Gaensler EA. Bronchiolitis obliterans organizing pneumonia. *N Engl J Med*. (1985) 312:152–8. doi: 10.1056/NEJM198501173120304

6. King TE, Lee JS. Cryptogenic organizing pneumonia. *NEJM*. (2022) 386:1058–69. doi: 10.1056/NEJMra2116777

7. Geddes DM, Corrin B, Brewerton DA, Davies RJ, Turner-Warwick M. Progressive airway obliteration in adults and its association with rheumatoid disease. *Q J Med*. (1977) 46:427–4.

8. Cordier JF, Loire R, Brune J. Idiopathic bronchiolitis obliterans organizing pneumonia. Definition of characteristic clinical profiles in a series of 16 patients. *Chest*. (1989) 96:999–04. doi: 10.1378/chest.96.5.999

9. Henriet AC, Diot E, Marchand-Adam S, de Muret A, Favelle O, Crestani B, et al. Organising pneumonia can be the inaugural manifestation in connective tissue diseases, including Sjogren's syndrome. *Eur Respir Rev*. (2010) 19:161–3. doi: 10.1183/09059180.00002410

10. Epler GR, Kelly EM. Post-breast cancer radiotherapy bronchiolitis Obliterans organizing pneumonia. *Respir Care*. (2020) 65:686–2. doi: 10.4187/respcare.07150

11. Radzikowska E, Nowicka U, Wiatr E, Jakubowska L, Langfort R, Chabowski M, et al. Organising pneumonia and lung cancer - case report and review of the literature. *Pneumonol Alergol Pol*. (2007) 75:394–7.

12. Radzikowska E, Szczepulska E, Chabowski M, Bestry I. Organising pneumonia caused by trastuzumab (Herceptin) therapy for breast cancer. *Eur Respir J*. (2003) 21:552–5. doi: 10.1183/09031936.03.00035502

13. Lebargy F, Picard D, Hagenburg J, Toubas O, Perotin JM, Sandu S, et al. Micronodular pattern of organizing pneumonia: case report and systematic literature review. *Medicine (Baltimore)*. (2017) 96:e5788. doi: 10.1097/MD.0000000000005788

14. Yoo JW, Song JW, Jang SJ, Lee CK, Kim MY, Lee HK, et al. Comparison between cryptogenic organizing pneumonia and connective tissue disease-related organizing pneumonia. *Rheumatology (Oxford)*. (2011) 50:932–8. doi: 10.1093/rheumatology/keq410

15. Crestani B, Valeyre D, Roden S, Wallaert B, Dalpin JC, Cordier JF. Bronchiolitis obliterans organizing pneumonia syndrome primed by radiation therapy to the breast. The Groupe dEtudes ET de Recherche sur les maladies Orphelines Pulmonaries (GERM"O"P). *Am J Respir Dis*. (1998) 158:1929–35. doi: 10.1164/ajrccm.158.6.9711036

16. Ishiwata T, Ebata T, Iwasawa S, Matsushima J, Ota S, Nakatani Y, et al. Nivolumab-induced acute Fibrinous and organizing pneumonia (AFOP). *Intern Med*. (2017) 56:2311–5. doi: 10.2169/internalmedicine.8271-16

17. López Pardo P, Sánchez Peña AM, Río Ramírez MT. Cryptogenic organizing pneumonia by alectinib. *Med Clin (Barc)*. (2021) 157:91–2. doi: 10.1016/j.medcli.2020.04.058

18. Tzortzi A, Kapetanstrataki M, Evangelopoulou V. Beghrakis P.a systematic literature review of E-cigarette-related illness and injury: not just for the Respirologist. *Int J Environ Res Public Health*. (2020) 17:2248. doi: 10.3390/ijerph17072248

19. Beasley MB, Franks TJ, Galvin JR, Gochuico B, Travis WD. Acute fibrinous and organizing pneumonia: a histological pattern of lung injury and possible variant of diffuse alveolar damage. *Arch Pathol Lab Med*. (2002) 126:1064–70. doi: 10.5858/2002-126-1064-AFAOP

20. Kim MC, Kim YW, Kwon BS, Kim J, Lee YJ, Cho YJ, et al. Clinical features and long-term prognosis of acute fibrinous and organizing pneumonia histologically confirmed by surgical lung biopsy. *BMC Pulm Med*. (2022) 22:56. doi: 10.1186/s12890-022-01852-z

21. Cohen AJ, King TE, Downey GP. Rapidly progressive bronchiolitis obliterans with organizing pneumonia. *Am J Respir Crit Care Med*. (1994) 149:1670–5. doi: 10.1164/ajrccm.149.6.8004328

22. Beardsley B, Rassl D. Fibrosing organising pneumonia. *J Clin Pathol*. (2013) 66:875–1. doi: 10.1136/jclinpath-2012-201342

23. Onishi Y, Kawamura T, Higashino T, Mimura R, Tsukamoto H, Sasaki S. Clinical features of acute fibrinous and organizing pneumonia: an early histologic pattern of various acute inflammatory lung diseases. *PLoS One*. (2021) 16:e0249300. doi: 10.1371/journal.pone.0249300

24. Chen H, Kuang Y, Huang X, Ye Z, Liu Y, Xie C, et al. Acute fibrinous and organizing pneumonia: two case reports and literature review. *Diagn Pathol*. (2021) 16:90. doi: 10.1186/s13000-021-01155-7

25. Yousem SA. Cicatricial variant of cryptogenic organizing pneumonia. *Human*. (2017) 64:76–82. doi: 10.1016/j.humpath.2017.03.018

26. Gudmundsson G, Sveinsson O, Jonsson IHJ, Frodadottir H, Aspelund T. Epidemiology of organising pneumonia in Iceland. *Thorax*. (2006) 61:805–8. doi: 10.1136/thx.2006.059469

27. Karakatsani A, Papakosta D, Rapti A, Antoniou KM, Dimadi M, Markopoulou A, et al. Hellenic interstitial lung diseases group. Epidemiology of interstitial lung diseases in Greece. *Respir Med*. (2009) 103:1122–9. doi: 10.1016/j.rmed.2009.03.001

28. Lazor R, Vandevenne A, Pelletier A, Leclerc P, Court-Fortune I, Cordier JF. Characteristics of relapses in a series of 48 patients. The Groupe d'Etudes et de Recherche sur les Maladles 'Orphelines' Pulmonaires (GERM'O'P). *Am J Respir Crit Care Med*. (2000) 162:571–7. doi: 10.1164/ajrccm.162.2.9909015

29. Radzikowska E, Wiatr E, Langfort R, Bestry I, Skoczylas A, Szczepulska-Wojcik E, et al. Cryptogenic organizing pneumonia – results of treatment with clarithromycin versus corticosteroids – observational study. *PLoS One*. (2017) 12:e0184739. doi: 10.1371/journal.pone.0184739

30. Izumi T, Kitaichi M, Nishimura K, Nagai S. Bronchiolitis obliterans organizing pneumonia. *Chest*. (1992) 102:715–9. doi: 10.1378/chest.102.3.715

31. King TE Jr, Mortenson RL. Cryptogenic organizing pneumonitis. *North Am Exp Chest*. (1992) 102:8S–13S.

32. Alasaly K, Muller N, Ostrow DN. Cryptogenic organizing pneumonia. A report of 25 cases and a review of the literature. *Medicine (Baltimore)*. (1995) 74:201–1. doi: 10.1097/00005792-199507000-00004

33. Cazzato S, Zompatori M, Baruzzi G, Schiattone ML, Burzi M, Rossi A, et al. Bronchiolitis obliterans-organizing pneumonia: an Italian experience. *Respir Med*. (2000) 94:702–8. doi: 10.1053/rmed.2000.0805

34. Oymak FS, Demirbaş HM, Mavili E, Akgun H, Gulmez I, Demir R, et al. Bronchiolitis obliterans organizing pneumonia. *Respiration*. (2005) 72:254–2. doi: 10.1159/000085366

35. Chang J, Han J, Kim DW, Lee I, Lee KY, Jung S, et al. Bronchiolitis obliterans organizing pneumonia: clinicopathologic review of a series of 45 Korean patients including rapidly progressive form. *J Korean Med Sci*. (2002) 17:179–6. doi: 10.3346/jkms.2002.17.2.179

36. Yılmaz S, Akıncı Özyürek B, Erdoğan Y, Cirit Koçer B, Demirağ F, Dadalı Y, et al. Retrospective evaluation of patients with organizing pneumonia: is cryptogenic organizing pneumonia different from secondary organizing pneumonia? *Tuberk Toraks*. (2017) 65:1–8. doi: 10.5578/tt.53938

37. Zhou Y, Wang L, Huang M, Ding J, Jiang H, Zhou K, et al. A long-term retrospective study of patients with biopsy-proven cryptogenic organizing pneumonia. *Chron Respir Dis*. (2019) 16:147997311985382. doi: 10.1177/1479973119853829

38. Drakopanagiotakis F, Paschalaki K, Abu-Hijleh M, Aswad B, Karagianidis N, Kastanakis E, et al. Cryptogenic and secondary organizing pneumonia. *Chest*. (2011) 139:893–0. doi: 10.1378/chest.10-0883

39. Onishi Y, Kawamura T, Nakahara Y, Kagami R, Sasaki S, Takahashi S, et al. Factors associated with the relapse of cryptogenic and secondary organizing pneumonia. *Respir Investig*. (2017) 55:10–5. doi: 10.1016/j.resinv.2016.09.001

40. Zhang Y, Li N, Li Q, Zhou Y, Zhang F, Chen T, et al. Analysis of the clinical characteristics of 176 patients with pathologically confirmed cryptogenic organizing pneumonia. *Ann Transl Med*. (2020) 8:763. doi: 10.21037/atm-20-4490

41. Colby TV. Pathologic aspects of bronchiolitis obliterans organizing pneumonia. *Chest*. (1992) 102:38S–343S. doi: 10.1378/chest.102.1.38S

42. Myers JL, Colby TV. Pathologic manifestations of bronchiolitis, constrictive bronchiolitis, cryptogenic organizing pneumonia, and diffuse panbronchiolitis. *Clin Chest Med*. (1993) 14:611–2. doi: 10.1016/S0272-5231(21)00925-4

43. Feinstein MB, DeSouza SA, Moreira AL, Stover DE, Heelan RT, Iyriboz TA, et al. A comparison of the pathological, clinical and radiographical, features of cryptogenic organising pneumonia, acute fibrinous and organising pneumonia and granulomatous organising pneumonia. *J Clin Pathol*. (2015) 68:441–7. doi: 10.1136/jclinpath-2014-202626

44. Lappi-Blanco E, Soini Y, Paakko P. Apoptotic activity is increased in the newly formed fibromyxoid connective tissue in bronchiolitis obliterans organizing pneumonia. *Lung*. (1999) 177:367–6. doi: 10.1007/PL00007654

45. Lappi-Blanco E, Kaarteenaho-Wiik R, Soini Y, Risteli J, Pääkkö P. Intraluminal fibromyxoid lesions in bronchiolitis obliterans organizing pneumonia are highly capillarized. *Hum Pathol*. (1999) 30:1192–6. doi: 10.1016/S0046-8177(99)90036-9

46. Cai M, Bonella F, Dai H, Sarria R, Guzman J, Costabel U. Macrolides inhibit cytokine production by alveolar macrophages in bronchiolitis obliterans organizing pneumonia. *Immunobiology*. (2013) 218:930–7. doi: 10.1016/j.imbio.2012.10.014

47. Radzikowska E, Roży A, Jaguś P, Wiatr E, Gawryluk D, Chorostowska-Wynimko J, et al. Cryptogenic organizing pneumonia: IL-1β, IL-6, IL-8, and TGF- β1 serum concentrations and response to clarithromycin treatment. *Adv Exp Med Biol*. (2016) 911:77–85. doi: 10.1007/5584_2016_223

48. Radzikowska E, Roży A, Jagus P, Polubiec-Kownacka M, Wiatr E, Chorostowska-Wynimko J, et al. Clarithromycin decreases IL-6 concentration in serum and BAL fluid in patients with cryptogenic organizing pneumonia. *Clin Exp Med*. (2016) 25:871–8. doi: 10.17219/acem/61953

49. Katoh S, Ikeda M, Shimizu H, Abe M, Ohue Y, Mouri K, et al. Increased Galectin-9 concentration and number of CD4+Foxp3high+cells in bronchoalveolar lavage fluid of patients with cryptogenic organizing pneumonia. *Lung*. (2015) 193:683–9. doi: 10.1007/s00408-015-9775-x

50. Todd NW, Marciniak ET, Sachdeva A, Kligerman SJ, Galvin JR, Luzina IG, et al. Organizing pneumonia/non-specific interstitial pneumonia overlap is associated with unfavorable lung disease progression. *Respir Med*. (2015) 109:1460–8. doi: 10.1016/j.rmed.2015.09.015

51. Yang DG, Kim KD, Shin DH, Choe KO, Kim SK, Lee WY. Idiopathic bronchiolitis obliterans with organizing pneumonia presenting with spontaneous hydropneumothorax and solitary pulmonary nodule. *Respirology*. (1999) 4:267–0. doi: 10.1046/j.1440-1843.1999.00187.x

52. Kang C, Vali Y, Naeem M, Reddy R. Cryptogenic organising pneumonia presenting with spontaneous pneumothorax and the value of procalcitonin: a case report. *Respir Med Case Rep*. (2017) 22:36–8. doi: 10.1016/j.rmcr.2017.06.006

53. Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM, et al. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. *Am J Respir Crit Care Med*. (2012) 185:1004–14. doi: 10.1164/rccm.201202-0320ST

54. Costabel U, Teschler H, Guzman J. Bronchiolitis obliterans organizing pneumonia (BOOP): the cytological and immunocytological profile of bronchoalveolar lavage. *Eur Respir J*. (1992) 5:791–7. doi: 10.1183/09031936.93.05070791

55. Poletti V, Cazzato S, Minicuci N, Zompatori M, Burzi M, Schiattone ML. The diagnostic value of bronchoalveolar lavage and transbronchial lung biopsy in cryptogenic organizing pneumonia. *Eur Respir J*. (1996) 9:2513–6. doi: 10.1183/09031936.96.09122513

56. Ravaglia C, Wells AU, Tomassetti S, Gurioli C, Gurioli C, Dubini A, et al. Diagnostic yield and risk/benefit analysis of trans-bronchial lung cryobiopsy in diffuse parenchymal lung diseases: a large cohort of 699 patients. *BMC Pulm Med*. (2019) 19:16. doi: 10.1186/s12890-019-0780-3

57. Roberton BJ, Hansell DM. Organizing pneumonia: a kaleidoscope of concepts and morphologies. *Eur Radiol*. (2011) 21:2244–54. doi: 10.1007/s00330-011-2191-6

58. Miao L, Wang Y, Li Y, Ding J, Chen L, Dai J, et al. Lesion with morphologic feature of organizing pneumonia (OP) in CT-guided lung biopsy samples for diagnosis of bronchiolitis obliterans organizing pneumonia (BOOP): a retrospective study of 134 cases in a single center. *J Thorac Dis*. (2014) 6:1251–60. doi: 10.3978/j.issn.2072-1439.2014.08.23

59. Guerry-Force ML, Müller NL, Wright JL, Wiggs B, Coppin C, Pare PD, et al. A comparison of bronchiolitis obliterans with organizing pneumonia, usual interstitial pneumonia, and small airways disease. *Am Rev Respir Dis*. (1987) 135:705–2.

60. Xaubet A, Ancochea J, Morell F, Rodriguez-Arias JM, Villena V, Blanquer R, et al. Spanish group on interstitial lung diseases, SEPAR. Report on the incidence of interstitial lung diseases in Spain. *Sarcoidosis Vasc Diffuse Lung Dis*. (2004) 21:64–70.

61. Yousem SA, Lohr RH, Colby TV. Idiopathic bronchiolitis obliterans organizing pneumonia/cryptogenic organizing pneumonia with unfavorable outcome: pathologic predictors. *Mod Pathol*. (1997) 10:864–1.

62. Lohr RH, Boland BJ, Douglas WW, Dockrell DH, Colby TV, Swensen SJ, et al. Organizing pneumonia. Features and prognosis of cryptogenic, secondary, and focal variants. *Arch Intern Med*. (1997) 157:1323–9. doi: 10.1001/archinte.1997.00440330057006

63. Barroso E, Hernandez L, Gil J, Garcia R, Aranda I, Romero S. Idiopathic organizing pneumonia: a relapsing disease. 19 years of experience in a hospital setting. *Respiration*. (2007) 74:624–1. doi: 10.1159/000103240

64. Sveinsson OA, Isaksson HJ, Sigvaldason A, Yngvason F, Aspelund T, Gudmundsson G. Clinical features in secondary and cryptogenic organising pneumonia. *Int J Tuberc Lung Dis*. (2007) 11:689–4.

65. Nizami IY, Kissner DG, Visscher DW, Dubaybo BA. Idiopathic bronchiolitis obliterans with organizing pneumonia an acute and life-threatening syndrome. *Chest*. (1995) 108:271–7.

66. Saito Z, Kaneko Y, Hasegawa T, Yoshida M, Odashima K, Horikiri T, et al. Predictive factors for relapse of cryptogenic organizing pneumonia. *BMC Pulm Med*. (2019) 19:10. doi: 10.1186/s12890-018-0764-8

67. Ichikawa Y, Ninomiya H, Katsuki M, Hotta M, Tanaka M, Oizumi K. Low-dose/long-term erythromycin for treatment of bronchiolitis obliterans organizing pneumonia (BOOP). *Kurume Med J*. (1993) 40:65–7. doi: 10.2739/kurumemedj.40.65

68. Radzikowska E, Wiatr E, Langfort R, Bestry I, Rudziński P, Roszkowski K. Organizing pneumonia-own experiences with clarithromycin treatment. *Pneumonol Alergol Pol*. (2004) 72:493–8. doi: 10.5603/ARM.28186

69. Stover DE, Mangino D. Macrolides: a treatment alternative for bronchiolitis obliterans organizing pneumonia? *Chest*. (2005) 128:3611–7. doi: 10.1378/chest.128.5.3611

70. Ding QL, Lv D, Wang BJ, Zhang QL, Yu YM, Sun SF, et al. Macrolide therapy in cryptogenic organizing pneumonia: a case report and literature review. *Exp Ther Med*. (2015) 9:829–4. doi: 10.3892/etm.2015.2183

71. Pathak V, Kuhn JM, Durham C, Funkhouser WK, Henke DC. Macrolide use leads to clinical and radiological improvement in patients with cryptogenic organizing pneumonia. *Ann Am Thorac Soc*. (2014) 11:87–91. doi: 10.1513/AnnalsATS.201308-261CR

72. Lee J, Cha SI, Park TI, Park JY, Jung TH, Kim CH. Adjunctive effects of cyclosporine and macrolide in rapidly progressive cryptogenic organizing pneumonia with no prompt response to steroid. *Intern Med*. (2011) 50:475–9. doi: 10.2169/internalmedicine.50.4237

73. Vieira AL, Vale A, Melo N, Caetano Mota P, Jesus JM, Cunha R, et al. Organizing pneumonia revisited: insights and uncertainties from a series of 67 patients. *Sarcoidosis Vasc Diffuse Lung Dis*. (2018) 35:129–8.

74. Ciftci F, Kumbasar ÖÖ. Seven patients with cryptogenic organizing pneumonia succesfully treated with clarithromycin. *Sarcoidosis Vasc Diffuse Lung Dis*. (2018) 35:165–0.

75. Petitpierre N, Cottin V, Marchand-Adam S, Hirschi S, Rigaud D, Court-Fortune I, et al. A 12-week combination of clarithromycin and prednisone compared to a 24-week prednisone alone treatment in cryptogenic and radiation-induced organizing pneumonia. *Sarcoidosis Vasc Diffuse Lung Dis*. (2018) 35:230–8.

76. Nishino M, Mathai SK, Schoenfeld D, Digumarthy SR, Kradin RL. Clinicopathologic features associated with relapse in cryptogenic organizing pneumonia. *Hum Pathol*. (2014) 45:342–1. doi: 10.1016/j.humpath.2013.09.010

77. Gomes R, Padrão E, Dabó H, Soares Pires F, Mota P, Melo N, et al. Acute fibrinous and organizing pneumonia: a report of 13 cases in a tertiary university hospital. *Medicine (Baltimore)*. (2016) 95:e4073. doi: 10.1097/MD.0000000000004073

78. Dai JH, Li H, Shen W, Miao LY, Xiao YL, Huang M, et al. Clinical and radiological profile of acute fibrinous and organizing pneumonia: a retrospective study. *Chin Med J*. (2015) 128:2701–6. doi: 10.4103/0366-6999.167293

79. Lu J, Yin Q, Zha Y, Deng S, Huang J, Guo Z, et al. Acute fibrinous and organizing pneumonia: two case reports and literature review. *BMC Pulm Med*. (2019) 19:141. doi: 10.1186/s12890-019-0861-3

80. Xu XY, Chen F, Chen C, Sun HM, Zhao BL. Acute fibrinous and organizing pneumonia: a case report and literature review. *Exp Ther Med*. (2016) 12:3958–62. doi: 10.3892/etm.2016.3865

81. Lee JH, Yum HK, Jamous F, Santos C, Campisi A, Surani S, et al. Diagnostic procedures and clinico-radiological findings of acute fibrinous and organizing pneumonia: a systematic review and pooled analysis. *Eur Radiol*. (2021) 31:7283–94. doi: 10.1007/s00330-021-07868-z

82. Zaizen Y, Tabata K, Yamano Y, Takei R, Kataoka K, Shiraki A, et al. Cicatricial organising pneumonia associated with fibrosing interstitial pneumonia - a clinicopathological study. *Histopathology*. (2022) 80:279–0. doi: 10.1111/his.14427

83. Woge MJ, Ryu JH, Bartholmai BJ, Yi ES. Cicatricial organizing pneumonia: a clinicopathologic and radiologic study on a cohort diagnosed by surgical lung biopsy at a single institution. *Hum Pathol*. (2020) 101:58–63. doi: 10.1016/j.humpath.2020.04.013

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 28 April 2023
DOI 10.3389/fmed.2023.1174443

OPEN ACCESS

EDITED BY
Shuibang Wang,
National Institutes of Health, United States

REVIEWED BY
Aurelien Justet,
Centre Hospitalier Universitaire de Caen, France
Irina Ruxandra Strambu,
Marius Nasta Institute of Pneumology, Romania

*CORRESPONDENCE
Laura M. Glenn
✉ lgle9041@uni.sydney.edu.au

RECEIVED 26 February 2023
ACCEPTED 10 April 2023
PUBLISHED 28 April 2023

CITATION
Glenn LM, Troy LK and Corte TJ (2023) Novel diagnostic techniques in interstitial lung disease.
Front. Med. 10:1174443.
doi: 10.3389/fmed.2023.1174443

Novel diagnostic techniques in interstitial lung disease

Laura M. Glenn[1,2,3]*, Lauren K. Troy[1,2,3] and Tamera J. Corte[1,2,3]

[1]Department of Respiratory and Sleep Medicine, Royal Prince Alfred Hospital, Camperdown, NSW, Australia, [2]Central Clinical School, The University of Sydney School of Medicine, Sydney, NSW, Australia, [3]NHMRC Centre of Research Excellence in Pulmonary Fibrosis, Camperdown, NSW, Australia

Research into novel diagnostic techniques and targeted therapeutics in interstitial lung disease (ILD) is moving the field toward increased precision and improved patient outcomes. An array of molecular techniques, machine learning approaches and other innovative methods including electronic nose technology and endobronchial optical coherence tomography are promising tools with potential to increase diagnostic accuracy. This review provides a comprehensive overview of the current evidence regarding evolving diagnostic methods in ILD and to consider their future role in routine clinical care.

KEYWORDS

interstitial lung disease, diagnostic techniques, biomarker, genomics, machine learning, optical coherence tomography, electronic nose

Introduction

Interstitial lung diseases (ILDs), despite being a large group of diverse disorders, are grouped together since they are invariably characterized by inflammation and/or fibrosis of the lung parenchyma (1). Affected patients often have similar symptoms including cough and breathlessness. While some ILDs share overlapping radiological and histopathological features, there is a wide range of natural histories and responses to treatment across the ILD spectrum. Delays in diagnosis can lead to missed opportunities to intervene early and potentially prevent progressive pulmonary fibrosis (PPF), a recently defined disease behavior entity which is associated with high morbidity and mortality (1, 2). Idiopathic pulmonary fibrosis (IPF), the most common ILD, is universally progressive; however, antifibrotic therapy with nintedanib or pirfenidone significantly reduces the rate of decline in lung function in patients with both early and moderate disease. A similar treatment effect with nintedanib has been demonstrated in other causes of PPF (3).

Unfortunately, a specific ILD diagnosis remains elusive in up to 20% of cases; and is often delayed (4). The reasons for this are multifactorial, including heterogeneous clinical presentation, radiological and pathological features even within diagnostic subgroups, the rarity of some ILDs, under-recognition of ILD features in the primary care setting; and importantly, global inequality of access to expert care (1, 5–10). The ILD multidisciplinary meeting (MDM) is the current recommended "gold standard" method for diagnosing ILDs, yet several studies have demonstrated suboptimal agreement between clinicians and ILD MDMs for individual diagnoses (11–18).

While this might initially seem discouraging, the future is promising. Recent research into novel diagnostic techniques and targeted therapeutics in ILD is moving the field toward increased precision and improved patient outcomes (19).

Conventional and emerging ILD diagnostic tools in use

Detailed clinical assessment through history-taking and examination, has always been important in ILD diagnosis and remains an essential first step in the modern systematic approach (1, 20). A history of exposures in the home and workplace should be obtained, including to mold, asbestos, and other relevant occupational dusts, which may point toward an underlying inciting agent. Recently, the use of a standardized questionnaire has been shown to increase both diagnostic confidence and antigen recognition in chronic hypersensitivity pneumonitis (21). Similarly, physical signs detected during the examination might support presence of an ILD (nail clubbing, fine inspiratory crackles) or suggest an underlying connective tissue disease diagnosis (sclerodactyly, inflammatory arthritis, typical skin rash).

Serological tests for autoantibodies may also point toward systemic autoimmune disease. Many ILD centers will screen for antinuclear antibodies, rheumatoid factor as well as more specific connective tissue disease-associated antibodies such as anti-double stranded DNA, anti-Ro and anti-La, anti-Scl-70, anti-ribonucleoprotein, anti-cyclic citrullinated peptide (CCP) and the anti-tRNA synthetase antibodies. New immunoassay platforms and an extended panel of highly specific myositis antibodies (including antibodies to PM-Scl, MDA-5, Mi2, Ku, TIF1γ, and NXP2) are now also part of routine testing in many expert centers worldwide. Similarly, nailfold capillaroscopy has recently been suggested as an adjunctive clinical test to assess for the presence of vascular changes associated with systemic sclerosis and other connective tissue diseases such as the idiopathic inflammatory myopathies and mixed connective tissue disease (22–24). The high specificity of abnormal nailfold capillaroscopy findings has rendered it an integral tool in the diagnosis of these diseases. Although generally performed using conventional microscopy, nailfold capillaroscopy has also been performed at the bedside using smartphone-dermatoscopy (23).

A critical technological advance was the development of computed tomography (CT) in the 1970s, enabling detailed axial imaging of the lung parenchyma for the first time (20). The Fleischner Society and international expert groups have subsequently published numerous glossaries, white papers and clinical practice guidelines describing classifications of interstitial lung disease patterns on high resolution CT (HRCT) chest imaging, including usual interstitial pneumonia (UIP), non-specific interstitial pneumonia (NSIP) and other patterns which are associated with specific ILDs (25–32). In over two-thirds of ILD cases, the synthesis of HRCT pattern and clinical information enables a diagnosis to be made at the ILD MDM (1).

For patients in whom clinical and HRCT findings are not sufficiently characteristic to allow confident diagnosis, further investigation including bronchoscopy or tissue sampling for histopathological classification may be required. Flexible bronchoscopic diagnostic techniques including bronchoalveolar lavage (BAL) and transbronchial biopsy were integrated into clinical practice around the same time as early CT imaging (20). BAL remains a routinely performed diagnostic test in patients with newly detected ILD who do not have a definite UIP pattern on HRCT. Cellular analysis of BAL fluid can reveal lymphocytosis helpful for distinguishing hypersensitivity pneumonitis from IPF or sarcoidosis; or high eosinophil counts suggesting a diagnosis of eosinophilic pneumonia. BAL cultures can help exclude infection as an alternate cause of lung infiltrates prior to institution of specific immunosuppressive or antifibrotic therapies. Transbronchial biopsies with forceps (TBB) are not routinely recommended for histopathological diagnosis due to having generally low diagnostic yield and moderate risk of pneumothorax (31). However, they might be considered on a case-by-case basis for diagnosis of airway-centered processes such as sarcoidosis or organizing pneumonia, where the sensitivity is much higher. There is still widespread use for these indications in centers where clinicians have expertise in TBB.

Historically, tissue sampling required surgical lung biopsy (via open thoracotomy in the 1950s and 1960s and via video-assisted thoracoscopic surgery (VATS) in more recent decades) if the patient was considered suitable for thoracic surgery (20). Within the last decade, transbronchial lung cryobiopsy (TBLC) has emerged as a less invasive tissue sampling procedure for ILD diagnosis, with good diagnostic yield of ~80% (33–35), and comparable accuracy to VATS biopsy in some studies when considered within the MDM (36). Although the quality of available evidence is low, current systematic review and meta-analysis data demonstrate TBLC to have a lower 30-day mortality rate than VATS (0.6% versus 1.7%), and low risk of severe complications such as prolonged air leak or acute ILD exacerbation. These factors make TBLC a favorable alternative to SLB for tissue sampling in centers with expertise, with uptake of the procedure by centers across the world (33).

These diagnostic test results are considered by a multidisciplinary team within the MDM to formulate an ILD diagnosis – the recommended approach in IPF clinical practice guidelines in the last decade (2, 31). In recent years, there has been divergence of opinion regarding the need for strict pursual of specific ILD diagnosis versus "lumping" patients based on their expected and observed disease behaviors including those with a progressive fibrotic phenotype and those with non-progressive disease (37–39). Indeed, this is reflected in the variable recommendations of different MDMs. While some MDMs will recommend referral for biopsy in the event of unclassifiable or low confidence diagnoses, others will generate a working diagnosis (of lower confidence) to facilitate treatment institution. This is frequently related to a patient's current degree of disability and burden of comorbidities, limiting ability to proceed to biopsy and therefore attainment of a high confidence diagnosis. In the future, standardization of the ILD MDM, including approach to clinical decision-making, will be required to reduce heterogeneity of outputs. Indeed, although the MDM is considered the current "gold standard" method for ILD diagnosis, there is a clear need for simpler, non-invasive, and more precise ILD diagnostic tests.

Emerging ILD diagnostic tests under investigation

Genetic testing

The advent of biobanks and evolution of methods for molecular analysis, including targeted next-generation sequencing and whole genome sequencing in the early 2000s revolutionized the concept of genetic testing in ILD (40–42). Single nucleotide polymorphisms (SNPs) in genes encoding for proteins expressed by airway epithelial cells, such as *MUC5B*, have been identified to have both diagnostic and prognostic significance in IPF and other fibrotic ILDs such as rheumatoid arthritis-associated ILD and chronic hypersensitivity

TABLE 1 Gene polymorphisms associated with ILD.

Gene(s)	Phenotype
MUC5B	Risk allele (rs35705950) associated with increased susceptibility to familial pulmonary fibrosis (heterozygous OR 6.8, 95% CI 3.9–12; homozygous OR 20.8, 95% CI 3.8–113.7), IPF (heterozygous OR 9.0, 95% CI 6.2–13.1; homozygous OR 21.8, 95% CI 5.1–93.5). Also associated with UIP-pattern ILD in rheumatoid arthritis, and hypersensitivity pneumonitis patients.
Surfactant-related genes (SFTPC, SFTPA2, SFTPA1)	Heterozygous mutations associated with familial pulmonary fibrosis, RA-ILD
Other genes associated with protein expression and function	AKAP13, ATP11A, DPP9, FAM13A, DSP, OBFC1 variants associated with increased susceptibility to IPF and other idiopathic interstitial pneumonias in genome-wide association studies
Telomere-related genes (TERT, TERC, PARN, RTEL1, NAF1, DKC1, TINF2)	Mutations identified in up to 1/4 of familial pulmonary fibrosis patients and 1/10 sporadic IPF patients. Up to ½ patients with heterozygous variant will have non-IPF diagnosis (CTD-ILD, HP, RA-ILD); however, prognosis is similar to patients with UIP. Fibrosis of other organs, including liver cirrhosis and myelofibrosis, premature hair graying and/or other features of a "short telomere syndrome" may also be seen.

pneumonitis. Patients with at least one *MUC5B* risk allele have a more than threefold increased risk of developing pulmonary fibrosis (41). However, IPF patients with at least one of these alleles have been shown to have slower disease progression and improved survival compared to patients without a risk allele in retrospective and post-hoc analyses (41). The first whole genome sequencing study of 2,180 IPF cases, recently published, found single rare variants in *TERT* and *RTEL1* genes to be significantly associated with IPF development, and confirmed previously studied association with other more common genetic variants. SNP-heritability in IPF was estimated to be 32% (43).

Importantly, mutations in telomere-related genes such as *TERT* and *PARN* confer substantial risk of familial pulmonary fibrosis and are associated with more rapidly progressive disease, as well as poorer outcomes with immunosuppressive therapy and with lung transplantation (40, 44). A recent genome-wide meta-analysis of IPF patients showed a variant in the RNA antisense gene of protein kinase N2 (PKN2), rs115982800, to be significantly associated with FVC decline. Interestingly, no other genetic variants were associated with lung function decline, however this may have been due to study underpowering (45). Variability in the association between specific genetic variants and IPF increasingly suggest that it is a more heterogenous disease than traditionally thought, with different underlying pathophysiological mechanisms (46, 47).

Genetic variants that have been associated with ILD are described in Table 1 (40–47). In addition to direct cellular injury mediated by these genetic variants, pulmonary fibrosis may also be a consequence of chronic lung inflammation due to genetic mutations causing systemic autoimmune disease; for example, CTLA-4 haploinsufficiency with autoimmune infiltration (CHAI syndrome), or chronic respiratory infections; for example, hyper-IgE syndrome.

Variable penetrance of ILD-associated alleles, limited data on specific treatment response and limited access to genetic counseling and testing outside of tertiary centers currently limit the widespread implementation of genetic testing. However, it is increasingly used within the ILD multidimensional diagnostic paradigm to inform risk of progressive fibrotic ILD and treatment response for affected individuals with suspected familial pulmonary fibrosis and their family members (40). Genetic testing should also be considered where there is suspicion for a "short telomere syndrome," and evidence is mounting that screening of unaffected family members may be reasonable to facilitate earlier diagnosis and treatment institution.

Key points

1. Genetic variants with diagnostic and prognostic importance have been identified in familial pulmonary fibrosis and other ILDs, including single nucleotide polymorphisms in telomere-related genes, surfactant-related genes and other genes associated with protein expression and function.
2. Where access is available, genetic testing should be considered for all patients with a family history of ILD or who have features of a telomeropathy.

Other molecular testing, including serum biomarkers

RNA sequencing and genomic classifier testing represent novel diagnostic methods of significant interest (48). RNA sequencing involves the use of high throughput sequencing technologies to identify and quantify RNA transcripts in either whole tissue or single cells (49). Analysis of the transcriptome can identify differentially expressed or regulated genes between diseased tissues compared with healthy tissues, with the aim of delineating biological mechanisms or pathways underlying disease pathogenesis. Multiple studies have evaluated the use of both bulk RNA sequencing and single-cell RNA-sequencing (scRNA-seq) to analyze fibrotic lung tissue samples (50).

A genomic classifier, developed from RNA sequencing data, has been proposed as a novel diagnostic tool that might increase diagnostic yield and accuracy in ILD. The Envisia™ genomic classifier, which employs a machine learning algorithm developed to classify UIP versus non-UIP histopathological pattern ILDs, uses bulk RNA sequencing data obtained from high throughput sequencing of exome-enriched RNA extracted from transbronchial lung biopsies or transbronchial lung cryobiopsies (TBLCs). Several subsequent studies have demonstrated the classifier to have high specificity (≥86%) to predict UIP, however its sensitivity was as low as 68% (51–54). When results were presented within the ILD multidisciplinary meeting, the genomic classifier increased diagnostic confidence for patients with probable UIP (55). However, the classifier had less impact on the proportion of high confidence diagnoses than the TBLC result; and the 2022 ATS/ERS/JRS/ALAT IPF clinical practice guideline update has made no recommendation for or against the use of genomic classifier testing in fibrotic ILD diagnosis (2).

The quest for a biomarker signature from peripheral blood to improve specific ILD diagnosis and accurate prediction of prognosis at first presentation has been world-wide. Multiple biomarkers have been identified and several have been validated across multiple cohorts. Peripheral blood biomarkers with promise for future translation to clinical use include matrix metalloproteinase 7 (MMP-7), Krebs von den Lungen (KL-6), osteopontin (OPN), periostin and surfactant protein D (SP-D); and various other cytokines, chemokines, growth factors, matrix metalloproteinases, extracellular matrix proteins and markers of epithelial injury and apoptosis (56) (Figure 1).

