PROTECTING THE ACUTELY INJURED LUNG: PHYSIOLOGIC, MECHANICAL, INFLAMMATORY, AND TRANSLATIONAL PERSPECTIVES

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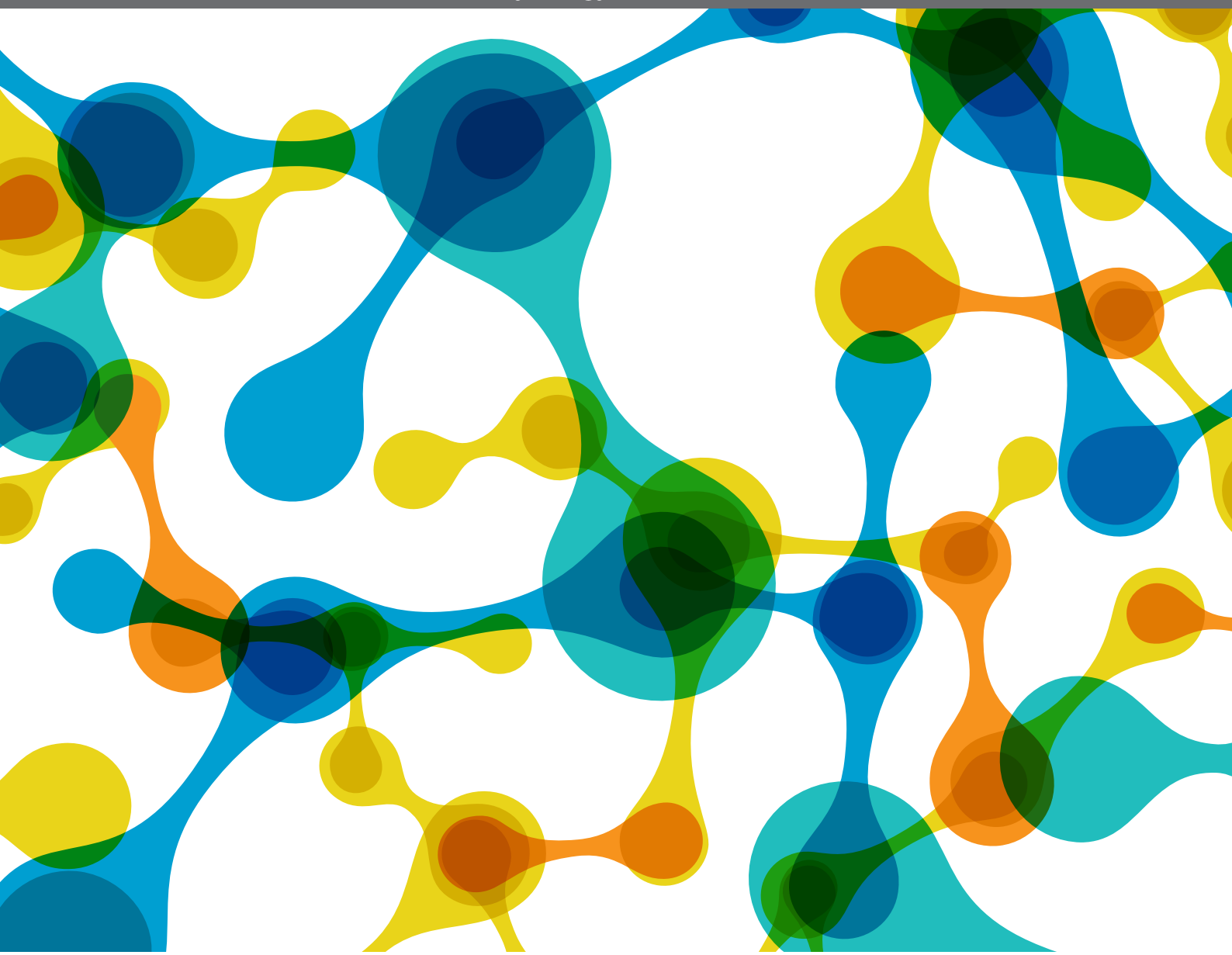

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PROTECTING THE ACUTELY INJURED LUNG: PHYSIOLOGIC, MECHANICAL, INFLAMMATORY, AND TRANSLATIONAL PERSPECTIVES

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Editorial: Protecting the acutely injured lung: Physiologic, mechanical, inflammatory, and translational perspectives

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Editorial on the Research Topic
Protecting the Acutely Injured Lung: Physiologic, Mechanical, Inflammatory, and Translational Perspectives

Introduction

Our Research Topic broadly reviewed multiple facets of treating the critically ill patient with acute lung injury (ALI) (Figure 1). There were nine papers discussing various aspects of protecting the acutely injured lung from ventilator induced lung injury (VILI), three papers covered inflammatory characteristics, three treatment strategy papers including veno-venous extracorporeal membrane oxygenation (vv- ECMO), gene therapy, and the physiology associated with successful and unsuccessful clinical trials on patients with ALI. Lastly, there was one paper reviewing lung fluid balance in the normal and acutely injured lung.

Protective mechanical ventilation

Acute respiratory distress syndrome (ARDS) was first identified in 1967 but remains a significant medical problem today (Ashbaugh, Bigelow et al., 1967). Fifty-five years later, the only treatments for ARDS are supportive, mainly in the form of mechanical ventilation (MV) (Matthay, Zemans et al., 2019). Unfortunately, inappropriately set MV can inadvertently cause VILI, significantly increasing ARDS-related mortality (Del Sorbo, Goligher et al., 2017) and

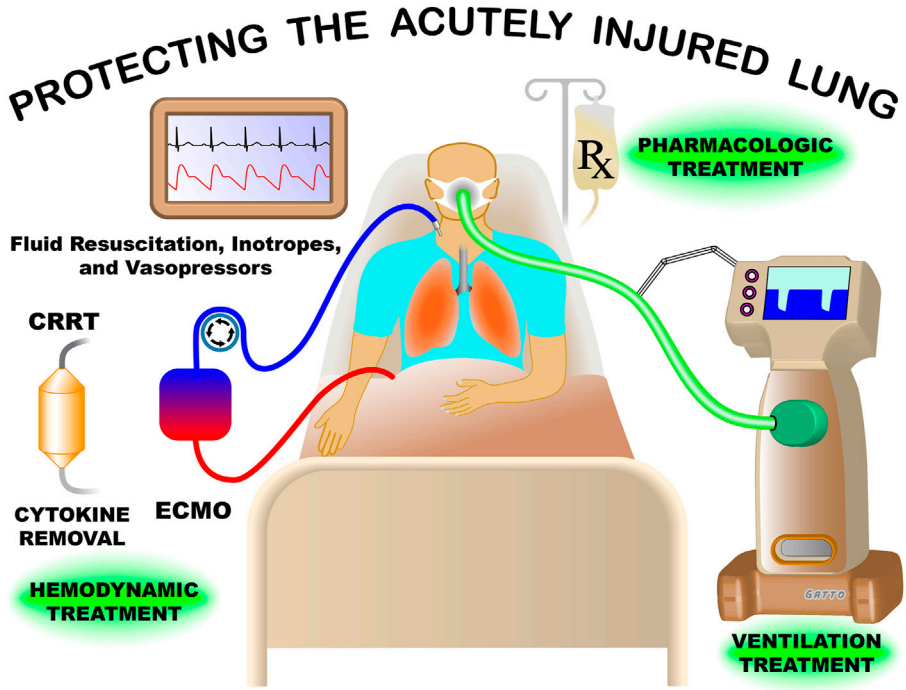

FIGURE 1
Treatment of the patient with acute lung injury (ALI) in the modern intensive care unit is extremely complex. Mechanical ventilation must be adjusted to maintain life supporting oxygenation and ventilation without causing an unintended ventilator induced lung injury (VILI). The impact of ventilation pressures must also be weight against the possible negative effect on hemodynamics including arterial blood pressure and cardiac output, which are supported with fluid resuscitation and vasoactive agents/inotropes. Currently, no pharmacologic treatments are available for the ALI patient, but development of drugs and gene therapy designed to reduce systemic inflammation, preserve air-blood barrier integrity, and remove edema from alveoli continues. Continuous renal replacement therapy (CRRT) can be used for acute kidney failure and can be combined with novel strategies to remove inflammatory cytokines and other mediator from the blood. Great advancements have been made in treating the ALI patients but much more work needs to be done, with the hope of significant breakthroughs in the near future.

the quest for a ventilator mode and method to minimize VILI is still being intensely studied. (Pelosi, Ball et al., 2021).

Rola and Daxon conducted a case series on COVID-19 induced ARDS (CARDS) patients. They showed that the time controlled adaptive ventilation (TCAV™) method to set and adjust the airway pressure release ventilation (APRV) mode was highly effective at stabilizing and then gradually reopening the collapsed and edema filled lungs of CARDS patients. The TCAV™ method [developed by Nader M. Habashi](Habashi 2005) is both personalized and adaptive and is the most studied method to set and adjust APRV (Nieman et al.). TCAV uses inspiratory and expiratory time to rapidly stabilize and then gradually open collapsed tissue over hours or days (Kollisch-Singule et al.). They showed the efficacy of this *Stabilize Lung Approach (SLA)* approach in CARDS patients using chest X-rays, ventilator parameters, and arterial blood gases (ABGs). They also discussed physiologic mechanisms for TCAV efficacy, fine points of TCAV settings, and complexities of training an entire intensive care unit (ICU) team on TCAV™ use. Lastly, they discussed the problem of the "apparent cure" when TCAV™ has a patient's lung fully recruited with near normal ABGs and respiratory system compliance (C_{RS}) such that they no longer meet the Berlin defined ARDS. If these patients are converted back to conventional mechanical ventilation (CMV) the lungs may rapidly re-collapse. (Nieman, Andrews et al., 2018). They presented a case in which a CARDS patient with a TCAV™-induced open lung ("apparent cure") was converted back to CMV and the lungs quickly derecruited.

APRV is controversial due to many publications discussing the harmful components of the mode without scientific support. Andrews et al. published an extensive review of 10 APRV Myths and Misconceptions, which are debunked using significant supporting scientific literature. Some of the discredited myths discussed include APRV can cause barotrauma, generates high tidal volumes, and creates unsafe auto-PEEP. The paper closes with a discussion on misconceptions dealing with APRV clinical trials. By exposing the truth concerning the physiologic impact of APRV, clinicians may better understand the mode leading to more effective use and improved patient care.

Suarez-Sipmann et al. used expired CO_2 kinetics to personalize lung protective ventilation for ARDS patients. Volumetric capnography (Vcap) that represents the volume of expired CO_2 in one signal breath can inform clinicians about pulmonary

perfusion, end-expiratory lung volume, dead space and ventilation inhomogeneities. Recent work has shown that Vcap can possibly be used to continuously measure end-expiratory lung volume (EELV), lung strain, and effective pulmonary blood flow. The ability to measure all of the above at the bedside would be an incredible tool in the clinicians' treatment options. Using these parameters, the clinician can modify the ventilator mode and the method necessary to maximize EELV and minimize lung strain that is individualized to each patient. With future development Vcap might be able to optimize lung protective ventilation strategies and reduce VILI-related mortality.

Most protective ventilation research efforts use CMV modes adjusting tidal volume (V_T) and positive end expiratory pressure (PEEP) with and without recruitment maneuvers (RM), in an attempt to reduce VILI. Ismaiel et al. used CMV in a rat ARDS model to differentiate the pathophysiologic impact of mechanical and inflammatory injury as VILI mechanisms. They showed that low tidal volume (LV_T) strategy reduces VILI by limiting mechanical damage, while hypercapnia limits pro-inflammatory and biochemical mechanisms of injury. They close by suggesting that LV_T combined with hypercapnia may work synergistically to reduce VILI.

A review of protective MV for the pediatric ARDS (PARDS) patient by Kollisch-Singule et al. discussed both traditional and novel modes of MV including APRV, high frequency oscillatory ventilation (HFOV), high frequency percussive ventilation (HFPV), and high frequency jet ventilation (HFJV). Unfortunately, results of this review show no consistent outcomes among modes, nor does it identify an optimal ventilator mode or method for the PARDS patient. They concluded that only high quality randomized controlled trials (RCTs) would be able identify if an optimal ventilation strategy does exist for the PARDS patient but caution that these trials are very difficult to conduct.

An extensive review of the HFOV mode was conducted by Miller et al. The review covers HFOV theory of operation, mechanics, and characteristics of all ventilators that can deliver the mode. Evidence of HFOV efficacy from bench models, animal studies and in both adult and pediatric patients are reviewed in detail. Although the physiologic rationale for HFOV is sound with many positive animal studies, the mode has lost popularity with both adult or pediatric patients following the failed 2013 OSCAR and OSCILLATE clinical HFOV RCTs (Ferguson, Cook et al., 2013; Young, Lamb et al., 2013). Possible reasons for the poor outcomes are discussed. There is continued study using HFOV in pediatric patients but the current RCTs are low quality. Since the mode is difficult to use it was suggested that staff education and competency are critical in future study.

In a crossover clinical trial in 20 patients with mild to moderate ARDS, Ball et al. studied the impact of multiple levels of variable pressure support ventilation (vPSV) on short term lung function. It has been shown that vPSV, compared with PSV, can improve oxygenation and patient-ventilator synchrony, but these studies were conducted using only one variability level at a fixed pressure support. In this study the addition of multiple levels of variability did not improve oxygenation and high variability levels increased patient-ventilator synchrony.

Accurate measurements of lung compliance (C_L) and driving pressure (ΔP) are critical when managing ventilator settings on patients with ARDS, which are often measured clinically using plateau pressure (Pplat). Although this 'stop flow' condition is valuable and universally accepted, it may underestimate the maximum stress that occurs in lung tissue under dynamic conditions. Tawfik et al. compared the static measurement of C_L and ΔP with another static method and two dynamic compliance measurement methods. The other static method to calculated C_L and ΔP the pressure at zero flow and the two dynamic methods used the inspiratory slope during inflation with a constant flow and the expiratory time constant method. They found that the static measurement using Pplat may underestimate the maximum pressure exposed to lung tissue during dynamic inflation. Whereas the static method using zero flow and the dynamic method using inspiratory slope gave a truer estimate of maximum pressure exposed to alveoli. This pilot study suggests further studies are necessary to identify the optimal method to measure C_L and ΔP necessary to improve patient outcomes.

Mechanical ventilation for patients with ALI can lead to ventilator induced diaphragmatic dysfunction (VIDD) (Vassilakopoulos and Petrof 2004). Currently, ultrasound and invasive phrenic nerve stimulation are the only bedside tools available to monitor diaphragm function and VIDD. Spadaro et al. measured fast and slow isoform of troponin I (fsTnL and ssTnl, respectively), which are specific markers of skeletal muscle damage. The goal was to identify the trend of skeletal troponin during weaning and compare it with fsTnL and ssTnl levels with diaphragmatic ultrasound in healthy volunteers. They found that fsTnL and ssTnl have specific and different trends during weaning with the fsTnL decreasing during the early phase of weaning, while high initial values of ssTnl were correlated with a larger diaphragmatic displacement over time. More work is necessary to identify if these markers can be used to identify diaphragm function in ALI patients.

Inflammation and biotrauma

There are three major mechanisms of VILI: Atelectrauma (alveolar recruitment/derecruitment–R/D), volutrauma (alveolar overdistension), and biotrauma (excessive release of inflammatory mediators caused by atelectrauma and volutrauma). Dysregulation of the renin-angiotensin system (RAS) is associated with both the development of ARDS and a known mechanism of VILI (Wang, Chai et al., 2019). Krenn et al. reviewed the impact of mechanical ventilation on RAS in the patients with ARDS with the intent of discovering novel

biomarkers and possible therapeutic targets (Krenn et al.). Many clinical trials have been conducted using RAS-modifying drugs in mechanically ventilated ARDS patients and patients with other medical issues, that resulted in positive outcomes. These positive studies suggest RAS-modifying drugs maybe effective treatment of both the primary cause of ARDS as well as the secondary VILI that is associated with increased ARDS-related mortality.

ARDS can cause a massive release of inflammatory mediators often referred to as the "cytokine storm". This hyperinflammatory response can lead to increase pulmonary microvascular permeability resulting in alveolar flooding with edema, a hallmark of ARDS lung pathology. There is growing evidence that cell-based therapies (mesenchymal stem cells) have therapeutic efficacy for ARDS (Lopes-Pacheco, Robba et al., 2020). Izrael et al. tested the treatment effect of human astrocytes therapy (AstroRx) in an endotoxin mouse ARDS model (Izrael et al.). They showed a significant reduction in multiple inflammatory mediators, improved lung histopathology and reduced mortality in AstroRx treated mice. This study demonstrated the immunosuppressive capacity of AstroRx cells and suggest an innovative ARDS treatment. In addition, this group is currently evaluating the therapeutic role of AstroRx in amyotrophic lateral sclerosis patients.

Impaired alveolar macrophage (AM) efferocytosis plays a role in ARDS pathogenesis, (Huang, Xiu et al., 2018), however, the ability to test AMs from ARDS patients is limited. Mahida et al. developed and *in vitro* model to assess the impact of ARDS on AMs (Mahida et al.). Normal AMs were harvested from lobectomy patients and then treated with bronchoalveolar lavage fluid (BALF) collected from ARDS patients. They found that ARDS BALF decreased AMs efferocytosis and Rac1 gene expression, but phagocytosis was not impaired. These findings are similar to that found in AMs from ARDS patients suggesting that this is an effective *in vitro* model to study the impact of ARDS on AMs. They also found that Rho-associated kinase partially resorted AM efferocytosis.

Treatment strategies

Severe ARDS can lead to respiratory failure as well as hemodynamic collapse, caused by right ventricular (RV) failure. Clinical options at this point include extracorporeal membrane oxygenation (ECMO) giving the lung more time to heal. Petit et al. reviewed the pathophysiology of RV function in ARDS and some potential treatments to improve function including ventilator adjustments, prone positioning, nitric oxide (NO) inhalation, and veno-venous ECMO (VV-ECMO) (Petit et al.). The impact of ventilator settings on RV failure and acute cor pulmonale was reviewed extensively. It was concluded that ventilator settings should be adjusted to a Pplat <27cmH_2O, inspiratory rate increased to reduce $PaCO_2$, limit PEEP level, and optimize O_2 delivery not the P/F ratio. They conclude that the RV protective approach should be evaluated in a future RCT with ECMO considered in extreme failure. Integrating the findings from this study to those by Andrews et al. and Rola and Daxon, pulmonary vascular resistance (PVR) is minimal at normal EELV (i.e., functional residual capacity) (Simmons et al., 1961). Thus, an important protective mechanism of the TCAV™ method may be to restore normal EELV, reduce PVR, which would improve RV function by decreasing afterload.

Few pharmacological approaches to treat ARDS are available. Liu and Dean reviewed the possible use of gene therapy that offers a highly controlled and targeted strategy to treat acute lung injury at the molecular level (Liu and Dean). Topics include delivery stategy, classes of targeted genes, and outcomes on ARDS pathogensis and resolution. They conclude that no combination of genes is responsible for ARDS, although several genes have been targeted up- and downregulation of genes with varying degrees of success. Early studies focused on increasing the expression of ion channels and tranporters to accelerate alveolar edema removal and have had minimal success. Current strategies targeting a reduced inflammatory response and repair or strengthening the alveolar-capillary barrier have shown more promise. Greater emphasis should be placed on studies using more clinically applicable large animal models designed to treat existing disease. The major limitation of effective gene therapy remains optimizing the gene delivery system.

Ultimately, treatment strategies for ALI and ARDS must be tested in RCTs to identify clinical efficacy. Villar et al. reviewed the physiologic-based gaps in 14 negative and positive RCTs (Villar et al.). Treatment strategies used in these RCTs included adjunct therapies (neuromuscular blockage (NMB) and prone position), optimal PEEP selection, HFOV, ECMO, and immune modulators. The findings from their study were as follows:

The ACURASYS trial investigated the hypothesis that by eliminating spontaneous breathing using NMB would improve lung mechanics and oxygenation in ARDS patients. However, it was concluded that NMB is not recommended in moderate to severe ARDS since patient-ventilator asynchronies increase. From a physiologic standpoint NMB should be used only if the patient has a ventilator pattern that could result in VILI.

Although the PROSEVA trial is controversial, the prone position has been shown to be highly effective as an ARDS treatment. There are 5-mechanisms suggested for the proning-induced improved oxygenation: (i) increased EELV, (ii) changes in regional diaphragm motion, (iii) improve ventilation/perfusion matching, (iv) increased secretion clearance, and (v) the weight of the heart is removed from the lung. A second clinical trial was suggested to confirm the large treatment effect seen in the first RCT.

Although there is strong physiologic evidence that heterogeneous lung collapse is a mechanism driving VILI there is currently no consensus on the use of RMs to open the lung nor the method to set PEEP to normalize EELV and stabilize alveoli. The ART and PHARLAP trials showed no benefit of RMs with the ART trial showing an increase in

ARDS related 6-months mortality. The EPVent study using esophageal-guided PEEP showed no difference in mortality as compared with the control group. Both studies had numerous design faults and thus with fine tuning both may 1 day improve outcomes but currently neither strategy is recommended.

Similar results were found in the EOLIA trial using ECMO vs. a control group which was stopped early. The main problems with the study were the expected 20% absolute risk reduction was unreasonable and 28% of the patients in the control group crossed over to receive ECMO. Also like the above ART and EPVent studies another ECMO RCT was recommended with a lower anticipated absolute risk and no cross over patients.

Inflammation causing disruption to both pulmonary endothelium and epithelium resulting in a high permeability pulmonary edema is a hallmark of ARDS pathophysiology. Thus, RCTs to reduced inflammation and edema have been conducted. However, a systematic review showed that there is currently no pharmacologic intervention that could reduce ARDS mortality (Santacruz, Pereira et al., 2019). The BALTI-2 study tested salbutamol as a method to increase alveolar edema clearance and the HARP-2 tested the anti-inflammatory simvastatin. Salbutamol actually increased mortality and simvastatin had no effect but like many of the RCTs discussed above there were serious concerns on the quality of the data for multiple reasons. The INTEREST trial tested interferon (IFN) β, which was designed to upregulate CD73 preventing vascular leakage. There was no reduction in mortality but once again the study design was flawed. Lastly, the recent DEXA-ARDS trial for moderate to severe ARDS using dexamethasone decreased the risk of 60-days mortality by and absolute 15%, paving the way to using steroids in COVID-19 patients.

This group concludes that future RCTs must be personalized to subclasses of ARDS patients that are physiologically able to respond to the treatment therapy. In addition, treatment must be personalized to the patient's specific lung physiology and morphology in order to improve patient outcomes.

Lung fluid balance

Inflammation and biotrauma can increase the permeability of air-blood barrier (ABB) resulting in pulmonary edema and plays a major role in both ARDS and VILI pathogenesis. (Del Sorbo, Goligher et al., 2017; Matthay, Zemans et al., 2019). Beretta et al. computationally modeled ABB disruption taking into account Starling forces, surfactant function, and edema safety factors including: (i) capacity of fluid accumulation on the thick side of the ABB, (ii) increased interstitial pressure, and (iii) increased lymph flow (Beretta et al.). This extensive review covers the mechanisms of ABB disruption, edema development time constants, factors preventing the development of edema, a "tipping point" in ABB injury followed by rapid alveolar flooding, and the role of spontaneous and mechanical ventilation. This study supports those of Rola and Daxon study (Rola and Daxon) showing that the TCAV™ method to set and adjust the APRV mode would favor reduced edema formation.

Conclusion

The papers in our Research Topic discuss novel and innovative treatment strategies for the patient with acute lung injury (ALI). Potential breakthrough methods of protective mechanical ventilation are discussed. Although there are currently no pharmacologic therapies for ARDS the recent DEXA-ARDS trial lends hope that pharmacologic interventions may be available in the near future. The mechanisms behind ALI-induced loss of lung fluid balance resulting in edema formation are discussed and analyzed using computational models. This work ties in with novel ventilation strategies that may also reduce edema formation. In addition, the innovative use of gene therapy to remove edema from flooded alveoli is reviewed. Lastly, success or failure of RCT's designed to treat ARDS are analyzed form physiologic basis with the goal to identify which clinical trials should be repeated and how to improve the quality of future RCTs.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

GN has lectured for Intensive Care On-line Network, Inc. (ICON). NH is the founder of ICON. NH holds patents on a method of initiating, managing and/or weaning airway pressure release ventilation, as well as controlling a ventilator in accordance with the same. GN has received an unrestricted educational grant from Dräger Medical Systems, Inc.

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

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ORIGINAL RESEARCH
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Assessment of Alveolar Macrophage Dysfunction Using an *in vitro* Model of Acute Respiratory Distress Syndrome

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Background: Impaired alveolar macrophage (AM) efferocytosis may contribute to acute respiratory distress syndrome (ARDS) pathogenesis; however, studies are limited by the difficulty in obtaining primary AMs from patients with ARDS. Our objective was to determine whether an *in vitro* model of ARDS can recapitulate the same AM functional defect observed *in vivo* and be used to further investigate pathophysiological mechanisms.

Methods: AMs were isolated from the lung tissue of patients undergoing lobectomy and then treated with pooled bronchoalveolar lavage (BAL) fluid previously collected from patients with ARDS. AM phenotype and effector functions (efferocytosis and phagocytosis) were assessed by flow cytometry. Rac1 gene expression was assessed using quantitative real-time PCR.

Results: ARDS BAL treatment of AMs decreased efferocytosis ($p = 0.0006$) and Rac1 gene expression ($p = 0.016$); however, bacterial phagocytosis was preserved. Expression of AM efferocytosis receptors MerTK ($p = 0.015$) and CD206 ($p = 0.006$) increased, whereas expression of the antiefferocytosis receptor SIRPα decreased following ARDS BAL treatment ($p = 0.036$). Rho-associated kinase (ROCK) inhibition partially restored AM efferocytosis in an *in vitro* model of ARDS ($p = 0.009$).

Conclusions: Treatment of lung resection tissue AMs with ARDS BAL fluid induces impairment in efferocytosis similar to that observed in patients with ARDS. However, AM phagocytosis is preserved following ARDS BAL treatment. This specific impairment in AM efferocytosis can be partially restored by inhibition of ROCK. This *in vitro* model of ARDS is a useful tool to investigate the mechanisms by which the inflammatory alveolar microenvironment of ARDS induces AM dysfunction.

Keywords: ARDS (acute respiratory disease syndrome), alveolar macrophage (AM), efferocytosis, BAL (bronchoalveolar lavage), Rac1, Rho-associated kinase (ROCK) inhibitor

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is an inflammatory pulmonary disorder, which results in hypoxemic respiratory failure. ARDS may develop in response to various insults, with sepsis being the underlying etiology in > 75% of cases (1). Since December 2019, the emergence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the ensuing pandemic has vastly increased the incidence of ARDS; initial studies showed that 41.8% of adult patients admitted with SARS-CoV-2 pneumonia developed ARDS (2, 3). Notwithstanding advances in supportive care and ventilation strategies, mortality for moderate to severe ARDS remains at 40–46%, and ARDS-specific treatment options are limited (1). Pharmacological therapies such as dexamethasone and tocilizumab have only been shown to be efficacious in SARS-CoV-2 ARDS (4, 5). We now understand more about how ARDS develops: It requires damage to the alveolar epithelium and endothelium (6), leading to reduced alveolar fluid clearance (7), increased permeability, exaggerated inflammation, and neutrophilic alveolar edema (8). However, the role of alveolar macrophages (AMs) in ARDS pathogenesis is not fully understood.

We have previously shown that AM efferocytosis is impaired in patients with sepsis-related ARDS, compared to a control group of ventilated sepsis patients without ARDS (9). Impaired AM efferocytosis is associated with increased alveolar neutrophil apoptosis and worse clinical outcomes (increased duration of mechanical ventilation and mortality), indicating this defect in efferocytosis plays a key role in the pathogenesis of ARDS (9). Further studies are required to investigate the pathophysiological role of AM dysfunction in ARDS; however, the difficulty in obtaining relevant cells from these patients is a major barrier to undertaking this work. Safety concerns preclude bronchoscopy in many patients with ARDS due to their ventilation status (9). For those patients in whom bronchoscopy can be performed, the bronchoalveolar lavage (BAL) fluid is highly neutrophilic, resulting in a relatively low AM yield, which is often insufficient to undertake all necessary experiments in every patient (9). This significantly limits the effectiveness of ARDS-related AM research.

Owing to these difficulties with isolating AMs from patients with ARDS, we sought to develop an *in vitro* model of ARDS. A previous study has shown that the treatment of monocyte-derived macrophages (MDMs) with ARDS patient BAL impairs macrophage efferocytosis (10). However, AMs are distinct from MDMs in terms of origin, function, and phenotype. Resident AMs develop from yolk sac progenitors at the embryonic stage (11) and can self-renew throughout life (12, 13), independently from monocytes and hematopoietic stem cells. AMs are crucial for maintaining alveolar immune homeostasis; exposure to the external environment requires AMs to finely balance inflammatory responses to infection against resolving functions to prevent immune-mediated tissue damage (14). The intrinsic protolerogenic characteristic of AMs has likely evolved to prevent excessive inflammation in the face of continuous low-level stimulation from a diverse range of foreign particles (15). Therefore, while previous studies utilizing MDMs are useful, they do not constitute the most representative model of ARDS and, therefore, would not be as appropriate to inform a mechanistic investigation. We postulated that by using AMs from lung resection tissue and treating with pooled ARDS patient BAL, we could develop a more accurate model of the AM defect in ARDS. Since ARDS patient BAL contains high concentrations of proinflammatory cytokines, we hypothesized that following treatment AMs will be driven away from a proresolving phenotype and toward a proinflammatory phenotype, which is associated with reduced efferocytosis capacity (16, 17).

Alveolar macrophage expression of the Mer tyrosine kinase (MerTK) receptor may be critical for efferocytosis (16, 18). MerTK signaling *via* phosphatidylinositol 3′-OH kinase (PI3K) results in activation of Rac1, which causes cytoskeletal rearrangement and engulfment of the apoptotic cell (19, 20). AM surface receptors, namely, CD206 and CD163, are also thought to mediate efferocytosis (14). AMs also express signal regulatory protein-α (SIRPα) on their surface, which binds surfactant proteins or CD47 on healthy cells (21). SIRPα signaling activates Rho-associated kinase (ROCK), and phosphatase and tensin homolog (PTEN), which oppose PI3K signaling, resulting in Rac1 inhibition and suppression of efferocytosis (22). The status of these important efferocytosis-related receptors in ARDS AMs remains unknown.