The landmark PROFILE study of incident cases of fibrotic interstitial lung disease involved a discovery analysis of concentrations of 123 previously described serum biomarkers using multiplex immunoassay, followed by a validation analysis employing independent immunoassays for each of the three identified biomarkers. SP-D, carbohydrate antigen 19.9 (CA19-9) and cancer antigen 125 (CA-125) were identified as prognostically important biomarkers, with higher baseline values of SP-D and CA 19–9 significantly associated with disease progression; and increasing levels of CA-125 over a three-month time period were associated with higher mortality risk (57). More recently, a panel of serum biomarkers was assessed in three separate IPF cohorts. Osteopontin, MMP-7, intercellular adhesion molecule-1 (ICAM-1) and periostin were differentially expressed between progressive and stable IPF. The investigators developed a statistical model incorporating these four biomarkers which was able to predict risk of disease progression in each cohort (58). Systematic review and meta-analysis data confirms the association between baseline MMP-7 levels and outcomes in untreated IPF, including disease progression and mortality risk (59).

Proteomic analysis of blood biomarkers for prediction of disease behavior holds promise as a future tool with potentially increased precision over individual biomarker measurement. Analysis of a cohort of connective tissue disease-associated ILD, chronic hypersensitivity pneumonitis and unclassifiable ILD patients' serum samples at diagnosis identified and validated 17 novel biomarkers associated with progressive pulmonary fibrosis, including CXCL17 (C-X-C motif chemokine ligand 17) and TGFA

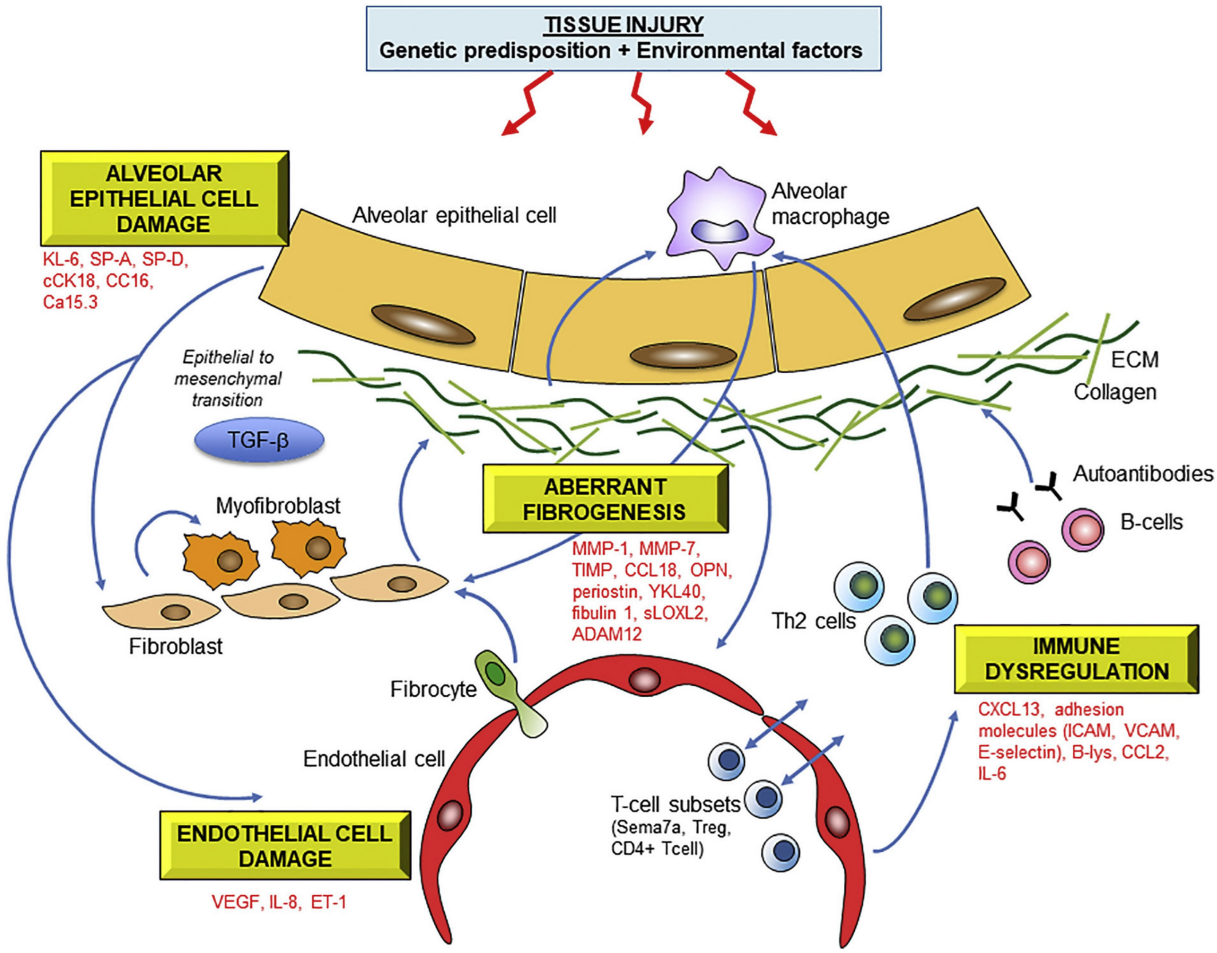

FIGURE 1
Molecular mechanisms underlying fibrotic lung injury and potential serum biomarkers of ILD. Abbreviations: KL-6 Krebs von lungen 6; SP-A/SP-D surfactant protein A/D; cCK18 cleaved cytokeratin 18; CC16 Clara cell protein 16; MMP matrix metalloproteinase; OPN osteopontin, HSP47 heat-shock protein 47; sLOXL2 serum lysyl oxidase-like 2; ADAM a disintegrin and metalloprotease; CCL18 chemokine ligand 18; ICAM intercellular adhesion molecule; VCAM vascular cell adhesion molecule; CXCL13 C-X-C motif chemokine 13; CCL2 CC chemokine 2; IL-6 interleukin-6. Reprinted from Pharmacology and Therapeutics, 202, Jee et al. (56), with permission from Elsevier.

TABLE 2 Outcomes of single cell RNA sequencing studies in fibrotic ILD.

Study	Year	Cell population studied	Insights into human fibrotic lung transcriptome
Nemeth et al. (50)	2020	Alveolar type II epithelial (AE2) cells, mesenchymal cells	Activation and proliferation of fibroblasts, excessive extracellular matrix deposition in IPF
Tsukui et al. (69)	2020	Fibroblasts	CTHRC1-expressing fibroblasts demonstrated in fibroblastic foci in fibrotic lungs from IPF and systemic sclerosis patients
Xu et al. (70)	2016	Alveolar epithelial cells	Frequent co-expression of multiple surface markers associated with indeterminate differentiation and aberrant activation of downstream signaling pathways, for example TGF-β, in IPF
Xi et al. (71)	2017	AE2 cells	Subpopulation of AE2 cells with HIF1α-driven impaired differentiation and migration in IPF
Morse et al. (72)	2019	Alveolar epithelial cells, macrophages	Increased fibroblasts and basal, ciliated, goblet and club cells in IPF lower lobes compared to healthy lungs; decreased alveolar epithelial cells and marked alterations in inflammatory cell populations, including discrete macrophage subsets highly expressing SPP1 and MERTK
Reyfman et al. (73)	2019	Alveolar epithelial cells, macrophages	Heterogeneity in epithelial cell and alveolar macrophage gene expression, and populations of profibrotic alveolar macrophages in fibrotic lungs
Valenzi et al. (74)	2019	Myofibroblasts	Upregulated expression of collagen and other profibrotic genes by myofibroblasts in systemic sclerosis-associated ILD lungs
Adams et al. (75)	2020	Epithelial and stromal cells, macrophages, vascular endothelial cells	Identified a population of aberrant cells at edge of fibroblast foci that co-express basal epithelial, mesenchymal, senescence and developmental markers; profibrotic macrophage populations in IPF lungs
Carraro et al. (76)	2020	Epithelial cells	Restriction of basal-to-ciliated differentiation in IPF
Habermann et al. (77)	2020	Epithelial cells	Extracellular-matrix producing epithelial cell population highly enriched in IPF lungs
Liu et al. (78)	2020	Fibroblasts	Altered gene expression in fibroblast populations from age-matched fibrotic lungs from mice and humans
Mayr et al. (79)	2021	Alveolar epithelial cells	Altered alveolar epithelial cell expression of CRTAC1, which encodes a glycosylated extracellular matrix protein; also reflected in bronchoalveolar lavage fluid and patient plasma

AE2, alveolar epithelial type 2 cells; IPF, idiopathic pulmonary fibrosis; CTHRC1, collagen triple helix repeat containing-1; TGF-β, transforming growth factor beta; HIF1α, hypoxia inducible factor 1 subunit alpha; SPP1, secreted phosphoprotein 1; MERTK, tyrosine-protein kinase Mer; ILD, interstitial lung disease; CRTAC1, cartilage acidic protein 1.

(transforming growth factor alpha) (60). A proteomic "signature" of PPF was then developed and validated using machine learning algorithms, incorporating 12 serum biomarkers, which had a sensitivity of 0.90 for identifying a progressive fibrosing ILD phenotype. Patients with a high-risk proteomic signature experienced significant deterioration in their forced vital capacity (FVC) of −227.1 mL (95% CI −286.7 mL to −167.5 mL); as opposed to patients with low-risk proteomic signature whose FVC did not decline over 12 months (60).

Liquid biopsy is another emerging molecular diagnostic technique, which involves the extraction and analysis of circulating cell-free (ccf) DNA fragments from blood. Levels of ccfDNA have been shown to be significantly increased in IPF patients compared with age- and sex-matched healthy volunteers, and were also significantly associated with disease severity as measured by the previously validated IPF GAP (gender-age-physiology) score. In that study, the median plasma expression of ccfDNA fragments, 104 ng/mL differentiated between cases of more advanced IPF (GAP score 2–3), versus more mild disease (GAP score 1) (61). Interestingly, discordance between ccfDNA and genomic DNA extracted from peripheral blood mononuclear cells in some IPF patients, but not healthy controls, was observed. It has been hypothesized that ccfDNA in peripheral blood might encode genetic information present in the diseased lungs which is not measurable in genomic DNA (61).

Bulk RNA sequencing analyses the average expression level of genes across all cells in a tissue, as opposed to single-cell RNA sequencing, which quantifies differential gene expression by specific cell populations within tissues. Bulk RNA sequencing studies have contributed to increased knowledge of the pathobiology of IPF and other fibrotic lung diseases (62–64). Bulk RNA extracted from IPF lung explants demonstrated the severe IPF transcriptome to be enriched in pathways of T-cell infiltration and activation and tumor development, and a specific subset of genes correlated with patients' forced vital capacities (FVC) (65). A 2019 exploratory study analyzed bulk RNA from IPF explants, and then subsequently performed micro-CT scanning and standard immunohistochemistry on cores of tissue taken from differentially affected lung regions (66). A core set of differentially expressed genes was identified to be present in the IPF lung before fibrosis was even histologically evident, and their profile was further altered in areas of more advanced fibrosis.

Single-cell RNA-sequencing studies, largely performed using explanted IPF lungs in comparison with control healthy donor lungs, have identified numerous upregulated and downregulated genes in fibrotic lungs expressed by specific cell populations (67). For example, altered alveolar type II epithelial cell and mesenchymal cell gene expression has been associated with the activation and proliferation of fibroblasts and excessive extracellular matrix deposition seen in IPF (50). Techniques such as confocal microscopy, immunohistochemistry, in-situ hybridization and/or proteomic analysis of lung tissues, BAL fluid and/or serum have been employed concurrently to ascertain the functional correlation of altered gene expression (68). For example, collagen-producing cell subpopulations in fibrotic lungs were observed to be concentrated within fibroblastic foci in fibrotic lungs (69). Table 2 summaries insights into the fibrotic lung transcriptome gained from single cell RNA sequencing studies performed in IPF and other fibrotic ILDs.

Clearly, RNA sequencing (RNA-seq) has advanced knowledge of the molecular and pathway alterations underlying fibrotic lung diseases. Additionally, RNA-seq and these other molecular tests have potential for future use in identification of novel biomarkers enabling "smart splitting" of ILD patients at diagnosis based on respective components of inflammation and fibrosis in their disease pathogenesis. In addition, this may lead to identification of more specific drug targets and improved disease behavior prediction.

Key points

1. RNA sequencing, involving the use of high throughput sequencing technologies to identify and quantify RNA transcripts in whole tissues or single cells, has identified differentially expressed genes between lung tissue obtained from ILD patients and healthy controls.
2. A genomic classifier developed from RNA sequencing data has demonstrated high specificity in differentiating UIP from non-UIP pattern ILDs, yet it has low sensitivity and little additional impact on diagnostic confidence at ILD MDM.
3. Peripheral blood biomarkers, including MMP-7 and SP-D, have been demonstrated to differentiate between specific ILD subtypes; and also predict disease behavior and response to treatment. These biomarkers need to be assessed for their utility in the integrated clinical setting.
4. Additional novel molecular diagnostic techniques include proteomic and metabolomic analysis of blood, bronchoalveolar lavage specimens and lung tissue samples; and "liquid biopsy" (analysis of cell-free circulating DNA fragments in blood). These techniques hold significant promise for improving future precision disease profiling and guiding therapeutic decision-making, yet require more extensive study before they are ready for implementation into routine clinical care.

Artificial intelligence technologies: deep-learning based radiologic and histopathological assessment

Computer-based deep learning techniques, consisting of convolutional neural network (CNN) algorithms able to autonomously detect features in images, have the potential to revolutionize ILD assessment by reducing human inter-observer variability in interpretation of diagnostic tests (80).

A 2021 systematic review, including 19 retrospective studies, demonstrated deep learning-based assessment of ILD CT scans to have good diagnostic accuracy for classification of ILD pattern, between 76.4–95.1%, when considering consensus radiologist assessment as the reference standard (81).

Walsh et al. (82) conducted a case-cohort study for deep learning algorithm development and assessment and included 1,157 high resolution CT scans obtained from patients with fibrotic ILD, separated into training, validation, and testing cohorts. An initial CNN segmented the lungs and then resampled them to create a maximum of 500 four-slice combinations per scan, creating montages which were fed into the training dataset. The final algorithm was able to classify each HRCT using the 2011 ATS/ERS/JRS/ALAT IPF guidelines with a diagnostic accuracy of 76.4% on the first testing set, and 73.3% on the second testing set which was comparable to the median accuracy of thoracic radiologists (70.7%) (82). In view of its ability to provide rapid and reproducible results, the investigators concluded that this technology could be beneficial for ILD assessment in centers without access to radiologists with ILD expertise. More recently, a retrospective multicenter study assessed the diagnostic ability of a deep-learning algorithm when applied to 1,239 high resolution CT scans of fibrotic ILD. The algorithm performed superiorly to two expert radiologists in predicting histopathologic UIP (area under the receiver-operating characteristic curve, 0.87 vs. 0.80, $p < 0.05$) (83).

Larger recent studies of HRCT scans obtained from participants in the Australian IPF Registry have also assessed the prognostic ability of deep learning algorithms. The extent of lung fibrosis on baseline HRCT, as assessed by data-driven texture analysis, a deep learning technique, significantly correlated with annual rate of decline in forced vital capacity and diffusing capacity for carbon monoxide (84). Another study employing the Systematic Objective Fibrotic Imaging Analysis Algorithm (SOFIA), which was also developed and validated in the identification of UIP-like features on HRCT in patients enrolled in the Australian IPF registry, demonstrated deep learning-based radiologic UIP probability to be predictive of survival in multivariable analysis, where radiologist-determined UIP probability was not (85). Figure 2 demonstrates SOFIA analysis of an HRCT montage.

The ability for deep learning algorithms to be applied in assessment of ILD histopathology specimens also requires consideration. Pilot studies have assessed automated digital quantification of extent of fibrosis in digital images of whole lung sections (86). One study of 71 IPF patients enrolled in the Finnish IPF registry developed and then tested the ability of a semi-supervised deep learning algorithm to identify and quantify specific ILD features in lung tissue samples. The most representative hematoxylin and eosin-stained slide for each patient was scanned at 40× magnification and 20 of the resulting whole slide images were used to train the algorithm. An expert pathologist manually annotated pathognomonic features in the images to train the model. In this cohort, increased number of fibroblastic foci were significantly associated with shortened survival; and high percentages of interstitial and intra-alveolar inflammatory cells were associated with prolonged survival (87).

Key points

1. Computer-based algorithmic analysis of radiology and histopathology in ILD, using deep-learning techniques, may be able to improve diagnostic and prognostic precision by reducing human inter-observer variability in their interpretation.
2. Deep-learning based assessment of radiologic UIP probability has been demonstrated to have excellent prognostic utility for mortality, and this technology may be integrated into ILD care, particularly in centers without access to expert radiologists (or pathologists).

Endobronchial optical coherence tomography

Optical coherence tomography (OCT) is a non-ionizing imaging technology that employs low coherence light waves to capture high resolution of soft tissues to a resolution of <10 μm. Endogenous tissue serves as an optical scattering media, enabling measurement of the time delay and magnitude of backscattered light to generate cross-sectional images (88, 89).

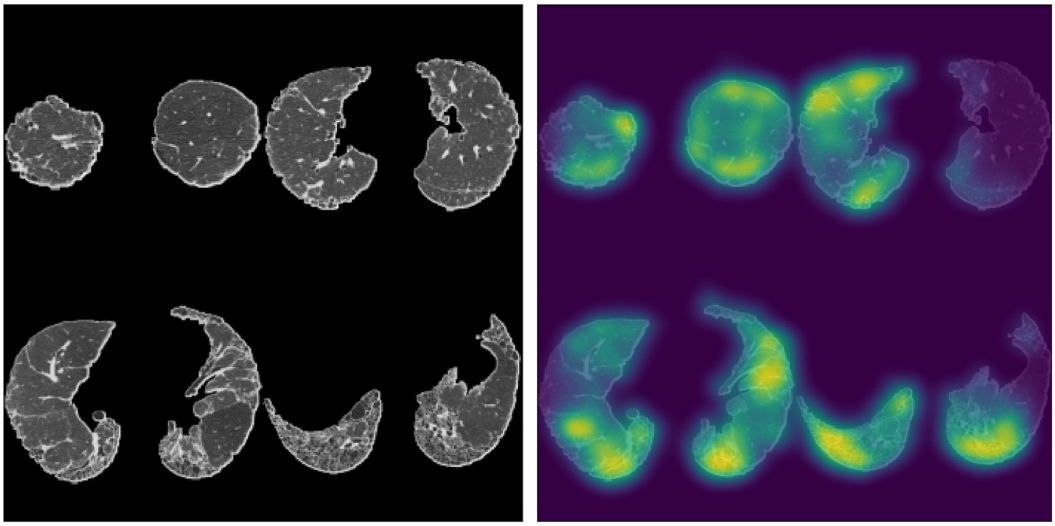

FIGURE 2
Four slice montage generated from a patient with an assigned HRCT diagnosis of probable UIP based on two expert thoracic radiologists, accompanied by a saliency map depicting parts of the lungs most influential in SOFIA-based decision-making. SOFIA probabilities for this montage were UIP: 0.224, probable UIP: 0.764, indeterminate for UIP 0.012, alternative diagnosis: 0.000. The SOFIA deep-learning algorithm generates up to 500 four axial slice montages from an HRCT scan by segmenting the lungs into quarters (excluding the apical 10%) and then randomly selecting slices from each quarter. The algorithm generates a set of numbers from 0 to 1, totalling 1.0, representing the probability of each of the UIP diagnosis categories (definite UIP, probable UIP, indeterminate, alternative diagnosis); which is the average probability for each diagnostic category across all the montages generated for each HRCT (85). Sourced and reproduced with permission from Simon L. F. Walsh, M.D., F.F.R.R.C.S.I., National Heart and Lung Institute, Imperical College London, London, United Kingdom.

Endobronchial OCT (EB-OCT) is performed by passing an OCT probe through the working channel of a flexible bronchoscope to evaluate peripheral and subpleural lung tissue in-vivo. By conducting helical scanning and subsequent pullback of the probe, a three-dimensional reconstruction of sequential images along the airway path is produced. Additional advantages include the ability to image large tissue volumes to microscopic resolution without the need for tissue removal, as in surgical lung biopsy or transbronchial lung cryobiopsy, both associated with significantly higher risk of patient morbidity and mortality than diagnostic bronchoscopy (88).

Like exhaled breath analysis and other novel ILD diagnostic techniques, studies investigating the application of endobronchial OCT in ILD are scarce. Historically, this technology has been used for assessment of asthma and other airways diseases, for example, to assess airway caliber and extent of airway remodeling and quantify airway mucous (90–93). It has also been employed during bronchoscopic interventions for real-time imaging during treatment of airway obstruction (94, 95), and to guide lymph node or peripheral pulmonary nodule sampling via transbronchial needle aspiration for lung cancer diagnosis (96). More recently, small studies have demonstrated utility of EB-OCT in ILD diagnosis for patients with non-diagnostic HRCT or indeterminate biopsy. For example, by detection of microscopic honeycombing not seen on HRCT or by distinguishing between traction bronchiectasis or bronchiolectasis and microscopic honeycombing in the setting of a false-positive radiologic UIP diagnosis (97).

A 2021 prospective diagnostic accuracy study comparing EB-OCT to surgical lung biopsy in 27 patients with unclassifiable or low confidence fibrotic ILD diagnoses, demonstrated EB-OCT to have a sensitivity of 100% (95% CI 75.8–100%) and specificity of 100% (95% CI 79.6–100%) for both histopathologic diagnosis of UIP, as assessed by expert pathologist assessment of surgical lung biopsy specimens; and clinical diagnosis of IPF, as determined by the treating respiratory physician (88). Importantly, high agreement was also demonstrated between EB-OCT (interpreted by both an EB-OCT expert pathologist and novice pathologists who were trained in EB-OCT interpretation during the study) and histopathologic ILD pattern (weighted k:0.87 [0.72–1.0]). EB-OCT criteria used to distinguish between ILD diagnoses were developed from review of previous studies in IPF, lung cancer and other pulmonary pathologies. Features included subpleural fibrosis replacing normal alveolar tissue and microscopic honeycombing in UIP, non-destructive interstitial fibrosis in NSIP and fibrosis around airways with preserved distal alveolar architecture in "airway-centered fibrosis" (88).

These results are certainly very promising and propose EB-OCT as both a novel diagnostic tool and for use in monitoring treatment response and/or disease progression through serial quantifications of extent of lung fibrosis. Yet, larger studies in multiple sites are needed before EB-OCT is ready for implementation in routine clinical practice. It will be important to evaluate inter-observer variability in EB-OCT interpretation; particularly since poor inter-clinician (and inter-meeting) agreement are problems frequently encountered in traditional histopathologic ILD diagnosis by surgical lung biopsy and in the current "gold standard" method, the ILD multidisciplinary meeting (98). Technical issues such as validated methods for confirmation of correct subpleural EB-OCT probe positioning will also require further consideration (99). The use of polarization-sensitive EB-OCT, which has the added ability to detect birefringence from collagen in fibrosis, has recently been investigated as a tool for quantification of fibrosis in ILD; with positive early results (100).

Key points

1. Endobronchial OCT is a non-invasive, non-ionizing radiation technology which uses low coherence light waves to generate high resolution images of soft tissues.

2. Preliminary diagnostic accuracy studies suggest OCT is a promising method with high sensitivity and specificity for identifying UIP, and for differentiating between ILD subtypes through assessment of features such as subpleural fibrosis with and without associated architectural distortion, microscopic honeycombing, and airway-centered fibrosis.

Exhaled breath analysis–electronic nose (eNose) technology

"Breathomics," a field of study involving the analysis of particles and molecules in exhaled breath, has also garnered significant interest in the last few years as an attractive potential ILD diagnostic tool since capturing exhaled breath is both simple and non-invasive (101).

Components of exhaled breath may be analyzed using real-time quantification of small volatile compounds like nitric oxide using chemiluminescence, electrochemical or laser analyzers (102). Alternatively, exhaled breath condensate can be obtained through cooling of exhalate in a collection device for subsequent laboratory analysis. Exhaled breath condensate contains water vapor plus small amounts of both volatile and non-volatile molecules arising from the alveoli and airways. Liquid chromatography-mass spectrometry or specific enzyme immunoassays can be performed for measurement of larger, non-volatile molecules; or analysis of volatile organic compounds (VOCs) can be performed using gas chromatography–mass spectrometry (GC–MS), a high-throughput technique which separates, then identifies and quantifies molecules in complex mixtures of compounds (102).

The thousands of VOCs detectable in exhaled breath (103) are either produced endogenously as by-products of cellular metabolism or may also reflect exposure to various exogenous compounds. For example, isoprene and acetone are commonly detectable endogenously produced VOCs; whereas acetonitrile is not produced endogenously but is found in the breath of cigarette smokers (103). Altered cellular metabolism in various disease states is supported by studies of VOCs in COPD (104), lung cancer (105, 106) and other respiratory illnesses (102). Significantly elevated levels of specific amino acids and other organic compounds have been demonstrated in the exhaled breath of IPF patients when compared with healthy controls (107–109). GC–MS analysis of exhaled breath samples obtained from ILD patients and healthy controls has also been able to distinguish between IPF and connective tissue disease-associated ILD (CTD-ILD) based on discriminating VOCs (110). Importantly, in this study, VOC profiles were also associated with measures of disease severity, including total lung capacity and six-minute walk distance (110).

Electronic nose ("eNose") technology has been developed as a novel method of exhaled breath analysis. eNoses are devices containing multiple cross-reactive gas sensors, each with partial specificity to various molecules. Upon exposure to specific VOCs, an electronic response is generated from each sensor; and a unique pattern of sensor responses is produced for the individual whose breath is being tested, known as the "breathprint" (101, 111). A preliminary eNose ILD study of 31 sarcoidosis and 25 healthy controls previously demonstrated the breathprint of patients with untreated sarcoidosis to be distinct from healthy control individuals (112). Similarly, electronic nose analysis of exhaled breath-derived VOC profiles has been shown to distinguish between IPF patients and healthy controls, and to inversely correlate with bronchoalveolar lavage fluid total cell count in a small study of 32 IPF patients, 33 COPD patients and 36 healthy controls recruited from 2 centers (113). A single center analysis of the breathprint of 174 ILD patients, 23 COPD patients and 33 healthy controls demonstrated the VOC signature of ILD patients as measured using an eNose device to be distinguishable from those of healthy controls and patients with COPD. However, the specificity of the eNose for distinguishing between different ILD subgroups was poor (114).

A larger single center, cross-sectional study undertaken in the Netherlands demonstrated that an eNose device, the SpiroNose, was able to differentiate between ILD patients and healthy control subjects based on machine learning algorithmic analysis of their breathprint (101). Additionally, these pattern recognition algorithms were able to distinguish between IPF and non-IPF ILDs, with an area under the curve (AUC) of 0.91(95% CI 0.85–0.96) in the training set and an AUC of 0.87 (95% CI 0.77–0.96) in the validation set. Furthermore, the model directly compared individual diagnoses' breathprints and was consistently able to distinguish between different ILD subgroups including IPF, chronic hypersensitivity pneumonitis, CTD-ILD, idiopathic non-specific interstitial pneumonia, interstitial pneumonia with autoimmune features and sarcoidosis; with AUCs ranging between 0.85–0.99 (101).

Another cross-sectional study performed in the Netherlands demonstrated eNose technology to be able to reliably differentiate sarcoidosis from control participants, and from other subgroups of ILD, including chronic hypersensitivity pneumonitis (115). Importantly, preliminary studies suggest that exhaled breath analysis using an eNose may also predict treatment response. A recent analysis of 42 treatment-naïve ILD patients, showed that patients who responded to both immunosuppressive and antifibrotic therapies (defined as FVC improvement of $\geq$5% after 1–3 months, or FVC decline of $\leq$2.5%, respectively) had distinguishable exhaled VOC profiles, compared with patients who did not respond to treatment (116).

Unlike GC–MS, eNose analysis of exhaled breath VOCs has been performed in real-time, by using cloud-connected collection devices connected to validated online analysis platforms (101) and has therefore been proposed as a novel point-of-care diagnostic tool that could readily be incorporated into clinical practice (111). Importantly, breath biomarkers such as FeNO (fractional exhaled nitric oxide) can potentially be obtained and analyzed remotely; and were thus considered a novel approach for diagnosis and monitoring of chronic lung diseases during the SARS-CoV-2 pandemic (117). It is clear however that their potential extends beyond the current pandemic and that the use of eNose technology could contribute meaningfully to ILD diagnosis and patient self-management in the future, should their use be validated in larger studies.

Key points

1. eNoses are a novel technology capable of analyzing volatile compounds present in exhaled breath via cross-reactive gas sensors.
2. Cross-sectional studies have demonstrated eNose exhaled breath analysis to consistently be able to distinguish between ILD patients and healthy controls; and to differentiate ILD subtypes including IPF, chronic hypersensitivity pneumonitis, sarcoidosis, NSIP and CTD-ILD.

Acoustic signatures and ultrasonography

Digital auscultation represents a novel tool to measure and digitally record lung sounds, a well-established clinical sign of ILD (118–121). Preliminary studies have evaluated multichannel lung sound analysis, using microphones placed on the anterior and posterior chest to acquire recording of lung sounds. The technique can distinguish the bibasal fine, "velcro-like" crackles associated with IPF from lung crackles due to other causes such as congestive cardiac failure (122). Clinician assessment in a tertiary ILD center with a traditional stethoscope has been shown to predict the presence of fibrotic ILD and UIP pattern as seen on HRCT, yet has clear limitations including inter-observer variability, particularly outside of this setting (123).

More recently, feasibility studies of digital lung sound recordings obtained using electronic stethoscopes have confirmed "velcro-like" crackles to be predictive of fibrotic ILD on HRCT, particularly UIP pattern (OR 19.8) and to positively correlate with specific radiologic features including reticulation, honeycombing and traction bronchiectasis (119, 120).

A 2019 pilot study of 19 IPF patients analyzing digital lung sound recordings at seven timepoints over 12 months identified a set of 19 acoustic features able to distinguish IPF patients from healthy subjects. These included features such as number of crackles, crackle onset timing and frequency range in hertz (120). Serial analysis of the digital sound recordings showed individual acoustic signatures to change over time and to correlate with markers of disease severity and progression, including visual scores of ILD extent on CT and volumetric analysis using Computer-Aided Lung Informatics for Pathology Evaluation and Rating (CALIPER) software (120). Additionally, a score integrating the acoustic signals and composite physiologic index (CPI), a well-established prognostic model in IPF (124), was better able to predict the extent of fibrosis on HRCT than the CPI alone.

Another Mexican study investigated the use of a mobile health application enabling clinicians to record and analyze respiratory sounds using a smartphone and acoustic sensors (121). Although this was only a small feasibility study, and further research into this technology is required, the prospect of this diagnostic tool is exciting since it is non-invasive and broadly accessible, potentially improving remote evaluation of ILD patients distant from tertiary centers.

There is also increasing interest in the use of thoracic ultrasound for diagnosis and monitoring of interstitial lung diseases. Currently, its use as a diagnostic tool is limited since features on ultrasound such as "B-lines," although very sensitive, ultrasonographic signs of interlobular septal thickening in ILD, are not a specific finding, and may be seen in many other conditions including cardiac failure, atelectasis, pneumonia, and other diagnoses (125, 126). The potential for ultrasound however, as a screening tool is compelling, particularly in patients with established risk factors for ILD such as connective tissue disease (126), and for monitoring disease progression (127). A recent study evaluating lung ultrasound findings in 24 ILD patients over 12 months found that the lung ultrasound (LUS) score, the sum of B-lines counted in each intercostal space, using a standard 56-lung intercostal space LUS protocol, to correlate with HRCT Warrick score, a score of extent of radiologic fibrosis (127).

Key points

1. Digital analysis of lung sounds and thoracic ultrasonography might be able to detect early features of ILD, and also represent simple, non-invasive methods for monitoring of disease extent.

Future directions

There is a currently unmet need for improved diagnostic biomarkers and tests in ILD. While there has been great progress in this field, the next steps globally are to integrate such tools into clinical care. Some biomarkers, including MMP-7 or integrated scores, genetic testing, and even some radiological AI tools are prime for clinical integration. Others, including digital auscultation and eNose technology are promising as potential screening tests for early detection of ILD in primary care and other non-expert settings, as well as offering opportunities for remote assessment and monitoring. eNose technology, EB-OCT and deep-learning based radiologic assessment are particularly attractive diagnostic tests since they do not require invasive tissue sampling. While genomic testing and deep learning-based histopathological assessment currently do require tissue sampling, this research continues to further our knowledge of the pathobiological mechanisms underlying the development and progression of fibrotic lung diseases.

The future of ILD diagnostics is promising. Feasibly, machine learning algorithms may be trained to generate virtual biopsies from radiologic or EB-OCT data, negating the need for invasive investigations altogether. Liquid biopsies from blood samples may also play a role in elucidating key transcriptomic signatures for precision disease profiling and therapeutic strategies. Regardless, further research is needed to develop and externally validate novel diagnostic techniques to improve access to timely and accurate diagnosis for ILD patients.

Author contributions

LG wrote the manuscript, with input from LT and TC. All authors reviewed the manuscript and agreed with regard to the contents.

Funding

This research was supported by a Lung Foundation Australia scholarship, with matched funding provided by the University of Sydney (Lung Foundation Australia/Diana Cox Idiopathic Pulmonary Fibrosis PhD Scholarship 2019).

Conflict of interest

LG has received travel and conference support from Boehringer Ingelheim. LT has provided paid consultancy for Erbe Elektromedezin and Boehringer Ingelheim. TC has received grant support, consultancy fees, and speaking honoraria from Boehringer Ingelheim and Hoffman-La Roche, consultancy fees from Bristol Myers Squibb; grant support from Biogen, and provides consultancy for DevPro and Ad Alta.

Publisher's note

References

1. Wijsenbeek M, Suzuki A, Maher TM. Interstitial lung diseases. *Lancet*. (2022) 400:769–86. doi: 10.1016/S0140-6736(22)01052-2

2. Raghu G, Remy-Jardin M, Richeldi L, Thomson CC, Inoue Y, Johkoh T, et al. Idiopathic pulmonary fibrosis (an update) and progressive pulmonary fibrosis in adults: an official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med*. (2022) 205:e18–47. doi: 10.1164/rccm.202202-0399ST

3. Flaherty K, Wells AU, Cottin V, Devaraj A, Walsh SLF, Inoue Y, et al. Nintedanib in progressive Fibrosing interstitial lung diseases. *N Engl J Med*. (2019) 381:1718–27. doi: 10.1056/NEJMoa1908681

4. Ryerson CJ, Corte TJ, Myers JL, Walsh SLF, Guler SA. A contemporary practical approach to the multidisciplinary management of unclassifiable interstitial lung disease. *Eur Respir J*. (2021) 58:2100276. doi: 10.1183/13993003.00276-2021

5. Ryerson CJ, Corte TJ, Lee JS, Richeldi L, Walsh SLF, Myers JL, et al. A standardized diagnostic ontology for fibrotic interstitial lung disease. An international working group perspective. *Am J Respir Crit Care Med*. (2017) 196:1249–54. doi: 10.1164/rccm.201702-0400PP

6. George PM, Spagnolo P, Kreuter M, Altinisik G, Bonifazi M, Martinez FJ, et al. Progressive fibrosing interstitial lung disease: clinical uncertainties, consensus recommendations, and research priorities. *Lancet Respir Med*. (2020) 8:925–34. doi: 10.1016/S2213-2600(20)30355-6

7. Richeldi L, Launders N, Martinez F, Walsh SLF, Myers J, Wang B, et al. The characterization of interstitial lung disease multidisciplinary team meetings: a global study. *ERJ Open Res*. (2019) 5:00209–2018. doi: 10.1183/23120541.00209-2018

8. Wijsenbeek M, Kreuter M, Olson A, Fischer A, Bendstrup E, Wells CD, et al. Progressive fibrosing interstitial lung diseases: current practice in diagnosis and management. *Curr Med Res Opin*. (2019) 35:2015–24. doi: 10.1080/03007995.2019.1647040

9. Pritchard D, Adegunsoye A, Lafond E, Pugashetti JV, DiGeronimo R, Boctor N, et al. Diagnostic test interpretation and referral delay in patients with interstitial lung disease. *Respir Res*. (2019) 20:253. doi: 10.1186/s12931-019-1228-2

10. Cosgrove GP, Bianchi P, Danese S, Lederer DJ. Barriers to timely diagnosis of interstitial lung disease in the real world: the INTENSITY survey. *BMC Pulm Med*. (2018) 18:9. doi: 10.1186/s12890-017-0560-x

11. Walsh SLF. Multidisciplinary evaluation of interstitial lung diseases: current insights. *Eur Respir Rev*. (2017) 26:17002. doi: 10.1183/16000617.0002-2017

12. Furini F, Carnevale A, Casoni GL, Guerrini G, Cavagna L, Govoni M, et al. The role of the multidisciplinary evaluation of interstitial lung diseases: systematic literature review of the current evidence and future perspectives. *Front Med*. (2019) 6:246. doi: 10.3389/fmed.2019.00246

13. Flaherty KR, King TE Jr, Raghu G, Lynch JP III, Colby TV, Travis WD, et al. Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis? *Am J Respir Crit Care Med*. (2004) 170:904–10. doi: 10.1164/rccm.200402-147OC

14. Thomeer M, Demedts M, Behr J, Buhl R, Costabel U, Flower CDR, et al. Multidisciplinary interobserver agreement in the diagnosis of idiopathic pulmonary fibrosis. *Eur Respir J*. (2008) 31:585–91. doi: 10.1183/09031936.00063706

15. Walsh SLF, Maher TM, Kolb M, Poletti V, Nusser R, Richeldi L, et al. Diagnostic accuracy of a clinical diagnosis of idiopathic pulmonary fibrosis: an international case-cohort study. *Eur Respir J*. (2017) 50:1700396. doi: 10.1183/13993003.00936-2017

16. Jo HE, Glaspole IN, Levin KC, McCormack SR, Mahar AM, Cooper WA, et al. Clinical impact of the interstitial lung disease multidisciplinary service. *Respirology*. (2016) 21:1438–44. doi: 10.1111/resp.12850

17. De Sadeleer LJ, Meert C, Yserbyt J, Slabbynck H, Verschakelen JA, Verbeken EK, et al. Diagnostic ability of a dynamic multidisciplinary discussion in interstitial lung diseases: a retrospective observational study of 938 cases. *Chest*. (2018) 153:1416–23. doi: 10.1016/j.chest.2018.03.026

18. Walsh SLF, Wells AU, Desai SR, Poletti V, Piciucchi S, Dubini A, et al. Multicentre evaluation of multidisciplinary team meeting agreement on diagnosis of parenchymal lung disease: a case-cohort study. *Lancet Respir Med*. (2016) 4:557–65. doi: 10.1016/S2213-2600(16)30033-9

19. Maher TM, Nambiar AM, Wells AU. The role of precision medicine in interstitial lung disease. *Eur Respir J*. (2022) 60:2102146. doi: 10.1183/13993003.02146-2021

20. Guler SA, Corte TJ. Interstitial lung disease in 2020: a history of Progress. *Clin Chest Med*. (2021) 42:229–39. doi: 10.1016/j.ccm.2021.03.001

21. Barnes H, Chambers D, Grainge C, Corte TJ, Bastiampillai S, Frenkel S, et al. Clinical utility of a standardized chronic hypersensitivity pneumonitis exposure questionnaire. *Respirology*. (2022) Online ahead of print). doi: 10.1111/resp.14404

22. Umashankar E, Abdel-Shaheed C, Plit M, Girgis L. Assessing the role for nailfold videocapillaroscopy in interstitial lung disease classification: a systematic review and meta-analysis. *Rheumatology*. (2022) 61:2221–34. doi: 10.1093/rheumatology/keab772

23. Jee AS, Parker MJS, McGill N, Bleasel JF, Webster S, Troy LK, et al. Nailfold capillaroscopy by smartphone-dermatoscope for connective tissue disease diagnosis in interstitial lung disease: a prospective observational study. *ERJ Open Res*. (2021) 7:00416–2021. doi: 10.1183/23120541.00416-2021

24. Smith V, Vanhaecke A, Guerra MG, Melsens K, Vandecasteele E, Paolino S, et al. May capillaroscopy be a candidate tool in future algorithms for SSC-ILD: are we looking for the holy grail? A systematic review. *Auto Immun Rev*. (2020) 19:102619. doi: 10.1016/j.autrev.2020.102619

25. Tuddenham WJ. Glossary of terms for thoracic radiology: recommendations of the nomenclature Committee of the Fleischner Society. *Am J Roentgenol*. (1984) 143:509–17. doi: 10.2214/ajr.143.3.509

26. Austin JH, Müller NL, Friedman PJ, Hansell DM, Naidich DP, Remy-Jardin M, et al. Glossary of terms for CT of the lungs: recommendations of the nomenclature Committee of the Fleischner Society. *Radiology*. (1996) 200:327–31. doi: 10.1148/radiology.200.2.8685321

27. Hansell DM, Bankier AA, Mac Mahon H, McLoud TC, Müller NL, Remy J. Fleischner society: glossary of terms for thoracic imaging. *Radiology*. (2008) 246:697–722. doi: 10.1148/radiol.2462070712

28. Travis WD, Costabel U, Hansell DM, King TE Jr, Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med*. (2013) 188:733–48. doi: 10.1164/rccm.201308-1483ST

29. American Thoracic Society, European Respiratory Society. American Thoracic Society/European Respiratory Society international multidisciplinary consensus classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med*. (2002) 165:277–304. doi: 10.1164/ajrccm.165.2.ats01

30. Lynch DA, Sverzellati N, Travis WD, Brown KK, Colby TV, Galvin JR, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner society white paper. *Lancet Respir Med*. (2018) 6:138–53. doi: 10.1016/S2213-2600(17)30433-2

31. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, et al. Diagnosis of idiopathic pulmonary fibrosis: an official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med*. (2018) 198:e44–68. doi: 10.1164/rccm.201807-1255ST

32. Raghu G, Remy-Jardin M, Ryerson CJ, Myers JL, Kreuter M, Vaskova M, et al. Diagnosis of hypersensitivity pneumonitis in adults. An official ATS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med*. (2020) 202:e36–69. doi: 10.1164/rccm.202005-2032ST

33. Rodrigues I, Gomes RE, Coutinho LM, Rego MT, Machado F, Morais A, et al. Diagnostic yield and safety of transbronchial lung cryobiopsy and surgical lung biopsy in interstitial lung diseases: a systematic review and meta-analysis. *Eur Respir Rev*. (2022) 31:210280. doi: 10.1183/16000617.0280-2021

34. Kheir F, Becerra JPU, Bissell B, Ghazipura M, Herman D, Hon SM, et al. Transbronchial lung Cryobiopsy in patients with interstitial lung disease: a systematic review. *Ann Am Thorac Soc*. (2022) 19:1193–202. doi: 10.1513/AnnalsATS.202102-198OC

35. Korevaar DA, Colella S, Fally M, Camuset J, Colby TV, Hagmeyer L, et al. European Respiratory Society guidelines on transbronchial lung cryobiopsy in the diagnosis of interstitial lung diseases. *Eur Respir J*. (2022) 60:2200425. doi: 10.1183/13993003.00425-2022

36. Troy LK, Grainge C, Corte TJ, Williamson JP, Vallely MP, Cooper WA, et al. Diagnostic accuracy of transbronchial lung cryobiopsy for interstitial lung disease diagnosis (COLDICE): a prospective, comparative study. *Lancet Respir Med*. (2020) 8:171–81. doi: 10.1016/S2213-2600(19)30342-X

37. Cottin V, Tomassetti S, Valenzuela C, Walsh SLF, Antoniou KM, Bonella F, et al. Integrating clinical probability into the diagnostic approach to idiopathic pulmonary fibrosis: an international working group perspective. *Am J Crit Care Med*. (2022) 206:247–59. doi: 10.1164/rccm.202111-2607PP

38. Spagnolo P, Ryerson CJ, Putman R, Oldham J, Salisbury M, Sverzellati N, et al. Early diagnosis of fibrotic interstitial lung disease: challenges and opportunities. *Lancet Respir Med*. (2021) 9:1065–76. doi: 10.1016/S2213-2600(21)00017-5

39. Wijsenbeek M, Cottin V. Spectrum of fibrotic lung diseases. *N Engl J Med*. (2020) 383:958–68. doi: 10.1056/NEJMra2005230

40. Zhang D, Newton CA. Familial pulmonary fibrosis: genetic features and clinical implications. *Chest*. (2021) 160:1764–73. doi: 10.1016/j.chest.2021.06.037

41. Newton CA, Molyneaux PL, Oldham JM. Clinical genetics in interstitial lung disease. *Front Med*. (2018) 5:116. doi: 10.3389/fmed.2018.00116

42. Borie R, Guen L, Ghanem M, Taillé C, Dupin C, Dieudé P, et al. The genetics of interstitial lung diseases. *Eur Respir Rev*. (2019) 28:190053. doi: 10.1183/16000617.0053-2019

43. Peljto AL, Blumhagen RZ, Walts AD, Cardwell J, Powers J, Corte TJ, et al. Idiopathic pulmonary fibrosis is associated with common genetic variants and limited rare variants. *Am J Respir Crit Care Med*. (2023). Online ahead of print). doi: 10.1164/rccm.202207-1331OC

44. Swaminathan AC, Neely ML, Frankel CW, Kelly FL, Petrovski S, Durheim MT, et al. Lung transplant outcomes in patients with pulmonary fibrosis with telomere-related gene variants. *Chest*. (2019) 156:477–85. doi: 10.1016/j.chest.2019.03.030

45. Allen RJ, Oldham JM, Jenkins DA, Leavy OC, Guillen-Guio B, Melbourne CA, et al. Longitudinal lung function and gas tranfer in individuals with idiopathic pulmonary fibrosis: a genome-wide association study. *Lancet Respir Med*. (2023) 11:65–73. doi: 10.1016/S2213-2600(22)00251-X

46. Mathai SK. Genetic associations and lung function in IPF: unexpected answers, persistent questions. *Lancet Respir Med*. (2023) 11:5–6. doi: 10.1016/S2213-2600(22)00270-3

47. Mackintosh JA, Chambers DC. Towards truly "idiopathic" pulmonary fibrosis. *Respirology*. (2022) 27:682–3. doi: 10.1111/resp.14320

48. Kheir F, Becerra JPU, Bissell B, Ghazipura M, Herman D, Hon SM, et al. Use of a genomic classifier in patients with interstitial lung disease: a systematic review and meta-analysis. *Ann Am Thorac Soc*. (2022) 19:827–32. doi: 10.1513/AnnalsATS.202102-197OC

49. Chambers DC, Carew AM, Lukowski SW, Powell JE. Transcriptomics and single-cell RNA-sequencing. *Respirology*. (2019) 24:29–36. doi: 10.1111/resp.13412

50. Nemeth J, Schundner A, Frick M. Insights into development and progression of idiopathic pulmonary fibrosis from single cell RNA studies. *Front Med*. (2020) 7:611728. doi: 10.3389/fmed.2020.611728

51. Pankratz DG, Choi Y, Imtiaz U, Fedorowicz GM, Anderson JD, Colby TV, et al. Usual interstitial pneumonia can be detected in Transbronchial biopsies using machine learning. *Ann Am Thorac Soc*. (2017) 14:1646–54. doi: 10.1513/AnnalsATS.201612-947OC

52. Choi Y, Liu TT, Pankratz DG, Colby TV, Barth NM, Lynch DA, et al. Identification of usual interstitial pneumonia using RNA-Seq and machine learning: challenges and solutions. *BMC Genomics*. (2018) 19:101. doi: 10.1186/s12864-018-4467-6

53. Raghu G, Flaherty KR, Lederer DJ, Lynch DA, Colby TV, Myers JL, et al. Use of a molecular classifier to identify usual interstitial pneumonia in conventional transbronchial lung biopsy samples: a prospective validation study. *Lancet Respir Med*. (2019) 7:487–96. doi: 10.1016/S2213-2600(19)30059-1

54. Richeldi L, Scholand MB, Lynch DA, Colby TV, Myers JL, Groshong SD, et al. Utility of a molecular classifier as a complement to high-resolution computed tomography to identify usual interstitial pneumonia. *Am J Respir Crit Care Med*. (2021) 23:211–20. doi: 10.1164/rccm.202003-0877OC

55. Kheir F, Alkhatib A, Berry GJ, Daroca P, Diethelm L, Rampolla R, et al. Using Bronchoscopic lung Cryobiopsy and a genomic classifier in the multidisciplinary diagnosis of diffuse interstitial lung diseases. *Chest*. (2020) 158:2015–25. doi: 10.1016/j.chest.2020.05.532

56. Jee AS, Sahhar J, Youssef P, Bleasel J, Adelstein S, Nguyen M, et al. Review: serum biomarkers in idiopathic pulmonary fibrosis and systemic sclerosis-associated interstitial lung disease – frontiers and horizons. *Pharmacol Ther*. (2019) 202:40–52. doi: 10.1016/j.pharmthera.2019.05.014

57. Maher TM, Oballa E, Simpson JK, Porte J, Habgood A, Fahy WA, et al. An epithelial biomarker signature for idiopathic pulmonary fibrosis: an analysis from the multicentre PROFILE cohort study. *Lancet Respir Med*. (2017) 12:946–55. doi: 10.1016/S2213-2600(17)30430-7

58. Clynick B, Corte TJ, Jo HE, Stewart I, Glaspole IN, Grainge C, et al. Biomarker signatures for progressive idiopathic pulmonary fibrosis. *Eur Respir J*. (2022) 59:2101181. doi: 10.1183/13993003.01181-2021

59. Khan FA, Stewart I, Saini G, Robinson KA, Jenkins RG. A systematic review of blood biomarkers with individual participant data meta-analysis of matrix metalloproteinase-7 in idiopathic pulmonary fibrosis. *Eur Respir J*. (2022) 59:2101612. doi: 10.1183/13993003.01612-2021

60. Bowman WS, Newton CA, Linderholm AL, Neely ML, Pugashetti JV, Kaul B, et al. Proteomic biomarkers of progressive fibrosing interstitial lung disease: a multicentre cohort analysis. *Lancet Respir Med*. (2022) 10:593–602. doi: 10.1016/S2213-2600(21)00503-8

61. Pallante P, Malapelle U, Nacchio M, Sgariglia R, Galati D, Capitelli L, et al. Liquid biopsy is a promising tool for genetic testing in idiopathic pulmonary fibrosis. *Diagnostics*. (2021) 11:1202. doi: 10.3390/diagnostics11071202

62. Boesch M, Baty F, Brutsche MH, Tamm M, Roux J, Knudsen L, et al. Transcriptomic profiling reveals disease-specific characteristics of epithelial cells in idiopathic pulmonary fibrosis. *Respir Res*. (2020) 21:165. doi: 10.1186/s12931-020-01414-z

63. Nance T, Smith KS, Anaya V, Richardson R, Ho L, Pala M, et al. Transcriptome analysis reveals differential splicing events in IPF lung tissue. *PLoS One*. (2014) 9:e97550. doi: 10.1371/journal.pone.0097550

64. Yu X, Gu P, Huang Z, Fang X, Jiang Y, Luo Q, et al. Reduced expression of BMP3 contributes to the development of pulmonary fibrosis and predicts the unfavourable prognosis in IIP patients. *Oncotarget*. (2017) 8:80531–44. doi: 10.18632/oncotarget.20083

65. Sivakumar P, Thompson JR, Ammar R, Porteous M, McCoubrey C, Cantu E 3rd, et al. RNA sequencing of transplant-stage idiopathic pulmonary fibrosis lung reveals unique pathway regulation. *ERJ Open Res*. (2019) 5:00117–2019. doi: 10.1183/23120541.00117-2019

66. McDonough JE, Ahangari F, Li Q, Jain S, Verleden SE, Herazo-Maya J, et al. Transcriptional regulatory model of fibrosis progression in the human lung. *JCI Insight*. (2019) 4:e131597. doi: 10.1172/jci.insight.131597

67. Beisang DJ, Smith K, Yang L, Benyumov A, Gilbertsen A, Herrera J, et al. Single-cell RNA sequencing reveals that lung mesenchymal progenitor cells in IPF exhibit pathological features early in their differentiation trajectory. *Sci Rep*. (2020) 10:11162. doi: 10.1038/s41598-020-66630-5

68. Hao Y, Bates S, Mou H, Yun JH, Pham B, Liu J, et al. Genome-wide association study: functional variant rs2076295 regulates Desmoplakin expression in airway epithelial cells. *Am J Respir Crit Care Med*. (2020) 202:1225–36. doi: 10.1164/rccm.201910-1958OC

69. Tsukui T, Sun KH, Wetter JB, Wilson-Kanamori JR, Hazelwood LA, Henderson NC, et al. Collagen-producing lung cell atlas identifies multiple subsets with distinct localization and relevance to fibrosis. *Nat Commun*. (2020) 11:1920. doi: 10.1038/s41467-020-15647-5

70. Xu Y, Mizuno T, Sridharan A, Du Y, Guo M, Tang J, et al. Single-cell RNA sequencing identifies diverse roles of epithelial cells in idiopathic pulmonary fibrosis. *JCI Insight*. (2016) 1:e90558. doi: 10.1172/jci.insight.90558

71. Xi Y, Kim T, Brumwell AN, Driver IH, Wei Y, Tan V, et al. Local lung hypoxia determines epithelial fate decisions during alveolar regeneration. *Nat Cell Biol*. (2017) 19:904–14. doi: 10.1038/ncb3580

72. Morse C, Tabib T, Sembrat J, Buschur KL, Bittar HT, Valenzi E, et al. Proliferating SPP1/MERTK-expressing macrophages in idiopathic pulmonary fibrosis. *Eur Respir J*. (2019) 54:1802441. doi: 10.1183/13993003.02441-2018

73. Reyfman PA, Walter JM, Joshi N, Anekalla KR, McQuattie-Pimentel AC, Chiu S, et al. Single-cell Transcriptomic analysis of human lung provides insights into the pathobiology of pulmonary fibrosis. *Am J Respir Crit Care Med*. (2019) 199:1517–36. doi: 10.1164/rccm.201712-2410OC

74. Valenzi E, Bulik M, Tabib T, Morse C, Sembrat J, Bittar HT, et al. Single-cell analysis reveals fibroblast heterogeneity and myofibroblasts in systemic sclerosis-associated interstitial lung disease. *Ann Rheum Dis*. (2019) 78:1379–87. doi: 10.1136/annrheumdis-2018-214865

75. Adams TS, Schupp JC, Poli S, Ayaub EA, Neumark N, Ahangari F, et al. Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. *Sci Adv*. (2020) 6:eaba1983. doi: 10.1126/sciadv.aba1983

76. Carraro G, Mulay A, Yao C, Mizuno T, Konda B, Petrov M, et al. Single-cell reconstruction of human basal cell diversity in Normal and idiopathic pulmonary fibrosis lungs. *Am J Respir Crit Care Med*. (2020) 202:1540–50. doi: 10.1164/rccm.201904-0792OC

77. Habermann AC, Gutierrez AJ, Bui LT, Yahn SL, Winters NI, Calvi CL, et al. Single-cell RNA sequencing reveals profibrotic roles of distinct epithelial and mesenchymal lineages in pulmonary fibrosis. *Sci Adv*. (2020) 6:eaba1972. doi: 10.1126/sciadv.aba1972

78. Liu X, Rowan SC, Liang J, Yao C, Huang G, Deng N, et al. Definition and signatures of lung fibroblast populations in development and fibrosis in mice and men. *bioRxiv* [Preprint. (2020). doi: 10.1101/2020.07.15.203141

79. Mayr CH, Simon LM, Leuschner G, Ansari M, Schniering J, Geyer PE, et al. Integrative analysis of cell state changes in lung fibrosis with peripheral protein biomarkers. *EMBO Mol Med*. (2021):e12871. doi: 10.15252/emmm.202012871

80. Kim SY, Diggans J, Pankratz D, Huang J, Pagan M, Sindy N, et al. Classification of usual interstitial pneumonia in patients with interstitial lung disease: assessment of a machine learning approach using high-dimensional transcriptional data. *Lancet Respir Med*. (2015) 3:473–82. doi: 10.1016/S2213-2600(15)00140-X

81. Soffer S, Morgenthau AS, Shimon O, Barash Y, Konen E, Glicksberg BS, et al. Artificial intelligence for interstitial lung disease analysis on chest computed tomography: a systematic review. *Acad Radiol*. (2022) 29:S226–35. doi: 10.1016/j.acra.2021.05.014

82. Walsh SLF, Calandriello L, Silva M, Sverzellati N. Deep learning for classifying fibrotic lung disease on high-resolution computed tomography: a case-cohort study. *Lancet Respir Med*. (2018) 6:837–45. doi: 10.1016/S2213-2600(18)30286-8

83. Bratt A, Williams JM, Liu G, Panda A, Patel PP, Walkoff L, et al. Predicting usual interstitial pneumonia histopathology from chest CT imaging with deep learning. *Chest*. (2022) 162:815–23. doi: 10.1016/j.chest.2022.03.044

84. Humphries SM, Mackintosh JA, Jo HE, Walsh SLF, Silva M, Calandriello L, et al. Quantitative computed tomography predicts outcomes in idiopathic pulmonary fibrosis. *Respirology*. (2022) 27:1045–53. doi: 10.1111/resp.14333

85. Walsh SLF, Mackintosh JA, Calandriello L, Silva M, Sverzellati N, Larici AR, et al. Deep learning-based outcome prediction in progressive fibrotic lung disease using high-resolution computed tomography. *Am J Respir Crit Care Med*. (2022) 206:883–91. doi: 10.1164/rccm.202112-2684OC

86. Testa LC, Jule Y, Lundh L, Bertotti K, Merideth MA, O'Brien KJ, et al. Automated digital quantification of pulmonary fibrosis in human histopathology specimens. *Front Med*. (2021) 8:607720. doi: 10.3389/fmed.2021.607720

87. Mäkelä K, Mäyränpää MI, Sihvo HK, Bergman P, Sutinen E, Ollila H, et al. Artificial intelligence identifies inflammation and confirms fibroblastic foci as prognostic tissue biomarkers in idiopathic pulmonary fibrosis. *Hum Pathol*. (2021) 107:58–68. doi: 10.1016/j.humpath.2020.10.008

88. Nandy S, Raphaely RA, Muniappan A, Shih A, Roop BW, Sharma A, et al. Diagnostic accuracy of Endobronchial optical coherence tomography for the microscopic diagnosis of usual interstitial pneumonia. *Am J Respir Crit Care Med*. (2021) 204:1164–79. doi: 10.1164/rccm.202104-0847OC

89. Popescu DP, Choo-Smith LP, Flueraru C, Mao Y, Chang S, Disano J, et al. Optical coherence tomography: fundamental principles, instrumental designs and biomedical applications. *Biophys Rev*. (2011) 3:155. doi: 10.1007/s12551-011-0054-7

90. Adams DC, Miller AJ, Applegate MB, Cho JL, Hamilos DL, Chee A, et al. Quantitative assessment of airway remodelling and response to allergen in asthma. *Respirology*. (2019) 24:1073–80. doi: 10.1111/resp.13521

91. Ding M, Chen Y, Guan WJ, Zhong CH, Jiang M, Luo WZ, et al. Measuring airway remodeling in patients with different COPD staging using Endobronchial optical coherence tomography. *Chest*. (2016) 150:1281–90. doi: 10.1016/j.chest.2016.07.033

92. Chen Y, Ding M, Guan WJ, Wang W, Luo WZ, Zhong CH, et al. Validation of human small airway measurements using endobronchial optical coherence tomography. *Respir Med*. (2015) 109:1146–53. doi: 10.1016/j.rmed.2015.09.006

93. Williamson JP, James AL, Phillips MJ, Sampson DD, Hillman DR, Eastwood PR. Quantifying tracheobronchial tree dimensions: methods, limitations and emerging techniques. *Eur Respir J*. (2009) 34:42–55. doi: 10.1183/09031936.00020408

94. Williamson JP, McLaughlin RA, Phillips MJ, Armstrong JJ, Becker S, Walsh JH, et al. Using optical coherence tomography to improve diagnostic and therapeutic bronchoscopy. *Chest*. (2009) 136:272–6. doi: 10.1378/chest.08-2800

95. Williamson JP, McLaughlin RA, Phillips MJ, Curatolo A, Armstrong JJ, Maddison KJ, et al. Feasibility of applying real-time optical imaging during Bronchoscopic interventions for central airway obstruction. *J Bronchology Interv Pulmonol*. (2010) 17:307–16. doi: 10.1097/LBR.0b013e3181f9eaae

96. Shostak E, Hariri LP, Cheng GZ, Adams DC, Suter MJ. Needle-based optical coherence tomography to guide Transbronchial lymph node biopsy. *J Bronchol Interv Pulmonol*. (2018) 25:189–97. doi: 10.1097/LBR.0000000000000491

97. Hariri LP, Adams DC, Wain JC, Lanuti M, Muniappan A, Sharma A, et al. Endobronchial optical coherence tomography for low-risk microscopic assessment and diagnosis of idiopathic pulmonary fibrosis *in vivo*. *Am J Respir Crit Care Med*. (2018) 197:949–52. doi: 10.1164/rccm.201707-1446LE

98. Marinescu DC, Ryerson CJ. Endobronchial optical coherence tomography for the diagnosis of fibrotic interstitial lung disease: a light at the end of the tunnel? *Am J Respir Crit Care Med*. (2021) 204:1122–4. doi: 10.1164/rccm.202108-1899ED

99. Kalverda KA, Vaselli M, Wijmans L, De Bruin DM, Jonkers RE, Poletti V, et al. Endobronchial optical coherence tomography: shining new light on diagnosing usual interstitial pneumonitis? *Am J Respir Crit Care Med*. (2022) 205:967–8. doi: 10.1164/rccm.202111-2619LE

100. Nandy S, Berigei SR, Keyes CM, Muniappan A, Auchincloss HG, Lanuti M, et al. Polarization-sensitive Endobronchial optical coherence tomography for microscopic imaging of fibrosis in interstitial lung disease. *Am J Respir Crit Care Med*. (2022) 206:905–10. doi: 10.1164/rccm.202112-2832LE

101. Moor CC, Oppenheimer JC, Nakshbandi G, Aerts JGJV, Brinkman P, Maitland-van der Zee A, et al. Exhaled breath analysis by use of eNose technology: a novel diagnostic tool for interstitial lung disease. *Eur Respir J*. (2021) 57:2002042. doi: 10.1183/13993003.02042-2020

102. Hayton C, Terrington D, Wilson AM, Chaudhuri N, Leonard C, Fowler SJ. Breath biomarkers in idiopathic pulmonary fibrosis: a systematic review. *Respir Res*. (2019) 20:7. doi: 10.1186/s12931-019-0971-8

103. Amann A, de Lacy CB, Miekisch W, Schubert J, Buszewski B, Pleil J, et al. The human volatilome: volatile organic compounds (VOCs) in exhaled breath, skin emanations, urine, feces and saliva. *J Breath Res*. (2014) 8:034001. doi: 10.1088/1752-7155/8/3/034001

104. Bos LD, Sterk PJ, Fowler SJ. Breathomics in the setting of asthma and chronic obstructive pulmonary disease. *J Allergy Clin Immunol*. (2016) 138:970–6. doi: 10.1016/j.jaci.2016.08.004

105. Zhou J, Huang ZA, Kumar U, Chen DDY. Review of recent developments in determining volatile organic compounds in exhaled breath as biomarkers for lung cancer diagnosis. *Anal Chim Acta*. (2017) 996:1–9. doi: 10.1016/j.aca.2017.09.021

106. Tsou PH, Lin ZL, Pan YC, Yang HC, Chang CJ, Liang SK, et al. Exploring volatile organic compounds in breath for high-accuracy prediction of lung cancer. *Cancers*. (2021) 13:1431. doi: 10.3390/cancers13061431

107. Gaugg MT, Engler A, Bregy L, Nussbaumer-Ochsner Y, Eiffert L, Bruderer T, et al. Molecular breath analysis supports altered amino acid metabolism in idiopathic pulmonary fibrosis. *Respirology*. (2019) 24:437–44. doi: 10.1111/resp.13465

108. Rindlisbacher B, Strebel C, Guler S, Kollár A, Geiser T, Fiedler GM, et al. Exhaled breath condensate as a potential biomarker tool for idiopathic pulmonary fibrosis – a pilot study. *J Breath Res*. (2018) 12:016003. doi: 10.1088/1752-7163/aa840a

109. Yamada YI, Yamada G, Otsuka M, Nishikiori H, Ikeda K, Umeda Y, et al. Volatile organic compounds in exhaled breath of idiopathic pulmonary fibrosis for discrimination from healthy subjects. *Lung*. (2017) 195:247–54. doi: 10.1007/s00408-017-9979-3

110. Plantier L, Smolinska A, Fijten R, Flamant M, Dallinga J, Mercadier JJ, et al. The use of exhaled air analysis in discriminating interstitial lung diseases: a pilot study. *Respir Res*. (2022) 23:12. doi: 10.1186/s12931-021-01923-5

111. Van der Sar IG, Wijbenga N, Nakshbandi G, Aerts JGJV, Manintveld OC, Wijsenbeek M, et al. The smell of lung disease: a review of the current status of electronic nose technology. *Respir Res*. (2021) 22:246. doi: 10.1186/s12931-021-01835-4

112. Dragonieri S, Brinkman P, Mouw E, Zwinderman AH, Carratú P, Resta O, et al. An electronic nose discriminates exhaled breath of patients with untreated pulmonary sarcoidosis from controls. *Respir Med*. (2013) 107:1073–8. doi: 10.1016/j.rmed.2013.03.011

113. Dragonieri S, Scioscia G, Quaranta VN, Carratu P, Venuti MP, Falcone M, et al. Exhaled volatile organic compounds analysis by e-nose can detect idiopathic pulmonary fibrosis. *J Breath Res*. (2020) 14:047101. doi: 10.1088/1752-7163/ab8c2e

114. Krauss E, Haberer J, Maurer O, Barreto G, Drakopanagiotakis F, Degen M, et al. Exploring the ability of electronic nose technology to recognize interstitial lung diseases (ILD) by non-invasive breath screening of exhaled volatile compounds (VOC): a pilot study from the European IPF registry (eurIPFreg) and biobank. *J Clin Med*. (2019) 8:1698. doi: 10.3390/jcm8101698

115. Van der Sar IG, Moor CC, Oppenheimer JC, Luijendijk ML, Van Daele PLA, Maitland-van der Zee AH, et al. Diagnostic performance of electronic nose Technology in Sarcoidosis. *Chest*. (2022) 161:738–47. doi: 10.1016/j.chest.2021.10.025

116. Van der Sar IG, Moor CC, Vellekoop BP, Wijsenbeek MS. Predicting treatment response in patients with interstitial lung disease using electronic nose technology. *Eur Respir J*. (2022) 60:345. doi: 10.1183/13993003.congress-2022.345

117. Kouri A, Gupta S, Yadollahi A, Ryan CM, Gershon AS, To T, et al. Addressing reduced laboratory-based pulmonary function testing during a pandemic. *Chest*. (2020) 158:2502–10. doi: 10.1016/j.chest.2020.06.065

118. Richeldi L, Cottin V, Würtemberger G, Kreuter M, Calvello M, Sgalla G. Digital lung auscultation: will early diagnosis of fibrotic interstitial lung disease become a reality? *Am J Respir Crit Care Med*. (2019) 200:261–3. doi: 10.1164/rccm.201902-0306LE

119. Fukumitsu T, Obase Y, Ishimatsu Y, Nakashima S, Ishimoto H, Sakamoto N, et al. The acoustic characteristics of fine crackles predict honeycombing on high-resolution computed tomography. *BMC Pulm Med*. (2019) 19:153. doi: 10.1186/s12890-019-0916-5

120. Sgalla G, Larici AR, Sverzella N, Bartholmai B, Walsh SLF, Nikolic D, et al. Quantitative analysis of lung sounds for monitoring idiopathic pulmonary fibrosis: a prospective pilot study. *Eur Respir J*. (2019) 53:1802093. doi: 10.1183/13993003.02093-2018

121. Reyes BA, Olvera-Montes N, Charleston-Villalobos S, González-Camarena R, Mejía-Ávila M, Aljama-Corrales T. A smartphone-based system for automated bedside detection of crackle sounds in diffuse interstitial pneumonia patients. *Sensors*. (2018) 18:3813. doi: 10.3390/s18113813

122. Vyshedskiy A, Bezares F, Paciej R, Ebril M, Shane J, Murphy R. Transmission of crackles in patients with interstitial pulmonary fibrosis, congestive heart failure, and pneumonia. *Chest*. (2005) 2005:1468–74. doi: 10.1378/chest.128.3.1468

123. Brito-Zerón P, Prieto-González S, Benegas M, Cuerpo S, Espinosa G, Ramírez J, et al. Auscultation of Velcro crackles is associated with usual interstitial pneumonia. *Medicine*. (2016) 95:e2573. doi: 10.1097/MD.0000000000002573

124. Wells AU, Desai SR, Rubens MB, Goh NSL, Cramer D, Nicholson AG, et al. Idiopathic pulmonary fibrosis: a composite physiologic index derived from disease extent observed by computed tomography. *Am J Respir Crit Care Med*. (2003) 167:962–9. doi: 10.1164/rccm.2111053

125. Gutierrez M, Tardella M, Rodriguez L, Mendoza J, Clavijo-Cornejo D, García A, et al. Ultrasound as a potential tool for the assessment of interstitial lung disease in rheumatic patients. Where are we now? *Radiol Med*. (2019) 124:989–99. doi: 10.1007/s11547-019-01053-5

126. Frongillo E, Gaudioso G, Feragelli B. Ultrasound and interstitial lung disease: use and limitations. *Radiol Med*. (2020) 125:66–7. doi: 10.1007/s11547-019-01094-y

127. Pitsidianakis G, Vassalou EE, Vasarmidi E, Bolaki M, Klontzas ME, Xirouchaki N, et al. Performance of lung ultrasound for monitoring interstitial lung disease. *J Ultrasound Med*. (2022) 41:1077–84. doi: 10.1002/jum.15790

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 03 May 2023
DOI 10.3389/fmed.2023.1145257

OPEN ACCESS

EDITED BY
John-David Aubert,
Centre Hospitalier Universitaire Vaudois (CHUV), Switzerland

REVIEWED BY
Sevim Bavbek,
Ankara University, Türkiye
Alessandra Bettiol,
University of Florence, Italy

*CORRESPONDENCE
Justyna Fijolek
✉ jfijolek@op.pl

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 15 January 2023
ACCEPTED 13 March 2023
PUBLISHED 03 May 2023

CITATION
Fijolek J and Radzikowska E (2023) Eosinophilic granulomatosis with polyangiitis – Advances in pathogenesis, diagnosis, and treatment.
Front. Med. 10:1145257.
doi: 10.3389/fmed.2023.1145257

Eosinophilic granulomatosis with polyangiitis – Advances in pathogenesis, diagnosis, and treatment

Justyna Fijolek*† and Elzbieta Radzikowska†

The Third Department of Pneumonology and Oncology, National Tuberculosis and Lung Diseases Research Institute, Warsaw, Poland

Eosinophilic granulomatosis with polyangiitis (EGPA) is a rare disease characterized by eosinophil-rich granulomatous inflammation and necrotizing vasculitis, pre-dominantly affecting small-to-medium-sized vessels. It is categorized as a primary antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs) but also shares features of hypereosinophilic syndrome (HES); therefore, both vessel inflammation and eosinophilic infiltration are suggested to cause organ damage. This dual nature of the disease causes variable clinical presentation. As a result, careful differentiation from mimicking conditions is needed, especially from HES, given the overlapping clinical, radiologic, and histologic features, and biomarker profile. EGPA also remains a diagnostic challenge, in part because of asthma, which may pre-dominate for years, and often requires chronic corticosteroids (CS), which can mask other disease features. The pathogenesis is still not fully understood, however, the interaction between eosinophils and lymphocytes B and T seems to play an important role. Furthermore, the role of ANCA is not clear, and only up to 40% of patients are ANCA-positive. Moreover, two ANCA-dependent clinically and genetically distinct subgroups have been identified. However, a gold standard test for establishing a diagnosis is not available. In practice, the disease is mainly diagnosed based on the clinical symptoms and results of non-invasive tests. The unmet needs include uniform diagnostic criteria and biomarkers to help distinguish EGPA from HESs. Despite its rarity, notable progress has been made in understanding the disease and in its management. A better understanding of the pathophysiology has provided new insights into the pathogenesis and therapeutic targets, which are reflected in novel biological agents. However, there remains an ongoing reliance on corticosteroid therapy. Therefore, there is a significant need for more effective and better-tolerated steroid-sparing treatment schemes.

KEYWORDS

eosinophils, lymphocytes, inflammatory disorders, granulomatous inflammation, blood vessels

1. Introduction

Eosinophilic granulomatosis with polyangiitis (EGPA) is a rare disease characterized by late-onset asthma, blood and tissue eosinophilia, and small-to-medium vessel vasculitis (1). It was first described in 1951 by two pathologists (J. Churg and L. Strauss), based on an analysis of autopsies of 13 patients with asthma, eosinophilia, and specific organ lesions, such as cardiac insufficiency, renal failure, and peripheral neuropathy (2). Its annual incidence and pre-valence range from 1 to 3 per 1,000,000 and 11 to 45 per 1,000,000, respectively, without gender dominance (3). However, the disease may be underdiagnosed because of restrictive pathomorphological criteria (2). Patients with asthma are a particular risk group, as they experience EGPA 34 times more frequently than those in the general population (4). The mean age at disease onset is approximately 50 years (5), although the disease can also occur in children (6).

Eosinophilic granulomatosis with polyangiitis is often diagnosed in pneumonological departments, where patients are referred due to asthma and lung lesions in chest computed tomography (CT) scans. In a recent study, among 46 consecutive patients hospitalized in a respiratory center because of peripheral eosinophilia and respiratory/lung symptoms (from 2017 to 2019), EGPA was the most common cause of these conditions (45.6%) (7). According to the current nomenclature classification, EGPA belongs to the group of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs), along with granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) (8), however, it is clearly distinct from GPA to MPA (9, 10). This is a unique disease sharing features of vasculitis and hypereosinophilic syndrome (HES) (11). In addition, these two processes are responsible for the heterogeneous clinical symptoms and phenotypes. Therefore, diagnosis is challenging and requires careful differentiation under mimicking conditions. ANCA are present less frequently than GPA and MPA (up to 30–40% of patients), and primarily target myeloperoxidase (MPO) (9, 10).

Given its rarity and unique features (such as eosinophilia and eosinophilic inflammation), EGPA has often been excluded from AAV studies, which has resulted in a delay in progress in knowledge about the disease compared to other AAVs. However, recently, increasing interest in EGPA as a subject of clinical trials has been observed, and new international projects concerning EGPA are being developed (12). Significant improvements in our understanding of the disease reflect meaningful progress in its early diagnosis and treatment. In this article, we discuss advances in EGPA, including its pathogenesis, diagnosis, and treatment, considering novel drugs that have or are being evaluated to improve patient outcomes.