We hypothesized that treatment of lung resection tissue AMs with pooled ARDS patient BAL will recapitulate the defect in efferocytosis we observed *in vivo* and allow us to determine the mechanism by which this defect occurs. Our study had the following aims:

(1) To determine whether treatment of lung resection tissue AMs with ARDS patient BAL can replicate the impaired efferocytosis observed in patients with ARDS.

(2) To determine whether treatment of lung resection tissue AMs with ARDS patient BAL decreases AM expression of MerTK and increases expression of SIRPα.

(3) To determine whether inhibition of ROCK-PTEN signaling can increase AM efferocytosis in this *in vitro* model of ARDS.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was obtained to recruit ventilated sepsis patients with and without ARDS (REC 16/WA/0169) and for the use of lung tissue samples from patients undergoing routine thoracic surgery (REC 17/WM/0272). For patients who lacked capacity, permission to enroll was sought from a personal legal representative following the UK Mental Capacity Act (2005). For patients with capacity, written informed consent was obtained from the patient.

Patient Recruitment

Invasively ventilated adult patients with ARDS and sepsis were recruited from the intensive care unit of the Queen Elizabeth Hospital, Birmingham, UK, from December 2016 to February 2019, and BAL was collected as previously described (9).

Demographic and physiological details of the patients can also be found in this prior publication. BAL fluid was rendered acellular by centrifuging at 500 g for 5 min. Acellular supernatant BAL was then pooled and stored at −80°C before use in this study.

Adult patients who underwent lung lobectomy as part of their clinical treatment plan for malignancy at Birmingham Heartlands Hospital from September 2017 to July 2019 were also recruited. Recruited patients were never-smokers or long-term ex-smokers (quit > 5 years), with normal spirometry and without airways disease. No patient received chemotherapy before surgery. Following lobectomy, lung tissue resection samples surplus to histopathological requirements were collected.

Alveolar Macrophage Isolation

Macroscopically normal lung tissue samples were perfused with 0.15 M saline *via* pressure bag by inserting a needle (21-gauge) in bronchioles. When saturated, the tissue was gently massaged to facilitate emptying lavage fluid from the tissue, ready for the next instillation. This process was repeated until the lavage fluid contained $<1 \times 10^4$ cells/ml (23).

Cells were pelleted from the lavage fluid by centrifugation at 500 g for 5 min. Mononuclear cells were then separated by gradient centrifugation using Lymphoprep (StemCell Technologies, Vancouver, BC, Canada), according to the instructions of the manufacturer. Mononuclear cells were then cultured in RPMI-1640 media supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (Sigma-Aldrich, Darmstadt, Germany) at 37°C and 5% CO_2 for 24 h to allow adherence. After 24-h culture, the wells were washed and media changed, thereby removing non-adherent mononuclear cells (24, 25). AMs were assessed for purity by cytospin (23); AM purity was consistently >95% across all samples.

Alveolar Macrophage Efferocytosis Assay

The efferocytosis assay was modified from published protocols (26–29). Neutrophils were isolated from the blood of healthy volunteers using Percoll density centrifugation (30) as previously described by our group (31). Neutrophil purity was >96% as assessed by cytospin and viability >97% as assessed by trypan blue exclusion. Neutrophils were suspended in a 5 μM solution of CellTracker™ Deep Red fluorescent dye (ThermoFisher, Waltham, MA, USA) in 10% FCS/RPMI at 4×10^6/ml, then incubated for 30 min at 37°C. Stained neutrophils were centrifuged at 1,500 g for 5 min then resuspended at 2×10^6/ml in serum-free RPMI and incubated at 37°C and 5% CO_2 for 24 h to allow apoptosis. Flow cytometric assessment of neutrophil apoptosis was performed using a fluorescein isothiocyanate (FITC)-conjugated Annexin V and 7-aminoactinomycin D (7-AAD) apoptosis detection kit (BioLegend, San Diego, CA, USA): mean neutrophil apoptosis of 93% with necrosis of <2% was observed.

Alveolar macrophages were cultured at 2.5×10^5/well in 24-well plates. As a negative control, 5 μg/ml Cytochalasin D (CytoD, Sigma-Aldrich, Darmstadt, Germany) was added for 30 min to inhibit actin filament polymerization required for efferocytosis. Stained apoptotic neutrophils (ANs) were added to AMs at a 4:1 ratio before incubation for 2 h at 37°C. The optimal assay duration of 2 h had previously been determined by time-course experiments. Media was removed and wells washed two times with ice-cold phosphate-buffered saline (PBS) to remove non-adherent/engulfed neutrophils. Cells were harvested using a 5-min TrypLE™ express (ThermoFisher, Waltham, MA, USA) incubation at 37°C, before acquisition using an Accuri C6 flow cytometer and software (BD Biosciences, Franklin Lakes, NJ, USA). AMs and ANs alone were used to set gates for their respective populations on forward and side-scatter plots. ANs alone were used to set a positive gate on the allophycocyanin (APC) plot, which was subsequently used to identify AMs which had engulfed ANs. Minimum 5,000 events gated as AMs were counted for each experimental condition and the percentage of APC^+ AMs calculated. CytoD-treated AMs (negative control) determined the background fluorescence present due to ANs adhering to the surface of AMs but not being engulfed. This background fluorescence was subtracted from the percentage of APC^+ AMs in other experimental conditions to give a corrected net efferocytosis index representative of neutrophil engulfment (**Supplementary Figure 1**). Steps were taken to avoid bias, including drawing gates based on single-cell populations (ANs and AMs) before assessing efferocytosis.

Alveolar Macrophage Phagocytosis Assay

Alveolar macrophage phagocytosis assays were performed using pHrodo™ red *Escherichia coli* and *Staphylococcus aureus* BioParticle® conjugates (ThermoFisher, Waltham, MA, USA) in a 96-well plate according to the instruction of the manufacturer and as previously described (23). The pHrodo™ beads were prepared according to the instructions of the manufacturer at a final concentration of 1 mg/ml. AMs were seeded at 50,000 cells per well in black well, clear bottomed 96-well plates, and cultured overnight. For negative control wells, 5 μg/ml CytoD (Sigma-Aldrich, Darmstadt, Germany) was added for 30 min. A total of 50 μl of pHrodo bead suspension was added per well and incubated for 6 h at 37°C. After 6 h, cells were washed three times with PBS before adding 100 μl fresh PBS. Fluorescence was measured using a microplate reader (Synergy 2, Bio-Tek, Winooski, VT, USA) set at the excitation/emission spectra of pHrodo™ red dye: 560/585 nm. The negative control (cytochalasin D treated) AMs were used to determine the background fluorescence present due to stained pHrodo™ red BioParticles® adhering to the outside of macrophages but not being engulfed. This background fluorescence value was subtracted from fluorescence values of other experimental conditions, to give the corrected net fluorescence value the representative of phagocytosis. Phagocytosis results were expressed as fold change in relative fluorescence unit from untreated AMs.

Use of Alveolar Macrophages in an *in vitro* Model of ARDS

Bronchoalveolar lavage from 14 recruited patients with sepsis-related ARDS was rendered acellular by centrifugation and then pooled. The acellular pooled BAL was mixed in a 1:1 ratio with 10% FCS/RPMI. To elicit functional changes associated with ARDS, AMs were treated with this 50% ARDS BAL mixture. AMs were also treated with a 1:1 mixture of

0.9% saline and 10% FCS/RPMI, as vehicle control (VC). Other treatments given in conjunction with 50% ARDS BAL or saline included 200 nM Y-27632 dihydrochloride (Rho-associated protein kinase inhibitor, Apexbio, Houston, TX, USA), 2 μM SF1670 (phosphatase and tensin homolog inhibitor, Selleckchem, Houston, TX, USA), and dimethyl sulfoxide (VC for Y-27632 and SF1670, Sigma-Aldrich, Darmstadt, Germany) at a 1:50,000 dilution. ROCK and PTEN inhibitor treatment doses were determined by dose response on untreated AM efferocytosis (**Supplementary Figure 2**). Other treatments not combined with 50% ARDS BAL or saline included 50 ng/ml interferon-γ (IFN-γ, Peprotech, UK), 1 μg/ml ultrapure lipopolysaccharide (LPS, Invitrogen, Waltham, MA, USA), 40 ng/ml interleukin-4 (IL-4, Peprotech, UK), and 40 ng/ml IL-13, (Peprotech, UK). Previous studies have shown that macrophage treatment with IFN-γ and LPS can induce a proinflammatory phenotype, whereas IL-4 and IL-13 treatment can induce a proresolving phenotype (17). Cytokine concentrations were based on published methods (32). The 1 μg/ml dose of LPS was based on the lowest dose required to elicit tumor necrosis factor-α (TNFα) production from AMs (**Supplementary Figure 3**). Efferocytosis, phagocytosis, apoptosis/viability, and RNA extraction for gene expression were performed 24 h after treatment with 50% ARDS BAL. Phenotyping was performed 48 h after treatment. AM apoptosis and viability were assessed using a flow cytometric apoptosis detection kit (BioLegend, San Diego, CA, USA).

Flow Cytometric Assessment of AM Surface Markers

Alveolar macrophages were labeled with the following antihuman antibodies or their isotype controls: CD206-APC, CD80-PE, CD163-FITC, Mer-APC, and SIRPα-FITC (see **Supplementary Table 1**). Surface marker expression was assessed by an Accuri C6 flow cytometer and software (BD Biosciences, Franklin Lakes, NJ, USA). AM population was gated on forward and side-scatter plot. The median fluorescence intensity (MFI) in relevant channels from isotype control AMs was subtracted from the MFIs of stained AMs, to give the net MFI for each antibody fluorophore. Results presented as fold change-corrected MFI, as a measure of change in cell surface expression, compared to VC (50% saline).

Assessment of AM Gene Expression

RNA was isolated from AMs using Nucleospin RNA kits (Machery-Nagel, Düren, Germany) as per the instructions of the manufacturer. RNA quantity was assessed with the NanoDrop 2000 UV-Vis Spectrophotometer (ThermoFisher, Waltham, MA, USA). One-Step Quantifast Probe RT-PCR Kits (Qiagen, Hilden, Germany) were used to assess gene expression with a CFX384 Touch Real-Time PCR Detection System (BioRad, Hercules, CA, USA). Taqman® gene expression assays (ThermoFisher, Waltham, MA, USA) were purchased for 18S on VIC-MGB (ref 4318839) and RAC1 on FAM-MGB (Hs01025984_m1). PCR conditions were used as per the recommendation of the manufacturer. Triplicate data were analyzed using CFX Maestro software (BioRad, Hercules, CA, USA). Relative quantification of target gene mRNA was calculated relative to expression of 18s endogenous control gene.

TABLE 1 | Characterization of pooled ARDS patient BAL.

Characterization of pooled ARDS patient BAL	
IL-6	453 pg/ml
IL-8	4,268 pg/ml
IL-1β	98 pg/ml
IL-1ra	3,023 pg/ml
IL-10	6 pg/ml
TNF-α	3 pg/ml
VEGF	209 pg/ml
MCP-1	1,045 pg/ml
IFN-γ	0 pg/ml
LPS	57 pg/ml
Total protein	2.89 mg/ml

IFN-γ, interferon-γ; IL, interleukin; TNF-α, tumor necrosis factor-α; MCP-1, macrophage chemoattractant protein-1; LPS, lipopolysaccharide; VEGF, vascular endothelial growth factor.

Bronchoalveolar Lavage Cytokine and Protein Quantification

Inflammatory cytokine (IL-6, IL-8, TNF-α, IL-1β, macrophage chemoattractant protein-1, IL-10, IL-1ra, and vascular endothelial growth factor) content of pooled patient BAL fluid was measured by a commercially available custom Magnetic Luminex® Performance Assay (R&D Systems, UK) as per the instructions of the manufacturer. Protein concentration in pooled patient BAL fluid was measured using the Pierce™ BCA (Bicinchoninic Acid) Protein Assay Kit (ThermoFisher Scientific, Waltham, MA, USA) as per the instructions of the manufacturer.

Statistical Analysis

Data were analyzed using Prism 8 software (GraphPad, San Diego, CA, USA). Parametric data are shown as mean and SD. Non-parametric data are shown as the median and interquartile range (IQR). Differences between continuously distributed data were assessed using Welch's *t*-tests for parametric data or Mann–Whitney tests for non-parametric data. Differences between non-parametric paired data were assessed using Wilcoxon matched-pairs signed-rank test. Differences between three or more unpaired parametric data sets were assessed using ANOVA followed by Dunn's multiple comparison tests. Differences between three or more paired parametric data sets assessed using the repeated measures ANOVA followed by Tukey's multiple comparison tests. Two-tailed *p*-values of ≤ 0.05 were considered significant.

RESULTS

Patient Characteristics

Samples of BAL from the first 14 patients with sepsis-related ARDS recruited to a previous study (9) were pooled and used to treat AMs isolated from lung resections. This pooled ARDS patient BAL was characterized with regards to inflammatory cytokine and LPS content (**Table 1**). AMs were isolated from the lung tissue of 16 patients who underwent lobectomy (mean yield of 9 million AMs per patient). The mean age of lobectomy

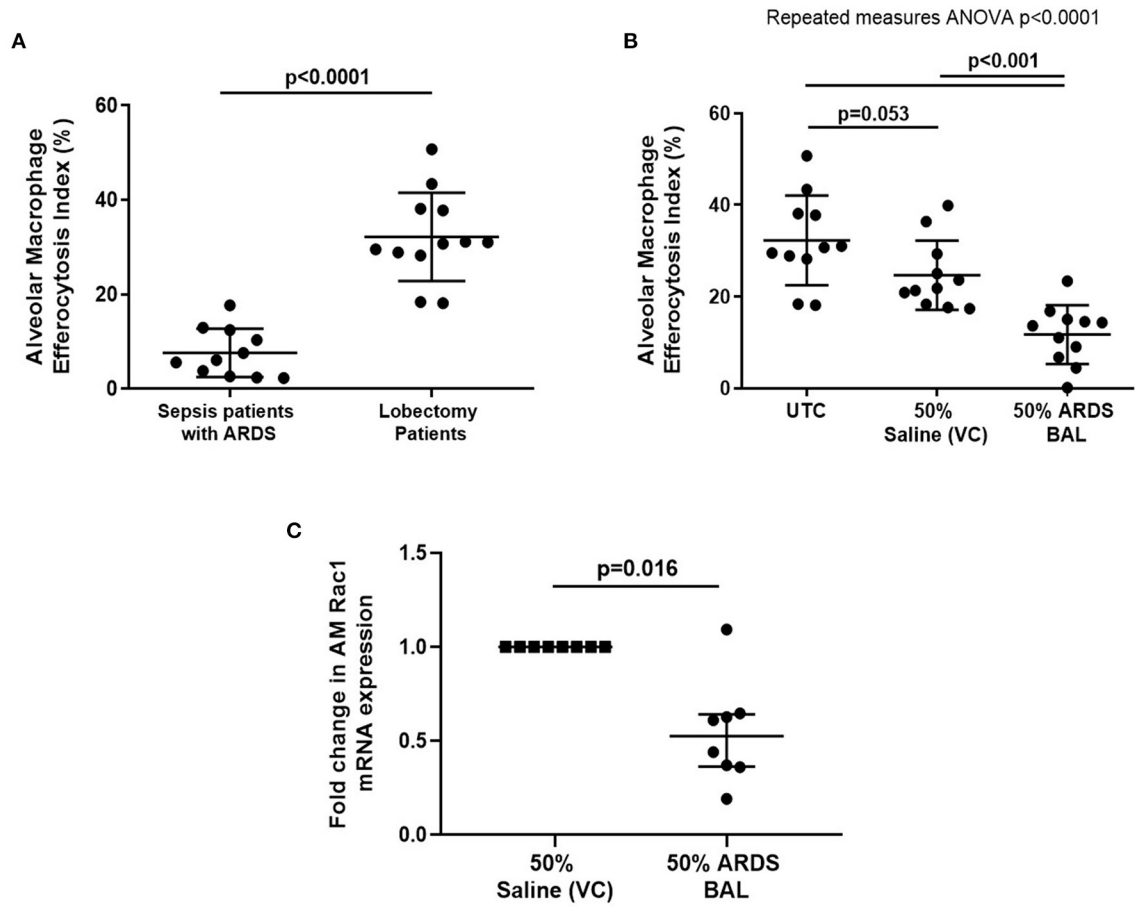

FIGURE 1 | Effect of pooled ARDS BAL treatment on lobectomy patient alveolar macrophage efferocytosis. **(A)** Alveolar macrophages (AMs) from sepsis patients with ARDS have significantly reduced efferocytosis index compared to AMs from lobectomy patients (means 7.6 vs. 32.2%, $p < 0.0001$). Statistical analysis by Welch's *t*-test, $n = 11$–12. **(B)** UTC, Untreated control (cultured in RPMI + 10% FBS); VC, vehicle control (50% saline). Effect of ARDS BAL treatment on lobectomy patient AM efferocytosis. Treatment with 50% ARDS BAL significantly reduced lobectomy patient AM efferocytosis compared to VC treatment (mean of differences 13.0%, $p = 0.0006$) and UTC (mean of differences 20.6%, $p = 0.0009$). Treatment of AMs with VC did not affect efferocytosis compared to UTC (mean of differences 7.6%, $p = 0.053$). Statistical analysis by the repeated measures ANOVA and Tukey's multiple comparisons tests, $n = 11$ for all groups. **(C)** Effect of ARDS BAL treatment on Rac1 gene transcription in lobectomy patient AMs. Data are shown as fold change in AM Rac1 mRNA expression from 50% saline treatment. Statistical analysis by the Wilcoxon matched-pairs signed-rank test, $n = 8$. VC, vehicle control (50% saline). Treatment with 50% ARDS BAL significantly reduced Rac1 mRNA expression in lobectomy patient AMs, compared to VC treatment (median of differences 0.48, $p = 0.016$). Error bars are shown as mean and SD. ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; FBS, fetal bovine serum.

patients was 70 years (SD = 6.9 years). The male:female split for lobectomy patients was 9:7.

ARDS BAL Treatment of Alveolar Macrophages Impairs Efferocytosis and Preserves Bacterial Phagocytosis

Alveolar macrophage efferocytosis was impaired in sepsis patients with ARDS compared to lobectomy patients (**Figure 1A**, mean 7.6% [SD = 5.1] vs. 32.2% [SD = 9.4], $p < 0.0001$). We established an *in vitro* model of ARDS, by treating lung resection tissue AMs with pooled ARDS BAL to induce AM dysfunction. ARDS BAL or saline VC treatment did not affect AM apoptosis or viability compared to standard culture (**Supplementary Figure 4**). Treatment of AMs with ARDS BAL reduced efferocytosis compared to VC treatment (**Figure 1B**, mean 11.7% [SD = 6.4] vs. 24.7% [SD = 7.6], $p = 0.0006$). Treatment of AMs with ARDS BAL reduced Rac1 mRNA expression compared to VC treatment (**Figure 1C**, median of differences 0.48, $p = 0.016$), which supports our finding that ARDS BAL inhibits AM efferocytosis.

Treatment of AMs with VC reduced phagocytosis of both *S. aureus* and *E. coli* pHrodo® bioparticles compared to untreated controls (**Figures 2A,B**, $p = 0.031$). Treatment of AMs with ARDS BAL increased phagocytosis of *S. aureus* pHrodo® bioparticles compared to VC treatment (**Figure 2A**, median of differences 0.32, $p = 0.031$). There was no difference in AM phagocytosis of *E. coli* pHrodo® bioparticles following ARDS BAL treatment compared to VC treatment (**Figure 3B**, $p =$

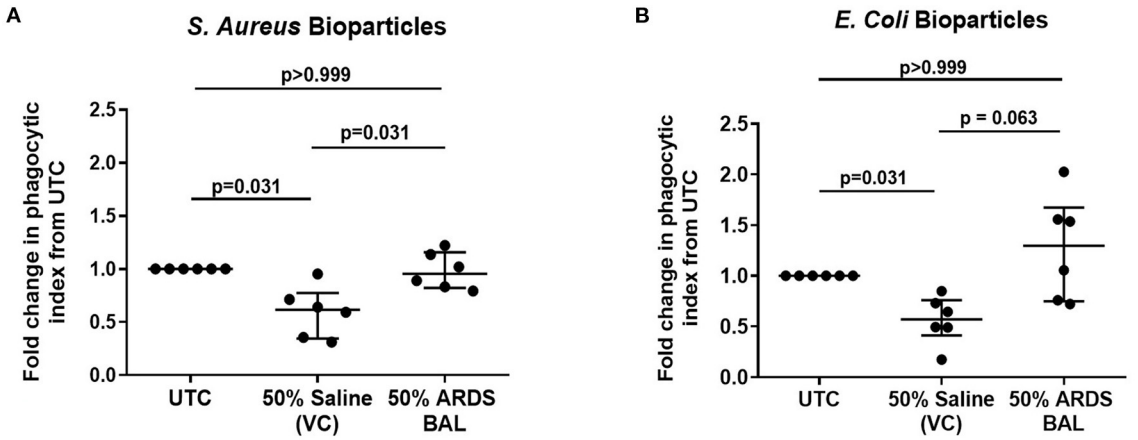

FIGURE 2 | Effect of pooled ARDS BAL treatment on lobectomy patient alveolar macrophage phagocytosis. Alveolar macrophage (AM) phagocytic index in lobectomy AMs treated with 50% ARDS BAL. Data corrected to fold change in the phagocytic index from untreated control (UTC—cultured in RPMI + 10% FBS). Statistical analysis by Wilcoxon matched-pairs signed-rank test, $n = 6$ for all groups. VC, vehicle control (50% saline). VC values are non-identical in graphs C and D. Data are shown as median and interquartile range. ARDS BAL or saline VC-treated AMs receive half the volume of culture media and FCS compared to UTC AMs. **(A)** Treatment of AMs with 50% saline VC reduced phagocytosis of *S. aureus* bioparticles (median of differences −0.38, $p = 0.031$). Treatment with 50% ARDS BAL caused a significant increase in AM phagocytosis of *S. aureus* bioparticles compared to VC (median of differences 0.32, $p = 0.031$). **(B)** Treatment of AMs with 50% saline VC reduced phagocytosis of *E. coli* bioparticles (median of differences −0.43, $p = 0.031$). No significant difference in AM phagocytosis of *E. coli* bioparticles was observed following treatment with 50% ARDS BAL compared to VC (median of differences 0.59, $p = 0.063$). ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; UTC, untreated control; FCS, fetal calf serum.

0.063). Thus, ARDS BAL treatment had divergent effects on AM efferocytosis and phagocytosis.

ARDS BAL Treatment of Alveolar Macrophages Alters Surface-Receptor Expression

Since ARDS BAL treatment of AMs had divergent effects on efferocytosis and phagocytosis, we used this *in vitro* model to investigate the association between AM phenotype and function. Treatment of AMs with ARDS BAL increased expression of CD206 (**Figure 3A**, mean fold change 0.39, $p = 0.006$) and MerTK (**Figure 3B**, mean fold change 0.3, $p = 0.028$) compared to VC treatment. Treatment of AMs with ARDS BAL decreased SIRPα expression compared to VC treatment (**Figure 3C**, mean fold change −0.26, $p = 0.006$). Treatment of AMs with ARDS BAL did not change the expression of CD163 or CD80 compared to VC treatment (**Figures 3D,E**, $p > 0.05$ for both). These changes in AM surface-receptor expression were incongruent with the observed defect in AM efferocytosis following ARDS BAL treatment.

Treatment of AMs with proinflammatory mediators IFN-γ and LPS also impaired efferocytosis (**Figure 4A**, mean difference 16.5%, $p = 0.008$). However, in contrast to ARDS BAL, proinflammatory mediator treatment decreased expression of both MerTK (**Figure 4B**, mean fold change −0.58, $p = 0.015$) and CD163 (**Figure 4C**, mean fold change −0.58, mean fold change −0.55, $p = 0.005$) while increasing expression of SIRPα (**Figure 4D**, mean fold change 2.48, $p = 0.036$). Proinflammatory mediator treatment had no significant effect on the expression of AM surface markers CD206 or CD80 (**Figures 4E,F**). Treatment with proresolving mediators IL-4 and IL-13 did not affect AM efferocytosis; however, their effect on AM surface-receptor expression was also assessed: expression of MerTK, CD163, and SIRPα was decreased while expression of CD206 was increased (**Figures 4A–E**). These findings indicate that the effect of ARDS BAL treatment on AMs cannot solely be explained by changes in surface-receptor expression.

Rho-Associated Kinase Inhibition Partially Restores Alveolar Macrophage Efferocytosis in an *in vitro* Model of ARDS

Since this model effectively replicated *in vitro* the efferocytosis defect in ARDS AMs evident *in vivo*, we next used this model to explore the mechanism driving this defect. Rac1 intracellular signaling pathways are summarized in **Figure 5**. From this pathway, we identified ROCK and PTEN as potential targets to modify activity. The addition of ROCK-inhibitor to ARDS BAL treatment increased AM efferocytosis compared to treatment with ARDS BAL plus VC (**Figure 6A**, mean fold change 0.17, $p = 0.009$). The addition of PTEN inhibitor to ARDS BAL treatment did not affect efferocytosis compared to treatment with ARDS BAL plus VC (**Figure 6B**). ROCK inhibition did not affect AM phagocytosis of *E. coli* or *S. aureus* bioparticles (**Supplementary Figure 5**). ROCK inhibition also did not affect AM surface marker expression of CD206, CD163, CD80, SIRPα, and MerTK (**Supplementary Figure 6**).

DISCUSSION

Herein, we have established a phenotypically and functionally accurate *in vitro* model through which we can model the effects of ARDS on AM function. In this model, ARDS BAL

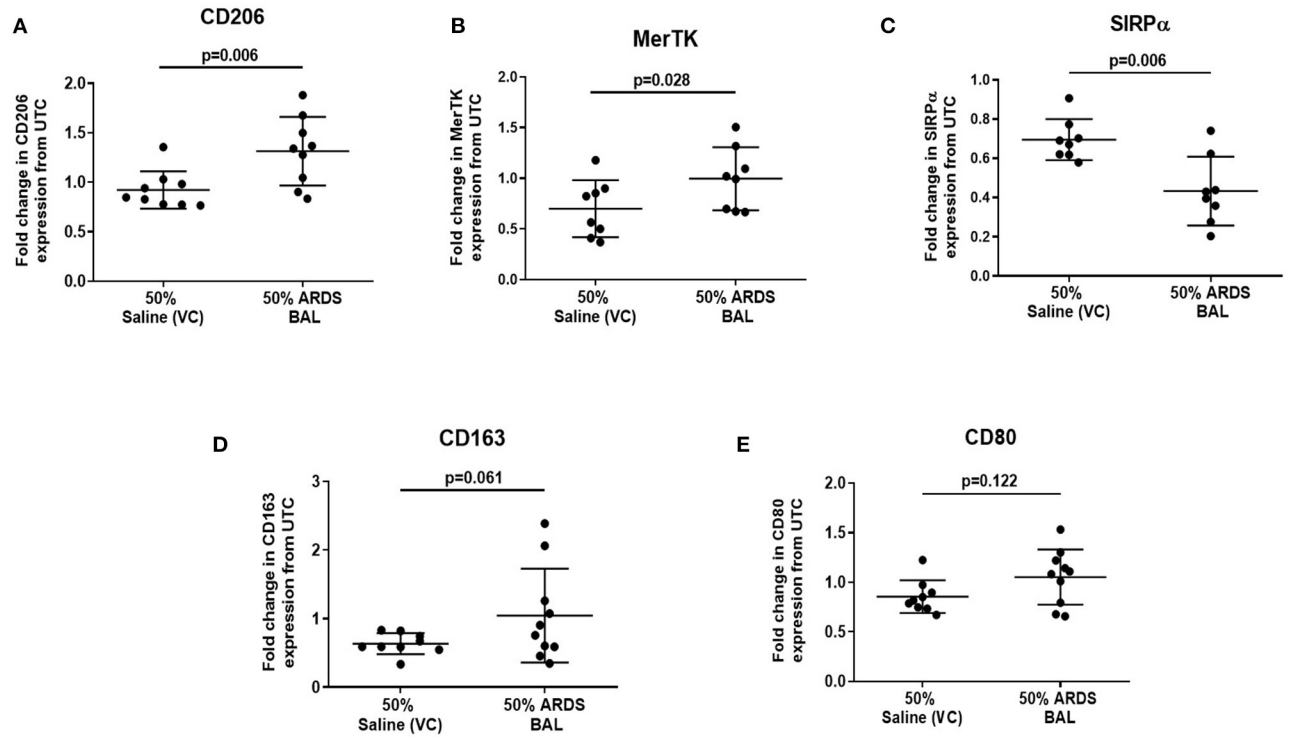

FIGURE 3 | Effect of ARDS BAL treatment on lobectomy patient alveolar macrophage surface-receptor expression. UTC, untreated control (RPMI + 10% FBS); VC, vehicle control (50% saline). Statistical analysis by paired *t*-test, $n \geq 8$ for all groups. **(A)** Treatment with 50% ARDS BAL significantly increased CD206 expression on AMs compared to VC treatment (mean of differences 0.39, $p = 0.006$), **(B)** 50% ARDS BAL treatment significantly increased MerTK expression on AMs compared to VC treatment (mean of differences 0.30, $p = 0.028$), **(C)** 50% ARDS BAL treatment significantly decreased SIRPα expression on AMs compared to VC treatment (mean of differences −0.26, $p = 0.006$), **(D)** 50% ARDS BAL treatment increased CD163 expression on AMs compared to VC treatment; AM, alveolar macrophage; however, this difference did not reach statistical significance (mean of differences 0.44, $p = 0.061$), and **(E)** 50% ARDS BAL treatment did not significantly change CD80 expression on AMs compared to VC treatment (mean of differences 0.19, $p = 0.122$). ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; FCS, fetal calf serum; MerTK, Mer receptor tyrosine kinase; SIRPα, signal regulatory protein alpha; AMs, alveolar macrophage.

treatment of lung resection tissue AMs induced an impairment in efferocytosis observed in ARDS patients, but preserved AM bacterial phagocytosis. Thus, the inflammatory contents of ARDS BAL do not induce a global impairment in AM function but rather a specific impairment in efferocytosis. The impairment in AM efferocytosis caused by ARDS BAL treatment is not mediated by changes in surface-receptor expression. ROCK inhibition partially restores AM efferocytosis in an *in vitro* model of ARDS. Modulation of the ROCK-PI3K-Rac1 intracellular signaling pathway may offer a therapeutic strategy to upregulate AM efferocytosis in ARDS.