Eosinophilic granulomatosis with polyangiitis has been defined mainly based on the histologic findings known since the first EGPA description by Churg and Strauss (2). According to the 1994 Chapel Hill Consensus Conference (CHCC), EGPA is defined as an eosinophil-rich and necrotizing granulomatous inflammation often involving the respiratory tract, with necrotizing vasculitis affecting small to medium vessels, and is associated with asthma and eosinophilia (13). In 2012, the nomenclature and classification system was revised. The former name "Churg-Strauss syndrome" was replaced with EGPA, and the disease was classified into a new group "ANCA-AAVs" alongside GPA and MPA (8). However, recent data indicate that the current terminology "EGPA" is not entirely appropriate and requires revision. Although it implies that EGPA is a genuine vasculitis *("polyangiitis")*, symptoms of vasculitis are not present in all patients, and it is still debated whether patients having asthma, hypereosinophilia, and eosinophil-rich granulomatous inflammation without necrotizing vasculitis, should be determined as having EGPA (14).

2. Pathogenesis and triggering factors

While the triggering factors for EGPA remain unknown, our understanding of its pathogenesis has significantly improved. The disease is considered an immune-inflammatory disorder based on the profound immunological dysregulation of both the innate and adaptive immune systems, including T and B lymphocytes, eosinophils, and neutrophils. In addition, genetic pre-dispositions have been reported (15).

2.1. T lymphocytes

In EGPA both Th1 and Th2 pathways are activated, and eosinophils contribute to organ damage (16). EGPA is mainly considered a Th2-response disease. This is evidenced by elevated serum levels of Th2-related cytokines (17, 18) and increased expression of Th2 and regulatory-type transcripts in bronchoalveolar lavage fluid (BALF) cells from patients with active EGPA (19). The T-cell receptors of patients with EGPA show a restricted repertoire (20), suggesting that an antigen-mediated process is likely responsible for their activation (1). Activated Th2 lymphocytes secrete many eosinophilotropic cytokines, including interleukins (IL) 3, 4, 5, 10, and 13, which enhance eosinophil maturation in the bone marrow and their peripheral activation (18, 19). Among these interleukins (ILs), IL-5 is the key cytokine that mediates the release of eosinophils into the bloodstream. It enhances eosinophil production, maturation, and activation and prolongs survival, mainly by inhibiting apoptosis (21), however, it is not responsible for fostering eosinophil infiltration of specific tissues (21). The relevance of the Th2 pathway is underlined by the efficacy of treatment based on the blocking of IL-5. IL-5 receptor (IL-5R) expression is specific to eosinophil differentiation, as it is almost exclusively expressed in eosinophils (22). Targeting IL-5 or IL-5R has become an attractive approach to treating eosinophil-related disorders, including EGPA (23).

Although the Th2 response plays a crucial role, Th17 and Th1 lymphocytes are also involved in EGPA pathogenesis. Th17 cells are specific lymphocytes that produce several proinflammatory cytokines (IL-17A, IL-17F, or IL-22) and are regulated by regulatory T-lymphocytes (Treg), which suppress the immune response and have a protective role in the development of autoimmune disorders (24). Elevated numbers of Th17 cells and decreased frequency

of Treg cells have been found in patients with EGPA; the Th17/Treg ratio correlates well with markers of disease activity, and CCR4-active chemokines contribute to eosinophilia (25). The involvement of the Th1 pathway is evidenced by the increased serum concentration of interferon-gamma (IFN-γ) in EGPA patients (26). This cytokine is involved in granuloma formation to protect against the cytotoxic effects of eosinophils. Moreover, Th1 cells were detected in skin lesion biopsies (27), and the gut mucosa of patients with EGPA; the latter has a positive correlation with disease activity (28). Clonally expanded CD8 + T cells have also been described in patients with EGPA, suggesting their pathogenic role in vascular damage (29).

2.2. Eosinophils

Evidence supports that eosinophils play a key role in the pathogenesis of EGPA, with abnormal proliferation, impaired apoptosis, and increased tissue toxicity attributed to eosinophil products (5). Their increased number and extracellular protein deposition have been observed in various tissue specimens, including skin (30) and endomyocardial samples (31). The direct toxic effect is associated with the release of cytoplasmic granules upon eosinophil activation (32, 33). However, it can also be an indirect toxic effect as a result of the recruitment and activation of other inflammatory cells (26). There were two types of granule-characterized eosinophils. The primary granule contains Charcot-Leyden crystal proteins and lipid bodies, which are complex inducible organelles that are the site of eicosanoid synthesis, while the secondary granule contains a variety of pre-formed proinflammatory cytokines, enzymes, and growth factors, as well as specific cationic proteins [major basic protein (MBP); eosinophilic cationic protein (ECP); eosinophil peroxidase (EPO); eosinophil-derived neurotoxin (EDN)], which are mainly responsible for specific organ damage (26, 34). The effect of eosinophils depends largely on the tissue involved, however, complications of their accumulation and activation include thrombosis (34, 35), fibrosis (36), and allergic inflammation (26, 34, 37). In addition to being activated, eosinophils secrete many cytokines which enhance the Th2 response, thereby maintaining a vicious circle. Eosinophils are a key source of IL-25. Its elevated concentrations have been found in patients with EGPA and are associated with disease activity and the degree of eosinophilia (38).

In addition to the Th-2 pathway, eotaxins (CCL11-eotaxin, CCL24-eotaxin 2, and CCL26-eotaxin 3) are potent eosinophil activators. They are eosinophil-selected chemokines mainly secreted by endothelial cells but also by T lymphocytes; for example, both IL-4 and 13 released by Th2 cells are synergic promoters of eotaxin synthesis (39). Furthermore, eotaxin 3 is a particularly potent chemoattractant that binds to a specific CCR3 receptor (highly expressed in eosinophils) (22). Increased levels of eotaxin 3 have been described in patients with EGPA and are correlated with disease activity (40, 41).

One case report of Fip1-like1-plateled-derived factor receptor A (FIP1L1-PDGFRA) – positive EGPA implicated the role of tyrosine kinase pathways as drivers for eosinophilia in EGPA (42). The efficacy of imatinib in FIP1L1-PDGFR A-unmutated EGPA has also been previously described (43, 44). These findings indicate a possible shared pathogenic mechanism of EGPA with HES.

2.3. The innate immune system

Increased IL-33, thymic stromal lymphopoietin (TSLP), and type 2 innate lymphoid cells (ILC2) have been found in patients with active EGPA, indicating that the pathogenesis of EGPA involves interactions between the innate and adaptive immune systems (45). TSLP is a critical mediator of the Th2 response, acting on multiple cell lineages, including eosinophils and ILC2, affecting their maturation, survival, and recruitment. One activator of TSLP is IL-4, which is significantly increased in patients with EGPA. ILC2 are characterized by high expression of transcription factor 3 (GATA3) and production of IL-5 and IL-13 (28), which are key factors involved in the recruitment of eosinophils.

2.4. B lymphocytes, ANCA, and neutrophils

The role of B lymphocytes in the pathogenesis of EGPA has also recently been highlighted, although not well established, however, the promising results of anti-CD 20 B-cells depleting therapy can support this idea (46). In addition, many patients exhibit an abnormal humoral response, reflecting B lymphocyte activation. Elevated serum concentrations of total immunoglobulin E (IgE) and IgE-containing immune complexes are often observed in patients with EGPA (26). It has also been reported that immunoglobulin G subclass 4 (IgG4) levels are essentially increased (47) and correlated with the number of affected organs and disease severity in EGPA (48). Tsurikisawa et al. (49) showed a significant increase in the proportion of B lymphocytes positive for CD80, CD27, and CD95 in the blood of EGPA patients with frequent relapses, while those with the seldom-relapsing disease had higher CD19-positive B-cell counts and higher serum IgG levels, suggesting that frequently relapsing EGPA is associated with induced B-cell apoptosis. Finally, a comparison of lymphocyte immunophenotypes in EGPA patients showed that, in addition to increased T lymphocyte activity, they correlated with increased plasmablasts and T follicular helper lymphocytes (Tfh), indicating that B-cell activation is involved in the development of EGPA (50).

The presence of ANCA also reflects the activation of B lymphocytes, however, the pathogenic role of these antibodies in EGPA has not been firmly established and is suspected to be similar to MPA. Animal models have shown that MPO-ANCA has a direct damaging effect on endothelial cells, resulting in the development of necrotizing crescentic glomerulonephritis and pulmonary hemorrhage (51). In a human case study, a newborn was reported to develop pulmonary-renal syndrome with the placental transmission of MPO-ANCA (52). A study conducted by Falk et al. (53) made a breakthrough regarding the pathogenic role of ANCA in AAVs. The study proved that ANCA can activate primed neutrophils to produce reactive oxygen species (ROS) and release lytic enzymes that cause necrosis of endothelial cells and adjacent matrix. Unlike GPA and MPA, where the role of ANCA is well established, in EGPA it is still not fully understood. First, ANCA is detected in only one-third of patients, less frequently than GPA and MPA (9, 54, 55). Second, although EGPA and MPA are characterized by the same type of ANCA (anti-MPO), the diseases differ significantly in their clinical phenotype [e.g., renal

involvement or diffuse alveolar hemorrhage (DAH) is much more frequent and more severe in MPA than in EGPA] (56). It has been suggested that alternative MPO epitopes, other than those in MPA, develop in ANCA-positive EGPA, contributing to mitigated vascular features (15). Finally, the presence of ANCA in EGPA does not always correlate with symptoms of vasculitis (14). Some authors speculate that for EGPA, a different targeted epitope, a change to the specific epitope conformation, or a failure in the masking process of this epitope by the ceruloplasmin fragment could explain the presence of MPO-ANCA (57).

In recent years, there has been much interest in neutrophil extracellular traps (NETs). NETs are defined as a network of chromatin threads containing histones and proteolytic enzymes (including MPO) that can be released by activated neutrophils to kill bacteria (58). Furthermore, NETs are considered to play an important role in the pathogenesis of AAVs and are a source of ANCA (59, 60). However, a recent study demonstrated enhanced NETs in patients with EGPA with no regard to ANCA status, significantly correlated with blood eosinophil count (61). Eosinophil extracellular traps (EETs) and eosinophil ETosis (EETosis) have also recently been studied in EGPA (62). Mukherjee et al. (63) demonstrated that immunoprecipitated immunoglobulins from ANCA (+) sputum derived from patients with EGPA allowed extensive EETs from both neutrophils and eosinophils *in vitro*. Direct evidence of EETs/EEtosis within the thrombus in patients with EGPA has been also provided (64).

2.5. Genetics

Several immunogenetic factors that pre-dispose patients to EGPA have been identified. It has been shown that the HLA-DRB1*07 and DRB1*04 alleles are associated with the development of EGPA, while DRB1*03 and DRB1*13 are protective (65). Another genetic risk factor is HLA-DRB4, which suggests a strong link with CD4 + T lymphocyte activation (66). In turn, functionally relevant variations in the IL-10 gene promoter (IL-10.2 haplotype) are associated with ANCA-negative EGPA (67).

Recently, a genome-wide association study (GWAS) demonstrated that ANCA status in EGPA is associated with a specific genetic background (56). EGPA with ANCA positivity is associated with human leukocyte antigen DQ (HLA-DQ), which shares both clinical and major histocompatibility complex (MHC) associations with anti-MPO AAV. In turn, ANCA-negative EGPA has a mucosal barrier origin and is associated with variants of the glycoprotein A33 (GPA33) and IL-5/interferon regulatory factor 1 (IRF1) (genotype sharing with asthma). There was an association of both EGPA subgroups (ANCA + and ANCA –) with variants at the TSLP, BCL2L11, and CDK6 loci and suggestive evidence for BACH2, Chromosome 10, and lipoma preferred partner (LPP), indicating that EGPA is characterized by certain genetic variants associated with the syndrome as a whole (56).

3. Triggering factors

There are no well-known triggering factors of EGPA, however, environmental factors, infections, and drugs have been speculated. Several cases of disease development following massive antigen inhalation (grain dust, flour dust, and cereal dust) (68) and exposure to pigeons have been described (69). Regarding infectious agents, *Aspergillus fumigatus* triggers EGPA. Some reports demonstrated that *Aspergillus* might be a pathogen common to both allergic bronchopulmonary aspergillosis (ABPA) and EGPA, and prolonged exposure to this fungus in some patients with ABPA may promote progression to EGPA (70). A case of concomitant ABPA and EGPA after *Aspergillus niger* infection has also been reported (71). Other infectious agents include viruses, among others. A case of EGPA following COVID-19 has been recently reported (72).

Other factors include drugs mainly used in asthma, such as leukotriene receptor antagonists (LTRAs) or anti-IgE antibodies, which are also suspected to induce EGPA (73, 74), however, the mechanism to induce vasculitis is not well-known. One hypothesis is that the administration of these drugs in the asthmatic phase of undiagnosed patients with EGPA may result in vasculitis burst due to reducing the steroid dose, previously masking symptoms of EGPA (5). Two case-controlled studies concluded that treatment with LTRAs did not increase the risk of EGPA (4, 75). However, a recent monocentric retrospective study found a significant correlation between LTRAs exposure and ANCA positivity in EGPA patients. The authors speculated that LTRAs could induce imbalanced stimulation of leukotriene receptors, which may cause neutrophil activation, NETs production, and subsequent ANCA stimulation, resulting in the development of vasculitis (76). Other suspected drugs include anti-IL therapies. Ikeda et al. (77) described a case of EGPA that became apparent following the discontinuation of dupilumab (anti-IL-4/IL-13 antibody). Additionally, Lim et al. (78) reported a case of EGPA during benralizumab (anti-IL5Rα) treatment.

As asthma is a major feature, allergy may also contribute to the development of EGPA. However, systematic allergy testing in patients with EGPA revealed evidence of allergy in less than one-third of patients (79). Other suspected factors include vaccination and desensitization (5). A case of EGPA that developed following a booster dose of the anti-SARS-CoV-2 vaccine has also been reported (80).

4. Clinical symptoms and disease stages

Classically, EGPA develops in three consecutive stages. The first is the prodromal phase dominated by asthma and allergic rhinosinusitis. After a variable period (mean 9.3 ± 10.8 years) (5), the eosinophilic phase develops—characterized by peripheral and tissue eosinophilia, which may result in pulmonary infiltrates, eosinophilic cardiomyopathy, or gastrointestinal involvement (GI). Next, the disease progresses into the vasculitic phase, in which organ manifestations consistent with vasculitis pre-dominate (81). However, disease succession does not always occur. In some patients, there is an overlap of these phases, or the disease may begin with the eosinophilic phase; in others, the absence of either eosinophilic or vasculitic phases is observed (1, 34). This complexity of the disease makes the clinical manifestation diverse. Interestingly, the spectrum of manifestations varies depending on the patient recruitment center, e.g., patients admitted to respiratory departments have more frequent cardiac involvement and limited

features of vasculitis (14). The frequencies of organ involvement and phenotypic features in the selected EGPA cohort are presented in **Table 1**.

4.1. The prodromal phase

Asthma is a major feature of EGPA usually preceding the symptoms of vasculitis (mean 9.3 ± 10.8 years) (5). It concerns 90–100% of patients (14, 54, 82–87) and is characterized by distinct features compared to asthmatic patients in the general population. First, it is usually late-onset asthma, which begins in adulthood at around 30–40 years of age. Second, an allergic background is present in less than one-third of patients with EGPA, compared with approximately 70% of patients with asthma in general, and there are no seasonal exacerbations (79). Atopy, if present, is associated with a better prognosis but with more severe or uncontrolled asthma manifestations in the year before the development of vasculitis (88). Third, asthma in EGPA is usually severe and often requires long-term treatment with oral corticosteroids (CS) despite the regression of systemic disease. In a retrospective study of 157 patients with EGPA, asthma was severe in 57% of cases, whereas persistent airflow obstruction was present in 38, 30, and 46% of patients at diagnosis, 3-year follow-up, and final visit, respectively (89). In another study, airflow obstruction was observed in approximately 40% of patients in clinical remission (90). It remains unclear why systemic therapy controls systemic manifestations in EGPA, but not asthma symptoms. Some authors speculate a dissociation between eosinophil bone marrow production and eosinophil recruitments in the airways which results that in sputum (but not blood), eosinophilia is still present in the group of EGPA patients in remission phase (91). Asthma, although often severe, may paradoxically improve during the full-blown vasculitic phase (92). However, it has recently been demonstrated that the severity of asthma increases 3–6 months before the onset of systemic symptoms (89). Furthermore, severe or uncontrolled asthma is associated with baseline pulmonary and ear, nose, and throat (ENT) manifestations but not with clear-cut vasculitic features (93).

Finally, asthma in EGPA is often accompanied by allergic manifestations in the upper respiratory tract, such as allergic rhinitis, chronic sinusitis (70–90%) (89), and nasal polyps (42–58%) (89, 94–96). At this stage of the disease, distinguishing prodromal ENT symptoms in the course of EGPA from chronic rhinosinusitis with nasal polyps (CRSwNP) is challenging; especially in the biopsy, both typical histological features of eosinophilic polyposis are present (96, 97). Lesions observed in GPA, such as destructive granulomatous inflammation or nasal crusting, are uncommon in EGPA. However, secretive otitis media, chronic ear drainage, sensorineural hearing loss, and facial nerve paralysis may occur (34, 98).

4.2. The eosinophilic phase

In this phase, clinical symptoms are due to eosinophilic infiltration of organs. Typically, the lungs, gastrointestinal tract, and heart are affected.

Lung involvement is present in 37–98% of patients with EGPA, depending on the study series (9, 55, 84, 99–102). In addition, a chest radiograph is abnormal in 70% of patients and shows bilateral pulmonary consolidative or reticulonodular opacities in a peripheral distribution (103). In high-resolution computed tomography (HRCT), which is a more precise method, pulmonary lesions can be classified as airspace and airway patterns (104), however, both types often coexist in one patient. Furthermore, all lung imaging changes observed in EGPA are not EGPA-specific and are frequently observed in other diseases (7, 105). The airspace pattern is mostly migrating patchy infiltrates with peripheral dominance corresponding to chronic eosinophilic pneumonia (EP), (104, 106) which antedate systemic vasculitis in 40% of cases (81). Other common findings are ground-glass opacities (39–53%), followed by consolidations (28–42%), and poorly defined nodules (24–63%) (89, 106). The airway pattern consists of small centrilobular nodules, tree-in-bud sign, bronchial dilatation, wall thickening, and mosaic perfusion pattern (89, 104, 106), which reflect airway involvement in the course of asthma generally, not only in EGPA (7). Greater severity and longer duration of asthma (>5 years) are significantly associated with a higher incidence of airway abnormalities on HRCT in patients with EGPA (107). Histologically, small nodules correspond to eosinophilic bronchiolitis and peribronchiolar vasculitis, whereas bronchial wall thickening is associated with airway wall eosinophil and lymphocyte infiltrations (106).

Other less frequent thoracic symptoms of EGPA include pleural effusion and hilar or mediastinal lymphadenopathy (108, 109). Pleural effusion may develop secondary to eosinophilic pleurisy as well as eosinophilic cardiomyopathy-associated congestive heart failure (1). Other HRCT findings may include interstitial edema, cardiac enlargement, or pericardial effusion, all of which are related to cardiac involvement. In some patients, these HRCT findings may be the only chest symptoms.

A small proportion of patients (3–4%) may experience DAH, which is a life-threatening vasculitic manifestation that can lead to acute respiratory distress (16).

GI is less common in EGPA, although it is significantly more frequent than in GPA or MPA (84). This organ manifestation is recognized in 24–78% of patients, depending on the series and diagnostic tests used (54, 55, 82–84, 100). Manifestations are non-specific and include abdominal pain, which is the most frequently reported symptom (30–91%) (54, 100, 110), followed by diarrhea (45%) (110) and minor bleeding (3–9%) (54, 82, 100). Cholecystitis, pancreatitis, intestinal infarction, and ischemic colitis have been described, but they are rarely present (1–3%) (54, 102). In a study of 383 patients with EGPA, symptoms of acute surgical abdomen occurred in approximately 6% of the cases (55). In another study, 22–45% experienced severe GI manifestations, potentially requiring surgery (16). In EGPA, clinical GI symptoms and findings on abdominal CT are non-specific and require differentiation from other diseases. Common CT features include bowel enlargement and pathologic enhancement (16), whereas histological examination demonstrates mainly eosinophilic infiltrations, sometimes with vasculitis and eosinophilic granulomas (5, 102, 111).

Among the three types of AAVs, cardiac involvement (CI) is most common in EGPA and is mostly present in ANCA-negative patients (9, 10, 55, 112). In Churg and Straus's original

TABLE 1 Organ involvement and phenotypic features of selected eosinophilic granulomatosis with polyangiitis (EGPA) cohorts.

	Guillevin et al. (99)	Comarmond et al. (55)	Durel et al. (100)	Sinico et al. (9)	Moosig et al. (82)	Tsurikisawa et al. (83)	Samson et al. (54)	Saku et al. (101)	Durel et al. (134)	Healy et al. (85)	Bettiol et al. (86)	Fijolek et al. (87)
No. of pts	96	383	101	93	150	121	118	188	63	93	573	86
M/F (n)	44/52	199/184	43/58	39/54	76/74	42/79	64/54	121/67	27/36	ND	276/297	35/51
Country	France	France	France, Italy, UK	Italy	Germany	Japan	France, Belgium, UK	Japan	France, Italy, Belgium, UK	New Zealand USA	Italy, Austria, UK	Poland
Study period	1963–1995	1957–2009	1990–2011	1989–2004	1990–2009	1999–2015	2005–2011	1996–2015	1990–2011	1997–2003	1988–2018	1992–2020
Center	Internal Medicine	FVSG	Internal Medicine, Allergology, Immunology	Nephrology, Immunology, Rheumatology, Pulmonology, Neurology	Rheumatology, Internal Medicine, Otorhinolaryngology, Ophthalmology, Cardiology	Allergology, Respirology	Internal Medicine, Immunology, Allergology, Pulmonology	Internal Medicine, Immunology, Rheumatology	Nephrology, Internal Medicine	Internal Medicine, Allergology, Immunology	Internal Medicine, Surgery, Rheumatology, Allergology, Pulmonology, Nephrology	Pulmonology
Age at onset of EGPA (mean or median; yrs)	48.2	50.3	49.2	51.6	49.1	53.3	51.9	59.7	60 (median)	ND	55.3 (median)	35 (median)
Eosinophil count/mm^3 (mean or median)	7,193	7,569	ND	4,400	1,100	8,528	8,231	8,775	3,650 (median)	ND	2,680	5,000 (median)
ANCA (+) (%)	47.6	31.0	42.6	37.0	30.0	35.0	41.0	47.0	84.0	16.1	50.1	14.0
Asthma (%)	100.0	91.1	100.0	95.7	92.7	98.3	94.0	95.2	100.0	100	96.3 (lower respiratory tract)	96.5
Sinusitis (%)	61.1	41.8	92.1	77.4	76.7	91.2	68.0	50.0	70.0	63.4	79.4 (ENT)	82.6
Organ manifestation (%)												
Lungs	37.5	91.4	54.5	50	61	67.6	98	34.6	38.0	65.6	ND	88.4
Nerve	78.1	55.1	66.3	64.5	76	98.3	74	88.3	46.0	52.0	63.2	54.6
Heart	13.5	27.4	20.8	16.1	46	73.9	38	11.2	14.0	28.0	21.3	76.7
GI	33.3	23.2	25.0	21.5	28	78.6	29	12.2	ND	17.2	10.1	19.8
Skin	51.0	39.7	46.5	52.7	49	67.9	48	41.5	40.0	67.7	36.6	43.0
Kidneys	26.0	21.75	26.0	26.9	18	35.2	27	18.1	86.0	17.2	13.8	16.3

ANCA, antineutrophil cytoplasmic antibodies; GI, gastrointestinal involvement; ENT, ear, nose, throat; FVSG, French Vasculitis Study Group; anti-MPO, antimyeloperoxidase; anti-PR3, antiproteinase 3; ND, no data.

10.3389/fmed.2023.1145257

cohort, it occurred in more than 50% of autopsies (2), however, its reported incidence varies from 11 to 74%, depending on the series and diagnostic techniques used (54, 55, 82, 84, 101, 113, 114). Clinical manifestations are variable and include myocarditis (often with thrombus formation), pericarditis, valvular insufficiency, or involvement of the conduction system, resulting in arrhythmia (5, 98, 115–117). The severity of clinical symptoms varies from mild to clinically overt and life-threatening. Patients most often complain of chest pain and dyspnea (116, 118, 119), but the first symptom may also be acute congestive heart failure, life-threatening arrhythmia, and cardiac death (119). In addition, cardiac involvement can be asymptomatic (83, 118, 120–123). In recent data of Polish 86 patients with EGPA, cardiac invasion was found in 76.7% of the cases, with almost 30% of the cases being asymptomatic (87).

Eosinophilia and its cytotoxicity play a crucial role in heart damage caused by EGPA (119). Patients with CI have been reported to have significantly higher eosinophil counts at diagnosis than those without this organ manifestation (118, 124); usually, they were younger, had negative ANCA, higher disease activity, and higher C-reactive protein (CRP) levels (118). Three successive stages of eosinophilic cardiac damage have been described. The first stage is necrosis due to the infiltration of eosinophils and the release of granular proteins. The second phase is characterized by thrombosis formation, whereas fibrosis of the endocardium and valves occurs in the final stage, resulting in restrictive cardiomyopathy and cardiac insufficiency (119). This phase corresponds to scarring of the endomyocardium and is irreversible; therefore, early detection of cardiac involvement is crucial for prognosis. This is because treatment at the earlier stages provides a chance to reverse the inflammatory process and limit myocardial necrosis.

CI of EGPA can also be derived from coronary vasculitis, which is a rare situation occurring in approximately 3% of patients and manifests as myocardial infarction with negative results on coronary angiography (82, 113).

4.3. The vasculitic phase

This phase manifests as a feature of vasculitis. Typically, the nervous system, skin, and kidneys are affected, with the latter being the rarest. However, every organ may be involved. This phase is often preceded by general symptoms such as fever, weakness, muscle pain, or arthritis.

Involvement of the nervous system is a prominent feature of the vasculitic phase. It affects 42–76% of EGPA patients (54, 55, 82, 84, 87, 102), mainly ANCA-positive (9, 10). Among other forms of AAVs, it is most prevalent in EGPA (65 vs. 23% in MPA, and 19% in GPA) (125). Frequently affected nerves include the peroneal, tibial, ulnar, and median nerves, but the typical presentation is mononeuritis multiplex, usually manifested by foot drop and symmetrical polyneuropathy, often progressing when left untreated (126). Patients complain of numbness, burning sensation, pain, limb weakness, and other sensory disturbances, which can be the first symptom, even in 63% of the cases (126, 127). Diagnosis is mainly based on clinical evaluation and may be confirmed by electromyography (EMG) or nerve biopsy. However, the latter procedure is infrequently performed in clinical practice. In a large study of 955 AAV patients, only 12% underwent nerve biopsies, of which 53% had definitive vasculitis (125). Pathophysiologically, nerve damage is caused by vasculitis and eosinophilic infiltrates, with the latter pre-dominating in ANCA-negative cases (128).

Central nervous system (CNS) involvement in EGPA is less common and is reported in 5–29% of cases with neurological symptoms (54, 55, 82, 83). The main neurological manifestations included ischemic cerebrovascular lesions (52%), intracerebral and/or subarachnoid hemorrhage (24%), loss of visual acuity (33%), and cranial nerve palsies (21%). The clinical course varies, with long-term neurological sequelae being common (43%). Intracerebral hemorrhages have the worst prognostic impact (129).

Skin involvement is the next most prominent feature of the vascular phase. Its frequency ranges from 23 to 68% in patients (54, 55, 83, 84, 87, 100, 101, 113), with vascular purpura being the most common (24–39%) (54, 55, 100, 113). Other findings include subcutaneous nodules that occur in 30% of cases (5) and less frequently, non-specific maculopapular rash, urticaria, petechiae, sterile pustules, livedo reticularis, vesicles, and pruritus (130). A wide range of histological changes is observed in the purpura of the skin, from eosinophilic vasculitis to leukocytoclastic vasculitis without eosinophilic infiltration, making diagnosis difficult (131). Other skin lesions in EGPA show extensive infiltration of eosinophils and surrounding inflamed small dermal blood vessels (132), however, eosinophil infiltration is not specific to EGPA and is a common finding in a broad spectrum of skin diseases (133).

In EGPA, renal involvement is less frequent and less severe than in other forms of AAV (134). In addition, its reported frequency depends on the profile of the medical facility. According to various studies from different centers, the frequency varies from 16.3 to 35% of patients (54, 55, 82–84, 87, 100, 113), with nephrological facilities even in 86% of patients presenting with renal diseases at vasculitis diagnosis (134). Renal involvement in EGPA pre-dominates in ANCA-positive patients, which is in line with the aforementioned study, in which 84% of patients had a positive ANCA test (134). The most common clinical symptom reported in different series was proteinuria (3.3–20%) (55, 82, 113), with renal insufficiency observed in 4.3–15% of cases (55, 83), and up to 75% of patients referred to nephrological facilities, in whom acute renal failure was the most common renal presentation (134). Histologically, the most typical pattern included pauci-immune necrotizing glomerulonephritis (78%), followed by membranous nephropathy (10%) and membranoproliferative glomerulonephritis (3%), both of which were ANCA-negative. Other findings include pure acute interstitial nephritis (10%) and interstitial eosinophilic inflammation in half of the patients, regardless of ANCA status (134).

5. Diagnosis, classification, and disease phenotypes

The diagnosis of EGPA is challenging and requires the correlation of clinical, laboratory, radiologic, and histopathologic findings, however, in cases with a history of asthma, eosinophilia, and both "vasculitic" and "eosinophilic" organ damage, the

suspicion of EGPA is quite straightforward - in contrast to those with incomplete manifestations, which can be difficult to recognize. In addition, some patients lack evidence of vasculitis or ANCA, and there is an ongoing debate over whether EGPA can be recognized in these cases. Histology can confirm the diagnosis of EGPA, but the simultaneous presence of all three typical lesions is rare (135). In clinical practice, the diagnosis of EGPA is mainly clinical, however, considering the rarity of the disease and the variety of symptoms, the accuracy of the diagnosis increases with a multidisciplinary discussion among experienced clinicians (16, 103).

5.1. Diagnostic and classification criteria

To date, there are no validated or universally accepted diagnostic criteria for EGPA. The aforementioned CHCC is a nomenclature classification and not a diagnostic classification (8, 13). The first diagnostic criteria were proposed by Lanham et al. (81) which included asthma, eosinophilia ≥ 1,500 cells/μL, and manifestations of vasculitis involving at least ≥2 extrapulmonary organs. These criteria were developed before classifying EGPA into AAVs and do not require histological examination. However, they have been widely used by clinicians owing to their simplicity in capturing the essence of the disease. Recently, the Joint Task Force of the European Respiratory Society (ERS) and the Foundation for the Development of Internal Medicine in Europe (Groupe d'Etudes et de Recherche sur les Maladies Orphelines Pulmonaires; GERM'O'P) proposed new diagnostic criteria (14, 136). They restricted the EGPA terminology to ANCA-positive cases and/or to those with genuine features of vasculitis (or with surrogates of vasculitis) that are precisely defined. In addition, they proposed that patients with asthma, blood eosinophilia, and systemic manifestations, but non-vasculitic and without ANCA, are referred to as having hypereosinophilic asthma with systemic manifestations (HASM), not EGPA. The next criteria are those used in the MIRRA study assessing the safety and efficacy of mepolizumab in patients with EGPA (23). In contrast to the above-mentioned criteria, they were very loose, with the majority of patients not having ANCA or features of vasculitis. However, these criteria were developed for the purposes of a clinical trial (as an eligibility criteria), and are not widely used in clinical practice.

Classification criteria are often mistakenly used as diagnostic criteria, although they are not. Classification criteria were designed to distinguish EGPA from other types of vasculitis; therefore, they should be used only when a diagnosis of small- or medium-sized vessel vasculitis has been established.

The first classification criteria for EGPA were published in 1990 by the American College of Rheumatology (ACR). They were developed by comparing 20 EGPA-diagnosed patients with 787 control patients with other forms of vasculitis and included six items: asthma, eosinophilia > 10%, neuropathy, pulmonary infiltrates, sinusitis, and extravascular eosinophils in the biopsy. The presence of ≥4 of these six criteria allowed the classification of vasculitis as EGPA (137). These criteria were characterized by low sensitivity (67.1%, with 17% of cases meeting the criteria for other vasculitides), and although the specificity was high (64–98.9%), up to 27% of the comparators fulfilled at least one of these criteria (12). Despite poor methodology and lack of validation, these criteria have remained unchanged for several decades. In 2022, the ACR/EAAR (European Alliance of Associations for Rheumatology) established new classification criteria based on a prospective international multisite observational study (Diagnostic and Classification Criteria in Vasculitis; DCVAS project) conducted at 136 sites from 32 countries, including 107 cases of EGPA and 450 comparators. These criteria highlight the significance of peripheral eosinophilia, asthma, and eosinophilic inflammation and specify other features that function as important disease classifiers (such as mononeuritis multiplex, obstructive airway disease, or nasal polyps). Moreover, unlike the previous 1990 criteria, these are validated, have excellent sensitivity (85%) and specificity (99%), and incorporate ANCA testing. The criteria include seven items that have been assigned a point weight (positive or negative), and vasculitis could be classified as EGPA if the cumulative score was ≥6 points (138). Although these criteria were developed primarily for clinical trial purposes, they represent a major advancement in clinical practice as well, however, they are only for EGPA classification and do not solve the problem with diagnosis. A summary of the proposed diagnostic and classification criteria for EGPA (including the definition of the disease) is presented in **Table 2**.

It is worth noting that owing to its dual nature, EGPA has been also listed as an "associated syndrome" in the classification of HESs (11).

5.2. Diagnostic tests and differential diagnoses

To date, there are no reliable biomarkers of EGPA. The results of these studies were inconclusive, with varying success rates (**Table 3**) (17, 40, 45, 139–151). Active EGPA is characterized by marked eosinophilia, usually ≥1,500 cells/μL or >10%, which correlates with disease activity (1, 5). It is a fixed feature of EGPA and an important diagnostic criterion, however, in patients treated with systemic CS (e.g., asthma), eosinophil count may rapidly decline within a few days, and the results may be falsely normal (5). A significant proportion of patients have elevated inflammatory markers, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), mainly at the onset of the disease (55). Non-specific elevations in IgE levels were detected in 75% of cases (26). MPO-ANCA should be tested with antigen-specific immunoassays in any patient with eosinophilic asthma and clinical features suggestive of EGPA (such as constitutional symptoms, purpura, polyneuropathy, unexplained heart, gastrointestinal or renal disease, and/or pulmonary infiltrates or hemorrhage) (152), however, only approximately one-third of patients are ANCA-positive (9). Recently, a novel observation of ANCA reactivity in the sputum of seronegative EGPA patients was reported (63). ANCA reactivity was associated with more severe respiratory symptoms and sputum eosinophilia. It is now being investigated whether ANCA sputum could be useful as a diagnostic tool for serum ANCA patients with EGPA as well as to identify a subset of patients with eosinophilic asthma who are at increased risk of developing EGPA in the future (63).

In EGPA, each organ may be affected; therefore, it is essential to conduct a thorough medical history interview and perform

TABLE 2 Eosinophilic granulomatosis with polyangiitis – definition, diagnosis and classification.

Definition Chapel Hill 2012	Diagnostic criteria			Classification criteria	
	Lanham criteria (1984)	Criteria proposed by the ERS-task force and GERM'O'P (2013, 2017)	Criteria proposed in the MIRRA trial (2017)	ACR 1990	ACR 2022
Eosinophil-rich and necrotizing granulomatous inflammation often involving the respiratory tract, and necrotizing vasculitis pre-dominantly affecting small to medium vessels, and associated with asthma and eosinophilia; ANCA is more frequent when glomerulonephritis is present Nasal polyps are common Limited expression of EGPA confined to the upper or lower respiratory tract may occur Granulomatous or non-granulomatous extravascular inflammation, such as non-granulomatous eosinophil-rich inflammation of lungs, myocardium, and gastrointestinal tract is common	1. Asthma 2. Blood eosinophilia >1,500 cells/mm^3 or >10% of WBC 3. Evidence of vasculitis involving two or more extrapulmonary organs (with or without biopsy) All 3 criteria must be meet	Asthma and eosinophilia >1,500 cells/mm^3 1. Definite vasculitis features, as: biopsy-proven necrotizing vasculitis of any organ, biopsy proven necrotizing glomerulonephritis or crescentic glomerulonephritis, DAH, palpable purpura, myocardial infarction due to proven coronaritis 2. Definite surrogates of vasculitis, as: hematuria associated with red casts or >10% dysmorphic erythrocytes or hematuria and 2 + proteinuria on urinalysis, or leukocytoclastic capillaritis and/or eosinophilic infiltration of the arterial wal lat biopsy 3. Mononeuritis or mononeuritis multiplex 4. ANCA and any systemic manifestation (extrapulmonary and non-ENT) At least one of the criteria is needed	A history or presence of asthma and eosinophilia >1,000 cells/mm^3 or >10% of WBC 1. Histo-pathological evidence of eosinophilic vasculitis, perivascular eosinophilic infiltration, or eosinophil-rich granulomatous inflammation 2. Neuropathy 3. Pulmonary infiltrates 4. Sinonasal abnormality 5. Cardiomyopathy 6. Glomerulonephritis 7. DAH 8. Palpable purpura 9. ANCA positivity At least two of the criteria are needed	1. Asthma 2. Eosinophilia >10% of WBC 3. Neuropathy, mono- or polyneuropathy 4. Pulmonary infiltrates 5. Paranasal sinus abnormality 5. Extravascular eosinophils in biopsy The presence of any 4 or more of these 6 criteria are needed	1. Obstructive airway disease (+3) 2. Nasal polyps (+3) 3. Mononeuritis multiplex (+1) 4. Eosinophilia ≥1z10^9/liter (+5) 5. Extravascular eosinophilic-pre-dominant inflammation on biopsy (+2) 6. Positive test for cANCA or anti-PR3 (−3) 7. Hematuria (−1) A score of ≥6 is needed

EGPA, eosinophilic granulomatosis with polyangiitis; ACR, American College of Rheumatology; ERS, European Respiratory Society; GERM'O'P, Groupe d'Etudes et de Recherche sur les Maladies Orphelines Pulmonaires; ANCA, antineutrophil cytoplasmic antibodies; DAH, diffuse alveolar hemorrhage; anti-PR3, anti-proteinase 3 antibodies; ENT, ear, nose, throat; WBC, white blood count.

diagnostic tests assessing the functions and/or organ lesions. In addition, it is important to detect life-threatening organ involvement, as it requires rapid implementation of treatment (153). Generally, once EGPA is diagnosed, evaluating possible lung, heart, kidney, GI, and peripheral nerve involvement is recommended (153). Regarding the lungs and respiratory manifestations, a complete pulmonary diagnostic evaluation, comprising chest imaging at baseline and pulmonary function tests, should be performed (153). Every patient should have at least one chest radiograph, however, a CT scan is more sensitive and can provide a more precise assessment of lung lesions (153). Bronchoscopy with an evaluation of inflammatory cells in BALF can confirm pulmonary eosinophilia (defined as ≥25% eosinophils at differential cell count) (108). When DAH is present, BALF is bloodier and contains hemosiderin-laden macrophages (5).

Cardiac involvement, in particular, is associated with poor prognosis (114, 154); therefore, basic cardiological examinations are recommended in all patients (at diagnosis and in case of relapse), irrespective of clinical symptoms (118, 153, 155). These examinations include resting electrocardiography (ECG), echocardiography (ECHO), and serum concentrations of brain natriuretic peptide (BNP) and troponin (118, 153, 155). The 24-h ECG monitoring can help detect arrhythmias that cannot be captured on resting ECG and may be life-threatening, leading to sudden death. Recently, cardiac magnetic resonance (CMR) imaging has been considered the gold standard technique for evaluating cardiomyopathies (118, 121, 156). It is a safe and non-invasive tool for the assessment of cardiac involvement in AAVs (118, 121, 122, 156–158). Furthermore, it can help identify the individual stages of myocarditis (with better visibility of endocavitary thrombosis) and determine the activity of the disease (121, 156, 158, 159), however, its particular diagnostic importance is in asymptomatic patients, in whom this manifestation can be easily overlooked (118, 120–123, 155, 158, 160, 161). CMR is also a useful tool for monitoring treatment efficacy and fibrosis (121, 161). Late gadolinium enhancement (LGE) by CMR (mostly of subendocardial location) is characterized by high sensitivity and specificity for the detection of cardiac inflammation and fibrosis (121), and its persistence following treatment has become a marker of cardiac disease severity (112). However, CMR abnormalities are detected in a high proportion of patients in clinical remission and their clinical and prognostic significance remains unclear (123, 161). Although endomyocardial biopsy (EMB) is still considered the gold standard for the diagnosis of myocarditis, it is not routinely performed due to the risk of complications and organizational difficulties. This procedure may be considered in doubtful cases, especially, when the diagnosis of EGPA has not been established (155). Signs of heart involvement in cardiological tests in EGPA are demonstrated in **Figure 1**. **Figure 2** presents chest imaging findings in patients with EGPA.

Renal involvement is the next poor prognostic factor; therefore, renal function tests and urinalysis should be performed in all cases at baseline and during follow-up (153). In asymptomatic patients, routine screening for GI and peripheral nerve involvement is not required, however, when symptoms are present, appropriate diagnostic procedures should be implemented (e.g., radiologic

TABLE 3 Selected studies investigated biomarkers in EGPA.

Investigated biomarker	Patients' cohort	Method	Results	Conclusion	References
Eotaxin-3	EGPA: 37 (15 active, 22 inactive), Healthy controls: 123 Disease controls: 138 (other AAV, HES, parasitic disease, SLE, SSc, CU other causes of eosinophilia).	Comparison of serum levels of eotaxin-3 in all groups, *ex vivo* stability of eotaxin-3 in serum samples testing, and determination of the association of SNPs in the eotaxin-3 gene.	1. Serum eotaxin-3 was highly elevated only in active EGPA (specificity: 87.5%, sensitivity: 98.6% at a cut-off level of 80 pg/ml). 2. None of the tested SNPs within the eotaxin-3 gene influenced the susceptibility to develop EGPA.	Serum eotaxin-3 is a sensitive and specific marker for the diagnosis of active EGPA. SNPs in the eotaxin-3 gene do not predict the risk of developing EGPA.	Zwerina et al. (41)
Eotaxin-1, Eotaxin-2, Eotaxin-3	EGPA: 40 (active) Healthy controls: 30 Disease controls: 57 (asthma, other AAV, HES)	Evaluation of serum eotaxin-1, 2, and 3 levels in all groups; identification of eotaxin-3 expression in tissue biopsies of EGPA.	Eotaxin-3 serum level was highly elevated only in active EGPA and correlated with blood eosinophil count, total IgE, and acute-phase parameters, with strong expression of eotaxin-3 in tissue biopsies of EGPA.	There is a significant association of eotaxin-3 with EGPA activity and blood eosinophil count.	Polzer et al. (40)
ECP	EGPA: 18 (11 active, 7 inactive) Healthy controls: 15	Serum levels of ECP evaluation in all groups.	Mean ECP serum level was significantly higher in active EGPA and correlated with blood eosinophil count.	ECP may be used as a disease activity marker in EGPA.	Guilpain et al. (139)
CCL17/TARC	EGPA: 25 (12 active, 13 inactive) HES: 18 Other AAV: 12 Other eosinophilia: 14 Healthy controls: 21	1. Serum levels of CCL17/TARC evaluation in all groups. 2. Identification of CCL17/TARC in tissue biopsies of EGPA.	1. Serum levels of CCL17/TARC were significantly elevated in active EGPA and correlated with blood eosinophil count, however, they are also noted in other eosinophilic diseases. 2. Expression of CCL17/TARC in the affected tissue of EGPA was found.	Serum levels of CCL17/TARC reflect EGPA activity. However, further studies to validate its use as an activity marker in EGPA are warranted.	Dallos et al. (140)
IgG4	EGPA: 46 (24 active, 22 inactive) GPA: 26 Atopic asthma: 25 Healthy controls: 20	Serum levels of IgG4 in all groups, assessment of tissue infiltration by IgG4 plasma cells	1. IgG4 levels were significantly higher in active EGPA and correlated with the number of disease manifestations and BVAS, and dropped during disease remission. 2. Tissue analysis did not show an increased IgG4 plasma cell infiltration.	Serum IgG4 levels are markedly elevated in active EGPA and correlate with the number of organ involvement and disease activity.	Vaglio et al. (48)
Blood eosinophil count, IgE, ESR, CRP	EGPA: 141 (mostly on treatment, during remission or mild disease activity; BVAS/WG = 1 or 2).	Parameters were measured quarterly (together 892 study visits)	1. Correlations between blood eosinophil count, IgE, ESR and CRP were mostly low or non-significant. 2. When BVAS/WG $\geq$1 defined active disease, the eosinophil blood count was weakly predictive of flare. 3. When BVAS/WG $\geq$3 defined active disease, ESR was weakly predictive of flare.	The blood eosinophil count, IgE, ESR and CRP have limitations as longitudinal biomarkers of disease activity or predictors of flare in EGPA.	Grayson et al. (141)
CCL17/TARC, eotaxin-3, IgG4, IgG4/IgG	EGPA: 25 (most patients on treatment with CS or IS, 18 disease flares during study period)	Evaluation of serum CCL17/TARC, eotaxin-3, IgG4 levels and IgG4/IgG ratio at each visit (together 105 study visits)	1. None of the biomarkers were useful to discriminate between active disease and remission. 2. Patients treated with CS had lower eotaxin-3 and blood eosinophil count levels compared to those not taking CS, irrespective of disease activity. 3. Use of IS was not associated with biomarkers levels.	Serum levels of CCL17/TARC, eotaxin-3, IgG4, and IgG4/IgG ratio do not clearly differentiate active and inactive EGPA.	Dejaco et al. (17)
Periostin	EGPA: 49 (46 had active disease within the past 28 days, 3 had active disease since the prior visit).	Evaluation of serum periostin levels at each visit (together 186 study visits)	1. No association between periostin level and presence or absence of disease flare was found. 2. An increase in periostin level was significantly associated with greater disease severity during a flare. 3. Periostin levels in EGPA were significantly higher than previously studied healthy controls and patients with asthma.	In EGPA, serum periostin level is modestly associated with greater disease severity during a flare, however, it does not discriminate active from inactive disease.	Rhee et al. (142)

(Continued)

TABLE 3 (Continued)

Investigated biomarker	Patients' cohort	Method	Results	Conclusion	References
A panel of 54 cytokines and chemokines	EGPA: 50 (40 active, 10 inactive) HES: 6 Asthma: 8 Healthy controls: 10	Evaluation of 54 cytokines and chemokines in the sera of all group, results were compared between disease and control groups.	1. Significant differences were only observed in serum levels of MDC, IL-8, MIP-1α and 1ß, and TNF-α, each of which were lower in active EGPA than in healthy controls, and differences between active EGPA and other disease groups did not reach significance. 2. Comparison between sera from active or inactive EGPA were not significance for any of the studied cytokines/chemokines.	No clear difference in the serum levels of measured cytokines and chemokines helped distinguish between active or inactive EGPA, or other disease or control groups.	Pagnoux et al. (143)
Anti-alpha-enolase antibodies	EGPA: 33 (24 active, 9 inactive)	Evaluation of anti-alpha-enolase antibodies, ANCA, ANA, RF, and anti-EPO in the sera.	1. Positive results in 82% EGPA patients with sensitivity and specificity of 82 and 44%, respectively, pre-dominated in males and associated with skin involvement. 2. Most positive patients had a negative IFT for ANCA. 3. There was no association between the presence and levels of anti-alpha-enolase antibodies and EGPA activity. 4. None of the EGPA patients and controls was positive for anti-EPO.	Alpha-enolase may be a target of autoimmunity in EGPA and usually shows negative ANCA IFT results.	Laskari et al. (144)
A panel of 22 proteins	Different types of vasculitis, including 37 patients with EGPA (most patients were on treatment).	A panel of 22 serum proteins was evaluated.	In EGPA G-CSF, GM-CSF, IL-6, IL-15 and sIL-2Rα showed significant increases during active disease, as did BCA-1/CXCL13 but only after adjustment for treatment.	1. G-CSF, GM-CSF, IL-6, IL-15, sIL-2Rα, and BCA-1/CXCL13 have been identified as a novel biomarkers of disease activity in GCA and EGPA. 2. Differences of biomarker levels between diseases independent of disease activity, were more apparent than differences related to disease activity.	Rodriguez-Pla et al. (145)
Anti-PTX 3 antibodies	EGPA: 38 GPA: 51 MPA: 12 SLE: 130 CTD: 97 Healthy controls: 97	Evaluation of anti-pentraxin 3 antibodies in the sera of all groups.	1. Anti-PTX3 antibodies were detected in 29.7% AAV patients, significantly more common in EGPA (44.7% vs. 25 and 19%). 2. The presence of anti-PTX3 was associated with a lower prevalence of systemic, ENT, and renal manifestations. 3. Among ANCA negative patients, 35.7% displayed positive anti-PTX3 antibodies. 4. The prevalence of anti-PTX3 antibodies was significantly higher in AAV patients than in healthy controls and other CDT patients, but lower than in SLE.	Anti-PTX3 antibodies appear a promising novel biomarker of AAV, especially of EGPA.	Padoan et al. (146)
Eicosanoid profile	EGPA: 23 Asthma: 30 HES: 12 Healthy controls: 54	Assessment of eicosanoid profile (18) in EBC of all groups; furthermore, in 21 of 23 EGPA patients and in 9 asthmatics eicosanoids were evaluated using BALF.	1. Markedly elevated levels of 12-HETE was found in EBC from EGPA compared to other groups 2. BALF was characterized by a significant elevation of 12-HETE and its metabolite 12-tetranor HETE in EGPA as compared with asthma, and correlated with disease activity.	12-HETE concentration in both EBC and BALF distinguish EGPA from asthma and HES.	Szczeklik W. et al. (147)
IL-33, sST2, TSLP, ILC2, blood eosinophil count	EGPA: 86 CEP: 25 Asthma: 11	Evaluation of serum levels of IL-33, sST2 and TSLP, and peripheral blood ILC2 count.	1. Blood eosinophil count or ILC2 and, sST2 or TSLP, and IL-33 were significantly higher in active EGPA than in inactive, at relapse, or in other diseases. 2. EGPA activity correlated with IL-33 and ILC2, but eosinophil count correlated with ILC2 TSLP (but not IL-33).	Increased ILC2 and IL-33 are associated with EGPA activity. Increases in IL-33 may indicate the presence of active vasculitis rather than peripheral or tissue eosinophilia.	Tsurikisawa et al. (45)

(Continued)

TABLE 3 (Continued)

Investigated biomarker	Patients' cohort	Method	Results	Conclusion	References
A panel of 160 protein	EGPA: 28 (13 active, 15 inactive).	The expression of 160 proteins was compared in sera from active and inactive EGPA	1. 12 out of 19 candidate markers were positively correlated with blood eosinophil count (FGF-7, SCF, GDNF, ß-NGF, IGFBP-4, Axl, PIGF, insulin, NT-4, ErbB3, OPN, BMP-4), while two, CD14 and MCP-3, were negatively correlated 2. The higher expression of Axl, OPN, HCC-4, GDNF, MCP-3 was found in active EGPA	The serum protein profiles were significantly different between active and inactive EGPA, however, Axl, OPN, HCC-4, GDNF and MCP-3 were consistently higher in active disease, with Axl having the largest AUC, indicating that it could be a candidate for a new biomarker of active EGPA.	Ma et al. (148)
Blood eosinophil count, ECP, IL-5, IL-4, IgG4, IgE, ANCA, periostin, IL-8, GM-CSF	EGPA: 30 (active) Severe eosinophilic asthma: 49	Evaluation of blood eosinophil count, and sera levels of ECP, IL-5, IL-4, IgE, IgG4, ANCA, and sputum biomarkers (eosinophils, periostin, IL-8, GM-CSF) to differentiate severe asthmatic patients from the prodromal phase of EGPA.	1. Patients with asthma had higher levels of sputum eosinophils, however, EGPA patients had higher levels of blood eosinophils in the past. 2. The GM-CSF was the only biomarker significantly increased in EGPA compared with asthma	Sputum GM-CSF might be a good biomarker of systemic eosinophilic disease.	Latorre et al. (149)
SAA1, FGA, SAP, CETP	EGPA: 58 Asthma: 33 Healthy controls: 25	Data-independent acquisition (DIA) followed by parallel reaction monitoring (PRM) analysis were performed to screen biomarkers for early diagnosis of EGPA and to differentiate asthma diagnosis.	1. Four candidate biomarkers were identified. SAA1, FGA, and SAP were upregulated in EGPA (sensitivity 82.3%, specificity 100%), while CETP was downregulated in EGPA compared to asthma. 2. The combination of SAA1, FGA, and SAP had a sensitivity and specificity of 82.35 and 100%, respectively, as biomarkers for early diagnosis of EGPA. 3. The combination of SAA1, FGA, SAP, and CETP had a sensitivity and specificity of 78 and 100%, respectively, as biomarkers for differential diagnosis of asthma.	SAA1, FGA, SAP, and CETP can be potentially useful biomarkers for early diagnosis of EGPA and differential diagnosis of asthma.	Xiao et al. (150)
FeNO, blood eosinophil count/percentage, total IgE	EGPA: 44 Allergic asthma: 44	Assessment of FeNO, eosinophil blood count/percentage, and total IgE for early diagnosis of EGPA and to distinguish EGPA from allergic asthma	1. FeNO level, blood eosinophil count/percentage, and total IgE were significantly higher in EGPA than in allergic asthma 2. Unlike the allergic asthma, there was no correlation between FeNO level and blood eosinophil count/percentage in EGPA	Patients with allergic asthma and high blood eosinophil count should be alert to the possibility of having EGPA For patients with infiltration of eosinophils into the airway, a diagnosis should not be based on peripheral blood eosinophil count (blood eosinophil count cannot predict eosinophilic airway inflammation and pulmonary function for patients with EGPA) FeNO level and PFTs should be monitored for patients who present with symptoms in other body systems	Zhao et al. (151)

EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; AAV, antineutrophil cytoplasmic antibodies vasculitis; BVAS, Birmingham Vasculitis Activity Score; BVAS/WG, Birmingham Vasculitis Activity Score/Wegener Granulomatosis; GCA, giant cell arteritis; HES, hypereosinophilic syndrome; SLE, systemic lupus erythematosus; SSc, scleroderma; CU, colitis ulcerosa; CTD, connective tissue disease; CEP, chronic idiopathic pneumonia; SNPs, single nucleotide polymorphism; IgE, immunoglobulin E; IgG4, immunoglobulin G4; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ENT, ear, nose, throat; CS, corticosteroids; IS, immunosuppressant; BALF, bronchoalveolar lavage fluid; EBC, exhaled breath condensate; 12-HETE, 12-hydroxy-eicosatetraenoic acid; ANCA, antineutrophil cytoplasmic antibodies; ANA, anti-nuclear antibodies; RF, rheumatoid factor; ECP, eosinophil cationic protein; IL4,5,6,8,15,33, interleukin, 4,5,6,8,15,33; sIL-2Rα, soluble IL-2 receptor alpha; MDC, macrophage-derived chemokine; MIP, 1α and 1ß-macrophage inflammatory protein 1alpha and 1beta; TSLP, thymic stromal lymphopoietin; CCL17/TARC, thymus and activation-regulated chemokine; TNF, α-tumor necrosis factor alpha; MCP-3, monocyte chemotactic protein 3; FGF-7, fibroblast growth factor 7; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte-colony stimulating factor; anti-PTX, anti-pentraxin 3 antibodies; anti-EPO, anti-eosinophil peroxidase antibodies; IFT, immunofluorescence test; BCA-1/CXCL13, B-lymphocyte chemoattractant; BMP-4, bone morphogenetic protein 4; sST2, soluble suppression of tumorigenicity 2 protein 2; ILC2, innate lymphoid cells 2; SCF, stem cell factor; GDNF, glial cell line-derived neurotrophic factor; ß-NGF, beta nerve growth factor; IGFBP-4, insuline-like growth factor-binding protein 4; PIGF, phosphatidylinositol-glycan biosynthesis class F protein; CD14, cluster of differentiation 14; OPN, osteopontin; NT-4, neurotrophin 4; ErbB3, receptor tyrosine kinase 3; Axl, receptor tyrosine kinase; HCC-4, human beta chemokine; SAA1, serum amyloid A1; FGA, fibrinogen alpha chain; SAP, serum amyloid P; CETP, cholesteryl ester transfer protein; FeNO, fractional exhaled nitric oxide; PFTs, pulmonary function tests.

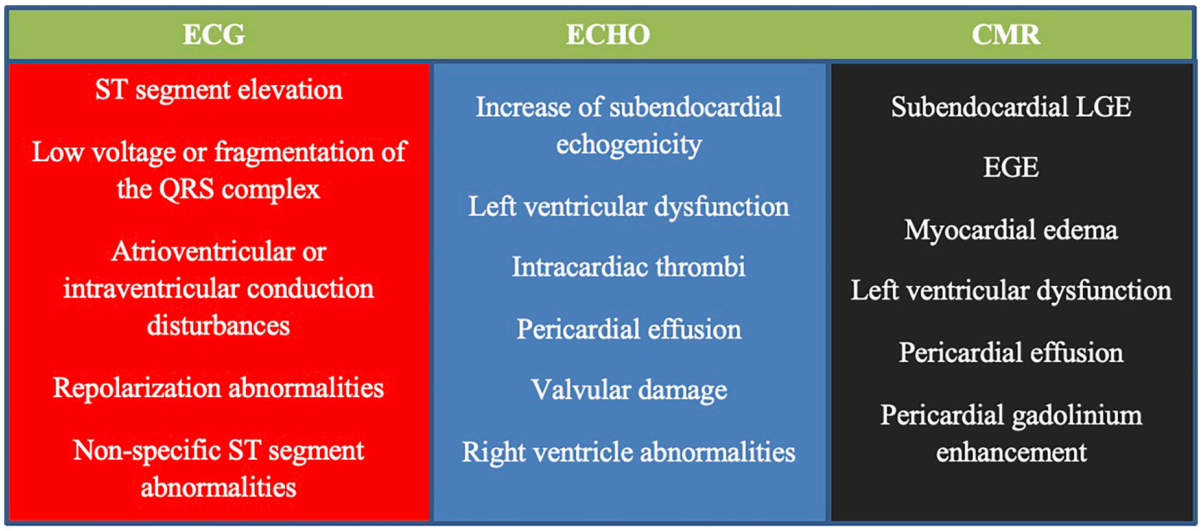

FIGURE 1
Signs of heart involvement in cardiological tests in patients with eosinophilic granulomatosis with polyangiitis (EGPA) [based on Bond et al. (155)]. ECG, electrocardiogram; ECHO, echocardiogram; CMR, cardiac magnetic resonance; LGE, late gadolinium enhancement; EGE, early gadolinium enhancement.

and/or endoscopic evaluation of the digestive tract in cases of gastrointestinal symptoms or electromyography and nerve conduction studies in cases suspected of nerve involvement). Other evaluations should be guided by clinical symptoms and physical examination (153).

In the presence of demonstrable lesions, biopsy procedures should be considered when feasible, and the patient's condition allows it, however, histological examination is not strictly necessary (153). Although pathomorphological lesions are well-defined (necrotizing vasculitis, extravascular granulomas, and eosinophil infiltration of arterial walls and adherent tissue), it is extremely rare to find all of them simultaneously (<20% of patients) (135). The most commonly biopsied organs are the skin, nerves, and muscles. Although EGPA is considered a multi-organ disease, it is well known that limited forms may also occur. When a single extrapulmonary manifestation attributable to systemic disease is present, the disease may be called "formes frustes" of EGPA (108). In such situations, diagnosis is only possible by organ biopsy (162).

While EGPA share features with eosinophilic inflammation and vasculitis, the primary differential diagnoses include other eosinophil-related disorders and vasculitides. First, other common causes of secondary eosinophilia should be excluded from the study. Eosinophilia can be reactive to drugs, and severe reactions may result in organ manifestations mimicking EGPA (e.g., drug rash with eosinophilia and systemic symptoms, DRESS syndrome) (16). A careful history of medication use is crucial to emphasize the association between drug use and symptom onset. Second, helminthic infections need to be ruled out. Serology of Toxocara and *Strongyloides stercoralis* is especially recommended (153). Both are associated with high eosinophilia and can be clinically inapparent (163, 164). Other parasite investigations depend on the patient's country of origin and travel history, however, stool culture, although it has low sensitivity, should also be performed (16). Next, screening for HIV should be performed, even though eosinophilia in this infection is usually mild (153). Lymphocytic variant reactive hypereosinophilia should also be considered, especially when skin manifestations dominate, with accompanying hypergammaglobulinemia. In such cases, lymphocyte immunophenotyping and T-cell receptor rearrangement analysis are indicated (153, 165).

Eosinophilic granulomatosis with polyangiitis often manifests as respiratory symptoms and lung infiltrates; therefore, it should be differentiated from eosinophilic lung disorders. ABPA and idiopathic EP share many features with EGPA, including eosinophilia, cough, dyspnea, and lung infiltrates. Moreover, a large proportion of patients with these diseases have asthma, which is a cardinal feature of EGPA (166, 167). ABPA is characterized by elevated serum *Aspergillus fumigatus*-specific IgE and IgG concentrations (149, 162) and often isolated fungal cultures in sputum or BALF (153, 166). However, distinguishing idiopathic EP from the second stage of EGPA remains challenging. The lack of organ symptoms and ANCA may help differentiate between the two (5), however, patients with idiopathic EP should be monitored for extrapulmonary symptoms because they may develop EGPA in the future.

Hypereosinophilic syndromes are the next most important consideration in the differential diagnosis of EGPA, given the overlapping clinical, radiologic, and histologic features, and biomarker profile (105, 168). Depending on the pathogenesis, three main types of HESs are distinguished: reactive (rHES), neoplastic (nHES), and idiopathic (iHES). In rHES, eosinophils are non-clonal and are thought to be driven by Th2 cytokines, mainly IL-5. This group includes patients with classified conditions associated with secondary eosinophilia, including EGPA (11, 165, 169) (however, eosinophilia in EGPA is not entirely secondary, as it has a partially genetic background related to the IRF1/IL5 gene variant) (56). In nHES, eosinophils are clonal and derived from eosinophil progenitors containing genetic alterations in oncogenic tyrosine kinase receptors, such as platelet-derived growth factor receptor A (PDGFRA) and B (PDGFRAB) and fibroblast growth

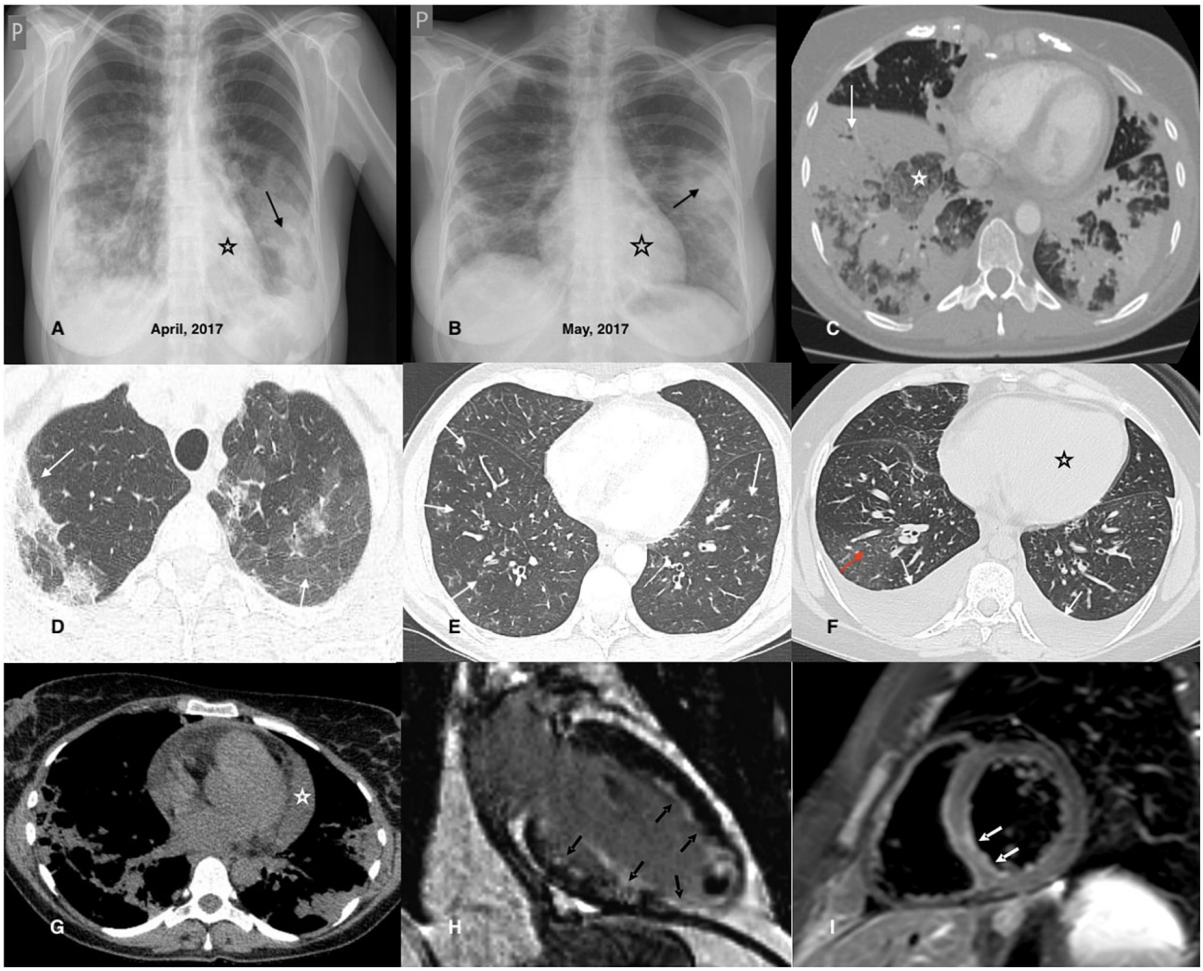

FIGURE 2
Chest imaging findings in patients with EGPA. **(A,B)** Chest X-rays of a 42-year-old female patient diagnosed with EGPA. They demonstrate migrating patchy infiltrates with peripheral dominance (black arrow), characteristic for eosinophilic infiltrates, and rapidly enlarging heart related to its acute injury in the course of EGPA. **(C)** Chest CT axial image (lung window) of a 40-year-old female EGPA patient showing pre-dominant massive bilateral ill-defined areas of airspace (white arrow) and ground-glass (white asterisk) opacities located in both lower lobes of the lungs. **(D)** Chest CT axial image (lung window) of a 37-year-old female patient presenting pre-dominant areas of ground-glass opacities of varying intensity in both upper lobes of the lung (black arrows); histological examination of the transbronchial biopsy specimen reveals features of eosinophilic pneumonia and eosinophilic vasculitis. **(E)** The image refers to a 46-year-old male patient admitted for worsening asthma and eosinophilia. Chest CT axial image (lung window) shows a pre-dominant airway pattern-bronchi wall thickening and small centrilobular nodules (white arrows). BALF examination indicated pulmonary eosinophilia (65% of eosinophils), and the patient complained of numbness of the feet, and for several days purpura-type skin lesions occurred; MPO-ANCA was detected in the sera. The patient was diagnosed with EGPA. **(F)** Chest CT axial image (lung window) of a 34-year-old female patient with EGPA and cardiac involvement showing pre-dominant features of cardiac insufficiency; the ground-glass opacities with interlobular septal thickening (red arrow) corresponding to interstitial edema; bilateral pleural effusion (white arrow) and enlarged heart is also present (black asterisk). **(G)** Chest CT axial image (mediastinal window) of a 38-year-old female patient diagnosed with EGPA and cardiac involvement. In addition to bilateral pulmonary infiltrates, an enlarged heart and pericardial effusion is visible (white asterisk). **(H)** CMR refers to a 32-year-old male patient with EGPA; late gadolinium enhancement (LGE) image in vertical long axis cross-section showing subendocardial enhancement pattern (typical for EGPA) of the anterior wall, subendocardial and transmural enhancement of the inferior wall, inferior papillary muscle and the left ventricle (LV) apex (black arrows); thrombus seen as an unenhanced mass in the apical part of the LV cavity. **(I)** CMR refers to a 26-year-old male patient diagnosed with EGPA with cardiac involvement; a T2-weighted turbo spin-echo (TSE) image with fat saturation in the short axis mid-cavity cross-section, presenting edema in the infero-lateral segment of the LV (white arrows).

factor receptor 1 (PGFR1) (105, 165, 169). This group also encompasses other myeloid neoplastic diseases with associated eosinophilia (with or without genetic abnormalities), as well as chronic eosinophilic leukemia. In turn, iHES is the largest type of HES (comprising about 50% of cases) and is a diagnosis of exclusion once reactive and neoplastic causes have been excluded (105, 165).

Although organ damage may be similar, some symptoms, such as hepatomegaly or splenomegaly, can be suggestive of clonal eosinophilia and nHES. In addition, a proportion of patients have abnormal peripheral blood counts, such as anemia (53%) or thrombocytopenia (31%), and patients with nHES usually do not respond to treatment with systemic CS (169). Screening for serum vitamin B12 and tryptase levels is sensitive to nHES and is recommended for all patients diagnosed with eosinophilia (153). In cases of suspected nHES, fusion gene testing is indicated. However, although only to be positive in nHES, a case of PDGFRA-positive EGPA has been described (42);

therefore, some authors believe that testing for PDGFRA mutation should be performed routinely in all cases with hypereosinophilia, regardless of clinical manifestation, suspected EGPA, or ANCA-status (170).

Idiopathic HES is the most difficult to distinguish from EGPA, especially in ANCA-negative cases without vasculitic symptoms (165, 169, 171, 172). Both clinical and radiological symptoms are similar, but HES is usually not considered to have asthma or nasal polyps. However, this is not a distinguishing feature. A case series of iHES with the first presenting asthma-like symptoms has been recently described (173). In addition, it has been reported that approximately 10% of patients with HES have rhinitis (169). Histological examination also showed no differentiation. HES is typically characterized by tissue infiltration by eosinophils, which is also often found in cases of EGPA (105). Other findings, such as vasculitis and granulomas, are not typical for HES but are considered hallmark features of EGPA (2). Recently, among patients with a diagnosis of HES lacking asthma, a group characterized by necrotizing eosinophilic vasculitis confirmed by biopsy has been distinguished (174). The distinction of EGPA from this entity is challenging, especially because it cannot be excluded that both may be a part of a common spectrum.

There is a need for further research on suitable features for distinguishing EGPA from HES. Finally, a comparative study of 166 patients with blood eosinophilia (>1.000 cells/μL) and systemic manifestations demonstrated that CRP level was a sound diagnostic biomarker that could accurately differentiate between HES and EGPA, with low levels (<36 mg/L) suggestive of HES (175). Other authors have proposed a HES-suggesting laboratory index (HSLI) based on white and eosinophil blood count, with values ≥ 4.25 exhibiting a significantly high relative risk for HES (176). Recently, a scoring system (E-CASE) for differentiating EGPA from other types of eosinophilic disorders, including HES, has been proposed. It was based on the clustering analysis of 19 parameters of 58 patients with eosinophil-related diseases at a tertiary hospital and was extensively validated in 40 patients at another tertiary institution. This system includes clinical (peripheral nerve disorder, asthma, lung, and skin involvement), laboratory (RF positivity, MPO-ANCA positivity, IgE, and CRP elevation), and histological features (vasculitis detected by pathological examination), which have been awarded a point weight. A score ≥ 12 was considered positive for EGPA (177).

The next diseases that should be differentiated include other forms of vasculitis, especially AAVs. GPA and MPA share several clinical and histological features with EGPA, however, there is usually a lack of asthma and eosinophilia. Nevertheless, eosinophilia may be present in GPA, although it is usually modest, and there are some clinical features distinguishing it from EGPA (**Table 4**) (178–180). EGPA may also need to be differentiated from polyarteritis nodosa (PAN), a rare form of necrotizing vasculitis that preferentially targets medium-sized arteries. Hypereosinophilia may occasionally be observed in PAN, and similar to EGPA, skin and peripheral nerves are the most frequently affected tissues. However, PAN is not associated with glomerulonephritis and small-vessel involvement, and ANCA is typically negative. In addition, it may be triggered by viral infections, particularly the hepatitis B virus; thus, patients may have positive viral serology and histological granulomas are usually absent (181).

Finally, because IgG4 may be elevated in a significant proportion of patients with EGPA (47, 48, 50), IgG4-RD has become an important differential diagnosis to consider. IgG4-RD may share some clinical features with EGPA, such as asthma, rhinitis, or peripheral eosinophilia (174, 182). Histopathologic examination is essential for diagnosis, which typically demonstrates lymphoplasmacytic infiltrate, storiform fibrosis, and obliterative phlebitis without vasculitis or granulomas (183).

5.3. Disease phenotypes

Although ANCA is detected in only 30–40% of patients, two main phenotypes of EGPA have been identified according to ANCA status, differing in clinical features, treatment response, and prognosis (**Figure 3**). First, the "vasculitic" phenotype (associated with ANCA-positivity and vasculitis symptoms), and second – the "tissular" phenotype (associated with ANCA-negativity and organ damage related to eosinophilic inflammation) (9, 10, 55, 100, 101, 184), both confirmed using GWAS, which found the distinct genetic background for each of them (56). However, these phenotypes rarely occur separately and tend to overlap in the same patient (128, 159). Generally, patients with positive MPO-ANCA have a more active disease with higher CRP levels, higher ratios of fever and myalgia (185), and significantly more common rhinosinusitis (184) than those with negative MPO-ANCA. However, the pre-valence of asthma does not appear to be dependent on ANCA status (184), although in some studies asthma was more common in ANCA-negative patients (185).

The next specific subgroup of EGPA patients was those with PR3-ANCA positivity. ANCA directed against PR3 is much less common in EGPA patients. In a recent large retrospective study of 734 patients with EGPA, PR3-ANCA was detected in 2% of cases and has been associated with a distinct clinical profile with features reminiscent of GPA (186). Compared to those with MPO-ANCA and ANCA-negative, patients with PR3-ANCA less frequently had asthma and peripheral neuropathy, while more frequently had skin symptoms, pulmonary nodules, and a lower median eosinophil count. Interestingly, myocarditis in this group was observed as frequently as in ANCA-negative patients and more frequently than in MPO-ANCA patients. In turn, long-term outcomes, such as relapse-free survival and overall survival in PR3-ANCA-positive EGPA patients were similar to those in patients with GPA PR3-ANCA (186).