In early ARDS, proinflammatory monocytes migrate to the alveoli and then differentiate into "recruited" AMs (33). A direct correlation was observed between alveolar monocyte influx, the severity of the respiratory failure, and mortality in ARDS (33). Murine models showed that following the initiation of lung injury, the majority of inflammatory cytokines (namely, tumor necrosis factor-alpha, IL-6, and IL-1β) were released by recruited AMs (34). Our study assessed total AM efferocytosis, and did not distinguish between resident and recruited AMs. Therefore, we initially postulated that the decreased AM efferocytosis in ARDS may be due to the polarization of AMs to a proinflammatory phenotype, which is associated with reduced efferocytosis (17). The concentrations of inflammatory cytokines within our pooled ARDS BAL are in keeping with those reported in previous studies (35, 36). We then undertook experiments using the *in vitro* model of ARDS to investigate the association between AM phenotype and function.

Intriguingly, ARDS BAL treatment of AMs increased expression of efferocytosis receptors (CD206 and MerTK) and decreased expression of the antiefferocytosis receptor SIRPα. These phenotypic changes were incongruent with the functional defect in AM efferocytosis induced by ARDS BAL. In comparison, treatment with proinflammatory mediators (IFN-γ and LPS) also decreased AM efferocytosis, but induced SIRPα expression and decreased MerTK expression. Although both ARDS BAL and proinflammatory mediator treatments impaired AM efferocytosis, they had opposite effects on efferocytosis receptor expression. A similar association was observed in cigarette smokers, between decreased AM efferocytosis (37), increased MerTK expression (38), and increased transcription

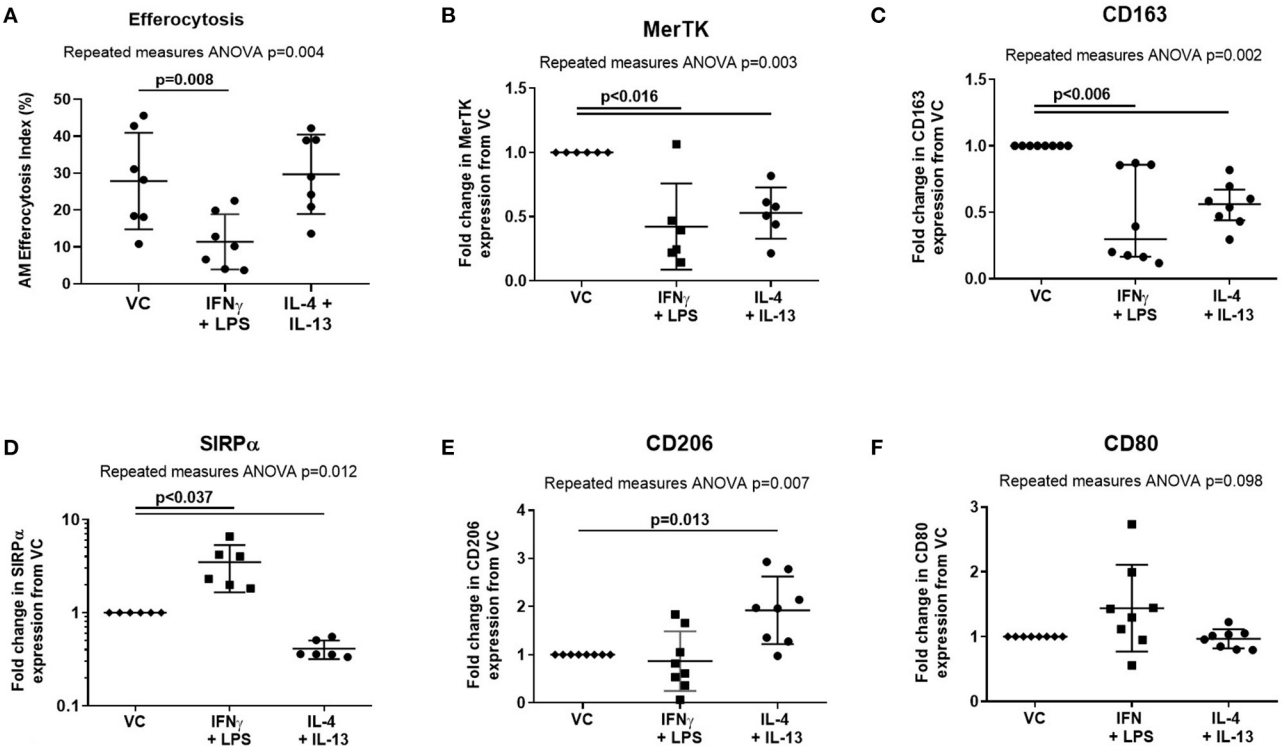

FIGURE 4 | Effect of pro- and anti-inflammatory mediators on alveolar macrophage efferocytosis and surface-receptor expression. VC, vehicle control (distilled water added at 1:500 to RPMI + 10% FBS). IL-4 + IL-13, interleukins 4 and 13 used at 40 ng/ml each; IFN-γ, 50 ng/ml interferon-γ; LPS, 1 μg/ml lipopolysaccharide. **(A)** Data are shown as mean and SD, $n = 7$ for all groups. Cytokine treatment significantly affected alveolar macrophage (AM) efferocytosis (repeated measures ANOVA $p = 0.004$). IFN-γ and LPS treatment significantly reduced AM efferocytosis compared to VC (Dunnett's multiple comparisons test mean difference 16.5%, $p = 0.008$). IL-4 and IL-13 had no significant effect on AM efferocytosis compared to VC (Dunnett's multiple comparisons test mean difference −1.8%, $p = 0.897$). **(B–F)** Statistical analysis by the repeated measures ANOVA with Dunnett's multiple comparison test, $n = 6$–8. Linear *y*-axis was used for all graphs, except SIRPα **(D)** for which a log scale *y*-axis was used. **(B)** Cytokine treatments significantly affected AM surface expression of MerTK (repeated measures ANOVA $p = 0.003$). Compared to treatment with VC, AM surface expression of MerTK was significantly decreased following treatment with IFN-γ + LPS (mean of differences −0.58, $p = 0.015$), and IL-4 + IL-13 (mean of differences −0.47, $p = 0.004$). **(C)** Cytokine treatments significantly affected AM surface expression of CD163 (repeated measures ANOVA $p = 0.002$). Compared to treatment with VC, AM surface expression of CD163 was significantly decreased following treatment with IFN-γ + LPS (mean of differences −0.55, $p = 0.005$), and IL-4 + IL-13 (mean of differences −0.45, $p = 0.002$). **(D)** Cytokine treatments significantly affected AM surface expression of SIRPα (repeated measures ANOVA $p = 0.012$). Compared to treatment with VC, AM surface expression of SIRPα was significantly increased following treatment with IFN-γ + LPS (median of differences 2.48, $p = 0.036$). Compared to treatment with VC, AM surface expression of SIRPα was significantly decreased following treatment with IL-4 + IL-13 (median of differences −0.59, $p < 0.0001$). **(E)** Cytokine treatments significantly affected AM surface expression of CD206 (repeated measures ANOVA $p = 0.007$). Compared to treatment with VC, AM surface expression of CD206 was significantly increased following treatment with IL-4 + IL-13 (mean of differences 0.92, $p = 0.013$). There were no significant changes in CD206 expression following treatment with IFN-γ + LPS (mean of differences −0.13, $p = 0.777$). **(F)** Cytokine treatments did not significantly affect AM surface expression of CD80 (repeated measures ANOVA $p = 0.098$). MerTK, Mer tyrosine kinase receptor; SIRPα, signal regulatory protein-α.

of genes associated with a proresolving phenotype (39). Patients with chronic obstructive pulmonary disease (COPD) also have impaired AM efferocytosis (40) with overexpression of efferocytosis receptors CD206 and CD163 (41). Our data, therefore, suggest that the AM efferocytosis defect induced by ARDS BAL treatment is not mediated by surface-receptor changes.

Strategies to upregulate AM efferocytosis may reduce secondary necrosis of alveolar neutrophils, thereby attenuating inflammation in ARDS. Since *in vitro* ARDS BAL treatment downregulated AM Rac1 gene expression, we sought to upregulate Rac1 expression and restore efferocytosis by inhibiting ROCK and PTEN. The addition of ROCK inhibitor to ARDS BAL treatment partially restored AM efferocytosis function and did not affect bacterial phagocytosis. However, the addition of PTEN inhibitor had no significant effect; this may be because the role of PTEN is less important in antagonizing the PI3K pathway. ROCK inhibitors have been shown to increase efferocytosis in MDMs and AMs from patients with COPD (42). Further studies to investigate the role of the ROCK-PTEN-Rac1 pathway in ARDS AM dysfunction are required. Measurements of Rac1 and PI3K proteins expression in our model, with and without ROCK inhibition, are required to support the hypothesis that Rac1 inhibition is partially responsible for

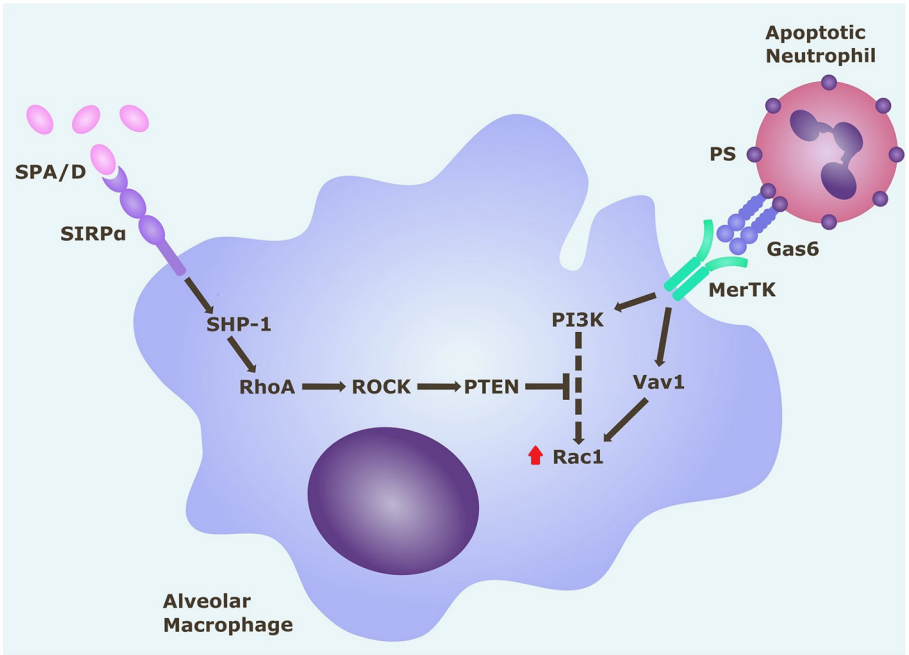

FIGURE 5 | Rac1 intracellular signaling pathways in alveolar macrophages. Alveolar macrophage efferocytosis is regulated by surface receptors MerTK and SIRPα. Gas6 binds to PS on the surface of apoptotic cells. Activation of MerTK by the PS opsonin Gas6 can trigger signaling cascades *via* PI3K and Vav1, which both upregulate Rac1. Activation of Rac1 results in cytoskeletal rearrangement and efferocytosis of the apoptotic cell. Activation of SIRPα by SP-A (or SP-D) triggers a signaling cascade along the SHP1/RhoA/ROCK/PTEN pathway, which inhibits PI3K signaling, and ultimately downregulates Rac1, thereby inhibiting efferocytosis. Gas6, growth arrest specific-6; MerTK, Mer tyrosine kinase receptor; PI3K, phosphatidylinositol 3′-OH kinase; PS, phosphatidylserine; PTEN, phosphatase and tensin homolog; ROCK, Rho-associated kinase; SHP-1, Src homology region 2 domain-containing phosphatase-1; SIRPα, signal regulatory protein-α; SPA/D, surfactant protein A/D.

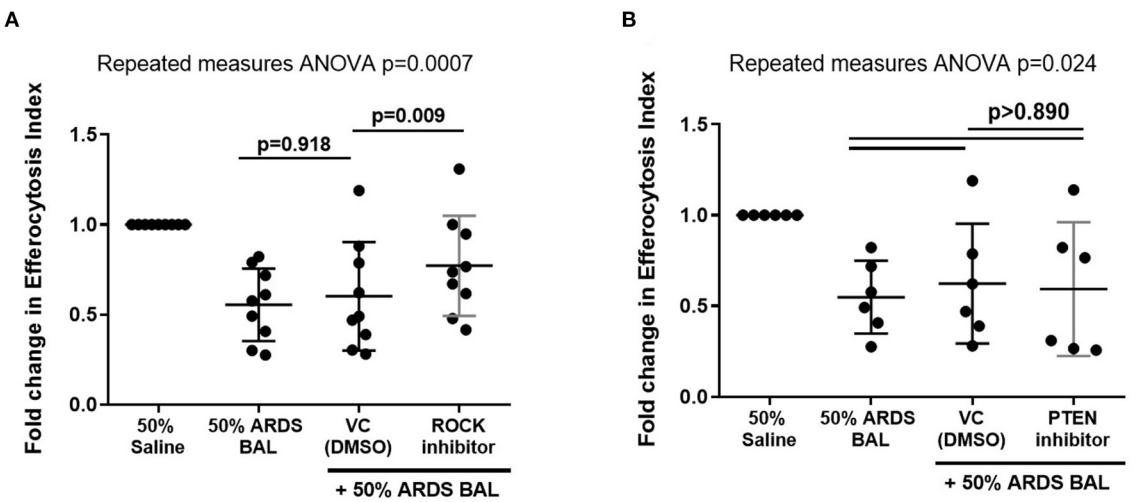

FIGURE 6 | Effect of ROCK and PTEN inhibitors on restoring alveolar macrophage efferocytosis following ARDS BAL treatment. Data are shown as fold change in AM efferocytosis index from 50% saline treatment. ROCK, Rho-associated protein kinase; PTEN, phosphatase and tensin homolog; VC, vehicle control [dimethyl sulfoxide (DMSO) at 1:50,000 dilution]. ROCK inhibitor = 200 nM Y-27632 dihydrochloride. PTEN inhibitor = 2 μM SF1670. Statistical analysis by repeated measures ANOVA with Tukey's multiple comparison test. **(A)** The addition of VC to ARDS BAL mixture had no significant effect on AM efferocytosis (mean of differences 0.05, $p = 0.918$, $n = 9$) compared to ARDS BAL alone. Addition of ROCK inhibitor to ARDS BAL treatment significantly increased efferocytosis compared to treatment with VC + ARDS BAL (mean of differences 0.17, $p = 0.009$, $n = 9$) **(B)** Addition of PTEN inhibitor to 50% ARDS BAL treatment had no significant effect on efferocytosis compared to treatment with VC + ARDS BAL (mean of differences 0.03, $p = 0.924$, $n = 6$).

impaired AM efferocytosis in ARDS. ROCK inhibition promotes PI3K signaling, which has multiple effects on a cellular function beyond upregulation of Rac1, namely, proliferation, chemotaxis, and migration (43). ROCK inhibition would have many off-target effects, thereby limiting its therapeutic potential as a strategy to upregulate AM function in ARDS. Existing medications could be tested using the *in vitro* model of ARDS, to determine if they can restore AM efferocytosis, e.g., N-acetylcysteine (44), macrolide antibiotics (45), statins (46), and glucocorticoids (47).

Studies utilizing an *ex-vivo* perfused human lung model of ARDS have shown that extracellular vesicles (EVs) are released following lung injury with *E. coli*; these isolated EVs subsequently mediated inflammatory lung injury when administered to uninjured lungs (48). Murine models of LPS lung injury have shown that EV transfer of microRNA cargo to AMs can increase inflammatory cytokine release (49). Further analysis of ARDS BAL and studies utilizing our *in vitro* model of ARDS are required to determine whether EV transfer of microRNA to AMs may affect intracellular pathways regulating efferocytosis (50).

Our study had some limitations. Due to logistical constraints, efferocytosis assays were undertaken with heterologous neutrophils, as opposed to autologous neutrophils, which would have more accurately reflected the environment *in vivo*. Although unaffected lung tissue was processed, we cannot rule out contamination with tumor-associated macrophages which are characterized by an immunosuppressive phenotype and may exhibit increased efferocytosis (51), which could account for some of the divergent effects observed. Expression of intracellular signaling mediators (e.g., Rac1) was only measured at the mRNA level. To draw definitive conclusions regarding the mechanism of impaired efferocytosis in ARDS, data on the protein expression of these mediators will be required. Ideally, the use of healthy human BAL would be a more appropriate VC instead of saline in this model; however, healthy BAL is a highly limited resource.

Another limitation to our study is that when assessing AM expression of TAM receptors (key mediators of macrophage efferocytosis), only MerTK was investigated (52). We had predominantly focused on MerTK, as this efferocytosis receptor was best characterized in the context of ARDS within the literature (18, 53–55). However, we omitted to investigate other important TAM receptors: Axl and Tyro3 (52). Impairment of the Axl signaling pathway has been associated with decreased AM efferocytosis in asthma (56). We report a contradictory increase in MerTK expression associated with decreased efferocytosis in AMs treated with ARDS BAL; however, this may, in part, be explained if the expression of TAM receptors Axl and/or Tyro3 were decreased. Further studies will be required to investigate this.

Studies have previously shown that the microenvironment can influence AM metabolism, inflammatory response, and gene expression (57, 58). *In vitro* culture of AMs can alter efferocytosis receptor expression profiles (56), therefore undertaking efferocytosis assays directly *in situ* on lung tissue may provide a more accurate representation of *in vivo* AM function (59). For future studies, precision-cut lung slices could be incubated with ARDS BAL before the assessment of efferocytosis directly on lung tissue (60); terminal deoxynucleotidyl transferase dUTP nick end labeling could be used to identify ANs in a double immunofluorescence method (59).

In conclusion, *in vitro* treatment of lung resection tissue AMs with pooled ARDS patient BAL can recapitulate the same functional defect observed *in vivo*. This dysfunction can be partially restored by ROCK inhibition. The *in vitro* model of ARDS is a useful tool to investigate the mechanisms by which the inflammatory alveolar microenvironment of ARDS induces AM dysfunction.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Wales Research Ethics Committee 1 (REC 16/WA/0169) and West Midlands - Solihull Research Ethics Committee (REC 17/WM/0272). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RM, AS, MM, and DT contributed to the study conception and design. RM, AS, DP, and SL contributed to data acquisition. RM, AS, and DT drafted the manuscript. All the authors contributed to the data analysis and interpretation, critically revised the manuscript for intellectual content, and approved the final version before submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.737859/full#supplementary-material

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Effects of Different Levels of Variability and Pressure Support Ventilation on Lung Function in Patients With Mild–Moderate Acute Respiratory Distress Syndrome

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Background: Variable pressure support ventilation (vPSV) is an assisted ventilation mode that varies the level of pressure support on a breath-by-breath basis to restore the physiological variability of breathing activity. We aimed to compare the effects of vPSV at different levels of variability and pressure support (ΔP_S) in patients with acute respiratory distress syndrome (ARDS).

Methods: This study was a crossover randomized clinical trial. We included patients with mild to moderate ARDS already ventilated in conventional pressure support ventilation (PSV). The study consisted of two blocks of interventions, and variability during vPSV was set as the coefficient of variation of the ΔP_S level. In the first block, the effects of three levels of variability were tested at constant ΔP_S: 0% ($PSV_{0\%}$, conventional PSV), 15% ($vPSV_{15\%}$), and 30% ($vPSV_{30\%}$). In the second block, two levels of variability (0% and variability set to achieve ±5 cmH_2O variability) were tested at two ΔP_S levels (baseline ΔP_S and ΔP_S reduced by 5 cmH_2O from baseline). The following four ventilation strategies were tested in the second block: PSV with baseline ΔP_S and 0% variability (PSV_{BL}) or ±5 cmH_2O variability ($vPSV_{BL}$), PSV with ΔP_S reduced by 5 cmH_2O and 0% variability (PSV_{-5}) or ±5 cmH_2O variability ($vPSV_{-5}$). Outcomes included gas exchange, respiratory mechanics, and patient-ventilator asynchronies.

Results: The study enrolled 20 patients. In the first block of interventions, oxygenation and respiratory mechanics parameters did not differ between $vPSV_{15\%}$ and $vPSV_{30\%}$

compared with $PSV_{0\%}$. The variability of tidal volume (V_T) was higher with $vPSV_{15\%}$ and $vPSV_{30\%}$ compared with $PSV_{0\%}$. The incidence of asynchronies and the variability of transpulmonary pressure (P_L) were higher with $vPSV_{30\%}$ compared with $PSV_{0\%}$. In the second block of interventions, different levels of pressure support with and without variability did not change oxygenation. The variability of V_T and P_L was higher with $vPSV_{-5}$ compared with PSV_{-5}, but not with $vPSV_{BL}$ compared with PSV_{BL}.

Conclusion: In patients with mild-moderate ARDS, the addition of variability did not improve oxygenation at different pressure support levels. Moreover, high variability levels were associated with worse patient-ventilator synchrony.

Clinical Trial Registration: www.clinicaltrials.gov, identifier: NCT01683669.

Keywords: variable pressure support ventilation, acute respiratory distress (ARDS), asynchronies, respiratory mechanic, assisted ventilation

INTRODUCTION

Pressure support ventilation (PSV) is an assisted ventilation mode commonly used in critically ill patients (Esteban et al., 2013). The maintenance of spontaneous respiratory activity in acute respiratory distress syndrome (ARDS) patients improves respiratory function and decreases the need for vasopressor and sedative drugs (Putensen et al., 2001). Assisted ventilation modes have been commonly used in the management of patients with ARDS, in particular those with mild to moderate hypoxemic respiratory failure (Bellani et al., 2016).

In the last years, researchers have proposed to vary the level of pressure support on a breath-by-breath basis to restore the physiological variability of breathing activity (Tobin et al., 1988). Variable pressure support ventilation (vPSV), compared with conventional PSV, improved oxygenation in the experimental models of ARDS (Gama de Abreu et al., 2008) and ventilator-patient synchrony in a small pilot study in critically ill patients with acute respiratory failure (Spieth et al., 2013). These effects could be mediated by an amelioration of the ventilation-perfusion matching (Huhle et al., 2016), as well as a recruitment effect due to the repetitive delivery of breaths with a higher tidal volume, which might also result in a reduction of lung inhomogeneity (Mauri et al., 2017). However, so far, the only clinical study published has used only one variability level at fixed pressure support (ΔP_S) (Spieth et al., 2013). Therefore, the effects of different levels of variability and the impact of variability at different ΔP_S levels remain unknown. Different levels of variability might modify differently the ventilation perfusion-matching and might affect differently gas exchange and respiratory mechanics.

The aim of this study was to evaluate the effects of vPSV, at different levels of variability and pressure support, on short-term lung function parameters in patients with mild to moderate ARDS. We tested the hypothesis that vPSV would improve gas exchange, respiratory mechanics, and patient-ventilator asynchrony. We also hypothesized that the degree of variability and the level of ΔP_S would influence the effects of vPSV.

Abbreviations: PSV, pressure support ventilation; ARDS, acute respiratory distress syndrome; vPSV, variable pressure support ventilation; ΔP_S, pressure support level; ICU, intensive care unit; PEEP, positive end-expiratory pressure; PBW, predicted body weight; SAPS, simplified acute physiology score; SOFA, sequential organ failure assessment score; RASS, richmond agitation-sedation scale; $PSV_{0\%}$, pressure support ventilation with no variability; $vPSV_{15\%}$, variable pressure support ventilation with 15% CV variability; $vPSV_{30\%}$, variable pressure support ventilation with 30% CV variability; V_T, tidal volume; PTP, esophageal pressure-time product; ΔP_{es}, esophageal pressure swings; P_L, peak transpulmonary pressure; PSV_{BL}, pressure support ventilation with no variability and baseline ΔP_S as per clinical indication; $vPSV_{BL}$, variable pressure support with variability set to achieve ± 5 cmH_2O and baseline ΔP_S as per clinical indication; PSV_{-5}, pressure support ventilation with no variability and ΔP_S reduced by 5 cmH_2O from the baseline value; $vPSV_{-5}$, variable pressure support ventilation with variability set to achieve ± 5 cmH_2O and ΔP_S reduced by 5 cmH_2O from the baseline value.

METHODS

Study Design

This was a prospective, crossover, randomized clinical trial conducted in a single university hospital intensive care unit (ICU).

Inclusion and Exclusion Criteria

Patients aged >18 years with mild to moderate ARDS (PaO_2/FIO_2 ratio between 100 and 300 mmHg with a positive end-expiratory pressure, PEEP $\geq$5 cmH_2O) already receiving PSV per clinical indication were screened for inclusion. Exclusion criteria were pregnancy, chronic obstructive pulmonary disease, presence of pneumothorax or chest tubes, and unavailability of research staff.

Interventions

According to the local clinical practice, conventional PSV was delivered by an Evita Infinity V500 ventilator (Dräger Medical AG, Lübeck, Germany) targeting a V_T of 6–8 ml/kg of predicted body weight, respiratory rate $\leq$25 min^{-1} with PEEP and FIO_2 titrated to achieve a peripheral oxygen saturation $\geq$92%. This ventilator can operate in vPSV mode setting the variability of the ΔP_S and delivers breaths with an approximately Gaussian distribution, truncated at 3 SDs from the mean ΔP_S. The parameter "variability" of this ventilator refers to the range of ΔP_S, e.g., 90% "variability" results in a 30% coefficient

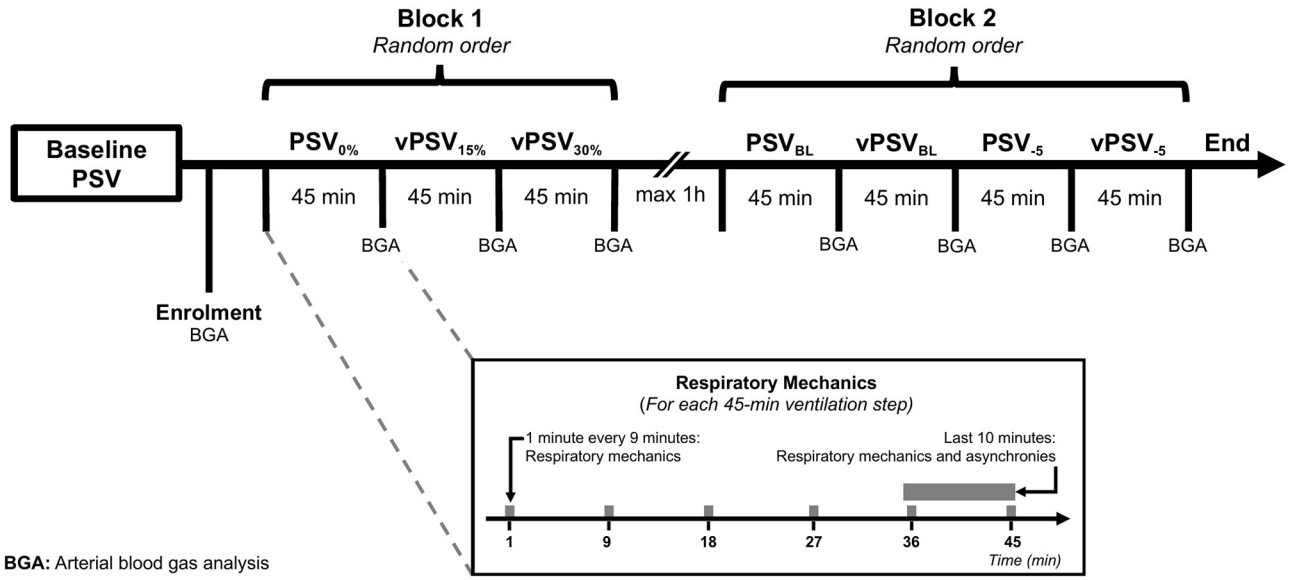

FIGURE 1 | Time course of interventions. Within each intervention block, different ventilation settings were delivered in random order. $PSV_{0\%}$, conventional PSV ventilation with no variability; $vPSV_{15\%}$, variable PSV with variability set to 15% CV; $vPSV_{30\%}$, variable PSV with variability set to 30% CV; PSV_{BL}, PSV with no variability and baseline ΔP_S as per clinical indication; $vPSV_{BL}$, variable pressure support with variability set to achieve ±5 cmH_2O and baseline ΔP_S as per clinical indication; PSV_{-5}, PSV with no variability and ΔP_S reduced by 5 cmH_2O from the baseline value; $vPSV_{-5}$, variable PSV with variability set to achieve ±5 cmH_2O and ΔP_S reduced by 5 cmH_2O from the baseline value; PSV, pressure support ventilation; CV, coefficient of variation.

of variation (CV). As illustrated in **Figure 1**, all patients underwent two blocks of interventions, receiving 45-min periods of ventilation with different settings. In the first block, the effects of three levels of variability were tested at constant ΔP_S to explore the effect of variability added to a fixed ΔP_S level, while in the second block, a variability of ±5 cmH_2O was added to ΔP_S set at either the baseline level or the baseline level minus 5 cmH_2O, to investigate the effects of variability at two ΔP_S levels. During the first block, the ΔP_S was set at a fixed value corresponding to the level chosen by the treating clinician before enrolment, and three different CV% levels were used: 0% ($PSV_{0\%}$), 15% ($vPSV_{15\%}$), and 30% ($vPSV_{30\%}$). During the second block, four ventilation settings were used: PSV with baseline ΔP_S (PSV_{BL}), baseline ΔP_S with variability set individually to ±5 cmH_2O ($vPSV_{BL}$), ΔP_S reduced by 5 cmH_2O compared with the baseline with either no variability (PSV_{-5}) or variability set to ±5 cmH_2O ($vPSV_{-5}$). The two blocks were performed sequentially, within 1 h from each other to allow for nursing assistance if required, and ventilation modes within each intervention block were assigned in random order with a Latin square design (as shown in **Figure 1**; **Supplementary Figures 1, 2**). The randomization sequence was generated with an online service, and a sealed envelope was opened at the moment of patient enrolment. Participants were blinded to the treatment assignment as were the operators involved in respiratory mechanics analysis.