6. Therapeutic management

The treatment strategy for EGPA depends on the severity of the disease (**Table 5**) and consists of induction and maintenance phases. The first phase aims to achieve remission of the disease, whereas the second phase prevents relapses. Other important objectives of treatment include limiting side effects and sequelae, improving the quality of life, and enabling the rapid return of the patient to normal activities (187). Prospective clinical trials specifically dedicated to EGPA are limited (188). Thus, treatment recommendations have been mostly derived from the results of trials involving other AAVs, rather than EGPA itself, and/or are based on expert opinion (153, 187).

TABLE 4 Differentiating EGPA from other AAVs [data based on the Samson et al. (54), Comarmond et al. (55), Tsurikisawa et al. (83), Fijolek et al. (87), Saku et al. (101), Puechal (178), Greco et al. (179), Nguyen et al. (180), Liu et al. (185), and Papo et al. (186)].

		EGPA	GPA	MPA
Serological features	Peripheral eosinophilia	++++	+	–
	ANCA	MPO 30–40% PR3 2% ANCA (–) >60%	PR3 80–95% MPO 5–20% ANCA (–) 0–20%	MPO 70–80% PR3 30% ANCA (–) 0–20%
Clinical features	ENT	70–90% allergic rhinosinusitis, nasal polyposis, usually without destruction	80–93% often destructive, with ulcers, crusting, septal perforation, hearing loss	–
	Lungs/airways	38–77% not-fixed pulmonary infiltrates, small nodules without cavitations asthma: 90–100%	53–83% nodules with cavitation, ground-glass opacities, consolidations, subglottic stenosis	25–55% pulmonary fibrosis DAH
	Heart	11–76% (mortality) ANCA positive 12-18.5% ANCA negative 30-38.7%	4–40%	10–21%
	Digestive tract	19.8–78% ANCA positive 22–24% ANCA negative 23–34.4%	11–24%	30–58%
	Skin	23–68% ANCA positive 36–45.4% ANCA negative 36.3–53.8%	33–45%	30–60%
	Nerve involvement	42–74% ANCA positive 64–66.7% ANCA negative 43–47.5%	20–50%	37–72%
	Kidneys	16–35% ANCA positive 27–80% ANCA negative 16–35.5%	50–80%	80–100% (severe)
	Eyes	<5%	28–50%	<5%
Histological features	Granulomas	++++ dominated by eosinophils	++++ dominated by neutrophils	–

AAVs, antineutrophil cytoplasmic antibody-associated vasculitides; EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; ANCA, antineutrophil cytoplasmic antibody; MPO, myeloperoxidase; PR3, proteinase 3; ENT, ear, nose, throat; DAH, diffuse alveolar hemorrhage.

6.1. Conventional agents

Induction therapy should be adapted according to disease severity (153, 187). Systemic CS is the cornerstone drug in EGPA, and treatment with CS alone is justified in patients with an Five-Factor Score (FFS) of 0 (153, 187). The initial recommended dose is 1 mg/kg/day of prednisolone equivalent with a maximum dose of 60 mg/kg/day for 2–3 weeks, followed by gradual reduction to the minimal effective dose or, if possible, until withdrawal (153). In severe cases with life-threatening manifestations, methylprednisolone pulses can be applied (at a dose of 7.5–15 mg/kg/day for 3 days, followed by oral CS) (153); however, there are no data to support favoring either intravenous pulse or high-dose oral CS for active severe EGPA (189). The French Vasculitis Study Group (FVSG) proposes a tapering-off schedule of CS between 12 and 18 months, of which the reference doses are around 20 mg/day, 10 mg/day, and 5 mg/day at 3 months, 6 months, and 1 year, respectively, of prednisolone equivalent (187), however, the threshold to which CS can be reduced without compromising asthma and/or ENT symptoms is unknown and varies from patient to patient. Optimization of local therapies may help reduce the risk of flares during oral CS tapering (e.g., increasing the dose of inhaled CS or nasal CS implementation) (190), while new biological therapies can make a significant contribution to lowering the dosage of maintenance CS therapy.

In cases with at least one poor prognostic factor (FFS $\geq$ 1), combined treatment with CS and IS is recommended (153, 187). No randomized controlled trial results are available to support this recommendation, however, the benefit of adding intravenous cyclophosphamide (CYC) to CS to achieve remission has been demonstrated (191). The preferred immunosuppressant is intravenously administered CYC at a dose adjusted for age and renal function (**Table 6**) (192). The FVSG guidelines recommend a dose of 0.6 g/m^2/per infusion on days 1, 15, and 30, followed by a dose of 0.7 mg/m^2/per infusion every 3 weeks, with a maximum of 1.2 g per infusion (153, 187). In cases with impaired kidney function (<65 years of age), treatment should be started with a lower dose of 0.5 g/m^2/per infusion, while in elderly patients, a rigid dose of 0.5 g/per infusions is recommended (regardless of kidney status) (153, 187). CYC infusion should be combined with antiemetic therapy and good hydration, with 2-mercaptoethanesulfonate sodium (MESNA) prophylaxis to limit bladder toxicity (190). In addition, CYC can also be administered orally at a dose of 2 mg/kg/day (without exceeding 200 mg/day) for 3–6 months, however, intravenous treatment is

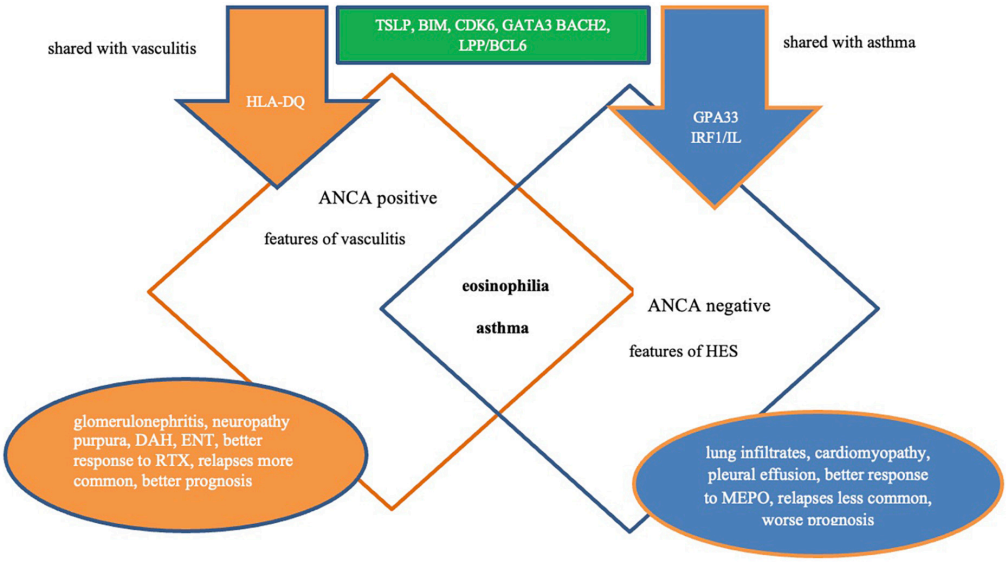

FIGURE 3
Phenotypes of EGPA. ANCA, antineutrophil cytoplasmic antibodies; DAH, diffuse alveolar hemorrhage; ENT, ear, nose, throat; MEPO, mepolizumab; RTX, rituximab; AAV, antineutrophil cytoplasmic antibody vasculitis; HES, hipereosinophilic syndrome.

preferred due to better compliance and lower cumulative drug dose (190). During the IS treatment, prophylaxis of *Pneumocystis jirovecii* is indicated (co-trimoxazole 400 mg/day or 980 mg thrice weekly), and screening for drug-induced neutropenia is necessary. The patient should be informed about the need for contraception and the possibility of egg/sperm freezing (153, 187, 190). If remission is achieved, maintenance treatment should be started 2–3 weeks following the last CYC pulse or a few days after oral CYC. The preferable drug is azathioprine (AZA), at a dose of 2–3 mg/kg/day, followed by methotrexate (MTX) at a dose of 0.3 mg/kg/week, for 18–24 months (153, 187, 190).

In cases of severe DAH, eye involvement, or fulminant mononeuritis multiplex, IS induction treatment should also be considered, although it is not listed in the FFS (153).

As previously mentioned, in patients without poor prognostic factors (FFS = 0), IS treatment in the induction phase is not indicated. This is supported by the results of the CHUSPAN 2 study, which demonstrated that adding AZA to CS in these patients did not improve remission rates, lower relapse risk, spare steroids, or diminish EGPA asthma or ENT exacerbation rates (193). However, treatment with IS as a second-line therapy can be considered in the group in two clinical situations: first, as a CS-sparing treatment in cases of CS dependence of >7.5–10 mg/day; second, in cases of CS intolerance. The preferred drugs are AZA and MTX, according to the scheme mentioned above (153, 187).

The recently published ACR guidelines differ from those of the FVSG (Table 7). According to these guidelines, the addition of an adjunctive IS is recommended in all patients with EGPA as the first-line therapy, regardless of the disease severity (not based on FFS), to reduce CS toxicity, however, no study results support this strategy (194).

6.2. Biological agents

In recent years, new treatment options for EGPA have emerged. The therapeutic array has expanded with the introduction of new biological drugs, which have been intensively studied (Table 8). Depending on the mechanism of action, these drugs can be divided into two groups: first, directed against eosinophilic inflammation; second, directed against the autoimmune component of EGPA and vasculitis. However, so far, no single agent allows complete control of EGPA, and the choice should be dictated by the clinical features (15).

6.2.1. Anti-eosinophil-driving cytokines agents

In the pathogenesis of EGPA, eosinophils play a key role; therefore, agents inhibiting these cells may be effective. IL-5 is the main cytokine that drives eosinophil maturation and proliferation (21). The first clinical evidence of the successful use of mepolizumab—a monoclonal antibody that prevents the binding of IL-5 to its receptor—was described in 2010 in two distinct studies of patients with EGPA treated with mepolizumab infusions at a dose of 750 mg monthly (195, 196). The breakthrough was the randomized MIRRA trial investigating the safety and efficacy of mepolizumab at a dose of 300 mg s.c. monthly as an add-on therapy in 136 EGPA patients with relapsing or refractory disease. In that study, compared to placebo, patients treated with mepolizumab had significantly more accrued weeks of remission (28 vs. 3%, OR 5.91 for ≥24 weeks of accrued remission; $p > 0.001$) and a higher rate of remission at both weeks 36 and 48 (32 vs. 3%, OR 16.74; $p < 0.001$). Relapses at 52 weeks were less frequent (56 vs. 82%; $p < 0.001$), and the average dose of oral CS was lower in the mepolizumab group between weeks 48 and 52 (44 vs. 7%; $p < 0.001$). Importantly, there were no differences in drug safety between the two arms (23). This trial led to the approval of mepolizumab by the Food and Drug

TABLE 5 Eosinophilic granulomatosis with polyangiitis severity criteria according to the FVSG and ACR.

FVSG		ACR	
FFS 1996	FFS 2009	Severe disease	Non-severe disease
1. Proteinuria > 1 g/d 2. GI bleeding, perforation, infarction, and/or pancreatitis 3. Renal insufficiency (Cr > 158 mg/dL) 4. CNS involvement 5. Cardiomyopathy The 5-years mortality rates: FS = 0 11.9% FFS = 125.9% FFS ≥ 245.95% study population: EGPA 82 MPA 52 PAN 260	1. Age > 65 years 2. Cardiac insufficiency 3. Renal insufficiency (stabilized peak Cr ≥ 150 μmol/L) 4. GI 5. Absence of ENT The 5-years mortality rates: FFS = 09.0% FFS = 121% FFS ≥ 240% study population: EGPA 230 MPA 218 PAN 349 GPA 311	Vasculitis with life- or organ-threatening manifestations, e.g., DAH, glomerulonephritis, central nervous system vasculitis, mononeuritis multiplex, cardiac involvement, mesenteric ischemia, limb/digit ischemia	Vasculitis without life- or organ-threatening manifestations, e.g., rhinosinusitis, asthma, mild systemic symptoms, uncomplicated cutaneous disease, mild inflammatory arthritis
FFS = 0 non-severe disease FFS ≥ 1 severe disease DAH, eye involvement, fulminant mononeuritis multiplex are also categorized as severe disease, although not listed in FFS			

FFS, Five-Factor Score; PAN, polyarteritis nodosa; EGPA, eosinophilic granulomatosis with polyangiitis; MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; GI, gastrointestinal involvement; Cr, creatinine; CNS, central nervous system; ENT, ear, nose, throat; DAH, diffuse alveolar hemorrhage; ACR, American College of Rheumatology; FVSG, French Vasculitis Study Group.

TABLE 6 Dosing of CYC pulses depending on age and renal function (EULAR).

Age (yrs)	Creatinine (umol/L)	
	<300	300–500
<60	15 mg/kg/pulse	12.5 mg/kg/pulse
60–70	12.5 mg/kg/pulse	10 mg/kg/pulse
>70	10 mg/kg/pulse	7.5 mg/kg/pulse

CYC, cyclophosphamide; EULAR, European League against rheumatism.

TABLE 7 Key recommendations for the treatment of EGPA according to the FVSG and ACR taking into account biologics.

	Severe disease	Non-severe disease	Severe relapse	Non-severe relapse
FVSG	CS + CYC 3–6 months (induction phase) AZA or MTX ≥18 months (maintenance phase)	CS alone to minimal dosage or withdrawal	RTX can be considered, especially after CYC failure (in induction phase) RTX can be considered after AZA or MTX failure (in maintenance phase)	CS + MEPO (first choice) CS + AZA or MTX
ACR	CS + CYC or RTX if remission on CYC, switch to MTX or AZA or MMF	CS + MEPO (first choice) CS + MTX/ AZA/MMF CS + RTX CS alone in selected patients	CS + RTX	CS + MEPO

EGPA, eosinophilic granulomatosis with polyangiitis; FVSG, French Vasculitis Study Group; ACR, American College of Rheumatology; CS, corticosteroids; CYC, cyclophosphamide; AZA, azathioprine; MTX, methotrexate; MEPO, mepolizumab; RTX, rituximab; MMF, mycophenolate mofetil.

Administration (FDA) in 2017 as the first biologic drug for the treatment of EGPA.

Despite these promising results, the MIRRA trial has several limitations that need to be outlined. First, about half of the patients treated with mepolizumab did not achieve protocol-defined remission as the Birmingham Vasculitis Activity Score (BVAS) = 0 and less than 4 mg of daily prednisone (however, a *post-hoc* analysis, in which a comprehensive definition of clinical benefit was applied, revealed that 78–87% of patients experienced benefit with mepolizumab) (197); second, the diagnostic criteria of EGPA were very loose and active asthma was considered a feature of EGPA relapse; third, none of the patients received mepolizumab as first-line therapy (all were treated with oral CS with or without IS); finally, only 10% of the patients included in the study were ANCA-positive; therefore, the ability of mepolizumab to limit vasculitis could not be reliably assessed.

Mepolizumab is now considered a potential treatment for non-severe relapsing and/or refractory EGPA, with limited data available on its impact on vasculitic manifestations (198). It has been demonstrated that its effectiveness is not affected by the baseline treatment of EGPA, duration of disease, or refractory status of the disease (199). The expert panel of the FVSG recommends mepolizumab to treat EGPA patients whose asthma is CS-dependent (>7.5 mg/day) and/or ENT manifestation, starting at a dose of 100 mg monthly, which has been approved for the treatment of severe eosinophilic asthma and is three times lower than that approved for the treatment of EGPA (200). The use of mepolizumab in Europe at this dosage is currently off-label (198), but many real-life studies have shown positive results with low-dose mepolizumab in patients with EGPA (201–203). More recently, a

TABLE 8 Completed and ongoing clinical trials with the use of biologics in EGPA.

Name	Start date	Aim	Cohort/intervention	Primary outcome	Results	Date of completion
MATOCSS NCT 00527566 open-label phase 1/2 $N = 7$	2007	Evaluation the safety and efficacy of MEPO as a steroid sparing treatment in patients with Churg-Strauss syndrome receiving stable steroid dose (at least 10 mg daily of prednisone or equivalent)	MEPO 750 mg iv every 4 weeks	Treatment-related side effects The lowest prednisone dose achieved at the end of the treatment phase	MEPO was well tolerated, and there were no severe AE There was a decrease in mean CS dose from 12.9 to 4.6 mg/day after 12 weeks of treatment	2009
MEPOCHUSS NCT 00716651 prospective open-label phase 2 $N = 10$	2008	Evaluation the efficacy and safety of MEPO for patients with refractory or relapsing Churg-Strauss syndrome	MEPO 750 mg iv every 4 weeks	Percentage of patients that attain remission (defined as BVAS = 0 and CS < 7.5 mg/day) at 32 week	Eight patients reached the remission at 32 week The daily CS dose was reduced in all patients No relapse occurred with MEPO therapy	2010
MIRRA NCT 02020889 prospective randomized double-blind phase 3 $N = 136$	2014	Investigating the efficacy and safety of MEPO in patients with EGPA receiving standard-of-care therapy.	MEPO 300 mg sc every 4 weeks vs. placebo	Number of patients in each group of the accrued duration of remission (defined as the number of weeks where BVAS = 0 and CS ≤ 4 mg/day over 52 weeks). The number of patients in remission at 36 and 48 weeks.	Accrued weeks of remission were significantly more in MEPO treated group than in the placebo group (28% vs. 3%). A higher percentage of MEPO-treated patients were in remission at both 36 and 48 weeks (32 vs. 3%). The annualized relapse rate was significantly lower in MEPO treated group than in the placebo group (1.14 vs. 2.27). 44% of MEPO-treated patients had a CS-sparing effect (vs. 7% in placebo). Remission did not occur in 47% of MEPO-treated patients.	2016 This study led to approval of MEPO for the treatment of EGPA
RITE NCT 02947945 prospective open-label phase 2 $N = 10$	2017	Evaluation of the efficacy and safety of reslizumab in the treatment of EGPA	All subjects received reslizumab at a dose of 3 mg/kg iv every 4 weeks for 28 weeks (in addition to standard of care therapy).	Safety of reslizumab in patients with EGPA.	Reslizumab was well tolerated and resulted in a significant reduction in daily oral CS. Of the 10 subjects, 3 experienced an EGPA exacerbation; one had a severe AE.	2018
BITE NCT 03010436 prospective open-label phase 2 $N = 10$	2017	Evaluation the efficacy and safety of benralizumab in the treatment of EGPA.	All patients received benralizumab at a dose of 30 mg sc every 4 weeks for 12 weeks, and then every 8 weeks for 16 weeks (in addition to standard-of-care therapy).	Safety and tolerability of benralizumab in patients with EGPA.	Benralizumab was well tolerated and resulted in reduction of median oral CS dose from 15 mg at the start to 2 mg at the end of treatment Mean annualized exacerbation rate was lowest during treatment compared with the pre- and post-treatment phases (1.5 vs. 4.6).	2019
NCT05030155 prospective randomized double-blind phase 3 $N = 100$	2022	Comparison of MEPO-based regimen to conventional treatment for remission induction in EGPA.	Newly diagnosed or relapsed active EGPA (BVAS ≥ 3), FFS = 0 lub ≥ 1, not exceeding the first 21 days of CS therapy; arms: MEPO 300 mg sc every 4 weeks vs. placebo (FFS = 0) MEPO 300 mg iv every 4 weeks vs. CYC iv followed by AZA p.o. (FFS ≥ 1), CYC iv followed by AZA p.o. vs. placebo group.	Percentage of patients who achieved a CS dose of ≤ 4 mg per day at day 168 without experiencing a relapse.	Ongoing	2025

(Continued)

TABLE 8 (Continued)

Name	Start date	Aim	Cohort/intervention	Primary outcome	Results	Date of completion
Long-term Access Program (LAP) of mepolizumab for subjects who participated in study MEA115921 NCT 03298061 open-label phase 3 $N = 104$	2015	Assessment a long-term efficacy of MEPO in the treatment of EGPA in patients receiving standard-of-care therapy.	Patients who require a dose of CS of 5 mg per day will receive MEPO at a dose of 300 mg sc every 4 weeks.	Number of patients with CS use (up to 3 years); number of AEs.	Ongoing	2023
MANDARA NCT 04157348 prospective randomized double-blind phase 3 $N = 140$	2019	Assessment the efficacy and safety of benralizumab compared to MEPO in the treatment of EGPA in patients receiving standard-of-care therapy.	Benralizumab 30 mg every 4 weeks sc vs. placebo and MEPO 300 mg sc every 4 weeks vs. placebo.	Proportion of patients who are in remission at both 36 and 48 weeks (BVAS = 0 and CS $\leq$ 4 mg per day, or BVAS = 0 and CS $\leq$ 7.5 mg per day).	Ongoing	2024
OCEAN NCT05263934 prospective randomized double-blind phase 3 $N = 160$	2022	Assessment the efficacy and safety of depemokimab compared with MEPO in relapsing or refractory EGPA in patients receiving standard-of-care therapy.	Depemokimab 200 mg sc every 26 weeks and placebo and MEPO 300 mg sc every 4 weeks.	Number of patients with remission at both 36 and 52 weeks (BVAS = 0 and CS $\leq$ 4 mg per day)	Ongoing	2025
REOVAS NCT 02807103 prospective randomized double-blind Phase 3 $N = 105$	2016	Comparison of RTX-based regimen to conventional treatment for remission induction in newly diagnosed or relapsing EGPA.	Arms: in FFS = 0: CS + RTX 1 g in D1 and 15 or CS + placebo; in FFS $\geq$ 1: CS + RTX or CS + CYC iv at a dose of 600 mg/m2 at 115, and 29 days, then 500 mg-fixed dose every 3 weeks (together 9 pulse).	The percentage of patients who obtained remission at day 180 (BVAS = 0 and CS $\leq$ 7.5 mg per day)	The remission rates in patients treated with RTX were comparable to those treated conventionally (63.5 vs. 60.4%). The mean duration of remission was comparable between two groups (10.37 vs. 11.68 weeks). VDI tended to be better in RTX-treated group.	2020
MAINRITSEG NCT 03164473 prospective randomized double-blind phase 4 $N = 98$	2018	Comparison of the efficacy and safety of RTX to AZA for maintenance remission in newly diagnosed or relapsing EGPA.	Arms: RTX at a dose of 500 mg iv every 6 months (4 infusions) and placebo vs. AZA 2 mg/kg per day for 24 months and placebo.	Duration of remission (BVAS = 0 and CS $\leq$ 7.5 mg per day) in weeks (time frame: 28 months).	Ongoing	2025

CS, corticosteroids; EGPA, eosinophilic granulomatosis with polyangiitis; FFS, Five-Factor Score; AZA, azathioprine; CYC, cyclophosphamide; MEPO, mepolizumab; RTX, rituximab; BVAS, Birmingham Vasculitis Activity Score; VDI, Vasculitis Damage Index; ENT, ear, nose, throat; AEs, adverse events.

retrospective collaborative study of 203 patients demonstrated that 100 mg can be an effective and safe dosage in EGPA and that its efficacy is comparable to that of 300 mg in remission rates, CS sparing effect, and rates of asthma/ENT exacerbations. In that study, improvement was observed in 10% of patients after dose escalation, suggesting that low-dose mepolizumab could be used as a first-line therapy with the possibility of an increase to 300 mg monthly in cases with an unsuitable response (204). Interestingly, out of 10 patients with CI treated with mepolizumab (at a dosage of either 100 or 300 mg/4 weeks), 9 achieved complete remission, suggesting that the inhibition of IL-5 signaling might be an effective novel treatment strategy for eosinophilic cardiac disease (204).

To date, no available data are evaluating the value of mepolizumab in the remission induction phase in patients with EGPA, however, some small retrospective studies have demonstrated that the use of mepolizumab as remission induction for severe EGPA might be safe and effective for controlling disease activity and reducing CS doses (205). Two randomized prospective trials of mepolizumab in EGPA are currently underway. One study assessed the long-term effectiveness of mepolizumab (at a dose of 300 mg) in patients with EGPA who required oral CS at a dose of $\geq$5 mg/day of prednisolone equivalent to control their symptoms (NCT 03298061). The second trial evaluated the efficacy of mepolizumab as a remission-inducing agent (at a dose of 300 mg) in comparison to the conventional therapeutic strategy guided by FFS (NCT 05030155).

Given the encouraging results of mepolizumab, other anti-IL5 therapies have also been investigated. These include reslizumab

TABLE 9 The scheme of asthma management to control symptoms according to GINA 2022.

Tracks of treatment	Step 1 symptoms less than 4–5 days a week	Step 2 symptoms less than 4-5 days a week	Step 3 symptoms most days or waking with asthma once a week or more	Step 4 daily symptoms or waking with asthma once a week or more and low lung function	Step 5 persistent symptoms and/or exacerbations despite optimized treatment with high dose controller medications (usually a high dose of ICS + LABA)
CONTROLLER and RELIEVER: As-needed low dose ICS-formoterol (PREFERRED RELIEVER)	As-needed low doseICS-formoterol		Low dose maintenance ICS-formoterol	Medium dose maintenance ICS-formoterol	Add-in LAMA Refer for assessment of phenotype Consider high dose maintenance ICS-formoterol, ± anti-IgE, anti-IL5/5R, anti-IL4R, anti-TSLP
CONTROLLER and RELIEVER: As-needed short-acting beta$_2$ agonist (ALTERNATIVE RELIEVER)	Take ICS whenever SABA taken	Low dose maintenance ICS	Low-dose maintenance ICS-LABA	Medium dose maintenance ICS-LABA	Add-in LAMA Refer for assessment of phenotype Consider high dose maintenance ICS-LABA, ± anti-IgE, anti-IL5/5R, anti-IL4R, anti-TSLP
Other controller options (less evidence for efficacy or safety, limited indications)		Low dose ICS whenever SABA taken or daily LTRA or HDM SLIT	Medium dose ICS or LTRA or HDM SLIT	Add LAMA or LTRA or HDM SLIT or switch to high dose ICS	Add azithromycin (three times per week) or LTRA; as last resort consider low dose oral CS but consider side effects
	Therapeutic education, skill training, and non-pharmacological treatment				

CS, corticosteroid; ICS, inhaled corticosteroid; LAMA, long-acting muscarinic antagonist; LABA, long-acting beta$_2$ agonist; SABA, short-acting beta$_2$ agonist; LTRA, leukotriene receptor antagonist; HDM SLIT, house dust mite sublingual immunotherapy; anti-IgE, anti-immunoglobulin E; anti-IL5/5R, anti-interleukin 5/interleukin 5-receptor; anti-IL4R, anti-interleukin 4-receptor; anti-TSLP, anti-cytokine thymic stromal lymphopoietin.

and benralizumab, which are anti-IL-5α receptors. Both were investigated in phase 2 open-label trials with a small number of patients, and the results were promising (206, 207). The efficacy and safety of benralizumab are currently evaluated in comparison to mepolizumab in patients with EGPA receiving standard care therapy (NCT 04157348). The other investigated agent was depemokimab, a long-acting (administered every 26 weeks at a dose of 200 mg) anti-IL-5α receptor drug (NCT05263934).

Since the Th2-pathway activation plays an important role in the pathogenesis of EGPA, drugs studied in asthma may open new possibilities for EGPA treatment. Dupilumab is a humanized monoclonal antibody to the IL-4α receptor that inhibits both IL-4 and IL-13 signaling (208) and is currently approved for moderate and severe uncontrolled asthma (209, 210), CRNP, and atopic dermatitis (21). Another promising drug is itepekimab, an anti-IL-33 monoclonal antibody. In the 2nd phase of a randomized trial, it led to a greater reduction in the mean blood eosinophil count, a lower incidence of asthma exacerbations, and improved lung function in patients with moderate-to-severe asthma with a good safety profile (211). Tezepelumab is the next most recently approved biologic drug (in December 2021) in the US for severe asthma, regardless of its phenotype or biomarkers. This human monoclonal antibody specifically binds to TSLP, preventing it from binding to its heterodimeric receptor (212). Blocking TSLP results in strong inhibition of the CCL2 - related eosinophilic pathway, as well as Th2- related cytokines (IL-4 and 13) and Th17 (212), all of which are involved in the pathogenesis of EGPA (16, 24, 44). Dexpramipexole is an orally bioavailable synthetic aminobenzothiazole that depletes eosinophils by inhibiting their maturation. In a phase 2 trial evaluating the effect of dexpramipexole in moderate-to-severe eosinophilic asthma, its administration led to a lowering of the absolute eosinophil count and improved the forced expiratory volume in 1 sec (FEV1) (213). The results of dexpramipexole in HES are also promising (214).

6.2.2. Anti-CD20 therapy

Rituximab (RTX) is a chimeric monoclonal antibody targeting the CD20 antigen present on B cells, resulting in its depletion (198). Clinical trials showed its effectiveness and safety in GPA and MPA both in the induction (215) and maintenance phases (216). Increasing experience in the treatment of other AAVs with RTX has also led to its use in EGPA. Several case reports and open-label studies have reported the efficacy of RTX in patients with EGPA (46, 217, 218). Recently published data from a retrospective European Collaborative Study involving patients with relapsing and/or refractory disease showed that in those receiving RTX ($N = 63$), the BVAS declined both at 6 and 12 months, and the frequency of remission, partial response, treatment failure, and stopping treatment due to adverse events was 49, 24, 24, and 3%, respectively, without statistically significant differences between ANCA-positive and ANCA-negative patients (219). In 2020, a randomized controlled trial (REOVAS) evaluating the efficacy and safety of RTX in comparison with conventional therapy for remission induction in EGPA was completed (220). In this study, patients with an FFS ≥ 1 (42/105) were randomized to receive CS and RTX/CYC for remission induction, followed by AZA for remission maintenance in both groups. Patients with FFS $= 0$ (63/105) were randomized to receive RTX with CS or CS as monotherapy. Remission rates for RTX and conventional treatment on days 180 and 360 were comparable in both groups (63.5 vs. 60.4%, and 59.6 vs. 64.2%, respectively). Similarly, the

mean duration of remission, relapse rates, and cumulative dose of prednisone was also comparable (220). The study showed that RTX was not superior to the conventional therapeutic strategy to induce vasculitis remission, however, it also did not show that it was inferior to standard therapy. Randomized trial investigating RTX in maintenance therapy compared with standard treatment is underway (MAINRITSEG; NCT 03164473). FVSG experts do not recommend using RTX as first-line induction therapy for EGPA, however, it can be considered for second-line-or-later treatment of severe refractory or relapsed disease, especially following CYC failure (200), at the dosage recommended for GPA and MPA (to induce remission, 375 mg/m^2 infused once a week for 4 weeks, or 1,000 mg twice at a 15-day interval, which is equally effective and safe; to maintain remission, renewed 500 mg infusions at 6-month intervals for at least 18 months) (200). The ACR guidelines are more flexible. According to these recommendations, RTX can be used in the first line of treatment on par with CYC (the choice is up to the physician), although it is especially preferred in patients with ANCA-positivity and glomerulonephritis. Among those with ANCA-negativity, heart involvement, GI, or severe nervous system involvement, CYC should be considered (200). The safety profile of RTX in patients with EGPA is similar to that of other AAVs, although some studies have reported more frequent allergic reactions to RTX infusion (217). However, recently, the case of non-ischemic cardiomyopathy following RTX treatment has been described (221), which suggests that RTX should be carefully used in case of heart failure, especially in patients with a previous history of cardiac disease. Interestingly, RTX in EGPA has been demonstrated to reduce the production of IL-5, probably by inhibiting B- to T-cell crosstalk (222). Some case series reports have shown the efficacy of RTX for asthma control in EGPA (223).

Recently, there have been reports on the effectiveness of a regimen based on sequential RTX and mepolizumab for the control of EPGA (224, 225). Bettiol et al. (226) published results of the European multicentre retrospective observational study and showed that sequential RTX and mepolizumab treatment (at a dose of 100 mg monthly) is effective to induce and maintain remission of both systemic and respiratory EGPA symptoms. These results seem to support the hypothesis that combining treatments with complementary mechanisms of action might lead to remission of both EGPA components.

6.2.3. Anti-IgE

Omalizumab is a monoclonal antibody that specifically binds to circulating IgE and blocks the inflammatory cascade, notably cell degranulation (mainly basophils and mastocytes), which causes a transient lowering of eosinophilia. It is currently used for the treatment of severe asthma with elevated IgE levels, chronic urticaria, and allergic rhinitis (198). In EGPA, data on the use of omalizumab are inconsistent and scarce and mostly come from case or case series reports. Some of them support the successful use of omalizumab as an adjunct therapy in EGPA patients with severe CS-resistant asthma, but not in those with extrapulmonary manifestations (227, 228). The results of a study comparing the efficacy of biologics in EGPA (RTX, mepolizumab, and omalizumab) showed that omalizumab was associated with significantly lower remission rates (15 vs. 78%) and significantly higher treatment failure (48 vs. 8%) than mepolizumab (219). Information on omalizumab efficacy against vasculitic features in EGPA is lacking, however, two life-threatening cases have been described, who were unresponsive to IS and eventually responded to omalizumab (229). In contrast, a relationship between omalizumab treatment and EGPA development has been described (230). Omalizumab is not recommended as a remission induction therapy for patients with EGPA. It may be considered only in patients who fail or are intolerant to conventional treatment and mepolizumab (200).

6.3. Other therapies

Other therapies include interferon α (IFNα), intravenous immunoglobulin (IVIg), and plasmapheresis. Data on the use of IFNα in EGPA are scarce and come mainly from case reports, suggesting that IFNα can induce EGPA remission (231). However, numerous adverse events (e.g., flu-like symptoms, cytopenia, hepatic toxicity, polyneuropathy, and depression) limit their use. Intravenous immunoglobulins may be used off-label in severe cases of refractory AAV in combination with other specific treatments, particularly in patients with severe infectious complications and secondary symptomatic immunological deficits (187). A multicenter double-blind trial showed the efficacy of IVIg as second-line therapy, especially in patients with neural involvement and residual peripheral neuropathy (232). A case of IVIg effectiveness in cardiac involvement in a patient with EGPA not responding to CYC has also been described (233). Regarding plasmapheresis, most data were based on studies that excluded EGPA. Recently published results of a large PEXOVAS study (involving GPA and MPA) showed that the use of plasmapheresis in patients with severe AAV did not reduce the incidence of death or end-stage kidney disease (234). Similar results were presented in a small prospective randomized study of 14 patients with EGPA showing any benefit in adding plasmapheresis to ongoing therapy (235).

7. Therapy of asthma

Therapy for asthma is indispensable in the treatment of patients with EGPA and does not differ from the treatment of asthma in the general population (190). Treatment should be adjusted to asthma severity using a stepwise pharmacological approach according to the current International Global Initiative for Asthma (GINA) recommendations (236). Anti-leukotriene drugs are not contraindicated if needed, however, patients require careful follow-up. The scheme of asthma management to control symptoms shows **Table 9**.

8. Therapy for ENT manifestations

Management of ENT manifestations in EGPA can be challenging. The main effective therapy remain intranasal CS. In addition, multiple daily nasal rinses with normal saline may provide

some benefit (237, 238). Other drugs, such antihistamines (for proven allergy) or long-term treatment of macrolides, can be tried in cases of intranasal CS inefficacy (190). In the last years, biologic treatment options have been proposed for ENT manifestations. Mepolizumab at a dose of 100 mg monthly has been approved by the FDA in 2021 as an add-on treatment option to standard of care in patients with CRSwNP. The approval has been based on data from the randomized trial (SYNAPSE) which explored the effect of mepolizumab vs. placebo in over 400 patients with CRSwNP, and showed that mepolizumab treatment significantly improved nasal polyp size and nasal obstruction; in addition, in the treated group there was a 57% reduction in the proportion of patients who had surgery compared with placebo group (239). In patients with EGPA, the MIRRA trial demonstrated that mepolizumab significantly reduced the frequency of disease relapses, including both asthma and sinonasal relapses (23).

Regarding to surgical treatment, the role of functional endoscopic sinus surgery (FESS) in EGPA is still a matter of debate. The results of the systematic review advise against FESS as a first-line treatment in EGPA and instead recommends a trial of maximal medication which is often successful in the initial treatment of nasal polyps (240). Surgical removal of nasal polyps can provide transient symptomatic relief but polyp recurrence is frequent (237). In the future, surgery in EGPA will probably collide with the introduction of new biologic drugs in the treatment regimens (238).

9. Prognosis and outcomes

Eosinophilic granulomatosis with polyangiitis is considered a milder form of AAV, with lower mortality compared to other AAVs (5, 95). It is now viewed as a chronic disease rather than a fatal condition due to the significant improvement in survival as a result of effective treatment based on CS and/or IS (55). In a monocentric study of 150 patients with EGPA, the 10-year survival rate was 89%, resulting in mortality comparable to that of the general population (82), while in others, overall survival reached 93% after a median follow-up of 6 years (100) and 90% at 7 years (54). However, the prognoses of patients with EGPA differ depending on the presence or absence of prognostic factors (FFS). In addition, relapses, asthma/ENT flares, and disease-related organ damage (sequelae) may severely impair the quality of life of patients with EGPA (54, 100).

Relapses in EGPA remain a major challenge. Their frequency rates vary between 25 and 49% (54, 55, 241). The factors predictive of relapse are not well established and are still under debate. Some studies have shown that peripheral eosinophil count at diagnosis ($<$3,000 cells/mm^3), cutaneous manifestation, and positive MPO-ANCA are associated with a higher risk of relapse (54, 55). In others, high IgE levels at onset (101) and FFS $>$ 1 were predictive of relapse (242). Recently, the FCGR3B polymorphism was described as a predictive factor for relapse among EGPA patients with ANCA positivity (243). As serial ANCA monitoring can have some utility in predicting relapses in GPA and MPA, EGPA data are limited because most studies did not provide complete results on repeat ANCA testing following treatment (244). However, according to the European EGPA Study Group recommendations, repeat ANCA testing is indicated in patients with MPO-ANCA-positive EGPA because persistence, rise, or reappearance of ANCA may justify more frequent clinical assessments (152).

The next problems in patients with EGPA are asthma and/or ENT flares, which are mostly independent of disease activity (82, 100). Although not life-threatening, both significantly contribute to patient morbidity, which is associated with persistent symptoms and exposure to long-term CS therapy and its associated adverse events (245). Among a multicenter cohort of 101 patients with EGPA with a median follow-up of 6 years, 92.5% still received systemic CS at the end of the study (100). In an American study involving 354 patients with a median follow-up of 7 years, at the last study visit, only 12.6% had been off all therapies for more than 2 years during their follow-up (241). While vasculitis relapses tend to ensue within the first 2 years following diagnosis, asthma and ENT manifestations tend to persist or relapse long after vasculitis has resolved (246). Sequeales are observed in 80% of patients with EGPA, regardless of the initial severity of the disease (54, 100). Next to asthma and ENT symptoms, it includes persistent polyneuropathy (45%), osteoporosis (30%), severe lung disease (17%), chronic kidney disease (13%), and chronic heart failure (11%) (54, 100).

A recent study from the US (247) excellently reflects the high burden of EGPA. The results showed that all-cause healthcare costs were 2.5-fold higher in patients with EGPA than in those with asthma alone (with similar geographic and insurance status). Furthermore, all-cause healthcare resource utilization and use of systemic CS were also significantly greater in EGPA, with more than one-third of these patients experiencing relapses (247). On the other hand, although the burden of the disease remains still high, the mortality and morbidity of patients have essentially decreased due to the change in approach toward the treatment over the years. A Spanish study analyzing the outcomes of AAV patients (including EGPA) showed improved results with a significant decrease in mortality and treatment-related morbidity in patients diagnosed after 2000 compared to those diagnosed prior to 2000, which was related to the use of less toxic regimens adapted to the disease activity and stage, and a drastic reduction in the cumulative CYC and CS dose (113). This trend in treatment continues with the implementation and search for new biologic therapies.

10. Conclusion

Despite notable progress in the understanding of its pathogenesis and disease management, EGPA remains a major diagnostic and therapeutic challenge. Its dual categorization with HESs and systemic vasculitides leads to varied clinical presentation, which requires careful differentiation from other mimicking disorders. To date, there are no universally approved diagnostic criteria, and diagnosis remains mainly clinical. The role of ANCA is not fully understood; but two phenotypes have been defined according to ANCA status, with consistently different genetic backgrounds, manifestations, prognoses, and treatment responses. However, ANCA is present only in approximately 30–40% of patients, and there is still an ongoing debate over whether EGPA should be recognized in cases without the presence of vasculitis and ANCA. Breakthroughs in clinical practice are

novel classification criteria that are expected to accelerate clinical studies on EGPA in the future. Although the prognosis is good, relapses in EGPA are frequent, and many patients have chronic symptoms that require long-term treatment with CS. Although effective, conventional therapy is not satisfactory for relapse prevention and resolution of chronic symptoms and is burdened with high toxicity. In this context, new biological agents are a valid therapeutic alternative, although more data are required.

Author contributions

JF and ER contributed to the conception and design of the work and drafted the manuscript. Both authors approved the final version of the manuscript.

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.

Publisher's note

References

1. White J, Dubey S. Eosinophilic granulomatosis with polyangiitis: a review. *Autoimmun Rev.* (2022) 22:103219. doi: 10.1016/j.autrev.2022.103219

2. Churg J, Strauss L. Allergic granulomatosis, allergic angiitis, and periarteritis nodosa. *Am J Pathol.* (1951) 27:277–301.

3. Springer J, Kalot M, Husainat N, Byram K, Dua A, James K, et al. Eosinophilic granulomatosis with polyangiitis: a systematic review and meta-analysis of test accuracy and benefits and harms of common treatments. *ACR Open Rheumatol.* (2021) 3:101–10. doi: 10.1002/acr2.11194

4. Harold L, Patterson M, Andrade S, Dube T, Go A, Buist A, et al. Asthma drug use and the development of Churg-Strauss syndrome (CSS). *Pharmacoepidemiol Drug Saf.* (2007) 16:620–6. doi: 10.1002/pds.1353

5. Nguyen Y, Guillevin L. Eosinophilic granulomatosis with polyangiitis (Churg-Strauss). *Semin Respir Crit Care Med.* (2018) 39:471–81. doi: 10.1055/s-0038-1669454

6. Jariwala M, Laxer R. Childhood GPA, EGPA, and MPA. *Clin Immunol.* (2020) 211:108325. doi: 10.1016/j.clim.2019.108325

7. Fijolek J, Wiatr E, Piotrowska-Kownacka D, Roszkowski-Sliz K. The role of peripheral eosinophilia in diagnosing lung disorders: experience from a single pneumonological center. *Multidiscip Respir Med.* (2021) 16:770. doi: 10.4081/mrm.2021.770

8. Jennette J, Falk R, Bacon P, Basu N, Cid M, Ferrario F, et al. 2012 revised international Chapel Hill consensus conference nomenclature of vasculitides. *Arthritis Rheum.* (2012) 65:1–11. doi: 10.1002/art.37715

9. Sinico R, Di Toma L, Maggiore U, Bottero P, Radice A, Tosoni C, et al. Prevalence and clinical significance of antineutrophil cytoplasmic antibodies in Churg-Strauss syndrome. *Arthritis Rheum.* (2005) 52:2926–35. doi: 10.1002/art.21250

10. Sable-Fourtassou R, Cohen P, Mahr A, Pagnoux C, Mouthon L, Jayne D, et al. Antineutrophil cytoplasmic antibodies and Churg-Strauss syndrome. *Ann Intern Med.* (2005) 143:632–8. doi: 10.7326/0003-4819-143-9-200511010-00006

11. Valent P, Klion A, Horny H, Roufosse F, Gotlib J. Contemporary consensus proposal on criteria and classification of eosinophilic disorders and related syndromes. *J Allergy Clin Immunol.* (2012) 130:607.e–12.e. doi: 10.1016/j.jaci.2012.02.019

12. Egan A, Kronbichler A, Neumann I, Bettiol A, Carlson N, Cid M, et al. The sound of interconnectivity; the European vasculitis society 2022 report. *Kidney Int Rep.* (2022) 7:1745–57. doi: 10.1016/j.ekir.2022.05.018

13. Jennette JC, Falk R, Andrassy K, Bacon P, Churg J, Gross W, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum.* (1994) 37:187–92. doi: 10.1002/art.1780370206

14. Cottin V, Bel E, Bottero P, Dalhoff K, Humbert M, Lazor R, et al. Revisiting the systemic vasculitis in eosinophilic granulomatosis with polyangiitis (Churg-Strauss). A study of 157 patients by the Groupe d'Etudes et de Recherche sur les Maladies Orphelines Pulmonaires and the European respiratory society taskforce on eosinophilic granulomatosis with polyangiitis (Churg-Strauss). *Autoimmun Rev.* (2017) 16:1–9. doi: 10.1016/j.autrev.2016.09.018

15. Fagni F, Bello F, Emmi G. Eosinophilic granulomatosis with polyangiitis: dissecting the pathophysiology. *Front Med.* (2021) 8:627776. doi: 10.3389/fmed.2021.627776

16. Trivioli G, Terrier B, Vaglio A. Eosinophilic granulomatosis with polyangiitis: understanding the disease and its management. *Rheumatology.* (2020) 59:iii84–94. doi: 10.1093/rheumatology/kez570

17. Dejaco C, Oppl B, Monach P, Cuthbertson D, Carette S, Hoffman G, et al. Serum biomarkers in patients with relapsing eosinophilic granulomatosis with polyangiitis (Churg-Strauss). *PLoS One.* (2015) 10:e0121737. doi: 10.1371/journal.pone.0121737

18. Kiene M, Csernok E, Muller A, Metzler C, Trabandt A, Gross W. Elevated interleukin-4 and interleukin-13 production by T cell lines from patients with Churg-Strauss syndrome. *Arthritis Rheum.* (2001) 44:469–73. doi: 10.1002/1529-0131(200102)44:23.0CO;2-0

19. Jakiela B, Szczeklik W, Plutecka H, Sokolowska B, Mastalerz L, Sanak M, et al. Increased production of IL-5 and dominant Th2-type response in airways of Churg-Strauss syndrome patients. *Rheumatology.* (2012) 51:1887–93. doi: 10.1093/rheumatology/kes171

20. Gioffredi A, Maritati F, Oliva E, Buzio C. Eosinophilic granulomatosis with polyangiitis: an overview. *Front Immunol.* (2014) 5:549. doi: 10.3389/fimmu.2014.00549

21. Nagase H, Ueki S, Fujieda S. The roles of IL-5 and anti-IL-5 treatment in eosinophilic diseases: asthma, eosinophilic granulomatosis with polyangiitis, and eosinophilic chronic rhinosinusitis. *Allergology Int.* (2020) 69:178–86. doi: 10.1016/j.alit.2020.02.002

22. Eng S, DeFelice M. The role and immunobiology of eosinophils in the respiratory system: a comprehensive review. *Clinic Rev Allergy Immunol.* (2016) 50:140–58. doi: 10.1007/s12016-015-8526-3

23. Wechsler M, Akuthota P, Jayne D, Khoury P, Klion A, Langford C, et al. Mepolizumab or placebo for eosinophilic granulomatosis with polyangiitis. *N Engl J Med.* (2017) 376:1921–32. doi: 10.1056/NEJMoa1702079

24. Louten J, Boniface K, de Waal Malefyt R. Development and function of Th17 cells in health and disease. *J Allergy Clin Immunol.* (2009) 123:1004–11. doi: 10.1016/j.jaci.2009.04.003

25. Jakiela B, Sanak M, Szczeklik W, Sokolowska B, Plutecka H, Mastalerz L, et al. Both Th2 and Th17 responses are involved in the pathogenesis of Churg-Strauss syndrome. *Clin Exp Rheumatol.* (2011) 29 (suppl.64):S23–34.

26. Khoury P, Grayson P, Klion A. Eosinophils in vasculitis: characteristics and roles in pathogenesis. *Nat Rev Rheumatol.* (2014) 10:474–83. doi: 10.1038/nrrheum.2014.98

27. Fujioka A, Yamamoto T, Takasu H, Kawano K, Masuzawa M, Katsuoka K, et al. The analysis of mRNA expression of cytokines from skin lesions in Churg-Strauss syndrome. *J Dermatol.* (1998) 25:171–7. doi: 10.1111/j.1346-8138.1998.tb02375.x

28. Ebina-Shibuya R, Leonard W. Role of thymic stromal lymphopoietin in allergy and beyond. *Nat Rev Immunol.* (2022) 1:1–14. doi: 10.1038/s41577-022-00735-y

29. Boita M, Guida G, Circosta P, Elia A, Stella S, Heffler E, et al. The molecular and functional characterization of clonally expanded CD8+ TCR BV T cells in eosinophilic granulomatosis with polyangiitis (EGPA). *Clin Immunol.* (2014) 152:152–63. doi: 10.1016/j.clim.2014.03.001

30. Drage L, Davis M, De Castro F, Van Keulen V, Weiss E, Gleich G, et al. Evidence for pathogenic involvement of eosinophils and neutrophils in Churg-Strauss syndrome. *J Am Acad Dermatol.* (2002) 47:209–16. doi: 10.1067/mjd.2002.124600

31. Arima M, Kanoh T. Eosinophilic myocarditis associated with dense deposits of eosinophil cationic protein (ECP) in endomyocardium with high serum ECP. *Heart.* (1999) 81:669–75. doi: 10.1136/hrt.81.6.669

32. Fettrelet T, Gigon L, Karaulov A, Yousefi S, Simon H. The enigma of eosinophil degranulation. *Int J Mol Sci.* (2021) 22:7091. doi: 10.3390/ojms22137091

33. Koike H, Nishi R, Furukawa S, Mouri N, Fukami Y, Iijima M, et al. In vivo visualization of eosinophil secretion in eosinophilic granulomatosis with polyangiitis: an ultrastructural study. *Allergol Int.* (2022) 71:373–82. doi: 10.1016/j.alit.2022.02.009

34. Vega Villanueva K, Espinoza L. Eosinophilic vasculitis. *Cur Rheumatol Rep.* (2020) 22:5. doi: 10.1007/s11926-020-0881-2

35. Emmi G, Silvestri E, Squatrito D, Amedei A, Niccolai E, D'Elios M, et al. Thrombosis in vasculitis: from pathogenesis to treatment. *Thrombosis J.* (2015) 13:15. doi: 10.1186/s12959-015-0047-z

36. Noguchi H, Kephart G, Colby T, Gleich G. Tissue eosinophilia and eosinophil degranulation in syndromes associated with fibrosis. *Am J Pathol.* (1992) 140:521–8.

37. Filley W, Holley K, Kephart G, Gleich G. Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. *Lancet.* (1982) 2:11–6. doi: 10.1016/S0140-6736(82)91152-7

38. Terrier B, Bieche I, Maisonobe T, Laurendeau I, Rosenzwajg M, Kahn J, et al. Interleukin -25: a cytokine linking eosinophils and adaptive immunity in Churg-Strauss syndrome. *Blood.* (2010) 16:4523–31. doi: 10.1182/blood-2010-02-267542

39. Moore P, Church T, Chism D, Panettieri R Jr, Shore S. IL-13 and IL-4 cause eotaxin release in human airway smooth muscle cells: a role for ERK. *Am J Physiol Lung Cell Mol Physiol.* (2002) 282:L847–53. doi: 10.1152/ajplung.00245.2001

40. Polzer K, Karonitsch T, Neumann T, Eger G, Haberler C, Soleiman A, et al. Eotaxin-3 is involved in Churg-Strauss syndrome – a serum marker closely correlating with disease activity. *Rheumatology.* (2008) 47:804–8. doi: 10.1093/rheumatology/ken033

41. Zwerina J, Bach C, Martorana D, Jatzwauk M, Hegasy G, Moosig F, et al. Eotaxin-3 in Churg-Strauss syndrome: a clinical and immunogenetic study. *Rheumatology.* (2011) 50:1823–7. doi: 10.1093/rheumatology/keq445

42. Emmi G, Silvestri E, Marconi R, Carrai V, Fanelli T, Zucchini P, et al. First report of FIP1L1-PDGFRalpha-positive eosinophilic granulomatosis with polyangiitis. *Rheumatology.* (2015) 54:1751–3. doi: 10.1093/rheumatology/kev242

43. Triggianese P, D'Antonio A, Conigliaro P, Buccisano F, Fonti G, Chimenti M, et al. A new focus on thyrosine kinases inhibitors in eosinophilic granulomatosis with polyangiitis. *Clin Exp Rheumatol.* (2021) 39 (suppl.129):S180–1. doi: 10.55563/clinexprheumatol/rrjt9m

44. Beketova T, Volkov M, Naryshkin E, Novoselova T, Nasonov E. Imatinib mesylate use in refractory eosinophilic granulomatosis with polyangiitis: a literature review and a case report. *Clin Rheumatol.* (2018) 37:1729–35. doi: 10.1007/s10067-018-4018-1

45. Tsurikisawa N, Oshikata C, Watanabe M, Tsuburai T, Kaneko T, Saito H. Innate immune response refelcts disease activity in eosinophilic granulomatosis with polyangiitis. *Clin Exp Allergy.* (2018) 48:1305–16. doi: 10.1111/cea.13209

46. Akiyama M, Kaneko Y, Takeuchi T. Rituximab for the treatmet of eosinophilic granulomatosis with polyangiitis: a systematic literature review. *Autoimmun Rev.* (2021) 20:102737. doi: 10.1016/j.autrev.2020.102737

47. Wu Z, Zhang S, Li P, Song N, Zhang F, Li Y. Elevated serum IgG4 was found in eosinophilic granulomatosis with polyangiitis. *J Clin Rheumatol.* (2021) 27:e501–4. doi: 10.1097/RHU.0000000000001606

48. Vaglio A, Strehl J, Manger B, Maritati F, Alberici F, Beyer C, et al. IgG4 immune response in Churg-Strauss syndrome. *Ann Rheum Dis.* (2012) 71:390–3. doi: 10.1136/ard.2011.155382

49. Tsurikisawa N, Saito H, Oshikata C, Tsuburai T, Akiyama K. Decreases in the numbers of peripheral blood regulatory T cells, and increases in the levels of memory and activated B cells in patients with active eosinophilic granulomatosis and polyangiitis. *J Clin Immunol.* (2013) 33:965–76. doi: 10.1007/s10875-013-9898-x

50. Kubo S, Kanda R, Nawata A, Miyazaki Y, Kawabe A, Hanami K, et al. Eosinophilic granulomatosis with polyangiitis exhibits T cell activation and IgG4 immune response in the tissue; comparison with IgG4-related disease. *RMD Open.* (2022) 8:e002086. doi: 10.1136/rmdopen-2021-002086

51. Shochet L, Holdsworth S, Kitching A. Animal models of ANCA associated vasculitis. *Front Immunol.* (2020) 11:525. doi: 10.3389/fimmu.2020.00525

52. Schlieben D, Korbet S, Kimura R, Schwartz M, Lewis E. Pulmonary-renal syndrome in a newborn with placental transmission of ANCAs. *Am J Kidney Dis.* (2005) 45:758–61. doi: 10.1053/j.ajkd.2005.01.001

53. Falk R, Terrel R, Charles L, Jennette J. Anti-neutrophil cytoplasmic antibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proct Natl Acad Sci U.S.A.* (1990) 87:4115–9. doi: 10.1073/pnas.87.11.4115

54. Samson M, Puechal X, Devilliers H, Ribi C, Cohen P, Stern M, et al. Long-term outcomes of 118 patients with eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome) enrolled in two prospective trials. *J Autoimmun.* (2013) 43:60–9. doi: 10.1016/j.jaut.2013.03.003

55. Comarmond C, Pagnoux C, Khellaf M, Cordier J, Hamidou M, Viallard J, et al. Eosinophilic granulomatosis with polyangiitis (Churg-Strauss). Clinical characteristics and long-term followup of the 383 patients enrolled in the French vasculitis study group Cohort. *Arthritis Rheum.* (2013) 65:270–81. doi: 10.1002/art.37721

56. Lyons P, Peters J, Alberici F, Liley J, Coulson R, Astle W, et al. Genome-wide association study of eosinophilic granulomatosis with polyangiitis reveals genomic loci stratified by ANCA status. *Nat Comm.* (2019) 10:5120. doi: 10.1038/s41467-019-12515-9

57. Chaigne B, Terrier B, Thieblemont N, Witko-Sarsat V, Mouthon L. Dividing the Janus vasculitis? Pathophysiology of eosinophilic granulomatosis with polyangiitis. *Autoimmun Rev.* (2016) 15:139–45. doi: 10.1016/j.autrey.2015.10.006

58. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss D, et al. Neutrophil extracellular traps kill bacteria. *Science.* (2004) 303:1532–5. doi: 10.1126/science.1092385

59. Arneth B, Arneth R. Neutrophil extracellular traps (NETs) and vasculitis. *In J Med Sci.* (2021) 18:1532–40. doi: 10.7150/ijms.53728

60. Sciascia S, Ponticelli C, Roccatello D. Pathogenesis-based new perspectives of management of ANCA-associated vasculitis. *Autoimmun Rev.* (2022) 21:103030. doi: 10.1016/j.autrev.2021.103030

61. Sangaletti S, Tripodo C, Chiodoni C, Guarnotta C, Cappetti B, Casalini P, et al. Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. *Blood.* (2012) 120:3007–18. doi: 10.1182/blood-2012-03-416156

62. Fukuchi M, Kamide Y, Ueki S, Miyabe Y, Konno Y, Oka N, et al. Eosinophil ETosis-mediated release of galectin-10 in eosinophilic granulomatosis with polyangiitis. *Arthritis Rheumatol.* (2021) 73:1683–93. doi: 10.1002/art.41727

63. Mukherjee M, Thomas S, Radford K, Dvorkin-Gheva A, Davydchenko S, Kjarsgaard M, et al. Sputum antineutrophil cytoplasmic antibodies in serum antineutrophil cytoplasmic antibody-negative eosinophilic granulomatosis with polyangiitis. *Am J Respir Crit Care Med.* (2019) 199:158–70. doi: 10.1164/rccm.201804-0809OC

64. Hashimoto T, Ueki S, Kamide Y, Miyabe Y, Fukuchi M, Yokoyama Y, et al. Increased circulating cell-free DNA in eosinophilic granulomatosis with polyangiitis: implications for eosinophil extracellular traps and immunothrombosis. *Front Immunol.* (2022) 12:801897. doi: 10.3389/fimmu.2021.801897

65. Wieczorek S, Hellmich B, Gross W, Epplen J. Associations of Churg-Strauss syndrome with the HLA-DRB1 locus, and relationship to the genetics of antineutrophil cytoplasmic antibody-associated vasculitides: comment on the article by Vaglio et al. *Arthritis Rheum.* (2008) 58:329–30. doi: 10.1002/art.23209

66. Vaglio A, Martorana D, Maggiore U, Grasselli C, Zanetti A, Pesci A, et al. HLA-DRB4 as a genetic risk factor for Churg-Strauss syndrome. *Arthritis Rheum.* (2007) 56:3159–66. doi: 10.1002/art.22834

67. Wieczorek S, Hellmich B, Arning L, Moosig F, Lamprecht P, Gross W, et al. Functionally relevant variations of the interleukin-10 gene associated with antineutrophil cytoplasmic antibody-negative Churg-Strauss syndrome, but not with Wegener's granulomatosis. *Arthritis Rheum.* (2008) 58:1839–48. doi: 10.1002/art.23496

68. Mouthon L, Khaled M, Cohen P, Guillevin L, Mouthon L, Subra J. Systemic small sized vessel vasculitis after massive antigen inhalation. *Ann Rheum Dis.* (2001) 60:1288–94.

69. Guillevin L, Amouroux J, Arbeille B, Boura R. Churg-Strauss angiitis. Arguments favoring the responsibility of inhaled antigens. *Chest.* (1991) 100:1472–3. doi: 10.1378/chest.100.5.1472

70. Harada M, Imokawa S, Miwa S, Nihashi F, Aono Y, Amano Y, et al. Chronic pulmonary aspergillosis may cause eosinophilic granulomatosis with polyangiitis via allergic bronchopulmonary aspergillosis. *Oxf Med Case Rep.* (2019) 19:omy126. doi: 10.1093/omcr/omy126

71. Coutinho I, Lopes M, Lima F, Ventura C, Rabadao E, Alfaro T, et al. Concomitant allergic bronchopulmonary aspergillosis and eosinophilic granulomatosis with polyangiitis after *Aspergillus niger* infection. *Pulmonology.* (2022) 28:231–4. doi: 10.1016/j.pulmoe.2021.12.004

72. Karampoor S, Afrashteh F, Rahmani S, Laali A. Eosinophilic granulomatosis with polyangiitis after COVID-19: a case report. *Respir Med Case Rep.* (2022) 38:101702. doi: 10.1016/j.rmer.2022.101702

73. Wechsler M, Finn D, Guanawardena D, Westlake R, Barker A, Haranath S, et al. Churg-Strauss syndrome in patients receiving montelukast as treatment for asthma. *Chest.* (2000) 117:708–13. doi: 10.1378/chest.117.3.708

74. Wechsler M, Wong D, Miller M, Lawrence-Miyasaki L. Churg-Strauss syndrome in patients treated with omalizumab. *Chest.* (2009) 136:507–18. doi: 10.1378/chest.08-2990

75. Hauser T, Mahr A, Metzler C, Coste J, Sommerstein R, Gross W, et al. The leucotriene receptor antagonist montelukast and the risk of Churg-Strauss syndrome: a case-crossover study. *Thorax.* (2008) 63:677–82. doi: 10.1136/thx.2007.087825

76. Schroeder J, Folci M, Losappio L, Chevallard M, Sinico R, Mirone C, et al. Anti-neutrophil cytoplasmic antibodies positivity and anti-leukotrienes in eosinophilic

granulomatosis with polyangiitis: a retrospective monocentric study on 134 Italian patients. *Int Arch Allergy Immunol.* (2019) 180:64–71. doi: 10.1159/000500544

77. Ikeda M, Ohshima N, Kawashima M, Shiina M, Kitani M, Suzukawa M. Severe asthma where eosinophilic granulomatosis with polyangiitis became apparent after the discontinuation of dupilumab. *Intern Med.* (2022) 61:755–9. doi: 10.2169/internalmedicine.7990-21

78. Lim A, Antony A, Gingold M, Simpson I, Looi W, MacDonald M. Emergence of extrathoracic manifestations of eosinophilic granulomatosis with polyangiitis during benralizumab treatment. *Rheumatology Adv Prac.* (2021) 5:rkab033. doi: 10.1093/rap/rkab033

79. Bottero P, Bonini M, Vecchio F, Grittini A, Patruno G, Colombo B, et al. The common allergens in the Churg-Strauss syndrome. *Allergy.* (2007) 62:1288–94. doi: 10.1111/j.1398-9995.2007.01486.x

80. Nappi E, De Santis M, Paoletti G, Pelaia C, Terenghi F, Pini D, et al. New onset of granulomatosis with polyangiitis following mRNA-based COVID-19 vaccine. *Vaccines (Basel).* (2022) 10:716. doi: 10.3390/vaccines10050716

81. Lanham J, Elkon K, Pusey C, Hughes G. Systemic vasculitis with asthma and eosinophilia: a clinical approach to the Churg-Strauss syndrome. *Medicine (Baltimore).* (1984) 63:65–81. doi: 10.1097/00005792-198403000-00001

82. Moosig F, Bremer J, Hellmich B, Holle J, Holl-Ulrich K, Laudien M, et al. A vasculitis centre based management strategy leads to improved outcome in eosinophilic granulomatosis and polyangiitis (Churg-Strauss, EGPA): monocentric experiences in 150 patients. *Ann Rheum Dis.* (2013) 72:1011–7. doi: 10.1136/annrheumdis-2012-201531

83. Tsurikisawa N, Oshikata C, Kinoshita A, Tsuburai T, Saito H. Longterm prognosis of 121 patients with eosinophilic granulomatosis with polyangiitis in Japan. *J Rheumatol.* (2017) 44:1206–15. doi: 10.3899/jrheum.161436

84. Wojcik K, Wawrzycka-Adamczyk K, Wludarczyk A, Sznajd J, Zdrojewski Z, Masiak A, et al. Clinical characteristics of polish patients with ANCA-associated vasculitides-retrospective analysis of POLVAS registry. *Clin Rheumatol.* (2019) 38:2553–63. doi: 10.1007/s10067-019-04538-w

85. Healy B, Bibby S, Steele R, Weatherall M, Nelson H, Beasley R. Antineutrophil cytoplasmic autoantibodies and myeloperoxidase autoantibodies in clinical expression of Churg-Strauss syndrome. *J Allergy Clin Immunol.* (2013) 131:571–6. doi: 10.1016/j.jaci.2012.05.058

86. Bettiol A, Sinico R, Schiavon F, Monti S, Bozzolo E, Franceschini F, et al. Risk of acute arterial and venous thromboembolic events in eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome). *Eur Respir J.* (2021) 57:2004158. doi: 10.1183/13993003.04158-2020

87. Fijolek, J, Wiatr E, Bujnowski P, Piotrowska-Kownacka D, Roszkowski-Sliz K. Evaluation of prognostic factors for patients with eosinophilic granulomatosis with polyangiitis recruited at the pneumonological centre and mainly ANCA negativity: a retrospective analysis of a single cohort in Poland. *Mod Rheumatol.* (2023) road001. doi: 10.1093/mr/road001

88. Berti A, Volcheck G, Cornec D, Smyth R, Specks U, Keogh K. Severe/uncontrolled asthma and overall survival in atopic patients with eosinophilic granulomatosis with polyangiitis. *Respir Med.* (2018) 142:66–72. doi: 10.1016/j.rmed.2018.07.017

89. Cottin C, Bel E, Bottero P, Dalhoff K, Humbert M, Lazor R, et al. Respiratory manifestations of eosinophilic granulomatosis with polyangiitis (Churg-Strauss). *Eur Respir J.* (2016) 48:1429–41. doi: 10.1183/13993003.00097-2016

90. Cottin C, Khouatra C, Dubost R, Glerant J, Cordier J. Persistent airflow obstruction in asthma of patients with Churg-Strauss syndrome and long-term follow-up. *Allergy.* (2009) 64:589–95. doi: 10.1111/j.1398-9995.2008.01854.x

91. Latorre M, Baldini C, Seccia V, Pepe P, Novelli F, Celi A, et al. Asthma control and airway inflammation in patients with eosinophilic granulomatosis with polyangiitis. *J Allergy Clin Immunol Pract.* (2016) 4:512–9. doi: 10.1016/j.jaip.2015.12.014

92. Szczeklik W, Sokolowska B, Zuk J, Mastalerz L, Szczeklik A, Musial J. The course of asthma in Churg-Strauss syndrome. *J Asthma.* (2011) 48:183–7. doi: 10.3109/02770903.2010.551796

93. Berti A, Cornec D, Moura M, Smyth R, Dagna L, Specks U, et al. Eosinophilic granulomatosis with polyangiitis. Clinical predictors of long-term asthma severity. *Chest.* (2020) 157:1086–99. doi: 10.1016/j.chest.2019.11.045

94. Bacciu A, Bacciu S, Mercante G, Ingegnoli F, Grasselli C, Vaglio A, et al. Ear, nose and throat manifestations of Churg-Strauss syndrome. *Acta Oto-Laryngol.* (2006) 126:503–9. doi: 10.1080/00016480500437435

95. Bacciu A, Buzio C, Giordano D, Pasanisi E, Vincenti V, Mercante G, et al. Nasal polyposis in Churg-Strauss syndrome. *Laryngoscope.* (2008) 118:325–9. doi: 10.1097/MLG.0b013e318159889d

96. Seccia V, Baldini C, Latorre M, Gelardi M, Dallan I, Cristofani-Mencacci L, et al. Focus on the involvement of the nose and paranasal sinuses in eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome): nasal cytology reveals infiltration of eosinophils as a very common feature. *Int Arch Allergy Immunol.* (2018) 175:61–9. doi: 10.1159/000484602

97. Brescia G, Padoan R, Schiavon F, Contro G, Parrino D, Tealdo G, et al. Nasal polyps in eosinophilic granulomatosis with polyangiitis: structured histopathology and CD105 expression. *Am J Otolaryngol.* (2020) 41:102661. doi: 10.1016/j.amjoto.2020.102661

98. Greco A, Rizzo M, De Virgilio A, Gallo A, Fusconi M, Ruoppolo G, et al. Churg-Strauss syndrome. *Autoimmun Rev.* (2015) 14:341–8. doi: 10.1016/j.autrev.2014.12.004

99. Guillevin L, Cohen P, Gayraud M, Lhote F, Jarrousse B, Casassus P. Churg-Strauss syndrome. Clinical study and long-term follow-up of 96 patients. *Medicine (Baltimore).* (1999) 78:26–37. doi: 10.1097/00005792-199901000-00003

100. Durel C, Berthiller J, Caboni S, Jayne D, Ninet J, Hot A. Long-term followup of a multicenter cohort of 101 patients with eosinophilic granulomatosis with polyangiitis (Churg-Strauss). *Arthritis Care Res.* (2016) 68:374–87. doi: 10.1002/acr.22686

101. Saku A, Furuta S, Hiraguri M, Ikeda K, Kobayashi Y, Kagami SI, et al. Longterm outcomes of 188 Japanese patients with eosinophilic granulomatosis with polyangiitis. *J Rheumatol.* (2018) 45:1159–66. doi: 10.3899/jrheum.171352

102. Fijolek J, Wiatr E, Gawryluk D, Langfort R, Kober J, Piotrowska-Kownacka D, et al. The basis of Churg-Strauss syndrome diagnosis in own material. *Pneumonol Alergol Pol.* (2012) 80:20–8.

103. Bernheim A, McLoud T. A review of clinical and imaging findings in eosinophilic lung diseases. *AJR Am J Roentgenol.* (2017) 208:1002–10. doi: 10.2214/AJR.16.17315

104. Choi Y, Im J, Han B, Kim J, Lee K, Myoung N. Thoracic manifestation of Churg-Strauss syndrome. Radiologic and clinical findings. *Chest.* (2000) 117:117–24. doi: 10.1378/chest.117.1.117

105. Zimmermann N, Wikenheiser-Brokamp K. Hypereosinophilic syndrome in the differential diagnosis of pulmonary infiltrates with eosinophilia. *Ann Allergy Asthma Immunol.* (2018) 121:179–85. doi: 10.1016/j.anai.2018.05.014

106. Kim Y, Lee K, Han J, Chong S, Chung M, Yi C, et al. Pulmonary involvement in Churg-Strauss syndrome: an analysis of CT, clinical, and pathologic findings. *Eur Radiol.* (2007) 17:3157–65. doi: 10.1007/s00330-007-0700-4

107. Nakamoto K, Saraya T, Ogawa Y, Ishii H, Takizawa H. Comparison of findings on thoracic computed tomography with the severity and duration of bronchial asthma in patients with eosinophilic granulomatosis with polyangiitis. *Respir Med.* (2018) 139:101–5. doi: 10.1016/j.rmed.2018.05.003

108. Cottin V. Eosinophilic lung diseases. *Clin Chest Med.* (2016) 37:535–56. doi: 10.1016/j.ccm.2016.04.015

109. Thompson G, Bourne M Jr, Moura M, Baqir M, Cartin-Ceba R, Makol A, et al. Pleuritis and pericarditis in antineutrophil cytoplasmic autoantibody-associated vasculitis. *Chest.* (2021) 160:572–81. doi: 10.1016/j.chest.2021.02.049

110. Pagnoux C, Mahr A, Cohen P, Guillevin L. Presentation and outcome of gastrointestinal involvement in systemic necrotizing vasculitides. Analysis of 62 patients with polyarteritis nodosa, microscopic polyangiitis, Wegener granulomatosis, Churg-Strauss syndrome, or rheumatoid arthritis-associated vasculitis. *Medicine.* (2005) 84:115–28. doi: 10.1097/01.md.0000158825.87055.0b

111. Furuta S, Iwamoto T, Nakajima H. Update on eosinophilic granulomatosis with polyangiitis. *Allergol Int.* (2019) 68:430–6. doi: 10.1016/j.alit.2019.06.004

112. Mavrogeni S, Karabela G, Gialafos E, Stavropoulos E, Spiliotis G, Katsifis G, et al. Cardiac involvement in ANCA (+) and ANCA (–) Churg-Strauss syndrome evaluated by cardiovascular magnetic resonance. *Inflamm Allergy Drug Targ.* (2013) 12:322–7. doi: 10.2174/18715281113129990054

113. Solans-Laque R, Fraile G, Rodrigues-Carballeira M, Caminal L, Castillo M, Martinez-Valle F, et al. Clinical characteristics and outcome of Spanish patients with ANCA-associated vasculitides. Impact of the vasculitis type, ANCA specificity, and treatment on mortality and morbidity. *Medicine.* (2017) 96:e6083. doi: 10.1097/MD.0000000000006083

114. Guillevin L, Pagnoux C, Seror R, Mahr A, Mouthon L, Le Toumelin P, et al. The Five-Factor Score revisited. Assessment of prognoses of systemic necrotizing vasculitides based on the French Vasculitis Study Group (FVSG) cohort. *Medicine.* (2011) 90:19–27. doi: 10.1097/MD.0b013e18205a4c6

115. Misra D, Shenoy S. Cardiac involvement in primary systemic vasculitis and potential drug therapies to reduce cardiovascular risk. *Rheumatol Int.* (2017) 37:151–67. doi: 10.1007/s00296-016-3435-1

116. Zampieri M, Emmi G, Beltrami M, Fumagalli C, Urban M, Dei L, et al. Cardiac involvement in eosinophilic granulomatosis with polyangiitis (formerly Churg-Strauss syndrome): prospective evaluation at a tertiary referral centre. *Eur J Int Med.* (2021) 85:68–79. doi: 10.1016/j.ejim.2020.12.008

117. Neumann T, Manger B, Schmid M, Kroegel C, Hansch A, Reinhardt D, et al. Cardiac involvement in Churg-Strauss syndrome. Impact of myocarditis. *Medicine.* (2009) 88:236–43. doi: 10.1097/MD.0b013e3181af35a5

118. Garcia-Vives E, Rodrigues-Palomares J, Harty L, Solans-Laque R, Jayne D. Heart disease in eosinophilic granulomatosis with polyangiitis (EGPA) patients: a screening approach proposal. *Rheumatology.* (2021) 60:4538–47. doi: 10.1093/rheumatology/keab027

119. Seguela P, Iriart X, Acar P, Montaudon M, Roudaut R, Thambo J. Eosinophilic cardiac disease: molecular, clinical and imaging aspects. *Arch Cardiovasc Dis.* (2015) 108:258–68. doi: 10.1016/j.acvd.2015.01.006

120. Hazebroek M, Kemna M, Schalla S, Sanders-van Wijk S, Gerretsen SC, Dennert R, et al. Prevalence and prognostic relevance of cardiac involvement in ANCA-associated vasculitis: eosinophilic granulomatosis with polyangiitis and granulomatosis with polyangiitis. *Int J Cardiol.* (2015) 199:170–9. doi: 10.1016/j.ijcard.2015.06.087

121. Fijolek J, Wiatr E, Gawryluk D, Nowicka U, Martusewicz-Boros M, Kober J, et al. The significance of cardiac magnetic resonance imaging in detection and monitoring of the treatment efficacy of heart involvement in eosinophilic granulomatosis with polyangiitis patients. *Sarcoidosis Vasc Diffuse Lung Dis.* (2016) 33:51–8.