Patient management procedures not related to mechanical ventilation, including sedation and fluid administration, were at the discretion of the treating clinician. When clinically feasible, we avoided changing FIO_2, PEEP, and ΔP_S during the study, and in case of desaturation below 92%, FIO_2 increase was prioritized over PEEP increase. After completion of the study protocol, ventilation was continued at the discretion of the treating physician.

Measurements

An esophageal balloon catheter (Compliance catheter, Microtek Medical B.V., Zutphen, The Netherlands) was inserted through the nose or mouth, filled with 1.5 ml, and correct positioning was verified with an occlusion maneuver (Akoumianaki et al., 2014). The flow was measured with a heated Fleisch-type pneumotachograph connected to a multi-channel transducer (ICU Lab, KleisTEK Engineering, Bari, Italy), while the tidal volume was measured as the integral of flow over time. Respiratory traces were recorded continuously throughout the study. An arterial blood gas analysis, heart rate, and invasive mean arterial pressure were recorded at baseline and the end of each ventilation step.

Pressure-time and flow-time curves were analyzed offline with a dedicated script written in MATLAB (MathWorks, MA, USA). The following parameters were computed breath by breath: V_T, PEEP, ΔP_S, mean airway pressure, inspiratory time to total time ratio (T_{insp}/T_{tot}), respiratory rate (RR), esophageal pressure swings (ΔP_{es}), and peak transpulmonary pressure (P_L). The respiratory muscle activity was quantified with the esophageal pressure-time product per min (PTP_{es}), calculated as follows (Mauri et al., 2016):

$$PTP_{es,min} = RR \cdot \int P_{mus}\, dt = RR \cdot \int (P_{cw,recoil} - P_{es})\, dt$$

where P_{mus} is the pressure generated by the respiratory muscles, and $P_{cw,recoil}$ is the chest wall recoil pressure, calculated assuming a fixed elastance of 5 cmH_2O/L. The asynchrony index was computed as the number of asynchronous events divided by the total number of ventilator cycles plus ineffective efforts during expiration multiplied by 100 (Blanch et al., 2015). Asynchronies were classified independently by two experienced operators (LB and MV), and discrepancies were resolved by consensus. The analysis of respiratory mechanics data was performed by three operators blinded to the ventilation settings (ADO, MF, and LM). Also, we measured the evolution of respiratory mechanics at min 1, 9, 18, 27, 36, and 45 from the start of each ventilation step. To allow sufficient time for patient adaptation, main analyses of respiratory mechanics and asynchronies were restricted to the last 10 min of each ventilation step.

Data Analysis and Sample Size Calculation

All variables are reported as medians [25th−75th percentile], if not otherwise specified. Measurements on multiple breaths were aggregated within-patients computing the median and the CV; then, between-patients medians [25th−75th percentile] were computed. Comparisons between continuous variables during the different ventilation steps were sought with Friedman's test and Dunn's *post-hoc* test. The primary endpoint was the partial pressure of arterial oxygen to FiO_2 ratio (PaO_2/FiO_2). From internal administrative data, we expected a baseline PaO_2/FiO_2 around 150 ± 50 mmHg. Using a Latin square crossover design, and assuming an intra-subject correlation of the PaO_2/FiO_2 between treatments with $\rho = 0.75$, we needed to enroll at least 16 patients to achieve 90% power (1-β) to detect a 20% relative increase in the PaO_2/FiO_2 ratio (Muller and Barton, 1989; Muller et al., 1992). To account for potential drop-off or missing respiratory mechanics data, we aimed to enroll 20 patients. Repeated measurement analysis of respiratory mechanics parameters at different timepoints within each ventilation block was performed using mixed-effects linear models using patients as random effects and timepoint, ventilation, and their interaction as fixed effects.

In one *post-hoc* analysis, associations were determined between the respiratory mechanics parameters of each breath and the ΔP_S received during the preceding breath in the $vPSV_{BL}$ and $vPSV_{-5}$ ventilation steps. For this purpose, mixed-effects linear models were used, using patients as random effects and the ΔP_S received during the preceding breath as the fixed effect.

All analyses were performed with R 3.2.3 (The R Foundation for Statistical Computing, www.r-project.org). Statistical significance was considered for two-tailed $p < 0.05$.

RESULTS

Twenty patients were enrolled and completed the study. Baseline characteristics are presented in **Table 1**. The FiO_2 and PEEP were kept constant during the study in all patients; one patient required ΔP_S reduction between ventilation block 1 and block 2 according to the treating clinician decision for reasons unrelated to the study procedures. **Tables 2, 3** show respiratory mechanics, hemodynamics, and arterial blood gas analysis in ventilation blocks 1 and 2, respectively. The distribution of key respiratory mechanics parameters in ventilation blocks 1 and 2 is illustrated in **Figures 2**, **3**, respectively. **Supplementary Figures 3–11** report details the evolution over time of the respiratory mechanics parameters in the different ventilation steps.

TABLE 1 | Baseline characteristics of patients.

Patient characteristics	
Number of patients	20
Age (years)	72 [59–79]
Female sex (N, %)	6/20 (30%)
Weight (kg)	80 [64–87]
Height (cm)	175 [165–180]
Body mass index (kg/m^2)	25.8 [22.7–29.5]
PBW (kg)	71 [58–75]
SAPS II	53 [38–60]
SOFA	7 [6–9]
RASS	−3 [−3 to −1]
Sedative drugs (N, %)	Propofol 5/20 (25%) Dexmedetomidine 3/20 (15%) Midazolam 4/20 (20%) None 8/20 (40%)
Analgesic drugs (N, %)	Fentanyl 8/20 (40%) Morphine 2/20 (10%) None 10/20 (50%)
Days of ventilation prior to inclusion	7 [5–9]
Primary reason for admission to the ICU	Acute respiratory failure: 10 (50%) Multiple trauma: 4 (20%) Brain hemorrhage: 3 (15%) Post-cardiac arrest: 3 (15%)
Risk factor for development of ARDS	Pneumonia: 11 (55%) Multiple fractures: 2 (10%) Sepsis: 4 (20%) Aspiration pneumonia: 3 (15%)
Blood gas analysis at enrolment PaO_2 (mmHg)	97 [79–120]
$PaCO_2$ (mmHg)	40 [36–46]
pHa	7.46 [7.44–7.51]
PaO_2/FIO_2 ratio (mmHg)	198 [154–250]
Ventilator settings at enrolment	
ΔP_S (cmH_2O)	15 [14–17]
PEEP (cmH_2O)	6 [5–8]
FIO_2 (%)	50 [43–58]
Tidal volume (mL/kg of PBW)	7.5 [7.0–8.5]
Respiratory rate (min^{-1})	15 [13–20]

PBW, predicted body weight; PEEP, positive end-expiratory pressure; ΔP_S, pressure support; SAPS, simplified acute physiology score; SOFA, sequential organ failure assessment score; RASS, richmond agitation-sedation scale; ICU, intensive care unit.

Block 1: Physiological Effects of Different Variability Levels at Constant ΔP_S

The PaO_2/FiO_2 did not differ between ventilation steps in block 1 ($p = 0.62$, **Table 2**). Median respiratory mechanics variables, other gas exchange, and hemodynamic parameters did not

TABLE 2 | Gas exchange, hemodynamics, and respiratory mechanics in patients during pressure support ventilation at different levels of variability (Block 1).

	Ventilation modes			*p*-values		
	$PSV_{0\%}$	$vPSV_{15\%}$	$vPSV_{30\%}$	Overall	$vPSV_{15\%}$ vs. $PSV_{0\%}$	$vPSV_{30\%}$ vs. $PSV_{0\%}$
Ventilation settings						
$\Delta P_{S,set}$ (cmH_2O)	15.0 [13.0–16.0]	15.0 [13.0–16.0]	15.0 [13.0–16.0]	>0.99		
ΔP_S set variability (CV %)	0	15	30	<0.001		
Gas exchange						
PaO_2/FIO_2 (mmHg)	209 [157–242]	214 [160–256]	210 [179–252]	0.62		
PaO_2 (mmHg)	96 [79–118]	98 [85–116]	108 [82–123]	0.62		
$PaCO_2$ (mmHg)	44 [37–46]	43 [38–46]	43 [37–48]	0.95		
pH	7.45 [7.43–7.49]	7.49 [7.43–7.50]	7.47 [7.44–7.50]	0.64		
Hemodynamics						
Heart rate (min^{-1})	85 [72–92]	83 [75–90]	83 [73–91]	0.46		
Mean arterial pressure (mmHg)	78 [68–96]	79 [73–91]	82 [70–90]	0.37		
Respiratory mechanics						
$\Delta P_{S,measured}$ (cmH_2O)	14.6 [12.5–15.7]	14.6 [12.7–16.0]	14.7 [12.6–16.5]	0.39		
$PS_{measured}$ (CV, %)	1.2 [0.7–2.0]	15.6 [15.0–17.2][a]	29.7 [27.5–31.5][a]	<0.001	0.006	<0.001
Total PEEP (cmH_2O)	7.2 [6.1–8.5]	7.4 [6.2–8.6]	7.5 [6.2–8.4]	0.09		
Total PEEP (CV, %)	2.2 [1.1–3.1]	2.2 [1.2–3.8]	2.6 [1.7–3.3][a]	0.034	0.40	0.026
P_{mean} (cmH_2O)	11.6 [9.9–12.6]	12.1 [10.1–12.9]	11.6 [10.4–12.8]	0.16		
P_{mean} (CV, %)	3.7 [3.0–6.0]	8.1 [5.8–9.6][a]	13.2 [10.2–15.5][a]	<0.001	0.016	<0.001
Respiratory rate (min^{-1})	16.7 [13.7–21.4]	16.8 [13.9–21.4]	15.6 [13.8–19.7]	0.86		
Respiratory rate (CV, %)	11.6 [9.2–15.8]	12.5 [10.3–22.4]	17.9 [15.8–24.9][a]	0.002	0.17	<0.001
V_T (ml/kg of PBW)	8.1 [7.3–10.0]	8.8 [7.0–10.7]	8.9 [7.2–10.1]	0.95		
V_T (CV, %)	6.7 [4.5–9.1]	13.1 [10.7–14.4][a]	23.8 [17.8–28.1][a]	<0.001	0.006	<0.001
T_{insp}/T_{tot}	0.34 [0.29–0.41]	0.37 [0.32–0.41]	0.37 [0.30–0.43]	0.35		
T_{insp}/T_{tot} (CV, %)	11.1 [7.4–15.1]	10.9 [9.6–16.3]	14.6 [12.3–21.0]	0.08		
PTP_{es} (cmH_2O s min^{-1})	126 [102–226]	154 [103–194]	136 [121–208]	0.95		
PTP_{es} (CV, %)	26.8 [15.6–39.2]	30.1 [17.0–47.6]	36.2 [25.9–58.2][a]	0.029	0.71	0.026
ΔP_{es} (cmH_2O)	5.0 [2.1–7.6]	3.0 [1.3–7.4]	2.7 [1.6–5.4]	0.10		
ΔP_{es} (CV, %)	23.2 [18.0–34.2]	26.8 [19.7–40.9]	26.8 [24.3–47.5]	0.07		
P_L (cmH_2O)	18.0 [16.9–21.4]	17.8 [16.0–21.9]	17.4 [15.9–20.2]	0.27		
P_L (CV, %)	4.5 [2.7–11.7]	14.7 [13.2–15.9][a]	25.8 [21.4–27.3][a]	<0.001	0.025	<0.001
Asynchrony index (%)	1.6 [0.6–10.5]	2.2 [0.5–16.3]	5.1 [1.0–17.4][a]	0.031	0.21	0.019

Values are computed during the last 10 min of a 45-min ventilation period. Data are reported as inter-subject median [25th−75th percentile] of the intra-subject median values.
[a]Significantly different from $PSV_{0\%}$. $PSV_{0\%}$, pressure support ventilation with no variability; $vPSV_{15\%}$, variable pressure support ventilation with 15% CV variability; $vPSV_{30\%}$, variable pressure support ventilation with 30% CV variability.
CV, coefficient of variation; PEEP, positive end-expiratory pressure; PBW, predicted body weight; ΔP_S, pressure support; V_T, tidal volume; PTP, esophageal pressure-time product; ΔP_{es}, esophageal pressure swings; P_L, peak transpulmonary pressure.

change between $vPSV_{15\%}$ and $vPSV_{30\%}$ compared with $PSV_{0\%}$ (**Table 2**). However, the variability of ΔP_S, $PEEP_{tot}$, P_{mean}, and V_T was higher with $PSV_{15\%}$ and $PSV_{30\%}$ compared with $PSV_{0\%}$ (**Table 2**). The RR and $PTP_{es,min}$ had higher variability only with $vPSV_{30\%}$ (**Table 2**). Moreover, asynchronies were more frequent with $vPSV_{30\%}$ compared with $PSV_{0\%}$ ($p = 0.019$, **Table 2**).

Block 2: Physiological Effects of Variability at Two Levels of ΔP_S

The PaO_2/FiO_2, as well as other gas exchange and hemodynamic parameters, did not differ between ventilation steps in block 2 (**Table 3**). Ventilation modes with ΔP_S reduced by 5 cmH_2O (PSV_{-5} and $vPSV_{-5}$) had lower P_{mean}, V_T, P_L, and higher RR (**Table 3**). Adding ±5 cmH_2O variability ($vPSV_{BL}$ and $vPSV_{-5}$ steps) increased the variability of ΔP_S and P_{mean} compared to PSV without variability at the corresponding ΔP_S level. Adding ±5 cmH_2O variability increased the variability of V_T and P_L only when using the baseline ΔP_S, but not when the ΔP_S was reduced by 5 cmH_2O. The incidence of asynchronies was not different between ventilation steps in block 2 (**Table 3**).

Tables 2, **3** report extensive details on respiratory mechanics, hemodynamics, and arterial blood gas analysis in ventilation blocks 1 and 2, respectively. The distribution of key respiratory mechanics parameters in ventilation blocks 1 and 2 is illustrated in **Figures 3**, **4**, respectively. **Supplemental Figures 3–11** report details the evolution over time of the respiratory mechanics parameters in the different ventilation steps.

TABLE 3 | Gas exchange, hemodynamics, and respiratory mechanics in patients during pressure support ventilation at different variability and pressure support level (Block 2).

	Ventilation modes				*p*-values			
	PSV_{BL}	**$vPSV_{BL}$**	**PSV_{-5}**	**$vPSV_{-5}$**	**Overall**	**$vPSV_{BL}$ vs. PSV_{BL}**	**PSV_{-5} vs. PSV_{BL}**	**$vPSV_{-5}$ vs. PSV_{-5}**
Ventilator Settings								
ΔP_S setting	Baseline		Baseline−5 cmH_2O					
$\Delta P_{S,set}$ (cmH_2O)	14.0 [12.0–16.0]	14.0 [12.0–16.0]	9.0 [7.0–11.0]	9.0 [7.0–11.0]	<0.001	0.99	<0.001	<0.001
Variability setting	None	± 5 cmH_2O	No variability	± 5 cmH_2O				
ΔP_S set variability (CV %)	0 [0–0]	11 [9–13]	0 [0–0]	15 [13–20]	<0.001	<0.001	0.99	<0.001
Gas exchange								
PaO_2/FIO_2 (mmHg)	213 [180–229]	194 [180–229]	215 [183–239]	197 [167–224]	0.61			
PaO_2 (mmHg)	95 [89–117]	99 [87–115]	99 [90–121]	98.8 [85–1112]	0.61			
$PaCO_2$ (mmHg)	42 [39–46]	43 [39–48]	44 [40–49]	44 [40–51]	0.18			
pH	7.47 [7.43–7.49]	7.48 [7.43–7.49]	7.47 [7.43–7.48]	7.47 [7.42–7.48]	0.21			
Hemodynamics								
Heart rate (min^{-1})	83 [77–93]	84 [77–92]	84 [77–92]	86 [76–92]	0.46			
Mean arterial pressure (mmHg)	88 [81–93]	82 [78–87]	80 [77–90]	86 [77–90]	0.37			
Respiratory mechanics								
$\Delta P_{S,measured}$ (cmH_2O)	13.2 [12.0–15.8]	13.6 [12.5–15.9]	8.4 [7.0–10.5][a]	8.5 [7.2–10.7]	<0.001	0.92	<0.001	0.67
$\Delta P_{Smeasured}$ (CV, %)	1.5 [0.9–4.8]	12.1 [11.1–14.3][a]	2.3 [1.7–6.6]	17.9 [15.2–18.6][b]	<0.001	0.001	0.99	<0.001
Total PEEP (cmH_2O)	7.6 [6.2–8.4]	7.5 [6.2–8.5]	7.7 [5.8–8.7][a]	7.7 [5.7–8.7]	0.001	0.99	0.009	0.92
Total PEEP (CV, %)	2.3 [1.4–4.9]	2.6 [1.8–8.0]	2.1 [1.6–4.3]	2.4 [1.4–5.9]	0.09			
P_{mean} (cmH_2O)	11.0 [9.7–12.6]	11.2 [9.7–13.1]	9.7 [8.4–11.9][a]	9.8 [8.4–11.8]	<0.001	0.67	<0.001	0.99
P_{mean} (CV, %)	5.4 [3.4–8.2]	8.1 [5.5–10.2][a]	2.5 [1.8–5.0]	6.0 [4.5–7.3][b]	<0.001	0.014	0.11	0.029
Respiratory rate (min^{-1})	14.7 [13.7–18.3]	17.6 [14.8–19.4]	22.9 [16.0–24.9][a]	20.1 [16.9–26.8]	0.022	0.96	0.041	0.95
Respiratory rate (CV, %)	18.8 [10.6–46.4]	32.0 [16.1–60.3]	13.1 [6.2–35.2]	14.6 [9.7–17.7]	0.003	0.43	0.43	0.99
V_T (ml/kg of PBW)	8.5 [7.2–9.4]	8.2 [7.0–9.1]	7.0 [5.9–7.6][a]	7.2 [6.0–7.7]	<0.001	0.92	<0.001	0.67
V_T (CV, %)	9.3 [5.1–15.7]	12.7 [11.1–15.3][a]	8.3 [4.2–13.6]	10.8 [9.4–14.6]	0.003	0.006	0.99	0.74
T_{insp}/T_{tot}	0.36 [0.30–0.37]	0.37 [0.30–0.39]	0.36 [0.32–0.39]	0.35 [0.32–0.38]	0.42			
T_{insp}/T_{tot} (CV, %)	9.3 [5.1–15.7]	12.7 [11.1–15.3][a]	8.3 [4.2–13.6]	10.8 [9.4–14.6]	0.058			
PTP_{es} (cmH_2O s min^{-1})	155.2 [118.4–262.8]	161.4 [87.1–248.3]	215.0 [128.1–357.9]	259.1 [151.1–422.8]	0.001	0.51	0.18	0.99
PTP_{es} (CV, %)	31.8 [20.6–52.3]	53.8 [23.9–71.1]	27.5 [16.0–40.5]	30.7 [18.5–44.7]	0.005	0.08	0.75	0.81
ΔP_{es} (cmH_2O)	4.4 [2.1–9.3]	5.3 [1.3–8.0]	5.6 [1.6–11.8]	10.4 [2.6–14.1]	<0.001	0.59	0.59	0.43
ΔP_{es} (CV, %)	32.7 [21.1–45.5]	40.9 [22.3–53.5]	22.8 [13.1–31.5]	20.6 [12.7–32.2]	<0.001	0.51	0.29	0.67
P_L (cmH_2O)	18.5 [15.9–23.2]	18.6 [16.1–23.4]	14.6 [11.5–20.8][a]	18.0 [14.0–21.6]	<0.001	0.99	<0.001	0.67
P_L (CV, %)	7.5 [3.8–12.6]	12.2 [11.1–17.0][a]	10.0 [4.2–13.1]	15.7 [12.4–17.3]	0.009	0.042	0.95	0.14
Asynchrony index (%)	1.5 [0.7–7.2]	2.4 [0.1–12.6]	0.9 [0.0–7.6]	1.3 [0–3.9]	0.21			

Values are computed during the last 10 min of a 45-min ventilation period. Data are reported as inter-subject median [25th−75th percentile] of the intra-subject median values.

[a] *Significant difference compared to PSV_{BL} ($p < 0.05$).*

[b] *Significant difference compared to PSV_{-5} ($p < 0.05$).*

PSV_{BL}, pressure support ventilation with no variability and baseline ΔP_S as per clinical indication; $vPSV_{BL}$, variable pressure support with variability set to achieve ±5 cmH_2O and baseline ΔP_S as per clinical indication; PSV_{-5}, pressure support ventilation with no variability and ΔP_S reduced by 5 cmH_2O from the baseline value; $vPSV_{-5}$, variable pressure support ventilation with variability set to achieve ±5 cmH_2O and ΔP_S reduced by 5 cmH_2O from the baseline value; CV, coefficient of variation; PEEP, positive end-expiratory pressure; PBW, predicted body weight; ΔP_S, pressure support; V_T, tidal volume; PTP, esophageal pressure-time product; ΔP_{es}, esophageal pressure swings; P_L, peak transpulmonary pressure.

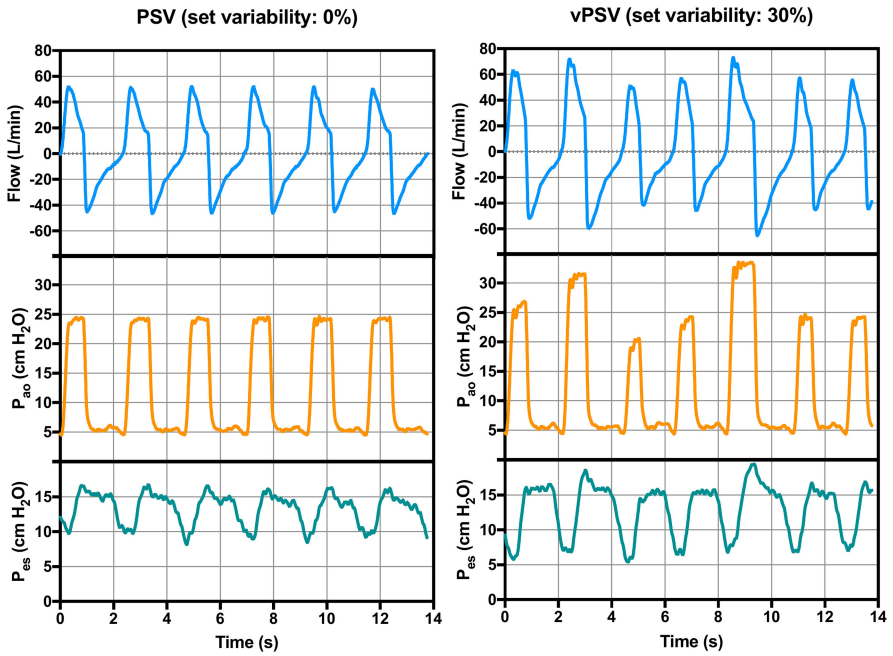

FIGURE 2 | Representative respiratory traces of a patient during conventional (left) and variable (right) pressure support ventilation. P_{ao}, pressure at the airway opening; P_{es}, esophageal pressure.

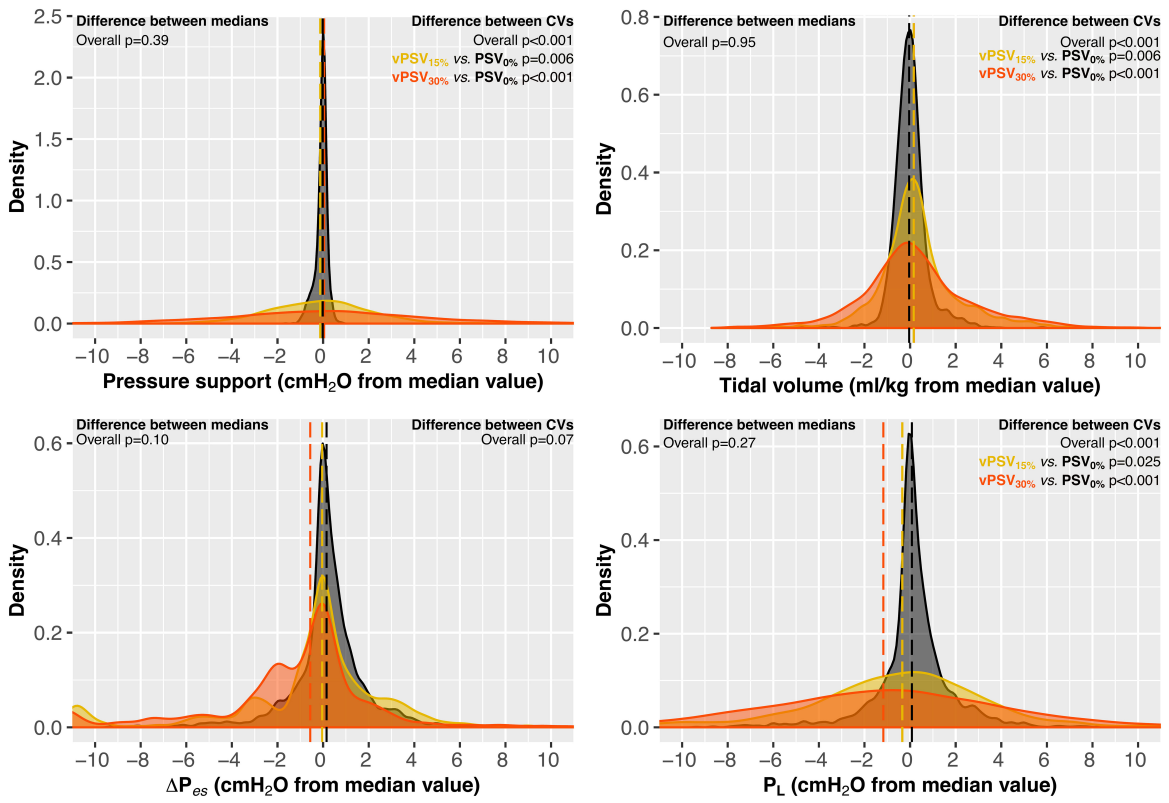

FIGURE 3 | Respiratory mechanics at different levels of variability (block 1). Variables are reported as the difference from the median value achieved during $PSV_{0\%}$ to allow between-patients visual comparisons. Dashed lines represent the medians of each ventilation step. $PSV_{0\%}$, conventional PSV ventilation with no variability; $vPSV_{15\%}$, variable PSV with variability set to 15% CV; $vPSV_{30\%}$, variable PSV with variability set to 30% CV; CV, coefficient of variation; ΔP_S, pressure support; ΔP_{es}, esophageal pressure swings; P_L, peak transpulmonary pressure.

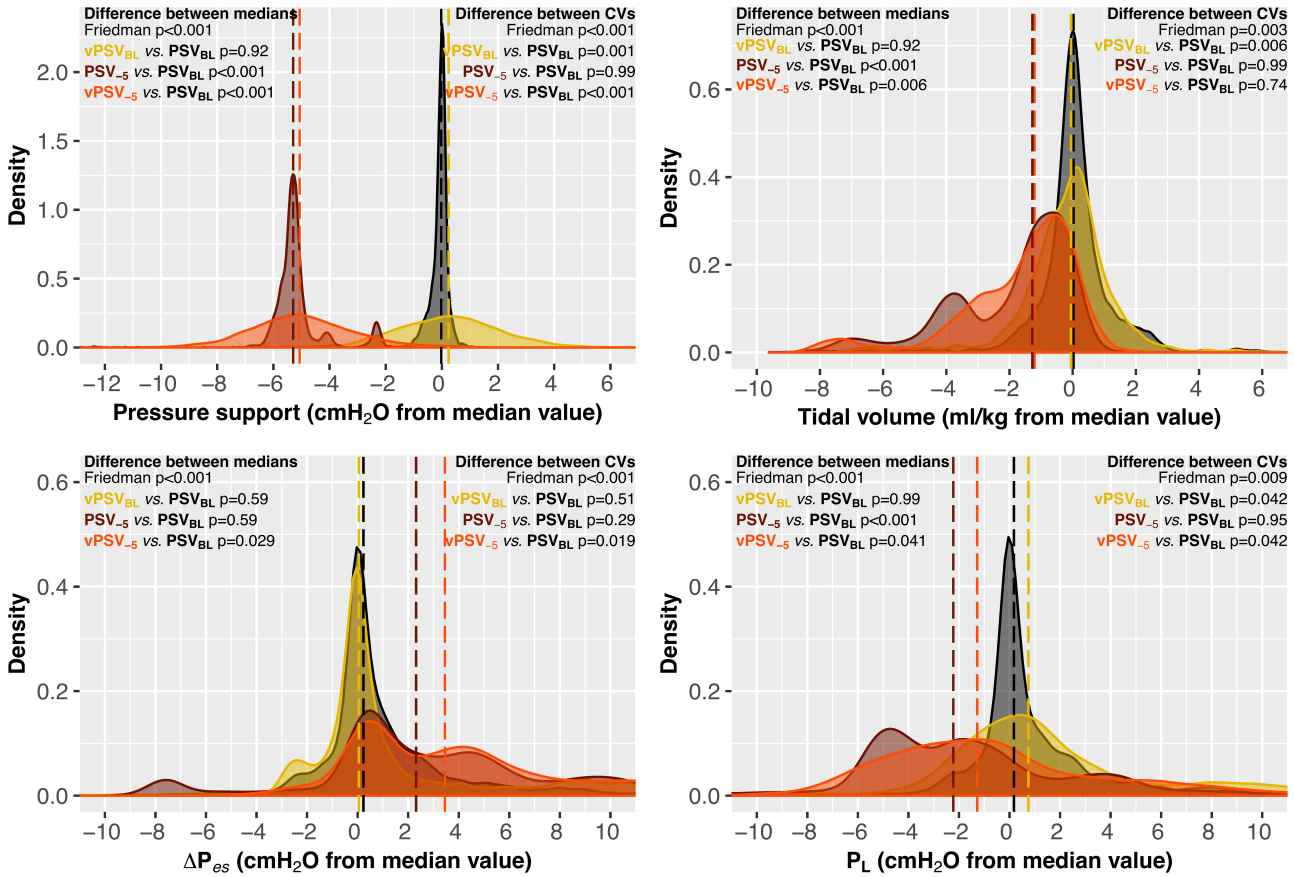

FIGURE 4 | Effects of variability on the distribution of respiratory mechanics parameters at different levels of pressure support (block 2). Variables are reported as the difference from the median value achieved during PSV_{BL} to allow between-patients visual comparisons. Dashed lines represent the medians of each ventilation step. PSV_{BL}, PSV with no variability and baseline ΔP_S as per clinical indication; $vPSV_{BL}$, variable pressure support with variability set to achieve ±5 cmH_2O and baseline ΔP_S as per clinical indication; PSV_{-5}, PSV with no variability and ΔP_S reduced by 5 cmH_2O from the baseline value; $vPSV_{-5}$, variable PSV with variability set to achieve ±5 cmH_2O and ΔP_S reduced by 5 cmH_2O from the baseline value; PSV, pressure support ventilation; CV, coefficient of variation; ΔP_S, pressure support; ΔP_{es}, esophageal pressure swings; P_L, peak transpulmonary pressure.

Post-hoc Analysis

Associations between respiratory mechanics parameters and the pressure level received in the preceding breath during $vPSV_{BL}$ and $vPSV_{-5}$ are reported in **Figure 5**. The ΔP_S received in the preceding breath was inversely associated with the magnitude of the inspiratory effort (ΔP_{es}) in the following breath, both during $vPSV_{BL}$ ($p = 0.003$) and $vPSV_{-5}$ ($p = 0.005$).

DISCUSSION

The main findings of this study are that in our mixed-ICU population of patients with mild to moderate ARDS: (1) vPSV with 15 or 30% variability did not influence gas exchange compared with conventional PSV; (2) at constant ΔP_S, vPSV increased the variability of V_T and P_L; (3) $vPSV_{30\%}$ increased the incidence of asynchronies; and (4) when the ΔP_S was reduced by 5 cmH_2O from the baseline value, adding variability did not increase the variability of V_T and P_L.

This is the first study comparing the short-term effects of vPSV at different levels of variability and ΔP_S in patients with ARDS. In previous studies, vPSV improved oxygenation in the experimental models of ARDS (Gama de Abreu et al., 2008; Spieth et al., 2011, 2012), but not in a cohort of hypoxemic critically ill patients (Spieth et al., 2013). However, that last study included mostly postoperative patients without a confirmed diagnosis of ARDS and investigated a single level of variability and pressure support. Opposite to what was found in preclinical studies in animals, vPSV had no effect on gas exchange, when the ΔP_S was set to the baseline value identified by the treating clinician and neither when it was reduced by 5 cmH_2O. This could be explained by several mechanisms; most importantly, the time investigated in each ventilation step was relatively short, and the fact that patients had an established diagnosis of ARDS mostly in their recovery phase and received mechanical ventilation for few days prior to the inclusion in this study. Under these conditions, patient lungs could have developed

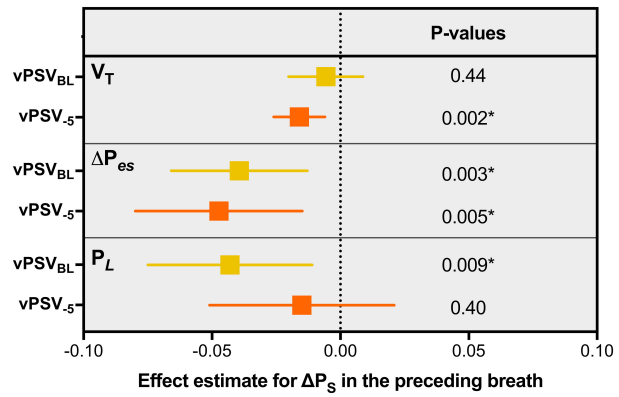

FIGURE 5 | Associations between respiratory mechanics parameters and the pressure level received in the preceding breath during variable PSV. Squares and confidence intervals refer to the effect estimate for ΔP_S in a mixed model comprising the ΔP_S received during the preceding breath as a fixed effect and the patient as a random effect with random intercept. The units of the estimates are expressed in the untransformed units of the variables, i.e., they represent the absolute change in V_T, ΔP_{es}, or P_L when the ΔP_S received during the preceding breath increases by 1 cmH_2O. $vPSV_{BL}$, variable PSV with variability set to achieve ±5 cmH_2O and baseline ΔP_S as per clinical indication; $vPSV_{-5}$, variable PSV ventilation with variability set to achieve ±5 cmH_2O and ΔP_S reduced by 5 cmH_2O from the baseline value; PBW, predicted body weight; PSV, pressure support ventilation; ΔP_S, pressure support; V_T, tidal volume; ΔP_{es}, esophageal pressure swings; P_L, peak transpulmonary pressure. *Significant association ($p < 0.05$).

consolidation, namely, the presence of lung regions scarcely responsive to recruitment (Cressoni et al., 2017). In this case, the breaths with higher ΔP_S received cyclically during variable pressure support might expose the patient to volutrauma in the aerated regions of the lung (Güldner et al., 2016; Pelosi et al., 2016) due to the reduced size of the lung aerated compartment. Another explanation for the possible lack of effect of variability on oxygenation might be that, different from what happens in PSV with a sigh, vPSV has no control over the time spent at higher pressure during tidal breathing. This might result in random breaths with higher P_S and short inspiratory time, both possibly insufficient to achieve recruitment. The tidal volume measured in this cohort was higher than the recommended targets, but this reflects the current clinical practice in patients with ARDS receiving assisted ventilation modes (Bellani et al., 2016; Writing Group for the PReVENT Investigators et al., 2018). During the second block of ventilations, the patients tolerated a ΔP_S reduction without worsening the gas exchange in the short term, at the price of a modest increase of the respiratory rate, suggesting that they were slightly over-assisted. This could have influenced patient-ventilator interaction (Kataoka et al., 2018) and the response to variability, as suggested by the finding that, during the second block of interventions, the variability of V_T was increased by vPSV compared with PSV only when the baseline ΔP_S was used. However, during ventilation steps with baseline ΔP_S, patients had a work of breathing estimated with the PTP_{es} of around 150 $cmH_2O \cdot s \cdot min^{-1}$, which is within the recommended range (Mauri et al., 2016). Interestingly, higher P_S resulted in a reduction in ΔP_{ES} in the following breath at both set ΔP_S levels, while the variability of V_T and P_L was increased by extrinsic variability only at higher ΔP_S. This seems to suggest that while a neural response to extrinsic variability is present independent of the level of assistance, its effects on the variability of V_T and P_L are influenced by the level of ΔP_S.

This study is underpowered to demonstrate the effects of vPSV on patient-centered outcomes like duration of ventilation. This is tested in another, yet ongoing clinical trial (Kiss et al., 2013). In the *post-hoc* analysis, the effects of vPSV on the response of patients in terms of inspiratory effort, transpulmonary pressure, and tidal volume developed in the following breath were studied. An inverse association between the ΔP_S received in the preceding breath and the inspiratory effort was observed. Different from other modified PSV modes such as the proportional assist ventilation (PAV) and the neurally adjusted ventilatory assist (NAVA), the variability of ΔP_S was random, i.e., is not related to the efforts of patients. This analysis suggests that there might be a complex interaction between the ventilator and a patient, in which the inspiratory effort and the adaptation of the patient to pressure support are influenced by the history of the previous breaths.

Limitations

This study has several limitations. The crossover design allowed the investigation of the effects of different levels of variability and ΔP_S in terms of gas exchange and respiratory mechanics in the short term but is intrinsically unable to investigate major clinical outcomes. The sample size is relatively low, no static measurements of respiratory mechanics were performed, and patients received heterogeneous sedation regimens that might have affected differently the respiratory drive. The population included in the study identifies a subgroup of critically ill patients meeting the criteria for mild to moderate ARDS who already received controlled or assisted mechanical ventilation for several days; however, the baseline patient characteristics were similar to those reported in a recent large observational study in patients with ARDS assisted non-invasively (Bellani et al., 2017). These patients with established respiratory failure, thus, possibly consolidated lung areas, might not benefit from the cyclic recruitment effect of vPSV, while patients with early ARDS might respond differently. However, the role of spontaneous breathing in the early management of ARDS is still unclear. This study could neither elucidate the mechanisms of the neural responses of the patients to variability nor the neuromuscular coupling of the respiratory muscles.

CONCLUSION

In our cohort of patients with mild to moderate ARDS, vPSV did not improve gas exchange at different levels of variability and pressure support. Compared with PSV, vPSV increased the variability of V_T, but not when low levels of variability were used in conjunction with lower pressure support. Moreover, vPSV did not exert a clinically relevant effect on the average inspiratory effort and work of breathing.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The study was approved by the Local Ethical Review Board (Comitato Etico Aziendale Policlinico San Martino protocol no. 1052/12) and prospectively registered on clinicaltrials.gov (study identifier: NCT01683669). According to the local ethical requirements, the next of kin provided written informed assent, followed by delayed written consent from patients in case of recovery of consciousness.

AUTHOR CONTRIBUTIONS

LB takes responsibility for the integrity of data. LB, PP, MV, and MG designed the study. LB, YS, MF, AD'O, DD'A, PRa, and IB conducted the study. LB, LM, MF, RH, AD'O, and CR analyzed the data. LB, MS, PP, PRo, and MG wrote the manuscript. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.725738/full#supplementary-material

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Conflict of Interest: MG was granted a patent on the variable pressure support ventilation mode of assisted ventilation (noisy PSV), which has been licensed to Dräger Medical AG (Lübeck, Germany).

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

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Astrocytes Downregulate Inflammation in Lipopolysaccharide-Induced Acute Respiratory Distress Syndrome: Applicability to COVID-19

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Background: An acute respiratory distress syndrome (ARDS) is caused by the increased amounts of pro-inflammatory cytokines and neutrophil-mediated tissue injury. To date, there is no effective treatment for the ARDS available, while the need for one is growing due to the most severe complications of the current coronavirus disease-2019 (COVID-19) pandemic. The human astrocytes (AstroRx) have shown immunomodulatory properties in the central nervous system (CNS). This study aimed to evaluate the capacity of astrocytes to decrease lung inflammation and to be applied as a treatment therapy in ARDS.

Methods: First, we assessed the ability of clinical-grade AstroRx to suppress T-cell proliferation in a mixed lymphocyte reaction test. Next, we tested the therapeutical potential of AstroRx cells in a lipopolysaccharide (LPS)-based ARDS mouse model by injecting AstroRx intravenously (i.v). We determined the degree of lung injury by using a severity scoring scale of 0–2, based on the American Thoracic Society. The scoring measured the presence of neutrophils, fibrin deposits, and the thickening of alveolar walls. The state of inflammation was further assessed by quantifying the immune-cell infiltration to the bronchoalveolar lavage fluid (BALF) and by the presence of proinflammatory cytokines and chemokines in the BALF and serum.

Results: We detected that AstroRx cells were capable to suppress T-cell proliferation *in vitro* after exposure to the mitogen concanavalin A (ConA). *In vivo,* AstroRx cells were able to lower the degree of lung injury in LPS-treated animals compared with the sham injected animals ($P = 0.039$). In this study, 30% of AstroRx treated mice showed no lung lesions (responder mice), these mice presented a steady number of eosinophils, T cells, and neutrophils comparable with the level of naïve control mice. The inflammatory cytokines and chemokines, such as TNFα, IL1b, IL-6, and CXCL1, were also kept in check in responder AstroRx-treated mice and were not upregulated as in the sham-injected mice ($P < 0.05$). As a result, the LPS-treated ARDS mice had a higher survival rate when they were treated with AstroRx.

Conclusions: Our results demonstrate that the immunosuppressive activity of AstroRx cells support the application of AstroRx cells as a cell therapy treatment for ARDS. The immunoregulatory activity may also be a part of the mechanism of action of AstroRx reported in the amyotrophic lateral sclerosis (ALS) neurodegenerative disease.

Keywords: ARDS, astrocytes, immune-modulation, inflammation, embryonic stem cells

INTRODUCTION

An acute respiratory distress syndrome (ARDS) is a form of progressive hypoxemia respiratory failure and pulmonary edema in the absence of heart failure (1). The etiology of ARDS varies (e.g., pneumonia, sepsis, or acute pancreatitis) and great efforts have been made in intensive care medicine, but the overall mortality is still high (2, 3). Recently, a significant proportion of patients infected with coronavirus disease-2019 (COVID-19) developed viral pneumonia that caused an acute lung injury (ALI) capable of rapid progression to viral sepsis and ARDS with a high fatality rate especially in older and comorbidities populations (4–6). The phases in the development of ARDS include the exudation stage characterized by the inflammatory cell infiltration and pulmonary edema (stage 1), the proliferation of myofibroblasts (stage 2), and extracellular matrix (ECM) over-deposited (stage 3) (7). The fibrosis process (stages 2 and 3) is rapid and occurs within 1 week (8, 9). This inflammatory response process referred to as cytokine storm or cytokine release syndrome (CRS), contributes to the development of ARDS and often irreversible multi-organ dysfunction syndrome (MODS) associated with the severe-critical forms of COVID-19 (10, 11). Another key process in the development of ARDS is neutrophil accumulation in high abundance in the pulmonary microcirculation, lung interstitium, and alveolar airspace of the patients with ARDS (12). In addition, an ARDS is associated with systemic neutrophil priming, delayed neutrophil apoptosis, and clearance of neutrophils from the lungs. In animal models, lung injury could be ameliorated by reducing the number of circulating neutrophils (13).

Current management for COVID-19 (severe acute respiratory syndrome-coronavirus 2, SARS-CoV-2) infected patients with severe pneumonia and ARDS remains supportive, such as the use of anti-infection drugs, intubated ventilator-assisted breathing therapy, and extracorporeal membrane oxygenation (ECMO) (14–16). Since ARDS is associated with high mortality and morbidity, it is vital to develop new effective therapeutic approaches capable of immunomodulating the immune system and inflammatory response. This could be of great benefit in preventing the disease progression and reducing the case mortality rate in the high-risk patients with COVID-19. A growing body of evidence has shown that cell-based therapies hold therapeutic effects for ARDS. Most of the studies have focused on the therapeutic effects of mesenchymal stem cells (MSCs) (17, 18) and some studies have also investigated the possible applications of other cell types, such as pulmonary epithelial progenitors (19, 20).

Another approach using astrocytes is presented as they are the most abundant glial cell in the central nervous system (CNS). Astrocytes regulate the concentration of different neurotransmitters and ions, supply various metabolites and energy, regulate osmolarity, modulate synaptic activity, secrete neurotrophic and neuroprotective factors, promote neurogenesis (21–23), and remyelination (24). Moreover, the astrocytes are essential players in immune-modulation (25, 26). Astrocytes have a dual role as key immune modulators of the immune response of the CNS to infections, neurodegenerative disorders, and injuries (27). Following injury and in disease, their ability to respond to, and commence initial responses to injury/disease is increasingly apparent. Astrocytes serve as a contact between the CNS and the peripheral immune system. In many pathological conditions, astrocytes either secrete anti-inflammatory or pro-inflammatory factors which modulate the immune system (28–30). Astrocytes, take part in both the recruitment and restriction of leukocytes in the CNS (31, 32). Neuroinflammation is increasingly recognized as an important mediator of disease progression in patients with amyotrophic lateral sclerosis (ALS), and similar to ARDS, it is characterized by reactive tissue and infiltrating peripheral monocytes and lymphocytes (33). The astrocytes derived from the pluripotent stem cells (AstroRx) demonstrated neuroprotective A2-type characteristics, such as glutamate uptake capabilities, secretion of neuroprotective factors, promotion of axon outgrowth, and protection of motor neurons (MNs) from oxidative stress (34). Intrathecal injection of astrocytes (AstroRx) in an ALS animal model demonstrated a therapeutic benefit (34) and is being tested first in the human clinical trial in the patients with ALS (clinicaltrial.gov ID NCT03482050). The immune-modulatory effect of astrocytes outside the CNS, to the best of our knowledge, is not reported yet and can shed more light on its anti-inflammatory activity in ALS disease.

In this current study, the effect of astrocytes on ARDS is investigated for the first time. We deciphered the immunomodulatory effect of AstroRx first *in vitro* by evaluating T-cell proliferation in a mixed lymphocyte reaction test. Then, we assessed the effect of intravenous (i.v.) AstroRx injection on lung injury, lung inflammation, and survival in a lipopolysaccharide (LPS)-based ARDS mouse model. Altogether our results indicate that AstroRx has the potential to maintain immune homeostasis in the lung and thereby increase the chance of survival.

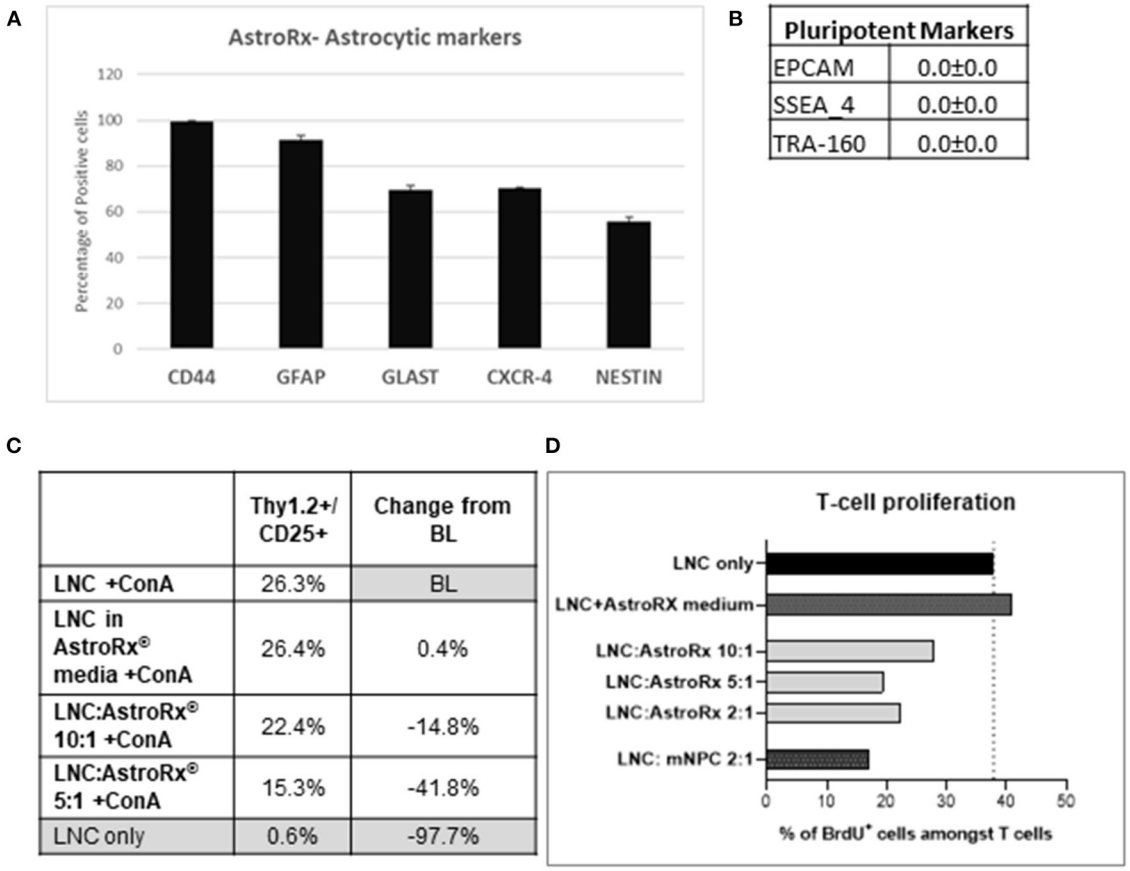

FIGURE 1 | Human astrocytes (AstroRx) demonstrate immunosuppressive capacities *in vitro*. **(A,B)** The AstroRx were analyzed by flow cytometry for astrocytic markers **(A)** and pluripotent stem cells markers **(B)**. **(C,D)** The immunosuppressive potential of AstroRx was assessed in a mixed lymphocyte reaction test. The AstroRx were co-cultured with murine lymph node cells (LNCs). **(C)** The LNCs were stained for T cell marker Thy1.2 and CD25 positive cells under ConA activation in the presence or absence of different amounts of AstroRx. **(D)** The proliferation state of T cells (CD3+) was determined by the proliferation marker BrdU in the presence of ConA. The LNCs only were used as negative control while the LNC with mouse neural precursor cells (mNPC) were used as a positive control. Average and ± SEM.

RESULTS

Suppression of T-cell Proliferation by AstroRx Cells

In a recent study, we have shown how human embryonic stem cell (hESC)-derived astrocytes (AstroRx) protect MNs *in vitro* and in the ALS animal models (34). In these studies, we demonstrated that AstroRx protected neurons by the secretion of neuroprotective and neurotrophic factors, uptake of glutamate, and regulation of oxidative stress. The immunomodulatory capacity of these human astrocytes (AstroRx) was not fully defined yet. To better understand if AstroRx can protect from inflammatory damage, we analyzed its immunosuppressive potential. For this, we used fully differentiated AstroRx, which expressed high levels of astrocytic markers (**Figure 1A**) and did not express pluripotent stem cells markers (**Figure 1B**). The AstroRx were co-cultured with murine lymph-node cells (LNC). The T-cell proliferation in this culture was induced by concanavalin A (ConA). When ConA was not added to the culture, the percentage of T cells in the culture was below 1%. The addition of either AstroRx cells or the conditioned medium of AstroRx cells did not affect the percentage of T cells in the culture (not shown).

In contrast, in the presence of ConA, the AstroRx cells profoundly influenced the T-cell population ($Thy1.2^+/CD25^+$), which decreased by about 15% in the LNC:AstroRx® (at a ratio of 10:1) co-culture. A greater inhibitory effect was achieved when using a ratio of 5:1 of LNC:AstroRx culture, which decreased T-cell population by 42%. This effect was not observed when only AstroRx-conditioned medium was added (**Figure 1C**), indicating that the effect was cell-contact dependent.

To evaluate the proliferation capacity of T cells in the presence of AstroRx, we performed a BrdU cell proliferation assay. The percentage of $CD3^+/BrdU^+$ cells in a culture of LNC with ConA but without AstroRx was chosen to serve as a baseline (BL). Without ConA, the percentage of human proliferating cells among the T cells was below 1%, the addition of different amounts of AstroRx cells to LNCs in the absence of ConA did not affect the percentage of proliferating T cells (not shown). In the presence of ConA, 40% of T cells were BrdU+ and proliferating.

As a positive inhibitory control, we used mouse neural precursor cells (mNPCs), which were previously described for reducing T-cell proliferation (35). Co-culture with mNPCs had no effect on T-cell proliferation in the absence of ConA (not shown) but reduced the number of proliferating cells among the T cells by more than a half when T cells were exposed to ConA (**Figure 1D**). Interestingly, when T cells were co-cultured with AstroRx, the proliferation rate among the T cells decreased by more than a third in the LNC:AstroRx® 10:1 culture. Higher ratios of AstroRx® to LNC resulted in a greater inhibitory effect, similar to the range of co-cultures with mNPCs (decrease by 49 or 43% in LNC:AstroRx® 5:1 or LNC:AstroRx® 2:1 cultures, respectively).

The effect was not observed when AstroRx® conditioned media was added to the culture, again suggesting that the AstroRx suppression is mediated by a direct cell-contact or communication between the AstroRx cells and LNC. Altogether, these results show that AstroRx has the potential to suppress the T-cell proliferation in a dose-dependent manner.

AstroRx Cells Alleviate LPS-Induced Pulmonary Inflammation and Fibrosis

Next, we tested the immune suppressive features of AstroRx in an *in vivo* model for ARDS. Intratracheal administration of LPS in the BALB/c mice induces severe lung damage and is a prevalent ARDS animal model (36). To test whether the AstroRx was capable of limiting the lung inflammation and lung damage, we injected i.v. two different doses of AstroRx after LPS-induction ($n = 10$, 2×10^5 and $n = 10$, 5×10^5 cells). Naïve ($n = 4$), untreated mice and sham-injected LPS-treated mice ($n = 10$) served as the negative and positive controls, respectively. The treatment with AstroRx at both cell concentrations increased the survival of LPS-induced mice compared with the sham-injected animals (20% loss vs. 10% loss, **Figure 2A**). To assess the degree of lung injury in the mice that survived, we performed the histological analysis of the lung sections 72 h after an LPS treatment (**Figure 2B**). The sham-injected mice showed significant thickened alveolar walls and cell infiltration, while treatment with AstroRx cells at a concentration of 5×10^5 cells alleviated the LPS-induced damage. An analysis of ALI was performed according to a method described by Matute-Bello et al. using a severity scoring scale of 0–2, based on the American Thoracic Society Documents (37) assessing the alveolar wall thickness, fibrin presence, and neutrophil accumulation which sums together to a total severity score (**Figure 2C**). The treatment with AstroRx cells significantly lowered the total severity score as compared with the sham-injected ARDS animals with 3.22 compared with 4.6 (**Figure 2C**, $P = 0.039$). According to lung score, the animals within one group were categorized into the responders (lung score ≤ 2) and non-responders (lung score >2). The percentage of responders was higher among the AstroRx-treated mice compared with the sham injected mice (**Figures 2D,E**), and higher among the mice that received a high dose of AstroRx compared with the mice that received a low number of AstroRx.

AstroRx Attenuates LPS-Induced Cytokine and Chemokine Storm in Bronchoalveolar Lavage Fluid and Serum

To understand the factors that contributed to the reduced lung damage following the AstroRx treatment, we analyzed the infiltration of B and T lymphocytes, eosinophils, neutrophils, and macrophages into the bronchoalveolar lavage fluid (BALF).

In the sham-injected ARDS mice, most of the cells in the BALF consisted of neutrophils (>92%), while the immune profile of high-dose of AstroRx shift toward naïve mice the immune profile (**Figure 3**, **Supplementary Figure 1**, $p = 0.0274$ for neutrophils and macrophages and $p = 0.0206$ for T cells). The effect of AstroRx on the immune cells became even clearer when we analyzed the immune profile differentially in the responder and non-responder mice (**Figure 4**, **Supplementary Figure 2**, $P = 0.0286$). The responder mice (lung score ≤ 2) among the AstroRx-treated LPS-injected mice had an immune profile that was similar to one of the naïve non-LPS-injected mice in terms of cell proportion and a general low-level of absolute cell number. While the non-responder mice displayed an immune profile that resembled the sham-injected mice with a general heavy infiltration of immune cells and a specifically massive invasion of neutrophils.

As the immune profile is not only determined by the cells but also by molecules, we next analyzed the presence of cytokines and chemokines in the BALF and serum. At first sight, the expression of inflammatory cytokines and chemokines was quite heterogeneous within the group of AstroRx-treated animals (**Figure 5**, $P = 0.13$ and higher). But when we dissected the group again into responders and non-responders, it became clear, that the responders among the AstroRx treated mice showed low expression of TNFa, IL1b, IL-6, IL-5, CC2, and CXCL1 (**Figure 6** naïve vs. sham: $P = 0.0121$ (for all cytokines); 500k responders vs. non-responders: $P = 0.0091$ (for TNFa), $P = 0.0286$ for all other cytokines). Strikingly, their levels were similar to the levels in naïve, healthy mice. At the same time, the non-responder mice displayed cytokine/chemokine levels in the range of sham-injected mice. The same phenomenon was observed in blood serum as well (**Supplementary Figures 3**, **4** naïve vs. sham: $P = 0.0121$ (for TNFa and CXCL1); 500k responders vs. non-responders $P = 0.0235$ [for TNFa and CXCL1)]. Altogether, our data showed that AstroRx had the capability to reduce the infiltration of immune cells to the lungs and to prevent the rising of a cytokine storm in the responder mice. It is reasonable to assume that this capability contributed to the better survival rate among the AstroRx-treated mice.

MATERIALS AND METHODS

AstroRx Derived From Clinical Grade Human Embryonic Stem Cells (AstroRx®)

The protocol for manufacturing the human astrocytes (AstroRx®) from the embryonic stem cells was performed according to the protocol detailed in Izrael et al. (34). In brief, hESC line HADC100 was used as starting material for the derivation of AstroRx cells. The hESCs were grown

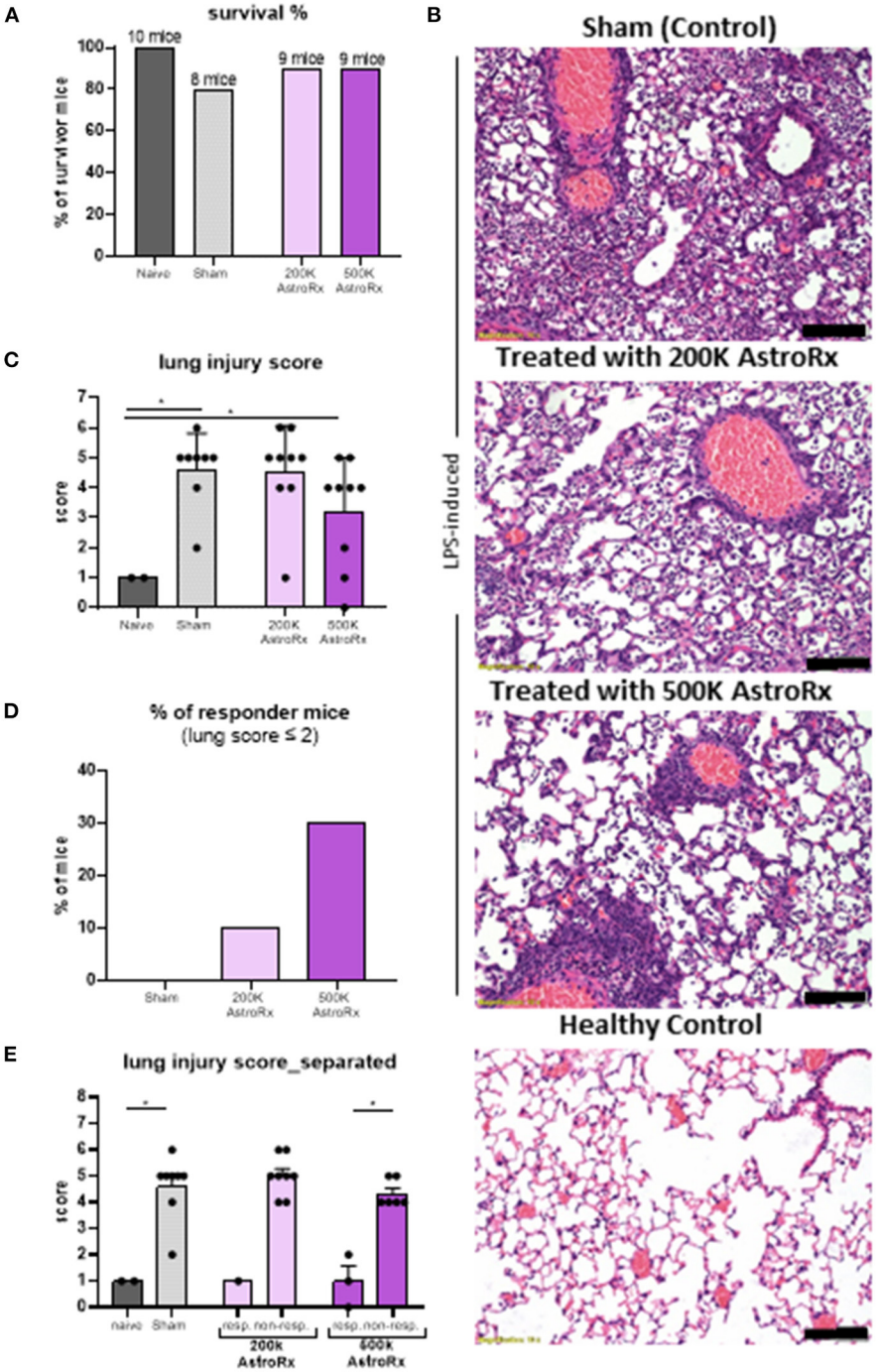

FIGURE 2 | The AstroRx cells alleviate lipopolysaccharide (LPS)-induced pulmonary inflammation and fibrosis. **(A)** The survival rate of animals treated with Plasmalyte (sham) and AstroRx cells (200K or 500K per animal). **(B)** H&E staining of lung sections from top to bottom: LPS-induced treated with vehicle (PlasmaLyte), LPS-induced treated with 200K AstroRx cells, LPS-induced treated with 500K AstroRx cells, or healthy controls (no-LPS). **(C)** An analysis for acute lung injury (ALI) was performed using a severity scoring scale of 0–2 (20 fields per animal were analyzed), based on the American Thoracic Society Documents, 2011 (37). **(D,E)** According to the lung score, the mice were categorized into responders (lung score ≤2) and non-responders (lung score >2), also considering the mice that had died from ARDS ($n = 10$), the results are expressed as mean ± SEM. Mann–Whitney comparison test. **(C,D)** Naïve vs. sham: $^*p = 0.0222$, **(C)** sham vs. 500k: $^*p = 0.0309$, **(E)** 500k responders vs. non-responders: $^*p = 0.0119$, $^*p < 0.05$. Scale bar: 100 μM.

in feeder free vitronectin coated flasks culture in essential 8™ (E8) medium (Thermo Fischer Scientific, MA, USA). Once enough cells were grown, hESC were detached to generate neurospheres (NS) in suspension (3D) cultures. The harvested hESC colonies were transferred into the 100-mm ultralow attachment culture plates (Corning) containing

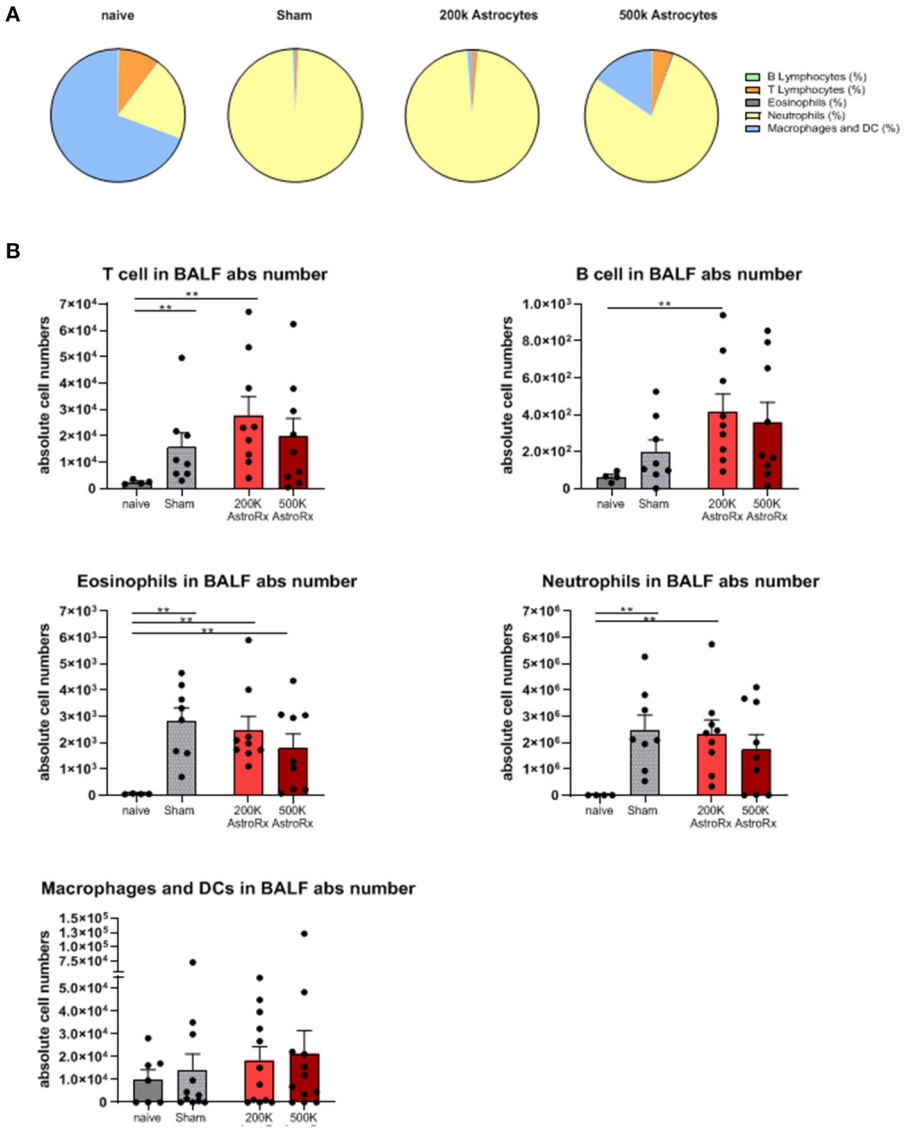

FIGURE 3 | Immune cells analyses of bronchoalveolar lavage. Bronchoalveolar lavage (BALF) was analyzed by flow cytometry for T cells, B cells, eosinophils, neutrophils, and macrophages/dendritic cells. **(A)** Proportions of each cell population by treatment. **(B)** An absolute number of each cell population in the BALF. The results are expressed as mean ± SEM. The Mann–Whitney comparison test. Naïve vs. sham: $^{**}p = 0.0081$, naïve vs. 200K astrocytes: $^{**}p = 0.0028$ (T cells, eosinophils, and neutrophils), $^{**}p = 0.0056$ (B cells), naïve vs. 500K astrocytes: $^{**}p = 0.0056$ (eosinophils), $^{**}p < 0.01$.

ITTSPP/B27 medium. The medium ITTSPP/B27 is a mixture of DMEM/F12 containing 1% B27 supplement, 1% glutamax, 1.5% HEPES at pH 7.4 (all from Thermo Fischer Scientific), 1% penicillin/streptomycin/amphotericin solution (Biological Industries, Israel), 25 µg/ml human insulin (ActRapid; Novo Nordisk, Denmark), 50 µg/ml human Apo-transferrin (Athens, GA, USA), 6.3 ng/ml progesterone, 10 µg/ml putrescine, 50 ng/ml sodium selenite, and 40 ng/ml triiodothyronine (T3) (all from Sigma, MO, USA). ITTSPP/B27 was supplemented with 20 ng/ml recombinant human epidermal growth factor (EGF) (R&D Systems, MN, USA). After 2 days, the medium was switched to ITTSPP/B27 supplemented with 20 ng/ml EGF and 10 µM all trans retinoic acid (Sigma, MO, USA). The culture was continued in suspension in the non-adherent plates for 7 days with daily replacement of the medium. During the last step, which allows for NS ripening, the culture was continued in ITTSPP/B27 medium supplemented with 20 ng/ml EGF for 18 days. The medium was replaced every other day. Then, round yellow NS were manually selected and transferred GMP-compliant laminin 521 (from Biolamina, Sweden) in ITTSPP/B27 supplemented with 20 ng/ml EGF. The medium was replaced every other day for 7–10 days (passage 0). To produce a monolayer of astrocyte progenitor cells (APC), the spheres were dissociated with TryplE (Thermo Fischer Scientific)

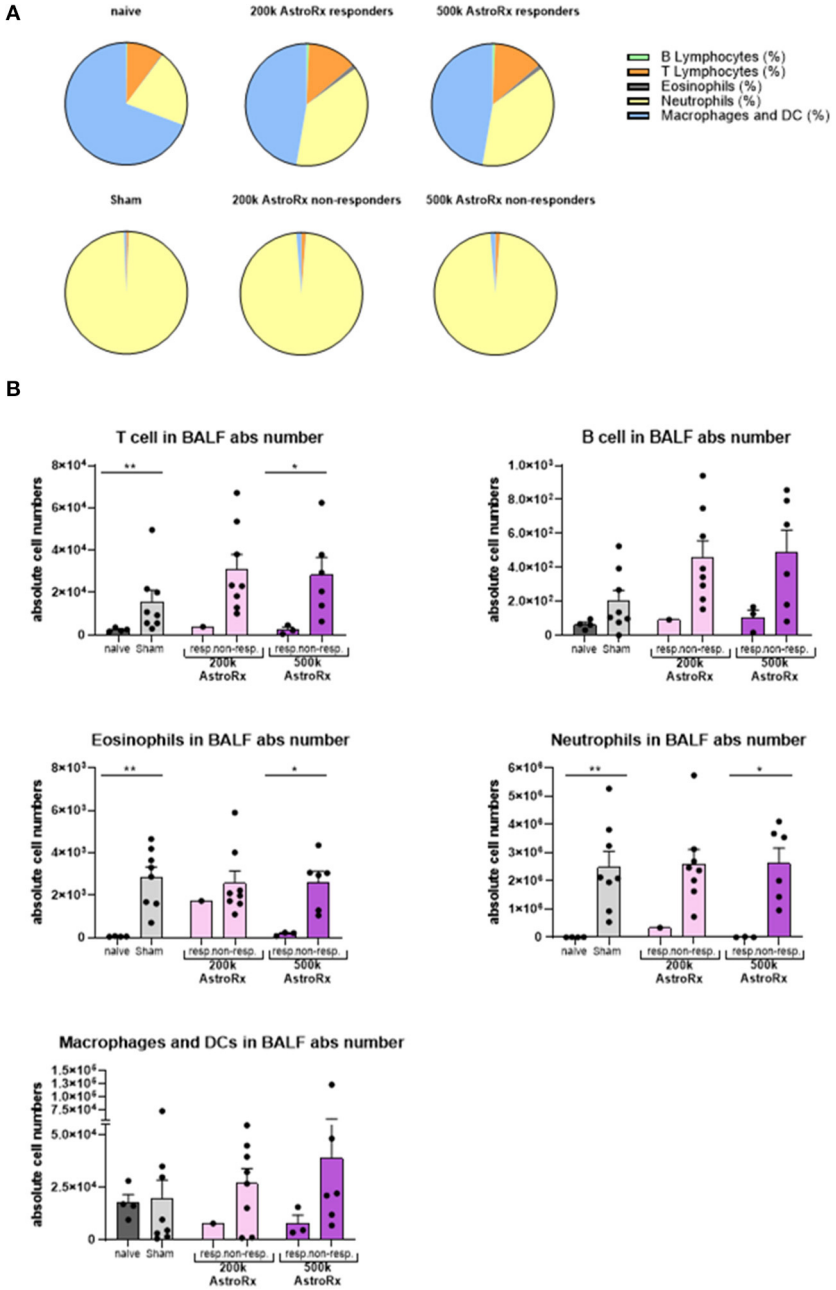

FIGURE 4 | Responder mice maintain immune homeostasis in the lungs after AstroRx treatment. Bronchoalveolar lavage was analyzed by flow cytometry for the immune cells. **(A)** The composition of cells in the BALF of responders and non-responders after AstroRx treatment. **(B)** An absolute number of immune cells in the BALF. The value of $n = 10$ mice for each experimental group (sham and both the groups of AstroRx treated mice) and $n = 4$ for naïve mice, the results are expressed as mean ± SEM. The Mann–Whitney comparison test. $^{*}p < 0.05$; $^{**}p < 0.01$; Naïve vs. sham: $^{**}p = 0.0081$ (T cells), $^{**}p = 0.0040$ (eosinophils and neutrophils), 500k responders vs. non-responders: $^{*}p = 0.0286$ (T cells, eosinophils, and neutrophils).

and reseeded on laminin coated flasks in N2/B27 medium consisting of DMEM/F12 with 0.5% (v/v) N_2 supplement, 1% (v/v) B27 supplement, 1% glutamax, and 1.5% HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) at pH 7.4 (all from Thermo Fischer Scientific). The growth factors EGF and basic fibroblast growth factor (bFGF, R&D Systems) were added at 10 ng/ml each. The monolayer cells were further passaged weekly until enough cells were generated. The cells were then frozen in liquid nitrogen and stored as banks of APCs. Thawed APCs were further expanded and allowed to differentiate into the committed AstroRx cells by the removal of growth factors (EGF and bFGF), 50 μg/ml ascorbic acid (Sigma)

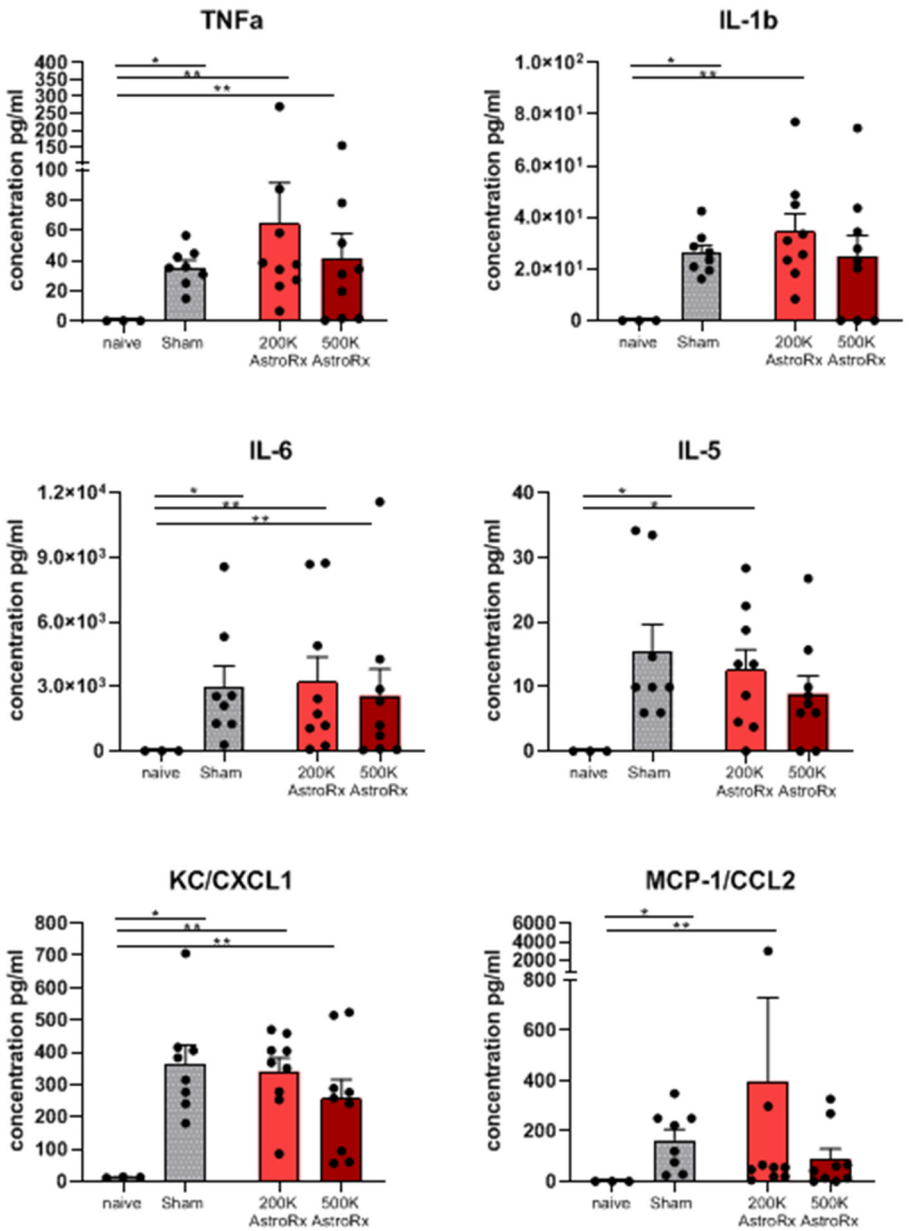

FIGURE 5 | Inflammatory cytokines/chemokines in lungs. The cytokines and chemokines in the BALF were quantified by ELISA (enzyme linked immunosorbent assay). The value of $n = 10$ mice for each experimental group (sham and both the groups of AstroRx treated mice) and $n = 4$ for naïve mice, the results are expressed as mean ± SEM. The Mann–Whitney comparison test. $^{*}p < 0.05$; $^{**}p < 0.01$; Naïve vs. sham: $^{*}p = 0.0121$, naïve vs. 200K or 500K astrocytes $p = 0.0091$ (TNFa, IL-6, CXCL1, and CCL2) and $^{*}p = 0.0318$ (IL-5).

was added, and the culture was continued for 7 days to yield AstroRx cells.

Animal Procedures

Female, 8 weeks old, BALB/C mice were obtained from Envigo (Israel) and maintained in SIA facility (Science in Action, Ness Ziona, Israel). Animal handling was performed according to the guidelines of the National Institute of Health (NIH) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The experiment was performed under the approval of "The Israel Board for Animal Experiments." A total of 30 BALB/C female mice were anesthetized using isoflurane and treated through intratracheal route of administration with LPS (IT, 800 μg of LPS—ChemCruz, The Netherlands, 055:B5) to induce ARDS, the animals were randomized to receive 200K, 500K AstroRx cells, or PlasmaLyte ($n = 10$ mice per experimental arm). Naive mice ($n = 4$, without LPS instillation) were used

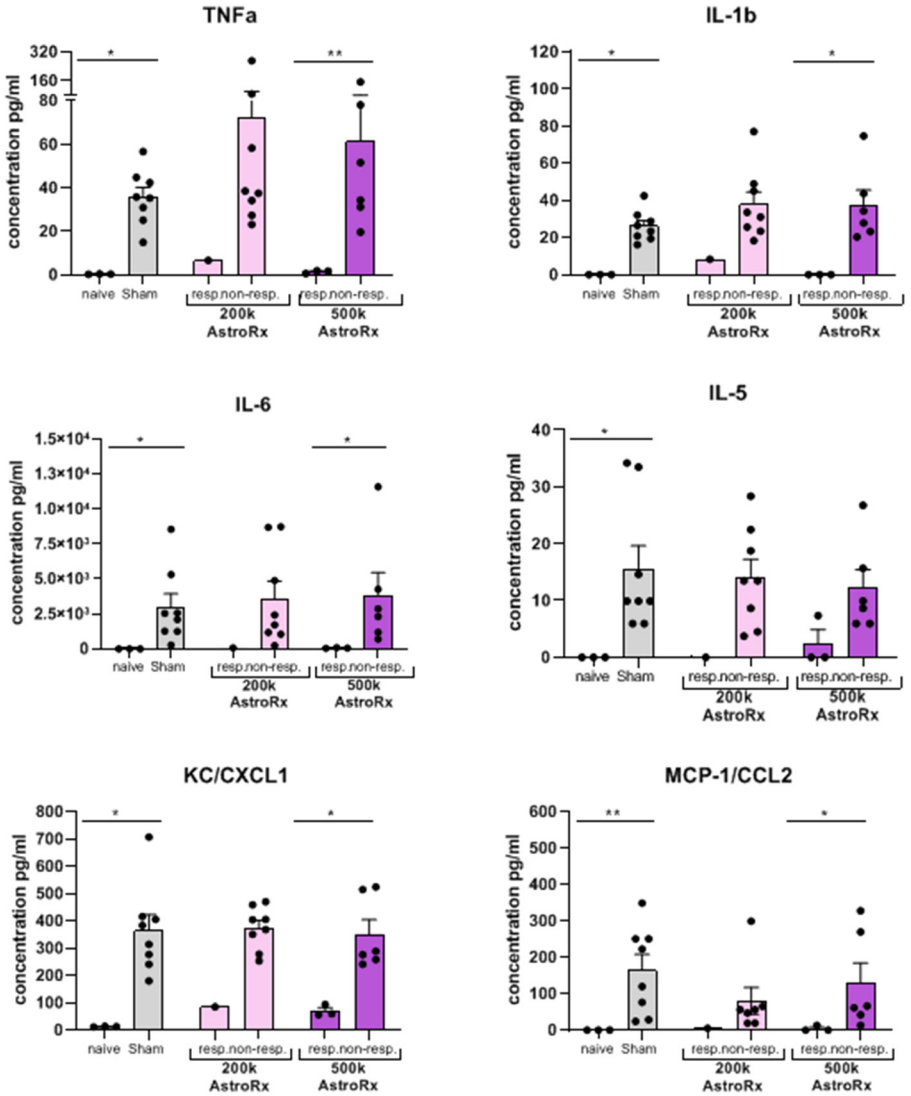

FIGURE 6 | Inflammatory cytokines/chemokines in the lungs are held in check in responder mice treated with AstroRx cells. The cytokines and chemokines in the BALF were quantified by ELISA. The value of $n = 10$ mice for each experimental group (sham and both the groups of AstroRx treated mice) and $n = 4$ for naïve mice, the results are expressed as mean ± SEM. The Mann–Whitney comparison test. $^{*}p < 0.05$; $^{**}p < 0.01$; naïve vs. sham: $^{*}p = 0.0121$ (for all cytokines/chemokines), 500K responders vs. non-responders: $^{**}p = 0.0091$ (TNFa), $^{*}p = 0.0286$ (IL-1b, IL-6, CXCL1, and CCL2).

as a control. Treated animals received i.v. injection of AstroRx cells or PlasmaLyte 6 h after LPS-induction. All the animals were sacrificed 72 h after the LPS instillation. The measurements of survival, body weight, hematology, lung histopathology, flow cytometry analysis of immune cells as well as cytokine and chemokines were collected from the BALF and serum. BALF was collected by intratracheal injection of 0.5 ml phosphate buffered saline (PBS) with 0.1 mM EDTA followed by a gentle aspiration three times. The recovered fluid was pooled and centrifuged. The BALF supernatant was preserved for the measurement of cytokines and chemokines. The sediment cells were resuspended and subjected to flow cytometry analysis.

Histology

Lungs were harvested and fixed in 4% formaldehyde. The tissues were then trimmed in a standard position and put in the embedding cassettes. One cassette per animal was prepared. The paraffin blocks were sectioned at ∼ 4 µm thickness, put on the glass slides, and stained with H&E. Pictures were taken using an Olympus microscope (BX60, serial NO. 7D04032) at an objective magnification of × 4 and × 10 and microscope's Camera (Olympus DP73, serial NO. OH05504, Tokyo, Japan).

An analysis for ALI was performed according to the method described in Matute-Bello et al. using a severity scoring scale of 0–2, based on the American Thoracic Society Documents, 2011 (37). An analysis was performed by an independently certified

veterinarian pathologist (Patho-logica Ltd., Ness Ziona, Israel) who was blinded to the experimental treatment.

Neutrophils

Not visible within the field—a score of 0; 1–5 neutrophils per field−1; more than 5 neutrophils per field−2.

Fibrin

Not visible within the field—a score of 0; a single well-formed band of fibrin within the airspace−1; multiple eosinophilic membranes−2.

Thickened Alveolar Walls

Due to technical artifacts, only septal thickening that is ≤two times the normal was considered. Less than ×2—score 0; ×2–×4—score 1; more than × 4—score 2.

The analysis was based on the measurements of 20 fields, using an objective magnification of ×4 and ×10 (high power fields, HPF).

Neutrophil cell count was performed using MATLAB color-based, brightness-based, and morphological-based segmentation. The cells were counted from a rectangle of 88,892 μm^2.

Cytokine and Chemokine Multiplex Measurements

The BALF cytokine concentrations were measured using the ProcartaPlex Luminex platform (Thermo Fischer Scientific, MA, USA). The measurements were performed two times (25 μl of each sample) with a custom multiplex panel detecting the following mouse cytokines: IFNγ, TNFα, RANTES, IL-6, IL-10, IL-1α, IL-1β, IP-10, MIP1α, and MCP-1/CCL2. The measurements were performed using the Luminex MAGPIX instrument, and results were analyzed with Xponent 4.2 software according to the instructions from the manufacturer.

Mixed lymphocyte Reaction Test

Three female C57/Bl6 mice at age of 6–8 weeks were euthanized by injection of a lethal dose of Pental (Pentobarbital Sodium). The lymph nodes were excised and drained to obtain lymph-node cells (LNCs). The LNCs from all the mice were pooled and then counted by a hemocytometer using trypan blue dye to exclude the dead cells. One million LNCs were seeded in RPMI-1640 medium (Biological Industries, 01-100-1A) supplemented with 2.5% fetal calf serum (Biological Industries, 04-121-1A), 1 mM L-glutamine (Biological Industries, 03-020-1C), and penicillin-streptomycin (Biological Industries, 03-031-1B). The LNC cultures under the multiple experimental conditions were maintained in flat-bottom plates, in duplicates, in a humidified atmosphere of 5% carbon dioxide at 37°C. The mNPCs were obtained using the method described in Fainstein and Ben-Hur (35) for isolation and growth of mNPCs. The mNPCs were kept in culture with the same media used to culture LNCs. The cells were harvested and analyzed for T-cell amount and proliferation by flow cytometry. Analysis of cells that expressed both Thy1.2 and CD25 determined the percentage of T cells in LNC culture, 24 h after activation with ConA.

BrdU T-cell Proliferation Assay

Co-expression of CD3/BrdU determined the percentage of proliferating T cells in co-cultures with LNC. The LNCs were kept in culture with and without 2.5 μg/ml ConA (Sigma, C5275) for 48 h. The percentage of T cells ($Thy1.2^+/CD25^+$) and proliferating T cells ($CD3^+/BrdU^+$) of the LNC cultures in the presence of ConA were set as baselines for the T-cell activation—without immunomodulation. The AstroRx® cells were added to LNC culture at ratios of 2:1, 5:1, and 10:1 LNC:AstroRx®, with and without 2.5 μg/ml ConA. Further, the LNCs in 0.5 ml RPMI-1640 were cultured with 0.5 ml conditioned medium with and without 2.5 μg/ml ConA. As a positive control, the mNPCs were added to the LNC culture at a ratio of 2:1 with and without 2.5 μg/ml ConA.

Flow Cytometry

The cells were analyzed by flow cytometry for identity and purity markers using the following antibodies: anti-GLAST (1:20; Miltenibiotec, Germany), anti-CD44 (1:20; BD Pharmingen, CA, USA), anti-CXCR4 (1:20; Biolegend, CA, USA), anti-TRA-1-60 (1:50; Biolegend), anti-EPCAM (1:50; Biolegend), anti-SSEA4 (1:50; Biolegend), anti-GFAP (1:2000; Sigma), anti-Nestin (1:500; BD Pharmingen), and anti-AQP-4 (1:2000; Abcam, UK). The Flow Cytometer FACS Canto II (BD, NJ, USA) was operated with FACSDIVA software (BD). At least 10,000 events were collected per sample. For immune cells identity, the following antibodies were used: FITC Rat Anti-Mouse I-A/I-E Clone 2G9 (RUO) (BD 553623), PerCP-Cy™5.5 Hamster Anti-Mouse CD3e (BD 551163), PerCP-Cy™5.5 Rat Anti-Mouse CD45R/B220 (552771), Mouse CCR3 PE-conjugated Antibody (R&D FAB729P), and APC-anti-mouse CD11 (Biolegend BLG-117310d).

Statistical Analyses

The statistical analyses were performed using GraphPad Prism 7 software (GraphPad Software, San Diego, CA, USA). The *p*-values were calculated by Mann–Whitney test. The values ≤0.05 were considered significant ($^* p < 0.05$; $^{**} p < 0.01$; and $^{***} p < 0.001$).

DISCUSSION

We are currently evaluating the curative role of AstroRx in patients with ALS. In a Phase I/IIa, open-label, dose-escalating clinical study, we studied the safety, tolerability, and therapeutic effects of AstroRx intrathecal transplantation.

Similarly to ARDS, the levels of circulating chemokines and cytokines in ALS are increased. The patients with a shorter diagnostic delay, which is a marker of more severe rapidly progressing disease (38) display higher levels of the inflammatory chemokine MCP-1/CCL-2. In addition, the increased levels of the inflammatory cytokines IL-17 and IL-6 were detected in the serum of the patients with ALS (39, 40). The increased levels of LPS in patients with ALS suggest systemic inflammation (41).

In preclinical studies, the AstroRx cells demonstrated a neuron-protective phenotype (A2 astrocytes) and secreted neurotrophic factors that were capable to uptake glutamate

and regulate oxidative stress (34). Depending on their mode of activation (A1/A0/A2) (23, 25), astrocytes may possibly play an anti-inflammatory (A2) or pro-inflammatory (A1) role.

To test the immunomodulatory effect of AstroRx cells, we chose a rapid and acute inflammation model. LPS lung instillation is one of the most used models for ARDS. This model shares a number of pathological features with COVID-19-related ARDS, such as hypoxemia, neutrophil accumulation, alveolar space thickening, fibrin and tissue pathology, and high levels of inflammatory cytokines (2, 10, 13). The similarities between the LPS-treated rodents and the patients with COVID-19, in terms of lung damage and the inflammatory response, make LPS-induced ARDS a reliable model to evaluate potential COVID-19 therapies.

Administration of cells, mainly MSC has been performed by either local or systemic routes in the different experimental models (42). Local administration via intratracheal or intrathoracic infusion delivers the cells directly to the site of injury, whereas systemic administration *via* intravenous infusion allows wide distribution throughout the body. The cells administered intravenously would encounter the first-class pulmonary effect (43), which might result in the significant retention of cells in the lung, thereby providing advantages for lung tissue repair. The ongoing clinical trials and most experimental studies have used intravenous route for MSC administration (44, 45). Therefore, intravenous infusion of AstroRx cells was selected as a route of administration for testing ARDS.

In the current study, the levels of TNF-alpha, IL-b1, and IL-6 were strongly elevated in LPS-induced animals but could be maintained in steady state in the responder mice of AstroRx and LPS-treated group. The expression of CCL2, a monocyte recruiter, and CXCL1, a neutrophil recruiter, was also increased in the LPS-induced sham animals, and significantly reduced in the AstroRx responder mice. The expression level of IL-6, IL1b, TNFα, and CXCL1 determined if the lung remained in a homeostatic state or became inflamed. The upregulated levels of CXCL1 and TNF were also detected in the peripheral blood. Astrocytes calcium signaling is implicated in regulating inflammation (46–49). Similar to the pathology reported in ALS disease, the levels of pro-inflammatory cytokines, such as TGF-β1, IL-10, IFNγ, and IL-6 are elevated in LPS-induced ARDS animals. Exposure of astrocytes to inflammatory stimuli (e.g., TGF-β1 and IFNγ) demonstrated that inflammatory stimuli can significantly alter the astrocyte calcium signaling elicited by multiple G-protein-coupled receptors (GPCRs) (50). Alterations in the GPCR-evoked astrocyte Ca^{2+} transients may represent mechanisms by which astrocytes regulate inflammation (51) and should be further investigated.

Our current study demonstrated the immunosuppressive capacity of AstroRx cells in the context of lung inflammation and presents AstroRx as an innovative approach for treating ARDS especially in light of the rising unmet need for a treatment for COVID-19-related ARDS.

To make AstroRx cell therapy clinically applicable, further research is needed to elucidate several issues, such as the optimal number of AstroRx cell doses, the time window of AstroRx cell administration, administration routes, and frequency (single vs. multiple-dose regimen). Comprehensive safety investigation on the fate of AstroRx cells was done once the cells were injected intrathecally into the cerebrospinal fluid (34). The safety profile of AstroRx cells upon i.v. injection requires further investigation in the terms of cell survival, homing capacity to different tissues (e.g., lungs, liver, and kidney), cell identity, and reactive state (A1/A2) after cell transplantation.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was performed according to guidelines of the National Institute of Health (NIH) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The experiment was performed under the approval of The Israel Board for Animal Experiments.

AUTHOR CONTRIBUTIONS

MR, MI, AR, and JMW conceived and designed the studies. MI, JMW, SGS, TS, and KM performed the experiments, analyzed the data, and interpreted the data. MR, MI, and JMW wrote 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.2021.740071/full#supplementary-material

Supplementary Figure 1 | The percentage analyses of immune cells in bronchoalveolar lavage. Bronchoalveolar lavage (BALF) of mice was analyzed by flow cytometry for lymphocytes and myeloid cells. The results are expressed as mean ± SEM. Mann–Whitney comparison test. $^{*}p < 0.05$; $^{**}p < 0.01$. Sham ($n = 10$) vs. 200k astrocytes treated mice ($n = 10$): $^{*}p = 0.0152$ (T cells), sham vs. 500k astrocytes treated mice ($n = 10$): $^{*}p = 0.0206$ (T cells), sham vs. 500k astrocytes treated mice: $^{*}p = 0.0274$ (neutrophils and eosinophils).

Supplementary Figure 2 | Immune cell distribution in the lungs resembles naïve, healthy mice. The BALF of mice was analyzed by flow cytometry for the percentage of lymphocytes and myeloid cells divided into responder and non-responders mice. The results are expressed as mean ± SEM. Mann–Whitney comparison test. $^{*}p < 0.05$; $^{**}p < 0.01$; Naïve vs. sham: $^{**}p = 0.0040$ (T cells, neutrophils, and macrophages), $^{**}p = 0.0028$ (B cells). 500k responders vs. non-responders: $^{*}p = 0.$ 0286 (all cell types).

Supplementary Figure 3 | CXCL1 and TNFa levels in the peripheral blood of AstroRx-cell treated mice mirror the situation in the lungs. CXCL1 and TNFa levels in the blood serum were quantified by ELISA. $n = 10$ mice for each experimental group [sham and both groups of human astrocytes (AstroRx) treated mice] and $n = 4$ for naïve mice, results are expressed as mean ± SEM. Mann–Whitney comparison test. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Supplementary Figure 4 | The CXCL1 and TNFa levels in the peripheral blood of AstroRx-cell treated mice mirror the situation in the lungs. The CXCL1 and TNFa levels divided by responder and non-responder mice in the blood serum were quantified by ELISA. The results are expressed as mean ± SEM. Mann–Whitney comparison test. $^{*}p < 0.05$.

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REVIEW
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Unsuccessful and Successful Clinical Trials in Acute Respiratory Distress Syndrome: Addressing Physiology-Based Gaps

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The acute respiratory distress syndrome (ARDS) is a severe form of acute hypoxemic respiratory failure caused by an insult to the alveolar-capillary membrane, resulting in a marked reduction of aerated alveoli, increased vascular permeability and subsequent interstitial and alveolar pulmonary edema, reduced lung compliance, increase of physiological dead space, and hypoxemia. Most ARDS patients improve their systemic oxygenation, as assessed by the ratio between arterial partial pressure of oxygen and inspired oxygen fraction, with conventional intensive care and the application of moderate-to-high levels of positive end-expiratory pressure. However, in some patients hypoxemia persisted because the lungs are markedly injured, remaining unresponsive to increasing the inspiratory fraction of oxygen and positive end-expiratory pressure. For decades, mechanical ventilation was the only standard support technique to provide acceptable oxygenation and carbon dioxide removal. Mechanical ventilation provides time for the specific therapy to reverse the disease-causing lung injury and for the recovery of the respiratory function. The adverse effects of mechanical ventilation are direct consequences of the changes in pulmonary airway pressures and intrathoracic volume changes induced by the repetitive mechanical cycles in a diseased lung. In this article, we review 14 major successful and unsuccessful randomized controlled trials conducted in patients with ARDS on a series of techniques to improve oxygenation and ventilation published since 2010. Those trials tested the effects of adjunctive therapies (neuromuscular blocking agents, prone positioning), methods for selecting the optimum positive end-expiratory pressure (after recruitment maneuvers, or guided by esophageal pressure), high-frequency oscillatory ventilation,

extracorporeal oxygenation, and pharmacologic immune modulators of the pulmonary and systemic inflammatory responses in patients affected by ARDS. We will briefly comment physiology-based gaps of negative trials and highlight the possible needs to address in future clinical trials in ARDS.

Keywords: acute respiratory distress syndrome, clinical trials, neuromuscular blockade, prone ventilation, high-frequency ventilation, positive end-expiratory pressure, extracorporeal oxygenation, anti-inflammatory drugs

BACKGROUND

The acute respiratory distress syndrome (ARDS) is a severe form of acute hypoxemic respiratory failure. Caused by an intense direct (pulmonary) or indirect (systemic) inflammatory insult to the alveolar-capillary membrane, it is characterized by the presence of diffuse, non-cardiogenic, high-permeability, protein-rich pulmonary edema, and hypoxemia unresponsive to the application of high inspiratory concentrations of oxygen (FiO_2) (Villar, 2011). The use of mechanical ventilation (MV) is the standard supportive therapy of patients with ARDS. Since the publication in 2000 of the milestone paper by the ARDS Network (Acute Respiratory Distress Syndrome Network, Brower et al., 2000), the aim of MV is to achieve adequate gas-exchange avoiding damaging the lungs by using physiological tidal volumes (VT) of 4–8 ml/kg predicted body weight (PBW), preventing alveolar collapse with positive end-expiratory pressure (PEEP), limiting end-inspiratory plateau pressure (Pplat) to less than 30 cmH_2O, and limiting FiO_2 to maintain an adequate PaO_2. These essential elements are the main components of the framework for lung-protective MV.

INTRODUCTION

Most ARDS patients improve their oxygenation, as assessed by the ratio between the arterial partial pressure of oxygen (PaO_2) and FiO_2 (PaO_2/FiO_2), disease-specific treatment and the application of adequate levels of PEEP. There is no typical ARDS patient. Over the years, hypoxemia has become an infrequent cause of death in ARDS (Slutsky et al., 2016). Physiologically, we are unaware of data linking a specific PaO_2/FiO_2 to predictable morphological changes in the alveolar-capillary membrane at ARDS onset. Recent evidence has shown an association between severity of lung damage and prediction of outcome when the PaO_2/FiO_2 is evaluated at 24 h under standardized ventilator settings using an enrichment strategy (Villar et al., 2019). We lack a standard definition for refractory or persisting hypoxemia, as a predetermined PaO_2 value under a particular FiO_2 and PEEP for a specific time-period. In this review, we will consider hypoxemia for enrolling patients into clinical trials when the PaO_2/FiO_2 is $\leq$ 200 mmHg on MV and PEEP $\geq$ 5 cmH_2O, in agreement with the Berlin criteria for moderate-to-severe ARDS (Ranieri et al., 2012). The purpose of this brief review is to summarize the current knowledge based on a number of major randomized controlled trials (RCTs) performed in ARDS patients on MV evaluating interventions applied during the acute phase and published since 2010, independent of whether they reported benefits or not in the primary or secondary outcomes when testing the experimental intervention. Those RCTs were conducted with the aim to improve oxygenation and mortality while the patient was in the intensive care unit (ICU) or after being discharged from ICU. We will discuss the physiology-based gaps of negative trials and highlight the possible needs to address in future trials design in ARDS.

REVIEW OF RANDOMIZED CONTROLLED TRIALS

Since 2010, 14 major RCTs (Papazian et al., 2010; Gao Smith et al., 2012; Ferguson et al., 2013; Guerin et al., 2013; Young et al., 2013; McAuley et al., 2014; Kacmarek et al., 2016; Working group for the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial (Art) Investigators et al., 2017; Combes et al., 2018; Beitler et al., 2019; Hodgson et al., 2019; The National Heart, Lung, and Blood Institute Petal Clinical Trials Network, Moss et al., 2019; Ranieri et al., 2020; Villar et al., 2020) have been published in patients with ARDS (**Table 1**). In those trials, patients received invasive MV and tested different adjunctive or rescue therapies for improving oxygenation, or different MV approaches to titrate PEEP, or several anti-inflammatory drugs for attenuating the pulmonary and systemic inflammatory responses.

Muscle Paralysis

There is sufficient evidence showing that MV can initiate or aggravate lung injury, a concept labeled as ventilator-induced lung injury (VILI) (Slutsky and Ranieri, 2013). Many of the consequences of VILI bear a resemblance to those of ARDS. Despite limiting VT and pressures during lung-protective MV, ARDS patients could develop tidal hyperinflation during spontaneous respiratory efforts while mechanically ventilated, especially in the early phases of ARDS. VT set in the ventilator does not always correspond to the exact VT delivered to the patient, due to the contribution of inspiratory muscle efforts to inflation pressure and more importantly in the presence of double triggering, reverse triggering, and other types of patient-ventilator asynchronies, which could develop despite receiving deep analgesia or sedation. Papazian et al. (2010) conducted an RCT, the *ARDS et Curarisation Systematique* (ACURASYS) trial, to examine the hypothesis that removing spontaneous respiratory efforts would improve lung mechanics and oxygenation in patients with persistent hypoxemia. It was known that neuromuscular blockade (NMB) decreases the work of breathing and asynchronies in ARDS (Light et al., 1975).

TABLE 1 | Successful and unsuccessful randomized clinical trials since 2010 in ventilated patients with acute respiratory distress syndrome (ARDS).

References publication year	Trial name	Study period, No. ICUs, Country	Criteria for enrollment	Patients	Intervention	Major findings	Remarks
Papazian et al. (2010)	ACURASYS	2006–2008 (24 months) 20 ICUs, France	MV, PaO_2/FiO_2 < 150 with PEEP ≥ 5 for < 48 h	340	Neuromuscular blockers (cisatracurium)	Improved adjusted 90-day mortality and VFDs	Control group was deeply sedated
Gao Smith et al. (2012)	BALTI-2	2006–2010 (40 months) 46 ICUs, UK	MV, within 72 h of ARDS onset (AECC criteria)	326	Salbutamol	Salbutamol worsen outcomes	Concerns for use of non-protective MV
Guerin et al. (2013)	PROSEVA	2008–2011 (41 months) 27 ICUs France, Spain	MV < 36 h, PaO_2/FiO_2 < 150 on FiO_2 ≥ 0.6 confirmed at 12–24 MV	466	Prone positioning for at least 12 h/daily	Decreased 28-day and 90-day mortality	Currently, it is standard of care in severe ARDS
Ferguson et al. (2013)	OSCILLATE	2009–2012 (38 months) 39 ICUs in Canada, United States, Saudi Arabia, Chile, India	MV, PaO_2/FiO_2 ≤ 200 on FiO_2 ≥ 0.5 and PEEP ≥ 10	548	High-frequency oscillation ventilation (HFOV)	Increased ICU and hospital mortality	Increasing harm from HFOV at higher PaO_2/FiO_2
Young et al. (2013)	OSCAR	2007–2012 (55 months) 29 ICUs, UK	MV, AECC criteria, PaO_2/FiO_2 ≤ 200 on PEEP ≥ 5	795	High-frequency oscillation (HFOV)	No change in 30-day mortality	HFOV increased harm at higher PaO_2/FiO_2
McAuley et al. (2014)	HARP-2	2010–2014 (39 months) 40 hospitals in UK and Ireland	MV, < 48 h from ARDS onset, PaO_2/FiO_2 ≤ 300 (AECC criteria)	540	Simvastatin	No effects on outcomes	Concerns for use of non-protective MV
Kacmarek et al. (2016)	OLA	2007–2013 (59 months) 20 ICUs in Spain, South Korea, Brazil	MV, PaO_2/FiO_2 ≤ 200 (AECC criteria). At 24 h, PaO_2/FiO_2 ≤ 200 on FiO_2 ≥ 0.5 and PEEP ≥ 10	200	Open lung approach (lung recruitment and PEEP titration)	Increased oxygenation and decreased driving pressure. No change in ICU mortality	Prognostic enrichment for enrollment at 12–36 h after ARDS onset
Working group for the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial (Art) Investigators et al. (2017)	ART	2011–2017 (65 months) 120 ICUs in Brazil, Argentina, Colombia, Italy, Poland, Portugal, Malaysia, Spain, Uruguay	MV, ARDS (AECC criteria) < 72 h, enrollment if PaO_2/FiO_2 ≤ 200 on PEEP ≥ 10 and FiO_2 = 1 for 30 min	1,010	Open lung approach (lung recruitment and PEEP titration)	Increased 28-day and 6-month mortality. Decreased VFDs. Increased risk of barotrauma	Concerns with study design, methodology, data analysis, and differences in health care systems
Combes et al. (2018)	EOLIA	2012–2017 (55 months) 23 ICUs in France, Canada, United States	Very severe ARDS: PaO_2/FiO_2 < 50 for > 3 h; or PaO_2/FiO_2 ≤ 80 for > 6 h; or pH < 7.25 with $PaCO_2$ ≥ 60 for > 6 h	249	Extracorporeal membrane oxygenation (ECMO)	No significant benefit in 60-day mortality	Control group included crossover to ECMO in 28% patients
The National Heart, Lung, and Blood Institute Petal Clinical Trials Network, Moss et al. (2019)	ROSE	2016–2018 (28 months) 48 hospitals in United States	MV, PaO_2/FiO_2 < 150 with PEEP ≥ 8 for < 48 h	1,006	Neuromuscular blockers (cisatracurium)	No significant benefit in 90-day mortality	Control group with lighter sedation
Beitler et al. (2019)	EPVent-2	2012–2017 (59 months) 14 hospitals in United States	MV, PaO_2/FiO_2 ≤ 200 within 36 h ARDS onset (Berlin criteria)	200	Esophageal pressure-guided for titrating PEEP	No significant benefit in 28-day mortality and VFDs	Median PEEP levels was similar in both groups over time
Hodgson et al. (2019)	PHARLAP	2012–2017 (59 months) 35 ICUs in Australia, New Zealand, Ireland, Saudi Arabia, UK	MV < 72 h, ARDS or other types of respiratory failure with PaO_2/FiO_2 ≤ 200 on PEEP ≥ 5	113	Lung recruitment maneuvers with PEEP titration	No benefits in VFDs or ICU/hospital mortality	Small sample size, PEEP titration used SpO_2, and treatment crossovers
Ranieri et al. (2020)	INTEREST	2015–2017 (25 months) 74 ICUs, 8 European countries	MV, PaO_2/FiO_2 ≤ 200 PEEP ≥ 5 (Berlin criteria) within 24 h	301	Interferon β-1a	No significant benefit in 28-day mortality and VFDs	Higher-than-expected use of corticosteroids
Villar et al. (2020)	DEXA-ARDS	2013–2018 (69 months) 17 ICUs, Spain	MV, PaO_2/FiO_2 ≤ 200 at ARDS onset; at 24 h, PaO_2/FiO_2 ≤ 200 on FiO_2 ≥ 0.5 and PEEP ≥ 10	277	Dexamethasone	Increased VFDs. Decreased 60-day mortality	Prognostic enrichment for enrollment at 24 h of ARDS onset

AECC, American-European Consensus Conference; ICU, intensive care unit; MV, mechanical ventilation; PEEP, positive end-expiratory pressure; SpO_2, oxygen saturation; UK, United Kingdom; VFDs, ventilator-free days.

The ACURASYS trial enrolled 340 ARDS patients with moderate-to-severe ARDS (defined by the investigators as a PaO_2/FiO_2 < 150 mmHg on PEEP ≥ 5 cmH_2O during the first 2 days of ARDS diagnosis). Patients were randomized to receive either cisatracurium or placebo for 48 h. Patients in the control arm were deeply sedated. The authors found that in patients assigned to NMB, the adjusted 90-day mortality was lower and ventilator free-days (VFDs) were higher than in those not receiving NMB.

The findings of the ACURASYS remained controversial for almost a decade for two reasons: the Kaplan-Meir survival curves of the two treatment arms separated only after 14 days of randomization, and, most importantly, the 90-day all-cause mortality only reached statistical significance with intensity adjustment (Yegneswaran and Murugan, 2011). Thus, additional validation of this trial was required. A RCT termed "*Reevaluation Of Systemic Early neuromuscular blockade (ROSE) trial*" (The National Heart, Lung, and Blood Institute Petal Clinical Trials Network, Moss et al., 2019) was unable to validate ACURASYS results. The ROSE trial was powered for evaluating the efficacy and safety of NMB in decreasing 90-day mortality. The trial stopped for futility when 1006 ARDS patients were enrolled. Although this trial was not an exact replication of ACURACYS (since both arms of the trial received PEEP ≥ 8 cmH_2O, and the control arm received lighter sedation targets), there were no differences between groups in the rate of barotrauma and in the number of VFDs, and 90-day mortality was virtually identical in the two groups.

What are the implications of these two RCTs? First, according to these results routine use of NMB agents are not recommended in moderate-to-severe ARDS patients. Contrary to expectations, the prevalence of patient-ventilator asynchronies in the form of reverse triggering (it occurs when a breath delivered by the ventilator triggers diaphragm contraction, initiating an assisted breath –the reverse of what occurs during assisted MV) increases with deeper sedation levels (Bourenne et al., 2019). Second, from a pathophysiological point of view, there is a basis to use NMB in any patient with ARDS when after ensuring an adequate ventilation and sedation, the patient has a ventilatory pattern that could promote VILI. Therefore, current data suggest that NMB agents can be used when they are physiologically and clinically indicated (Slutsky and Villar, 2019).

Prone Ventilation

Patho-physiologically and histo-pathologically, ARDS is a heterogeneous inflammatory process produced by a variety of insults with collapsed and consolidated areas mainly in the dependent regions, and more healthy units in the non-dependent regions (Villar, 2011). Thus, recruitability of alveolar spaces in ARDS lungs with PEEP is also heterogeneous, both between patients and within the lungs. Changes in body posture could have marked effects on pulmonary function in patients with acute respiratory failure. As normal practice, the critically ill patient is cared for in supine. The usual reduction in function residual capacity (FRC) when adopting the supine position is increased in ARDS ventilated patients, resulting in ventilation-perfusion mismatching and a fall in the PaO_2. Five mechanisms have been proposed to explain the improved oxygenation during prone positioning: (i) increased FRC, (ii) changes in regional diaphragm motion, (iii) improved ventilation-perfusion matching due to a redistribution of regional ventilation to dorsal regions of the lung, (iv) improved clearance of secretions, and (v) removal of the weight of the heart from the lung (Lamm et al., 1994).

Prone ventilation is considered an adjunctive intervention to the ventilatory management of ARDS patients with refractory hypoxemia. Prone ventilation can be performed safely if ICU teams are adequately trained. After several studies reported conflicting results about the efficacy of prone ventilation in persistent hypoxemia, a large RCT, the "*Proning Severe ARDS patients*" *(PROSEVA)* trial (Guerin et al., 2013), reported survival benefit in moderate-to-severe ARDS. In that trial, 466 patients with persistent ARDS (as defined by a PaO_2/FiO_2 < 150 mmHg with FiO_2 ≥ 0.6 and PEEP ≥ 5 cmH_2O) were randomly assigned to prone position for at least 16 h or to supine position. Patients in both groups were mechanically ventilated following the low PEEP-FiO_2 table from the ARDSnet trial (Acute Respiratory Distress Syndrome Network, Brower et al., 2000). All-cause mortality at 28 days was 33% in the supine group and 16% in the prone group, a highly significant difference that persisted 90 days after randomization.

Proponents of prone ventilation advise that the approach of the PROSEVA trial was a modification of a technique that finally became right at a time when patients were mechanically ventilated with low VT (Beitler et al., 2014). However, although prone positioning improves oxygenation in ARDS patients and could help in recruiting lung regions, there is controversy over its use in clinical practice (Villar et al., 2014). The large treatment effect seems too good to be true: a 28-day mortality of 16% is the lowest, ever reported in a trial, or in any observational study on ARDS. Furthermore, patients assigned to the supine group were mechanically ventilated during the first 72 h with low levels of PEEP (9 ± 3 cmH_2O). Therefore, as with NMB, an additional RCT is required to confirm these findings. Such a trial should guarantee that the control group will receive a higher PEEP approach.

High Frequency Oscillatory Ventilation

Knowledge on the mechanisms and importance of VILI has advanced over the years. Theoretically, from the perspective of lung-protective ventilation, high frequency oscillatory ventilation (HFOV) would be an ideal mode of ventilation for ARDS patients (Ferguson et al., 2007). By definition, it accomplishes adequate gas-exchange by providing very small VT that are typically 1–3 ml/kg, often less than the anatomic dead space, at frequencies from 3 to 15 cycles per second at a constant mean airway pressure. Since HFOV incorporates fewer and simpler controls that are not interrelated, it is an easier technique than conventional MV. However, there is a lack of evidence showing that HFOV is less harmful that using MV with low VT, moderate-to-high PEEP, and limitation of Pplat.

A critical examination of two RCTs comparing HFOV with lung-protective MV published in 2013 (enrolling 1343 ARDS patients) demonstrated no benefits of HFOV. In the OSCILLATE (*OSCILLation for Acute respiratory distress syndrome Treated*

Early) trial (Ferguson et al., 2013), the investigators stopped the trial after 548 of the 1,200 planned patients were randomized. Patients were eligible for inclusion if they had a $PaO_2/FiO_2 \leq 200$ mmHg on $FiO_2 \geq 0.5$. However, only patients with a $PaO_2/FiO_2 \leq 200$ after 30 min of assessment on a VT of 6 ml/kg PBW, PEEP ≥ 10 and FiO_2 of 0.6 were randomized. Patients in the HFOV group received HFOV after lung recruitment with an "open lung approach" (OLA) consisting in the application of a recruitment maneuver (RM) of 40 cmH_2O of pressure for 40 s for a median of 3 days. However, almost 15% of patients in the control group received HFOV as a rescue therapy. The absolute hospital mortality was 12% higher in the HFOV group (relative risk of death 1.33, $p = 0.005$). The relatively high mortality in the HFOV group could be explained by the higher prevalence of hemodynamic compromise (hypotension) after the initiation of HFOV, contributing to extrapulmonary organ dysfunction, and by increased use of sedative agents. In the OSCAR (*OSCillation in ARDS*) trial (Young et al., 2013), the study design was more pragmatic. The authors randomized 795 patients with a $PaO_2/FiO_2 \leq 200$ on PEEP ≥ 5 cmH_2O. Patients in the HFOV arm were ventilated by increasing mean airway pressure and FiO_2. The control group was treated according to local practice in participating ICUs, applying the low PEEP-FiO_2 table from the ARDSnet trial (Acute Respiratory Distress Syndrome Network, Brower et al., 2000). There were no differences in 30-day mortality between the groups.

What are the implications of these two trials? In a recent meta-analysis of 1,552 ARDS patients from four RCTs (Meade et al., 2017), including those from OSCILLATE and OSCAR trials, the investigators reported a significant interaction between PaO_2/FiO_2 at randomization and the effects of HFOV, revealing increased harm from HFOV at higher PaO_2/FiO_2. Although HFOV still have a place as a rescue therapy, especially in centers without access or experience in extracorporeal oxygenation, this meta-analysis strongly question its future use in ARDS (Vincent, 2017).

Recruitment Maneuvers and Transpulmonary Pressure

Therapeutic variation in the distribution of inspired gas for attenuating VILI is the foundation of both prone ventilation and RMs. With the use of computed tomography (CT), it was discovered that radiographically some lung regions in ARDS look relatively normal but other areas are partially collapsed and do not participate in gas-exchange. RMs are applied to reopen collapsed alveolar units and to attenuate the injurious effects of repetitive opening and closing of alveoli. In general, ARDS is a heterogeneous injury in three lung compartments (Nieman et al., 2020): (i) normal alveoli that are inflated at end of expiration –and is referred to as the "baby lung" (Gattinoni and Pesenti, 2005); (ii) alveoli that are collapsed and/or fluid filled; (iii) and alveoli that are in between normal and unstable, and that open and collapse with every breath. Of note, the "baby lung" concept led to the understanding of potential interaction between MV settings and outcome, and to the frequent use of CT as a standard tool for a more precise ventilatory management in ARDS patients. As a result, lung-protective MV is constrained by ventilating this heterogeneous lung to avoid VILI, by protecting the baby lung without overdistending the compliant lung, and by stabilizing the lung using PEEP. Compliance is a property that describes lung distensibility and is calculated as the change in lung volume divided by the change in pressure. Lung compliance of the respiratory system is calculated as the VT divided by the transpulmonary pressure, the pressure across the lung (or alveolar pressure minus pleural pressure) (Kacmarek et al., 2021). Atelectatic areas of the lungs can be expanded by a brief application of high transpulmonary pressure, followed by a PEEP level that maintains open the new re-aerated region (Suárez-Sipmann et al., 2007). PEEP prevents lung collapse at end expiration. Although there are three commonly used RMs (sighs, sustained inflations, and extended sighs) (Guerin et al., 2011), none of these three maneuvers are currently recommended. In fact, none of the above trials used those maneuvers, but favored the use of incremental PEEP steps maintaining a constant driving pressure (Pplat minus PEEP) in a pressure-controlled mode until the recruitment pressures are reached.

There is controversy about the outcome benefits of RMs in patients with ARDS. In a pilot RCT in 200 patients with persistent ARDS (Kacmarek et al., 2016) comparing the ARDSnet protocol (Acute Respiratory Distress Syndrome Network, Brower et al., 2000) with an OLA approach involving RMs and a decremental PEEP trial for identifying the level of PEEP associated with maximum dynamic compliance, OLA improved oxygenation and lung mechanics without harmful effects on all-cause mortality at 60 days, VFDs, or barotrauma. In a large RCT (Working group for the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial (Art) Investigators et al., 2017), the *Alveolar Recruitment for acute respiratory distress syndrome Trial (ART)* conducted at 120 ICUs in 9 countries (mostly from Brazil) and involving 1010 patients with moderate to severe ARDS, RMs and PEEP titration according to best respiratory system compliance did not show reduced 28-day mortality when compared to patients treated with lower levels of PEEP. By contrary, patients in the RMs group had higher 6-month mortality, lower VFDs, and increased risk of barotrauma. After the publication of the negative results of the ART trial, the *Permissive Hypercapnia, Alveolar Recruitment, and Low Airway Pressure (PHARLAP)* trial (Hodgson et al., 2019) evaluating the effects of an OLA strategy with RMs vs. lung-protective MV was stopped very early when only 113 patients with moderate-to-severe ARDS were randomized (of a planned 340 patients). This small sample size trial (57 patients in the RM group vs. 56 in the control group) had no power to identify differences in VFDs, risk of barotrauma, or 180-day mortality.

Although the findings of the ART trial do not support the routine application of RMs, major concerns about study design, methodology, data analyses, and important differences with health care systems in participating countries, provided solid reasons to distrust the results of the trial and their generalizability to other settings (Villar et al., 2017). Patho-physiologically, there is a rationale for using RM and PEEP titration in ARDS since the ART results are in conflict with previous physiological and

clinical data. There is a need for another RCT designed and implemented more appropriately to examine that the principle that "never give the lungs a chance to collapse" (Villar et al., 2017) is associated with better clinical benefits, although all-cause fatality rate might not be reduced further.

A newer technique for titrating PEEP is optimizing the transpulmonary pressure at end of expiration (PEEP minus pleural pressure) (Talmor et al., 2008). Pleural pressure is estimated *via* esophageal manometry employing the measurement of esophageal pressure at the end of expiration, as a surrogate estimate of pleural pressure. It differs among patients with hypoxemic acute respiratory failure, suggesting that lung and chest wall mechanics contribute to respiratory system mechanics, as measured by the mechanical ventilator (Kacmarek et al., 2021). In general, the transpulmonary end-expiratory pressure is equal to zero: the more negative the transpulmonary pressure, the greater the collapse caused by a reduction in lung compliance. A negative transpulmonary pressure reflects that the forces trying to collapse the lung are stronger than the forces maintaining the lung open. This process may reverse if the end-expiratory transpulmonary pressure becomes zero or positive (Fumagalli et al., 2017). That is the main rationale for applying PEEP. PEEP increases the alveolar pressure. When alveolar pressure is equal to or exceeds pleural pressure, the resulting transpulmonary pressure is positive. This mechanism reduces lung collapse if the lung is opened before applying PEEP. That is why performing a RM and setting the appropriate level of PEEP by a decremental titration, augments FRC, decreases atelectasis, improves oxygenation, and increases compliance when compared with the same incremental PEEP level without recruitment (Pirrone et al., 2016).

A multicenter trial (EPVent-2) (Beitler et al., 2019) to validate preliminary results of a previous pilot study on esophageal pressure-guided ventilation (Talmor et al., 2008) was conducted in 200 patients with moderate-to-severe ARDS and examined whether PEEP titration guided by esophageal pressure is more effective than PEEP using a modified PEEP-FiO_2 table adopted from the OSCILLATE trial (Ferguson et al., 2013) using higher PEEP values. The primary outcome was a composite score including mortality and VFDs, calculated in a way that death was a worse outcome than fewer days free of MV. However, values for esophageal end-expiratory pressure, PEEP, Pplat, driving pressure, and PaO_2/FiO_2 were similar between both groups within the first 7 days. Although patients allocated to esophageal pressure-guided PEEP received fewer rescue therapies, the primary endpoint was not different between groups. Although for the investigators these findings did not support the use of PEEP titration using esophageal manometry, there are several reasons that could explain a lack of benefits of this trial (Suarez-Sipmann et al., 2019). First, in the multicenter EPVent-2 trial, participating centers had a wide range of expertise in implementing this novel method, as contrary to the high expertise of investigators in the previous EPVent trial. Second, the study design was not a true validation of the tested hypothesis in the EPVent since the EPVent-2 incorporated the PEEP-FiO_2 table using much higher PEEP levels, and oxygenation was not the primary outcome. This led to similar mean values of PEEP and transpulmonary pressures in both groups, suggesting that, on average, both groups were ventilated similarly! Third, the trial was underpowered: the calculation of the trial sample size was based on an overestimated 22% absolute difference in 28-day mortality. Fourth, a major critique relates to the PEEP titration method. Linking the transpulmonary pressure to the oxygenation level by a non-physiological PEEP-FiO_2 table was an erroneous decision to assess the effects of PEEP in the esophageal manometry group. Changes in oxygenation could not parallel changes in lung mechanics. For example, in the control group patients requiring $FiO_2 \geq 0.5$ could be ventilated with PEEP > 16 cmH_2O, a level that exceeds by far what is considered usual care. On the other hand, there is no guarantee that esophageal pressure recordings were checked for quality control in most participating centers. Fifth, it is plausible that the trial was biased in favor of the control group since both groups were monitored with an esophageal catheter.

A recent *post-hoc* reanalysis of the EPVent-2 trial by the same authors (Sarge et al., 2021) showed that the effect of PEEP strategy on mortality depended on pre-intervention severity of multiorgan dysfunction. Esophageal manometry-guided PEEP was associated with lower mortality among patients with less severe multiorgan dysfunction. Intriguingly, the higher end-inspiratory transpulmonary pressure, a marker of tidal overdistension, was independently correlated with risk of circulatory shock. Future clinical trials should be designed to consider baseline both, esophageal pressure at the end of expiration (to minimize the development of atelectasis) and esophageal pressure at the end of inspiration (to minimize the development of overdistension).

Extracorporeal Membrane Oxygenation

Oxygenation using extracorporeal life support was initially used in patients with severe acute respiratory failure in whom it was impossible to provide adequate gas-exchange by MV (Egan et al., 1988). MV is dependent on the presence of functional lung units for gas diffusion. However, when the number of functional alveoli is markedly reduced, MV is unable to sustain gas-exchange. In those cases, replacing the alveolar gas-exchange by extracorporeal membrane oxygenation (ECMO) can substantially reduce VT, respiratory rate, and FiO_2, and the risk of developing VILI. Most adult ECMO for respiratory support are performed with a veno-venous technique (Munshi et al., 2019). To deliver gas-exchange during ECMO, part of the cardiac output goes through the extracorporeal circuit *via* the femoral, saphenous, or jugular veins. CO_2 is removed by the ECMO circuit while MV is applied at low respiratory rates, high levels of PEEP, and Pplat below 30 cmH_2O by applying very low VTs.

Today, ECMO equipment are simpler, cheaper, and safer. Despite advances in extracorporeal life support and worldwide clinical use of ECMO, the results of a recent RCT in ARDS patients (Combes et al., 2018) has led to a diminished enthusiasm

for using it in severe ARDS. Referred to as the EOLIA (*ECMO to rescue Lung Injury in severe ARDS*) trial, this international RCT examined the effects of early use of ECMO in patients with very severe ARDS (**Table 1**). Patients assigned to the control group received MV, and the use of NMB agents, prone positioning, RMs, inhaled nitric oxide, or prostacyclin were strongly encouraged for oxygenation objectives by protocol. Patients assigned to ECMO underwent percutaneous veno-venous cannulation. The data safety monitoring board of the study decided to stop the trial after 75% of the planned patient population had been enrolled because the lower boundary of the predefined stopping rule for futility (defined as $< 20\%$ absolute risk reduction in mortality at 60 days) was achieved. The planned sample size was 331 patients, but the trial was terminated when 249 patients were randomized. In retrospect, a 20% absolute risk reduction is an unreasonable very large effect size when calculating sample size. Had the trial been designed with less strict stopping rules and continued to full enrollment, EOLIA may would have reached statistical significance. A major flaw of the trial was that 28% of patients allocated to the control arm (lung protective MV) crossed over to receive ECMO after randomization. This high crossover rate makes it extremely problematic to draw definitive conclusions about the benefits of ECMO in ARDS patients with very severe hypoxemia. Furthermore, the crossover of patients randomized to ECMO in participating ECMO centers could be seen as a weakness since ECMO is not available in most cities of modern countries for treating very severe hypoxemic ARDS.

Despite the results of the EOLIA trial, many clinicians still believe that there is a role for ECMO in severe ARDS patients with single organ failure and potentially reversible pulmonary dysfunction when MV and other adjunctive therapies have failed. Ideally, a comparison among patients who crossed over to ECMO with patients with similar severity randomized to the ECMO group would be an appropriate task to do. However, no such subsets of patients were identified in the ECMO group after a careful systematic review of the EOLIA trial (Munshi et al., 2019).

Pharmacologic Modulators of the Pulmonary and Systemic Inflammatory Responses

Anti-inflammatory drugs have been tested as prophylaxis and/or treatment of ARDS. In 2019, a systematic review provided no conclusive evidence that any pharmacologic intervention could reduce mortality in ARDS patients (Santacruz et al., 2019).

In the last decade in the United Kingdom (UK), two large RCTs (7,11) evaluated pharmacologic therapies to improve outcome in ARDS patients. The BALTI-2 (*beta-2 agonist lung injury trial*) (7) investigated the effects of increasing alveolar water clearance using intravenous salbutamol. This was a multicenter, placebo-controlled, randomized trial at 46 ICUs between 2006 and 2010, where patients within 72 h of ARDS onset, as defined by the American-European Consensus Conference (AECC) criteria (Bernard et al., 1994), were allocated to receive either salbutamol or placebo for 7 days. The primary outcome was death at 28 days of randomization. The trial reported harm in the treatment group and was stopped after the second interim analysis. A total of 324 patients (161 in the salbutamol group vs. 163 in the placebo group) were analyzed. Treatment with salbutamol significantly increased 28-day mortality, and was poorly tolerated. The HARP-2 (*Hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in Acute lung injury to Reduce Pulmonary dysfunction-2*) (11) study tested the use of the anti-inflammatory effects of simvastatin in ARDS. This was a multicenter, double-blind, randomized trial at 40 ICUs from UK and Ireland between 2010 and 2014, where 540 patients within 48 h of ARDS onset, as defined by AECC criteria, were randomized. The primary outcome was the number of VFDs, and secondary outcomes included mortality at 28 days. Treatment with simvastatin was safe, but it did not improve clinical outcomes.

Although the pharmacological mechanisms underlying the increased mortality in patients receiving salbutamol and the no effects of simvastatin are unclear, there are serious concerns about the approach for ventilating ARDS patients in both trials. Despite that leading investigators of both trials recommended the use of lung-protective MV, an audit (Poole et al., 2017) examining the ventilation practice in BALTI-2 and HARP-2 trials found that compliance with the ARDSnet guidelines for VT was very poor across all time points. There was no feedback to participating centers for the MV management in these studies. Baseline data was only available in 49% of patients in the BALTI-2 trial and PEEP levels were not recorded in both trials. This audit analysis revealed that considerably less than half of patients in BALTI-2 and HARP-2 trials received lung protective MV. Given the importance of low VT ventilation in improving outcomes ARDS, the results of those two pharmacologic trials need to be re-examined.

The key pathophysiological event underlying ARDS is injury to both the lung capillary endothelium and the alveolar epithelium with increased pulmonary vascular leakage. It turns out that adenosine has anti-inflammatory properties and reduces endothelial permeability. The enzyme termed cluster of differentiation 73 (CD73) is expressed on endothelial and epithelial cells, and regulates adenosine production by converting extracellular adenosine to active adenosine (Thompson et al., 2004). Since interferon (IFN) β-1a upregulates CD73, preventing vascular leakage, a pilot nonrandomized study reported that treatment with recombinant human IFN-β was associated with a reduction of 28-day mortality in patients with ARDS (Bellingan et al., 2014). In the INTEREST trial (*Efficacy and Safety of Interferon beta-1a in patients with ARDS*), a multicenter, randomized, double-blind, parallel-group in 8 countries, 301 patients with moderate or severe ARDS were randomized to treatment with 10 μg of IFN-1a once daily for 6 days or to placebo (Ranieri et al., 2020). The primary outcome was a composite score combining number of VFDs and number of deaths at 28 days. A total of 296 patients completed the trial, which did not lead to fewer deaths and VFDs when compared to the placebo group. Apart from the lack of efficacy, the trial has two important limitations: (i) the

study was underpowered since no enrichment approach was considered to enroll patients; (ii) more than one third of patients received steroids, which inhibit the effects of IFN-β-1a signaling, raising the possibility that the treatment with IFN- β-1a in combination with corticosteroids could increase mortality in ARDS.

Since the first clinical description of ARDS, there has been a great interest in the role of corticosteroids for attenuating the underlying inflammatory state of ARDS because of their potent anti-inflammatory and antifibrotic effects on multiple signaling pathways (Rhen and Cidlowski, 2005). Several doses and types of corticosteroids have been evaluated in the context of ARDS with inconclusive results (Annane et al., 2017). Nevertheless, it is plausible that corticosteroids may benefit ARDS in the early stages of the disease process, a situation that was not evaluated in most RCTs. In addition, dexamethasone was never evaluated in a randomized controlled fashion in ARDS patients, despite its potent anti-inflammatory and weak mineralocorticoid effects. Dexamethasone is 20–30 times more potent than the naturally occurring hormone cortisol and 4–5 times more potent than prednisolone (Rhen and Cidlowski, 2005). In addition, dexamethasone has long-lasting pharmacological effects, allowing for a regime of one dose per day (Meijvis et al., 2011). The beneficial effects of dexamethasone in ARDS were unknown until recently. The DEXA-ARDS (*Dexamethasone in ARDS*) trial, was a multicenter, RCT performed in 277 patients with established moderate-to-severe ARDS (defined by a $PaO_2/FiO_2 \leq 200$ assessed with a PEEP $\geq$ 10 cmH_2O and $FiO_2 \geq 0.5$ at 24 h after diagnosis of ARDS) (Villar et al., 2020). Patients were randomized to receive treatment with dexamethasone (20 mg once daily from days 1 to 5, which was reduced to 10 mg from days 6 to 10) or continued routine intensive care (control group). The primary outcome was VFDs, and the secondary outcome was mortality at 60 days after randomization. Treatment with dexamethasone markedly increased the number of VFDs and decreased the risk of 60-day mortality by an absolute 15%. These findings paved the road of using steroids to improve survival among critically ill patients with coronavirus disease 19 (COVID-19) (WHO Rapid Evidence Appraisal for Covid-19 Therapies (React) Working Group et al., 2020).

FUTURE TRIAL DESIGN: PERSONALIZED MECHANICAL VENTILATION

The history of interventional clinical trials for ARDS is fraught with many failures and only a few successes in the last decade. Since there is no typical ARDS patient (Villar, 2011), the risk of developing ARDS depends on the underlying disease process but also augments with the number of predisposing factors. Ideally, therapies in ARDS should be personalized to the specific predisposing clinical condition or mechanism of organ injury at any given point in time, rather than being provided uniformly to all patients. The most critical factor in managing ARDS patients is the initiation of lung-protective ventilation instantaneously after endotracheal intubation. However, the development of therapeutic strategies for ARDS is complicated because ARDS is not a disease but a very heterogeneous syndrome (Juschten et al., 2021). The optimal ventilation strategy for ARDS still remains to be refined. For example, the physiological meaning of the end-expiratory transpulmonary pressure could be very useful in managing patients with increased chest wall stiffness or to set optimal PEEP during ventilation and weaning in morbidly obese patients (Kacmarek et al., 2021).

Current lung-protective MV strategies have not significantly decreased ARDS-associated all-cause mortality since the ARDSnet trial (Acute Respiratory Distress Syndrome Network, Brower et al., 2000), possibly because those strategies are reserved to ventilating heterogeneous atelectatic and stable lungs in severe ARDS (Nieman et al., 2020). Furthermore, in most RCTs, an unselected, mixed population of ARDS have been studied, missing the opportunity to test whether the experimental MV approach or adjunctive/pharmacological therapy is beneficial in patients having a single etiology or after assessing their "true" degree of hypoxemia under standardized ventilatory settings prior to randomization (Villar et al., 2007). It is highly plausible that in a substantial proportion of patients in recent RCTs in ARDS, the severity of lung injury was modest. Current definition for ARDS (Ranieri et al., 2012) did not require any specific FiO_2, and only a minimum PEEP level of 5 mH_2O to calculate the PaO_2/FiO_2 for stratifying patients as mild, moderate or severe ARDS. Any change in PEEP or FiO_2 can result in a modification of lung imaging and PaO_2/FiO_2 ratio. Therefore, depending on the applied MV approach, patients may or may not meet criteria for the diagnosis of ARDS. For example, a septic patient with a PaO_2 of 69 mmHg on a FiO_2 of 0.35 and 5 cmH_2O of PEEP satisfies the criteria for moderate ARDS, but there is no need to enroll this type of patients in a trial testing the effects of high PEEP levels and RMs. In addition, systemic inflammation seen in ARDS patients is not specific for ARDS, especially in patients with sepsis, and MV -although it could be considered a curative therapeutic approach in critically ill patients- it is not the cure for sepsis!

Medicine cannot always be provided by a protocol (Florio et al., 2020). Hopefully, it is plausible that in the near future, we will have mechanisms to identify classes or subclasses of ARDS patients that might respond to targeted therapy, including MV, adjunctive therapies, or pharmacological approaches (Sevransky et al., 2021). Research breakthroughs are not enough. To date, attempts to personalize or stratify the care of ARDS patients enrolled in clinical trials using physiologic measures have not been successful, except in the case of the DEXA-ARDS trial where the authors used an enrichment strategy under standardized ventilatory settings to identify patients for enrollment (Villar et al., 2020). If patients enrolled in a trial have a low risk of the condition to prevent, the trial –irrespective of sample size- will not validate the value of the intervention under study (Villar et al., 2005). MV for patients with ARDS would be personalized based on etiology, lung physiology and morphology, and clinical and biological classes or subclasses because explicit numerical values might not apply to individual patients

(Villar et al., 2019; Pelosi et al., 2021). Optimization of patient selection is central to the likelihood of success in future trial design for ARDS (Villar et al., 2020; Pelosi et al., 2021). Both prognostic and predictive enrichment strategies can improve the signal-to-noise ratio, allowing smaller sample sizes and increased effects sizes (Villar et al., 2019; Ware et al., 2020).

AUTHOR CONTRIBUTIONS

JV drafted the first version of the manuscript. JV, CF, GT, and FS-S contributed to the initial concept and design of this review. JV, CF, GT, LB, PR-S, and FS-S critically revised the manuscript. JV, CF, and PR-S obtained funding for the study. All authors read and approved the final manuscript.

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frontiers in Physiology

REVIEW
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Pulmonary Interstitial Matrix and Lung Fluid Balance From Normal to the Acutely Injured Lung

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This review analyses the mechanisms by which lung fluid balance is strictly controlled in the air-blood barrier (ABB). Relatively large trans-endothelial and trans-epithelial Starling pressure gradients result in a minimal flow across the ABB thanks to low microvascular permeability aided by the macromolecular structure of the interstitial matrix. These edema safety factors are lost when the integrity of the interstitial matrix is damaged. The result is that small Starling pressure gradients, acting on a progressively expanding alveolar barrier with high permeability, generate a high transvascular flow that causes alveolar flooding in minutes. We modeled the trans-endothelial and trans-epithelial Starling pressure gradients under control conditions, as well as under increasing alveolar pressure (Palv) conditions of up to 25 cmH_2O. We referred to the wet-to-dry weight (W/D) ratio, a specific index of lung water balance, to be correlated with the functional state of the interstitial structure. W/D averages ∼5 in control and might increase by up to ∼9 in severe edema, corresponding to ∼70% loss in the integrity of the native matrix. Factors buffering edemagenic conditions include: (i) an interstitial capacity for fluid accumulation located in the thick portion of ABB, (ii) the increase in interstitial pressure due to water binding by hyaluronan (the "safety factor" opposing the filtration gradient), and (iii) increased lymphatic flow. Inflammatory factors causing lung tissue damage include those of bacterial/viral and those of sterile nature. Production of reactive oxygen species (ROS) during hypoxia or hyperoxia, or excessive parenchymal stress/strain [lung overdistension caused by patient self-induced lung injury (P-SILI)] can all cause excessive inflammation. We discuss the heterogeneity of intrapulmonary distribution of W/D ratios. A W/D ∼6.5 has been identified as being critical for the transition to severe edema formation. Increasing Palv for W/D > 6.5, both trans-endothelial and trans-epithelial gradients favor filtration leading to alveolar flooding. Neither CT scan nor ultrasound can identify this initial level of lung fluid balance perturbation. A suggestion is put forward to identify a non-invasive tool to detect the earliest stages of perturbation of lung fluid balance before the condition becomes life-threatening.

Keywords: SARS-CoV-2, ARDS, edema, alveolar pressure, P-SILI, computational model

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INTRODUCTION

Efficient gas diffusion in the air-blood barrier is only possible by strict control of extravascular water volume to prevent edema-induced tissue swelling and alveolar flooding. In normal lungs, the thickness of the air-blood barrier does not exceed ∼0.2 μm. Edema is prevented by an extremely low microvascular permeability and dynamic remodeling of the interstitial matrix (Miserocchi and Rivolta, 2012). The physiologic parameters controlling lung fluid balance are dynamic and change over the life span of individuals to ensure a healthy match of regional ventilation to the metabolic requirement.

This study merges the understanding of normal physiologic fluid balance control with the ramifications of what can occur when this control is lost. We will first review how a healthy lung can tightly control extravascular lung water by specific mechanical alterations to parenchymal structure that will efficiently minimize edema. Next, we will investigate how progressive loss of this anti-edemagenic control inevitably leads to severe edema. We present a detailed analysis of Starling pressure gradients sustaining flows across the endothelial and epithelial barriers. We stress the point that the heterogeneity in edema distribution allows us to distinguish between lung regions that can still provide control of fluid balance to allow gas exchanges and other regions that have lost this capacity because of breakage of the interstitial matrix. We correlate the Starling pressure gradients with the specific regional edema formation measured as the lung tissue wet-to-dry weight ratio (W/D). This study provides contribution to the understanding of acute lung injury pathophysiology and the role of mechanical ventilation in edema formation. In particular, this study is one of the first to discuss trans-endothelial and trans-epithelial flows as distinct events for a given lung distension pressure. It is hoped that this review might provide indications aimed at avoiding an unintentional ventilator-induced lung injury (VILI).

THE AIR-BLOOD BARRIER AND THE MOLECULAR STRUCTURE OF THE INTERSTITIAL COMPARTMENT

The air–blood barrier (ABB) represents the interface for gas exchanges; the capillary endothelium is in close apposition with the alveolar epithelium so that the thickness of ABB is as low as 0.3 μm and its surface is in the range of 100 m^2 in the human lung (Weibel, 2015).

The ABB includes two portions: a thin side (**Figure 1A**) that accounts for more than 50% of alveolar surface that contains minimal interstitial space (Weibel, 2017), and a thick side (**Figure 1B**) that includes most of the parenchymal extra cellular matrix (ECM) and lymphatics. It has been suggested that the thick portion acts as a reservoir capacity where fluid filtered at the level of the thin portion might accumulate to be drained down a pressure gradient generated by lymphatics (Unruh et al., 1984; Conforti et al., 2002).

The special arrangement of the endothelial and epithelial cells is seen in **Figure 2**. Type-1 epithelial (Epi1) cells cover about 95% of the surface of each alveolus, with the rest being covered by globular Epi2 cells.

The body of Epi1 cells forms a series of interconnected structures that envelop, through several stalks, approximately 40 capillaries. There are only about 40 Epi1 cells lining a single alveolus facing about 170 endothelial cells, so any Epi1 cell covers about four endothelial cells. An average volume of ∼1,700 μm^3 of an Epi1 cell spreads over a surface area of ∼5,000 μm^2, and allows for a thickness of as low as 0.3 μm. Such a low number results from the combination of low number of Epi1 cells and extended plasma membrane surface. On the whole, the overall surfaces of Epi1 and endothelial cells are comparable.

Two important functional features of ABB are the following: very strict control of water balance by minimizing fluid exchanges across the endothelial and epithelial barriers, and remarkable mechanical resistance to the increase in tension related to change in lung volumes. These two important features rely on the structure-function relationship between the epithelial and endothelial cells and the macromolecular structure of the interstitial compartment separating the epithelial and endothelial layers.

The structure of the interstitial compartment includes a mesh of collagen and elastic fibers whose main role is to provide mechanical support and elasticity (Miserocchi and Rivolta, 2012). These molecules are chemically very stable and resistant to proteolysis. Within the space between these molecules, there is a dense mesh of molecules, belonging to the proteoglycan family, whose main role is to exert a strict control on the amount of extravascular water that must be kept to a minimum. Microvascular permeability is controlled by the low molecular weight heparan-sulfate proteoglycan (HS-PG, 300–500 kDa) of the basement membrane and by small peptidoglycans of the glycocalyx (PDGL), normally assuring high resistivity of the paracellular route (Reitsma et al., 2007). Large molecular weight chondroitin-sulfate proteoglycans (CS-PG > 1,000 kDa) bound to hyaluronan act as link proteins through low energy non-covalent bonds with other molecules and cells providing rigidity to the parenchymal mesh.

THE CONTROL OF EXTRAVASCULAR WATER UNDER PHYSIOLOGICAL CONDITIONS

The hydraulic pressure (Pint) in lung interstitial compartment is in the sub-atmospheric range, averaging ∼10 cmH_2O (Miserocchi et al., 1990), and a negative Pint value represents the fluid dynamic equilibrium between capillary filtration down a high resistance pathway (low microvascular permeability) and a lower resistance pathway represented by lymphatic drainage. The equilibrium is also favored by the transcapillary oncotic pressure gradient (favoring absorption) that exceeds the hydraulic gradient (favoring filtration) (Parker et al., 1978).

Figure 3 presents the fluid exchange models for the thin the thick portions of the ABB, and the parameters defining the pressure gradients that sustain potential water and solute fluxes

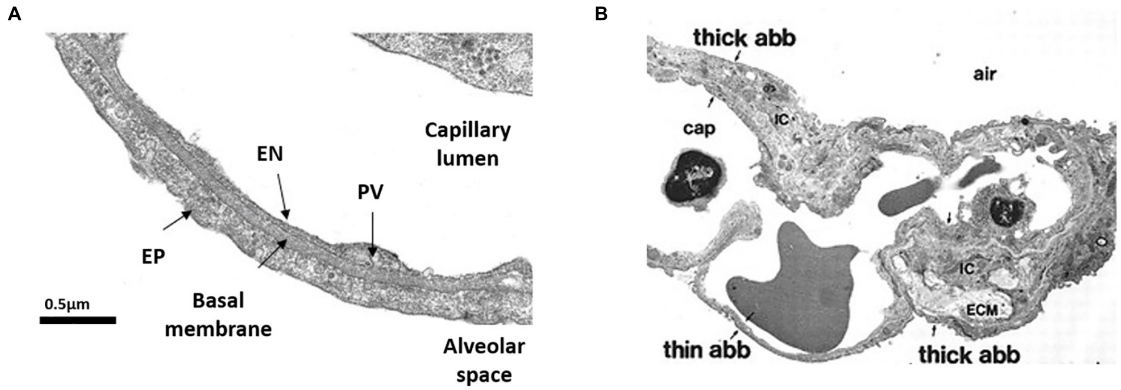

FIGURE 1 | Morphology of the air-blood barrier (ABB). **(A)** Thin portion of ABB showing the close apposition between alveolar epithelium (EP) and capillary endothelium (EN). PV is plasmalemmal vesicle (from Botto et al., 2006). **(B)** Microscopy image showing the ABB and its thin and thick portions. ECM, extra cellular matrix; cap, lung capillary; ic, interstitial cell (from Conforti et al., 2002).

across the endothelium and the epithelium. Note that lymphatics are only present in the thick portion of the ABB.

Transcapillary and transepithelial water exchanges are governed by the Starling law where P and Π are the hydraulic and the colloid osmotic pressures across any two compartments. Water flow (J_v) is defined as:

$$J_v = Kf \cdot [(P_1 - P_2) - \sigma(\Pi_1 - \Pi_2)] \quad (1)$$

where $Kf\ \left(filtration\ coefficient\right) = Lp \cdot A$, with Lp being the hydraulic conductance, A the surface area available for flow, and $[(P_1 - P_2) - \sigma(\Pi_1 - \Pi_2)]$ the Starling pressure gradient generating flow.

In this study, we will focus on the Starling pressure gradients, and based on experimentally measured values, will analyze how they are modified to cause severe perturbation in lung fluid balance. Relevance will also be given to the estimate of the effect of increasing alveolar pressure on these gradients, a point that, so far, has remained unexplored but of utmost importance in mechanical ventilation. In a companion study, the Starling pressure gradients presented in this study will be used to model the transcapillary, transepithelial, and lymphatic flows in physiological condition as well as in developing severe lung edema.

Our whole analysis considers W/D as the more precise and reliable index of the lung fluid balance that has been related to experimentally measured values of variables and coefficients appearing in Eq. 1. The validity of W/D as an index of water balance was confirmed by Parker and Townsley (2004) who reported its excellent correlation with albumin and total proteins collected from the bronchoalveolar lavage fluid, an index of derangement of fluid balance in experimental models of graded lung injury.

Table 1 reports the Starling pressure gradients in a healthy lung at end-expiration and at end- inspiration. End-expiration corresponds to functional residual capacity (FRC) ∼22% TLC in supine position, while end-inspiration corresponds to ∼32% TLC (Agostoni and Mead, 1964). Water balance under physiological condition is defined by a lung wet weight/dry weight ratio (W/D) of ∼5 (Conforti et al., 2002). In **Table 1** and from here on, positive values of a Starling gradient across the endothelium and the epithelium indicate fluid filtration into the interstitial space and the alveolar compartment, respectively; negative values of a Starling gradient indicate fluid clearance from these compartments.

Table 1 reports the expected values of hydraulic pressure for the capillary, interstitial, and alveolar fluids (Pcap, Pint, and $Pliq_{alv}$, respectively) as well as those of oncotic pressure (Πcap, Πint, and Πliq_{alv}, respectively). The value of $Pliq_{alv}$, the pressure of the liquid phase coating the alveolar surface, was estimated from the equation $Pliq_{alv} = Palv - \frac{2\gamma}{R}$, (Beck and Lai-Fook, 1983). We considered Palv = 0 at end-inspiration and at end-expiration (FRC) during spontaneous breathing, and we assumed a physiological value for γ = 1 dynes/cm substantially unchanged up to 70% TLC (Bachofen et al., 1970). We assumed an alveolar radius of ∼ 50 μm at TLC (Beck and Lai-Fook, 1983) and its decrease at FRC being proportional to $(FRC/TLC)^{1/3}$ (equal to 0.6). Accordingly, given the low value of γ, the ratio $2\gamma/R = Pliq_{alv}$ is ∼ 0 cmH_2O. Clearly, the large change in trans-epithelial Starling gradient reflects the remarkable change in Pint.

Endothelial and epithelial protein reflection coefficients (σ_{endo} and σ_{epi}, respectively) are also reported. Hydraulic (ΔP) and oncotic (σΠP) pressure gradients and the total Starling pressure gradient are reported in bold. Concerning σ, it was experimentally derived at high flow rates as $\sigma = 1 - \phi$ (ϕ being the lymph/plasma protein partition coefficient). However, this condition might not provide a σ value corresponding to the physiological condition of very low flow rates. For both endothelium and epithelium, we assumed σ = 0.85 (Parker et al., 2006), a higher value compared to those previously provided by Parker et al. (1978). Furthermore, under healthy conditions, the epithelium is almost totally impermeable to proteins (