122. Dennert R, van Paassen P, Schalla S, Kuznetsova T, Alzand B, Staessen J, et al. Cardiac involvement in Churg-Strauss syndrome. *Arthritis Rheum.* (2010) 62:627–34. doi: 10.1002/art.27263

123. Dunogue B, Terrier B, Cohen P, Marmursztejn J, Legmann P, Mouthon L, et al. Impact of cardiac magnetic resonance imaging on eosinophilic granulomatosis with polyangiitis outcomes: a long-term retrospective study on 42 patients. *Autoimmun Rev.* (2015) 14:774–80. doi: 10.1016/j.autrev.2015.04.013

124. Liu S, Guo L, Zhang Z, Li M, Zeng X, Wang L, et al. Cardiac manifestations of eosinophilic granulomatosis with polyangiitis from a single-center cohort in China:clinical features and associated factors. *Ther Adv Chronic Dis.* (2021) 12:1–12. doi: 10.1177/2040622320907051

125. Bischof A, Jaeger V, Hadden R, Luqmani R, Probstel A, Merkel P, et al. Peripheral neuropathy in antineutrophil cytoplasmic antibody-associated vasculitides. Insights from the DCVAS study. *Neurol Neuroimmunol Neuroinflamm.* (2019) 6:615. doi: 10.1212/NXI.0000000000000615

126. Wludarczyk A, Szczeklik W. Neurological manifestations in ANCA-associated vasculitis – assessment and treatment. *Expert Rev Neurother.* (2016) 16:861–3. doi: 10.1586/14737175.2016.1165095

127. Oiwa H, Mokuda S, Matsubara T, Funaki M, Takeda I, Yamawaki T, et al. Neurological complications in eosinophilic granulomatosis with polyangiitis (EGPA): the roles of history and physical examinations in the diagnosis of EGPA. *Int Med.* (2017) 56:3003–8. doi: 10.2169/internalmedicine.8457-16

128. Nishi R, Koike H, Ohyama K, Fukami Y, Ikeda S, Kawagashira Y, et al. Differential clinicopathologic features of EGPA-associated neuropathy with and without ANCA. *Neurology.* (2020) 94:e1726–37. doi: 10.1212/WNL.0000000000009309

129. Andre R, Cottin V, Saraux J, Blaison G, Bienvenu B, Cathebras P, et al. Central nervous system involvement in eosinophilic granulomatosis with polyangiitis (Churg-Strauss): report of 26 patients and review of the literature. *Autoimmun Rev.* (2017) 16:963–9. doi: 10.1016/j.autrev.2017.07.007

130. Micheletti R, Fuxench Z, Craven A, Watts R, Luqmani R, Merkel P, et al. Cutaneous manifestations of antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheumatol.* (2020) 72:1741–7. doi: 10.1002/art.41310

131. Ishibashi M, Kawahara Y, Chen K. Spectrum of cutaneous vasculitis in eosinophilic granulomatosis with polyangiitis (Churg-Strauss): a case series. *Am J Dermatopathol.* (2015) 37:214–21. doi: 10.1097/DAD.0000000000000192

132. Abdel-Halim M, Mahmoud A, Ragab G. Cutaneous manifestations of anti-neutrophil cytoplasmic antibody associated vasculitis. *Vessel Plus.* (2022) 6:8. doi: 10.20517/2574-1209.2021.40

133. Radonjic-Hoesli S, Bruggen M, Feldmeyer L, Simon H, Simon D. Eosinophils in skin diseases. *Semin Immunopathol.* (2021) 43:393–409. doi: 10.1007/s00281-021-00868-7

134. Durel C, Sinico R, Teixeira V, Jayne D, Belenfant X, Marchand-Adam S, et al. Renal involvement in eosinophilic granulomatosis with polyangiitis (EGPA): a multicentric retrospective study of 63 biopsy-proven cases. *Rheumatology.* (2020) 0:1–7. doi: 10.1093/rheumatology/keaa416

135. Churg A. Recent advances in the diagnosis of Churg-Strauss syndrome. *Mod Pathol.* (2001) 14:1284–93. doi: 10.1038/modpathol.3880475

136. Cordier J, Cottin V, Guillevin L, Bel E, Bottero P, Dalhoff K, et al. Eosinophilic granulomatosis with polyangiitis (Churg-Strauss). *Presse Med.* (2013) 42:507–10.

137. Masi A, Hunder G, Lie J, Michel B, Bloch D, Arend W, et al. The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). *Arthritis Rheum.* (1990) 33:1094–100. doi: 10.1002/art.1780330806

138. Grayson P, Ponte C, Suppiah R, Robson J, Craven A, Judge A, et al. 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology Classification Criteria for eosinophilic granulomatosis with polyangiitis. *Arthritis Rheumatol.* (2022) 74:386–92. doi: 10.1002/art.41982

139. Guilpain P, Auclair J, Tamby M, Servettaz A, Mahr A, Weill B, et al. Serum eosinophil cationic protein: a marker of disease activity in Churg-Strauss syndrome. *Ann N Y Acad Sci.* (2007) 1107:392–9. doi: 10.1196/annals.1381.041

140. Dallos T, Heiland G, Strehl J, Karonitsch T, Gross W, Moosig F, et al. CCL17/thymus and activation – related chemokine in Churg-Strauss syndrome. *Arthritis Rheum.* (2010) 62:3496–503. doi: 10.1002/art.27678

141. Grayson P, Monach P, Pagnoux C, Cuthbertson D, Carette S, Hoffman G, et al. Values of commonly measured laboratory tests as biomarkers of disease activity and predictors of relapse in eosinophilic granulomatosis with polyangiitis. *Rheumatology.* (2015) 54:1351–9. doi: 10.1093/rheumatology/keu427

142. Rhee R, Holweg C, Wong K, Cuthbertson D, Carette S, Khalidi N, et al. Serum periostin as a biomarker in eosinophilic granulomatosis with polyangiitis. *PLoS One.* (2018) 13:e0205768. doi: 10.1371/journal.pone.0205768

143. Pagnoux C, Nair P, Xi Y, Khalidi N, Carette S, Cuthbertson D, et al. Serum cytokine and chemokine levels in patients with eosinophilic granulomatosis with polyangiitis, hypereosinophilic syndrome, or eosinophilic asthma. *Clin Exp Rheumatol.* (2019) 37(Suppl. 117):40–4.

144. Laskari K, Hellmich B, Adamus G, Csernok E. Autoantibody profile in eosinophilic granulomatosis and polyangiitis: predominance of anti-alpha-enolase antibodies. *Clin Exp Rheumatol.* (2021) 39(Suppl.129):S83–7. doi: 10.55563/clinexprheumatol/08k9af

145. Rodriguez-Pla A, Warner R, Cuthbertson D, Carette S, Khalidi N, Koening C, et al. Evaluation of potential serum biomarkers of disease activity in diverse forms of vasculitis. *J Rheumatol.* (2020) 47:1001–10. doi: 10.3899/jrheum.190093

146. Padoan R, Gatto M, Ghirardello A, Tonello M, Franco C, Felicetti M, et al. IgG anti-pentraxin 3 antibodies are a novel biomarker of ANCA-associated vasculitis and better identify patients with eosinophilic granulomatosis with polyangiitis. *J Autoimmun.* (2021) 124:102725. doi: 10.1016/j.jaut.2021.102725

147. Szczeklik W, Sanak M, Mastalerz L, Sokolowska B, Gielicz A, Soja J, et al. 12-hydroxy-eicosatetraenoic acid (12-HETE): a biomarker of Churg-Strauss syndrome. *Clin Exp Allergy.* (2012) 42:513–22. doi: 10.1111/j.1365-2222.2011.03943.x

148. Ma J, Dong C, Wei S, Qiu M, Wu P, Ou C, et al. Serum cytokine profiling identifies Axl as a new biomarker candidate for active eosinophilic granulomatosis with polyangiitis. *Front Mol Biosci.* (2021) 8:653461. doi: 10.3389/fmolb.2021.653461

149. Latorre M, Bacci E, Seccia V, Dente F, Brighindi S, Di Franco A, et al. New biomarkers for early diagnosis of eosinophilic granulomatosis with polyangiitis (EGPA). *Euro Respir J.* (2017) 50:A3560. doi: 10.1183/1393003

150. Xiao J, Lu S, Wang X, Liang M, Dong C, Zhang X, et al. Serum proteomic analysis identifies SAA1, FGA, SAP, and CETP as new biomarkers for eosinophilic granulomatosis with polyangiitis. *Front Immunol.* (2022) 13:866035. doi: 10.3389/fimmu.2022.866035

151. Zhao B, Zheng H, Yang T, Zheng R. Eosinophilic granulomatosis with polyangiitis in allergic asthma: efforts to make early diagnosis possible. *Allergy Asthma Proc.* (2023) 44:59–63. doi: 10.2500/aap.2023.44.220072

152. Moiseev S, Bossuyt X, Arimura Y, Blockmans D, Csernok E, Damoiseaux J, et al. International Consensus on antineutrophil cytoplasm antibodies testing in eosinophilic granulomatosis with polyangiitis. *Am J Respir Crit Care Med.* (2020) 202:1360–72. doi: 10.1164/rccm.202005-1628SO

153. Groh M, Pagnoux C, Baldini C, Bel E, Bottero P, Cottin V, et al. Eosinophilic granulomatosis with polyangiitis (Churg-Strauss) (EGPA). Consensus Task Force recommendations for evaluation and management. *Eur J Int Med.* (2015) 26:545–53. doi: 10.1016/j.ejim.2015.04.022

154. Guillevin L, Lhote F, Gayraud M, Cohen P, Jarrousse B, Lortholary O, et al. Prognostic factors in polyarteritis nodosa and Churg-Strauss syndrome. A prospective study in 342 patients. *Medicine.* (1996) 75:17–28. doi: 10.1097/00005792-199601000-00003

155. Bond M, Fagni F, Moretti M, Bello F, Egan A, Vaglio A, et al. At the heart of eosinophilic granulomatosis with polyangiitis: into cardiac and vascular involvement. *Cur Rheumatol Rep.* (2022) 24:337–51. doi: 10.1007/s11926-022-01087-1

156. Cereda A, Pedrotti P, De Capitani L, Giannattasio C, Roghi A. Comprehensive evaluation of cardiac involvement in eosinophilic granulomatosis with polyangiitis (EGPA) with cardiac magnetic resonance. *Eur J Int Med.* (2017) 39:51–6. doi: 10.1016/j.ejim.2016.09.014

157. Mavrogeni S, Dimitroulas T, Kitas G. Cardiovascular magnetic resonance in the diagnosis and management of cardiac and vascular involvement in the systemic vasculitides. *Curr Opin Rheumatol.* (2019) 31:16–24.

158. Yune S, Choi D, Lee B, Lee J, Jeon E, Kim S, et al. Detecting cardiac involvement with magnetic resonance in patients with active eosinophilic granulomatosis with polyangiitis. *Int J Cardiovasc Imaging.* (2016) 32(Suppl 1):S155–62. doi: 10.1007/s10554-016-0843-y

159. Fijolek J, Wiatr E, Blasinska K, Piotrowska-Kownacka D, Roszkowski-Sliz K. Cardiac involvement and absence of asthma-what is phenotype specificity of EGPA: a case report. *Clin Med Res.* (2022) 20:170–6. doi: 10.3121/cmr.2022.1683

160. Marmursztejn J, Vignaux O, Cohen P, Guilpain P, Pagnoux C, Gouya H, et al. Impact of cardiac magnetic resonance imaging for assessment of Churg-Strauss syndrome: a cross-sectional study in 20 patients. *Clin Exp Rheumatol.* (2009) 27(Suppl. 52):S70–6.

161. Miszalski-Jamka T, Szczeklik W, Sokolowska B, Karwat K, Belzak K, Mazur W, et al. Standard and feature tracking magnetic resonance evidence of myocardial involvement in Churg-Strauss syndrome and granulomatosis with polyangiitis (Wegener's) in patients with normal electrocardiograms and transthoracic echocardiography. *Int J Cardiovasc Imaging.* (2013) 29:843–53. doi: 10.1007/s10554-012-0158-6

162. Nasser M, Thivolet-Bejui F, Seve P, Cottin V. Lung-limited or lung-dominant variant of eosinophilic granulomatosis with polyangiitis. *J Allergy Clin Immunol Pract.* (2020) 8:2092–5. doi: 10.1016/j.jaip.2020.01.058

163. Taylor M, Keane C, O'Connor P, Mulvihill E, Holland C. The expanded spectrum of toxocaral disease. *Lancet.* (1988) 1:692–5. doi: 10.1016/s0140-6736(88)91486-9

164. Siddiqui A, Berk S. Diagnosis of Strongyloides stercoralis infection. *Clin Infect Dis.* (2001) 33:1040–7. doi: 10.1086/322707

165. Kahn J, Groh M, Lefevre G. (A critical appraisal of) classification of hypereosinophilic disorders. *Front Immunol.* (2016) 4:216. doi: 10.3389/fmed.2017.00216

166. Asano K, Hebisawa A, Ishiguro T, Takayanagi N, Nakamura Y, Suzuki Y, et al. New clinical diagnostic criteria for allergic bronchopulmonary aspergillosis/mycosis and its validation. *J Allergy Clin Immunol.* (2021) 147:1261–8. doi: 10.1016/j.jaci.2020.08.029

167. Crowe M, Robinson D, Sagar M, Chen L, Ghamande S. Chronic eosinophilic pneumonia: clinical perspectives. *Ther Clin Risk Manag.* (2019) 15:397–403. doi: 10.2147/TCRM.S157882

168. Khoury P, Zagallo P, Talar-Williams C, Santos C, Dinerman E, Holland N, et al. Serum biomarkers are similar in Churg-Strauss syndrome and hypereosinophilic syndrome. *Allergy.* (2012) 67:1149–56. doi: 10.1111/j.1398-9995.2012.02873.x

169. Shomali W, Gotlib J. World Health Organization-defined eosinophilic disorders: 2022 update on diagnosis, risk stratification, and management. *Am J Hematol.* (2022) 97:129–48. doi: 10.1002/ajh.26352

170. Groh M, Kahn J, Puechal X, Guillevin L. Comment on: first report of FIP1L1-PDGFRalpha-positive eosinophilic granulomatosis with polyangiitis. *Rheumatology.* (2016) 55:384–5. doi: 10.1093/rheumatology/kev425

171. Leru P. Eosinophilic disorders: evaluation of current classification and diagnostic criteria, proposal of a practical diagnostic algorithm. *Clin Transl Allergy.* (2019) 9:36. doi: 10.1186/s13601-019-0277-4

172. Curtis C, Ogbogu P. Hypereosinophilic syndrome. *Clin Rev Allergy Immunol.* (2016) 50:240–51. doi: 10.1007/s12016-015-8506-7

173. Wei X, Li X, Wei Z, Zhang H, Deng J, Xing S, et al. Clinical analysis of hypereosinophilic syndrome first presenting with asthma-like symptoms. *Ann Med.* (2022) 54:11–21. doi: 10.1080/07853890.2021.2014555

174. Lefevre G, Leurs A, Gibier J, Copin M, Staumont-Salle D, Dezoteux F, et al. "Idiopathic eosinophilic vasculitis": Another side of hypereosinophilic syndrome? A comprehensive analysis of 117 cases in asthma-free patients. *J Allergy Clin Immunol Pract.* (2020) 8:1329–40.e3. doi: 10.1016/j.jaip.2019.12.011

175. Leurs A, Chenivesse C, Lopez B, Gibier J, Clement G, Groh M, et al. C-reactive protein as a diagnostic tool in differential diagnosis of hypereosinophilic syndrome and ANCA-negative eosinophilic granulomatosis with polyangiitis. *J Allergy Clin Immunol Pract.* (2019) 7:1347–51.e3. doi: 10.1016/j.jaip.2018.10.002

176. Ahn, S, Yoo J, Park Y, Park J, Lee J, Lee S. A new index for distinguishing hypereosinophilic syndrome and antineutrophil cytoplasmic antibody-negative eosinophilic granulomatosis with polyangiitis. *Asian Pac J Allergy Immunol.* (2020). doi: 10.12932/AP-080420-0805

177. Takahashi H, Komai T, Setoguchi K, Shoda H. A diagnostic score for eosinophilic granulomatosis with polyangiitis among eosinophilic disorders. *Allergol Int.* (2023) 72:316–23. doi: 10.1016/j.alit.2022.08.008

178. Puechal X. Granulomatosis with polyangiitis (Wegener's). *Joint Bone Spine.* (2020) 87:572–8. doi: 10.1016/j.jbspin.2020.06.005

179. Greco A, De Virgilio A, Rizzo M, Gallo A, Magliulo G, Fusconi M, et al. Microscopic polyangiitis: advances in diagnostic and therapeutic approaches. *Autoimmun Rev.* (2015) 14:837–44. doi: 10.1016/j.autrev.2015.05.005

180. Nguyen Y, Pagnoux C, Karras A, Quemeneur T, Maurier F, Hamidou M, et al. Microscopic polyangiitis: clinical characteristics amd long-term outcomes of 378 patients from the French Vasculitis Study Group Registry. *J Autoimmun.* (2020) 112:102467. doi: 10.1016/j.aut.2020.102467

181. Hernandez-Rodriguez J, Alba M, Prieto-Gonales S, Cid M. Diagnosis and classification of polyarteritis nodosa. *J Autoimmun.* (2014) 48-49:84–9. doi: 10.1016/j.jaut.2014.01.029

182. Morales, A, Cignarella A, Jabeen I, Barkin J, Mirsaeidi M. An update on IgG4-related lung disease. *Eur J Int Med.* (2019) 66:18–24. doi: 10.16/j.ejim.2019.06.010

183. Simo-Perdigo M, Martinez-Valle F. IgG4-related disease. *Rev Esp Med Nucl Imagen Mol. (Engl Ed).* (2021) 40:107–14. doi: 10.1016/j.remn.2020.12.001

184. Chang H, Chou P, Lai C, Tsai H. Antineutrophil cytoplasmic antibodies and organ-specific manifestations on eosinophilic granulomatosis with polyangiitis: a systematic review and meta-analysis. *J Allergy Clin Immunol Pract.* (2021) 9:445–52. doi: 10.1016/j.jaip.2020.07.038

185. Liu S, Han L, Liu Y, Yang J, Zhang Y, Li M, et al. Clinical significance of MPO-ANCA in eosinophilic granulomatosis with polyangiitis: experience from a longitudinal Chinese cohort. *Front Immunol.* (2022) 13:885198. doi: 10.3389/fimmu.2022.885198

186. Papo M, Sinico R, Teixeira V, Venhoff N, Urban M, Iudici M, et al. Significance of PR3-ANCA positivity in eosinophilic granulomatosis with polyangiitis (Churg-Strauss). *Rheumatology.* (2020) 60:4355–60. doi: 10.1093/rheumatology/keaa805

187. Terrier B, Darbon R, Durel C, Hachulla E, Karras A, Maillard H, et al. French recommendations for the management of systemic necrotizing vasculitides (polyarteritis nodosa and ANCA-associated vasculitides). *Orphanet J Rare Dis.* (2020) 15:351. doi: 10.1186/s13023-020-01621-3

188. Goglin S, Chung S. New developments in treatments for systemic vasculitis. *Cur Opin Pharmacol.* (2022) 66:102270. doi: 10.116/j.coph.2022.102270

189. Asano K, Suzuki Y, Tanaka J, Kobayashi K, Kamide Y. Treatments of refractory eosinophilic lung diseases with biologics. *Allergol Int.* (2023) 72:31–40. doi: 10.1016/j.alit.2022.10.004

190. Raffray L, Guillevin L. Updated for the treatment of EGPA. *Presse Med.* (2020) 49:104036. doi: 10.1016/j.lpm.2020.104036

191. Ribi C, Cohen P, Pagnoux C, Mahr A, Arene A, Lauque J, et al. Treatment of Churg-Strauss syndrome without poor-prognosis factors: a multicenter, prospective, randomized, open – label study of seventy-two patients. *Arthritis Rheum.* (2008) 58:586–94. doi: 10.1002/art.23198

192. Mukhtyar C, Guillevin L, Cid M, Dasgupta B, de Groot K, Gross W, et al. EULAR recommendations for the management of primary small and medium vessel vasculitis. *Ann Rheum Dis.* (2009) 68:310–7. doi: 10.1136/ard.2008.088096

193. Puechal X, Pagnoux C, Baron G, Quemeneur T, Neel A, Agard C, et al. Adding azathioprine to remission-induction glucocorticoids for eosinophilic granulomatosis with polyangiitis (Churg-Strauss), microscopic polyangiitis, or polyarteritis nodosa without poor prognosis factors: a randomized, controlled trial. *Arthritis Rheumathol.* (2017) 69:2175–86. doi: 10.1002/art.40205

194. Chung S, Langford C, Maz M, Abril A, Gorelik M, Guyatt G, et al. 2021 American college of rheumatology/vasculitis foundation guidelines for the management of antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheumatol.* (2021) 0:1–18. doi: 10.1002/art.41773

195. Kim S, Marigowda G, Oren E, Israel E, Wechsler M. Mepolizumab as a steroid – sparing treatment option in patients with Churg-Strauss syndrome. *J Allergy Clin Immunol.* (2010) 125:1336–43. doi: 10.1016/j.jaci.2010.03.028

196. Moosig F, Gross W, Hermann K, Bremer J, Hellmich B. Targeting interleukin-5 in refractory and relapsing Churg-Strauss syndrome. *Ann Intern Med.* (2011) 155:341–3. doi: 10.7326/0003-4819-155-5-201109060-00026

197. Steinfeld J, Bradford E, Brown J, Mallett S, Yancey S, Akuthota P, et al. Evaluation of clinical benefit from treatment with mepolizumab for patients with eosinophilic granulomatosis with polyangiitis. *J Allergy Clin Immunol.* (2019) 143:2170–7. doi: 10.1016/j.jaci.2018.11.041

198. Uzzo M, Regola F, Trezzi B, Toniati P, Franceschini F, Sinico R. Novel targets for drug use in eosinophilic granulomatosis with polyangiitis. *Front Med.* (2021) 8:754434. doi: 10.3389/fmed.2021.754434

199. Khoury P, Akuthota P, Baylis L, Chang S, Bentley J, Wechsler M. Impact of baseline treatment, duration of disease, and refractory status on outcomes in mepolizumab-treated patients with EGPA. *J Allergy Clin Immunol.* (2022) 149(Suppl.):AB52.

200. Terrier B, Charles P, Aumaitre O, Belot A, Bonnotte B, Crabol Y, et al. ANCA-associated vasculitides: recommendations of the French Vasculitis Study Group on the use of immunosuppressants and biotherapies for remission induction and maintenance. *Presse Med.* (2020) 49:104031. doi: 10.1016/j.Jpm.2020.104031

201. Detoraki A, Tremante E, Poto R, Morelli E, Quaremba G, Granata F, et al. Real-life evidence of low-dose mepolizumab efficacy in EGPA: a case series. *Respir Res.* (2021) 22:185. doi: 10.1186/s12931-021-01775-z

202. Vultaggio A, Nencini F, Bormioli S, Vivarelli E, Dies L, Rossi O, et al. Low-dose mepolizumab effectiveness in patients suffering from eosinophilic granulomatosis with polyangiitis. *Allergy Asthma Immunol Res.* (2020) 12:885–93. doi: 10.4168/aair.2020.12.5.885

203. Özdel Öztürk B, Yavuz Z, Aydin Ö, Mungan D, Sin B, Demirel Y, et al. Effectiveness of low-dose mepolizumab in the treatment of eosinophilic granulomatosis with polyangiitis (EGPA): a real-life experience. *Int Arch Allergy Immunol.* (2022) 183:1280–90. doi: 10.1159/000526410

204. Bettiol A, Urban M, Dagna L, Cottin V, Franceschini F, Del Giacco S, et al. Mepolizumab for eosinophilic granulomatosis with polyangiitis: a European multicenter observational study. *Arthritis Rheumatol.* (2022) 74:295–306. doi: 10.1002/art.41943

205. Ueno M, Miyagawa I, Nakano K, Iwata S, Hanami K, Fukuyo S, et al. Effectiveness and safety of mepolizumab in combination with corticosteroids in patients with eosinophilic granulomatosis with polyangiitis. *Arthritis Res Ther.* (2021) 23:86. doi: 10.1186/s13075-021-02462-6

206. Manka L, Guntur V, Denson J, Dunn R, Dollin Y, Strand M, et al. Efficacy and safety of reslizumab in the treatment of eosinophilic granulomatosis with polyangiitis. *Ann Allergy Asthma Immunol.* (2021) 126:696–701. doi: 10.1016/j.anai.2021.01.035

207. Guntur V, Manka L, Denson J, Dunn R, Dollin Y, Gill M, et al. Benralizumab as a steroid-sparing treatment option in eosinophilic granulomatosis with polyangiitis. *J Allergy Clin Immunol Pract.* (2021) 9:1186–93.e1. doi: 10.1016/j.jaip.2020.09.054

208. Steinke J, Borish L. Th2 cytokines and asthma. Interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. *Respir Res.* (2001) 2:66–70. doi: 10.1186/rr40

209. Castro M, Corren J, Pavord I, Maspero J, Wenzell S, Rabe K, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. *N Engl J Med.* (2018) 378:2486–96. doi: 10.1056/NEJMoa1804092

210. Wechsler M, Ford L, Maspero J, Pavord I, Papi A, Bourdin A, et al. Long-term safety and efficacy of dupilumab in patients with moderate-to-severe asthma (TRAVERSE): an open-label extension study. *Lancet Respir Med.* (2022) 10:11–25. doi: 10.1016/S2213-2600(21)00322-2

211. Wechsler M, Ruddy M, Pavord I, Israel E, Rabe K, Ford L, et al. Efficacy and safety of itepekimab in patients with moderate-to-severe asthma. *N Engl J Med.* (2021) 385:1656–68. doi: 10.1056/NEJMoa2024257

212. Menzies-Gow A, Colice G, Griffiths J, Almqvist G, Ponnarambil S, Kaur P, et al. NAVIGATOR: a phase 3 multicentre, randomized, double-blind, placebo-controlled, paralel-group trial to evaluate the efficacy and safety of tezepelumab in adults and adolescents with severe, uncontrolled asthma. *Respir Res.* (2020) 21:266. doi: 10.1186/s12931-020-01526-6

213. Siddiqui S, Bozik M, Archibald D, Dworetzky S, Mather J, Killingsworth R, et al. Late breaking abstract – phase 2 trial evaluating the effects of dexpramipexole on blood eosinophils, lung function, and airway biomarkers in eosinophilic asthma. *Eur Respir J.* (2021) 58(Suppl. 65):RCT2900. doi: 10.1183/13993003

214. Panch S, Bozik M, Brown T, Makiya M, Prussin C, Archibald D, et al. Dexpramipexole as an oral steroid-sparing agent in hypereosinophilic syndromes. *Blood.* (2018) 132:501–9. doi: 10.1182/blood-2018-02-835330

215. Jones R, Tervaert J, Hauser T, Luqmani R, Morgan M, Peh C, et al. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med.* (2010) 363:211–20. doi: 10.1056/NEJMoa0909169

216. Terrier B, Pagnoux C, Perrodeau E, Karras A, Khouatra C, Aumaitre O, et al. Long-term efficacy of remission – maintenance regimens for ANCA-associated vasculitides. *Ann Rheum Dis.* (2018) 77:1150–6. doi: 10.1136/annrheumdis-2017-212768

217. Mohammad A, Hot A, Arndt F, Moosig F, Guerry M, Amudala N, et al. Rituximab for the treatment of eosinophilic granulomatosis with polyangiitis (Churg-Strauss). *Ann Rheum Dis.* (2016) 75:396–401. doi: 10.1136/annrheumdis-2014-206095

218. Teixeira V, Mohammad A, Jones R, Smith R, Jayne D. Efficacy and safety of rituximab in the treatment of eosinophilic granulomatosis with polyangiitis. *RMD Open.* (2019) 5:e000905. doi: 10.1136/rmdopen-2019-000905

219. Canzian A, Venhoff N, Urban M, Sartorelli S, Ruppert A, Groh M, et al. Use of biologics to treat relapsing and/or refractory eosinophilic granulomatosis with polyangiitis: data from a European Collaborative Study. *Arthritis Rheumatol.* (2021) 73:498–503. doi: 10.1002/art.41534

220. Terrier, B, Pugnet G, de Moreuil C, Bonnotte B, Benhamou Y, Diot E, et al. Rituximab versus conventional therapeutic strategy for remission induction in eosinophilic granulomatosis with polyangiitis: a double-blind, randomized, controlled trial. *Arthritis Rheumatol.* (2021) 73 (Suppl. 9). Available online at: https://acrabstracts.org/abstract/rituximab-versus-conventional-therapeutic-strategy-for-remission-induction-in-eosinophilic-granulomatosis-with-polyangiitis-a-double-blind-randomized-controlled-trial/

221. Cheungpasitporn W, Kopecky S, Specks U, Bharucha K, Fervenza C. Non-ischemic cardiomyopathy after rituximab treatment for membranous nephropathy. *J Renal Inj Prev.* (2017) 6:18–25. doi: 10.15171/jrip.2017.04

222. Pepper R, Fabre M, Pavesio C, Gaskin G, Jones R, Jayne D, et al. Rituximab is effective in the treatment of refractory Churg-Strauss syndrome and is associated with diminished T-cell interleukin-5 production. *Rheumatology.* (2008) 47:1104–5. doi: 10.1093/rheumatology/ken175

223. Moura M, Berti A, Keogh K, Volcheck G, Specks U, Baqir M. Asthma control in eosinophilic granulomatosis with polyangiitis treated with rituximab. *Clin Rheumatol.* (2020) 39:1581–90. doi: 10.1007/s10067-019-04891-w

224. Higashitani K, Yoshimi R, Sato Y, Watanabe T, Ihata A. Rituximab and mepolizumab combination therapy for glucocorticoid-resistant myocarditis related to eosinophilic granulomatosis with polyangiitis. *Mod Rheumatol Case Rep.* (2022) 6:87–92. doi: 10.1093/mrcr/rxab022

225. Afiari A, Gabriel A, Gaiki M. Concurrent use of mepolizumab and rituximab for eosinophilic granulomatosis with polyangiitis and multisystem involvement. *Cureus.* (2020) 12:e9242. doi: 10.7759/cureus.9242

226. Bettiol A, Urban M, Bello F, Fiori D, Mattioli I, Lopalco G, et al. Sequential rituximab and mepolizumab in eosinophilic granulomatosis with polyangiitis (EGPA): a European multicenter observational study. *Ann Rheum Dis.* (2022) 81:1769–72. doi: 10.1136/annrheumdis-2022-222776

227. Basta F, Mazzuca C, Nucera E, Schiavino D, Afeltra A, Incalzi R. Omalizumab in eosinophilic granulomatosis with polyangiitis: friend or foe? A systematic literature review. *Clin Exp Rheumatol.* (2020) 38(Suppl 124):S214–20.

228. Celebi Sozener Z, Gorgulu B, Mungan D, Sin B, Misirligil Z, Aydin O, et al. Omalizumab in the treatment of eosinophilic granulomatosis with polyangiitis (EGPA): single-center experience in 18 cases. *World Allergy Organ J.* (2018) 11:39. doi: 10.1186/s40413-018-0217-0

229. Iglesias E, Camacho Lovillo M, Delgado Pecellin I, Lirola Cruz M, Falcon Neyra M, Salazar Quero J, et al. Successful management of Churg-Strauss syndrome using omalizumab as adjuvant immunomodulatory therapy: first documented pediatric case. *Pediatr Pulmonol.* (2014) 49:E78–81. doi: 10.1002/ppul.22884

230. Nazir S, Tachamo N, Fareedy S, Khan M, Lohani S. Omalizumab – associated eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome). *Ann Allergy Asthma Immunol.* (2017) 118:372–4. doi: 10.1016/j.anai.2016.12.003

231. Metzler C, Schnabel A, Gross W, Hellmich B. A phase II study of interferon-alpha for the treatment of refractory Churg-Strauss syndrome. *Clin Exp Rheumatol.* (2008) 26(Suppl. 49):S35–40.

232. Koike H, Akiyama K, Saito T, Sobue G, Research Group for IVIg for Egpa/Css in Japan. Intravenous immunoglobulin for chronic residua peripheral neuropathy in eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome): a multicenter, double-blind trial. *J Neurol.* (2015) 262:757–9. doi: 10.1007/s00415-014-7618-y

233. Pecoraro A, Crescenzi L, Carucci L, Genovese A, Spadaro G. Heart failure not responsive to standard immunosuppressive therapy is successfully treated with high dose intravenous immunoglobulin therapy in a patient with eosinophilic granulomatosis with polyangiitis (EGPA). *Int Immunopharmacol.* (2017) 45:13–5. doi: 10.1016/j.intimp.2017.01.025

234. Walsh M, Merkel P, Peh C, Szpirt W, Puechal X, Fujimoto S, et al. Plasma exchange and glucocorticoids in severe ANCA-associated vasculitis. *N Engl J Med.* (2020) 382:622–31. doi: 10.1056/NEJMoa1803537

235. Guillevin L, Lhote E, Cohen P, Jarrousse B, Lortholary O, Genereau T, et al. Corticosteroids plus pulse cyclophosphamide and plasma exchanges versus corticosteroids plus pulse cyclophosphamide alone in the treatment of polyarteritis nodosa and Churg-Strauss syndrome patients with factors predicting poor prognosis. A prospective, randomized trial in sixty-two patients. *Arthritis Rheum.* (1995) 38:1638–45. doi: 10.1002/art.1780381116

236. Global Initiative for Asthma [GINA]. *2022 GINA Report: Global Strategy for Asthma Management and Prevention*. (n.d.). Available online at: https://ginasthma.org/wp-content/uploads/2022/07/GINA-Main-Report-2022-FINAL-22-07-01-WMS.pdf (accessed July 7, 2022).

237. Baldwin C, Wolter N, Pagnoux C. Ear, nose, and throat involvement in eosinophilic granulomatosis with polyangiitis. *Adv Cell Mol Otolaryngol.* (2015) 3:2781. doi: 10.3402/acmo.v3.27181

238. Padoan R, Campaniello D, Felicetti M, Cazzador D, Schiavon F. Ear, nose, and throat in ANCA-associated vasculitis: a comprehensive review. *Vessel Plus.* (2021) 5:41. doi: 10.20517/2574-1209.2021.41

239. Han J, Bachert C, Fokkens W, Desrosiers M, Wagenmann M, Lee S, et al. Mepolizumab for chronic rhinosinusitis with nasal polyps (SYNAPSE): a randomized, double-blind, placebo-controlled, phase 3 trial. *Lancet Respir Med.* (2021) 9:1141–53. doi: 10.1016/S2213-2600(21)00097-7

240. Pendolino A, Unadkat S, Zhang H, Pendolino M, Bianchi G, Randhawa P, et al. The role of surgery in antineutrophil cytoplasmic antibody-associated vasculitides affecting the nose and sinuses: a systematic review. *SAGE Open Med.* (2020) 8:1–12. doi: 10.1177/2050312120936731

241. Doubelt I, Cuthbertson D, Carette S, Chung SA, Forbess LJ, Khalidi NA, et al. Clinical manifestations and long-term outcomes of eosinophilic granulomatosis with polyangiitis in North America. *ACR Open Rheumatol.* (2021) 3:404–12. doi: 10.1002/acr2.11263

242. Kim D, Song J, Park Y, Lee S. Five factor score of more than 1 is associated with relapse during the first 2 year – follow up in patients with eosinophilic granulomatosis with polyangiitis. *Int J Rheum Dis.* (2017) 20:1261–8. doi: 10.1111/1756-185X.13056

243. Alberici F, Bonatti F, Adorni A, Daminelli G, Sinico R, Gregorini G, et al. FCGR3B polymorphism predicts relapse risk in eosinophilic granulomatosis with polyangiitis. *Rheumatology.* (2020) 59:3563–6. doi: 10.1093/rheumatology/keaa134

244. Keogh K, Specks U. Churg-Strauss syndrome: clinical presentation, antineutrophil cytoplasmic antibodies, and leukotriene receptor antagonists. *Am J Med.* (2003) 115:284–90. doi: 10.1016/s0002-9343(03)00359-0

245. King C, Harper L, Little M. The complications of vasculitis and its treatment. *Best Pract Res Clin Rheumatol.* (2018) 32:125–36. doi: 10.1016/j.berh.2018.07.009

246. Puechal X, Pagnoux C, Baron G, Lifermann F, Geffray L, Quemeneur T, et al. Non-severe eosinophilic granulomatosis with polyangiitis: long-term outcomes after remission-induction trial. *Rheumatology.* (2019) 58:2107–16. doi: 10.1093/rheumatology/kez139

247. Bell C, Blauer-Peterson C, Mao J. Burden of illnes and costs associated with eosinophilic granulomatosis with polyangiitis: evidence from a managed care database in the United States. *J Manag Care Spec Pharm.* (2021) 27:1249–59. doi: 10.18553/jmcp.2021.21002

Frontiers in Medicine

Translating medical research and innovation into improved patient care

A multidisciplinary journal which advances our medical knowledge. It supports the translation of scientific advances into new therapies and diagnostic tools that will improve patient care.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact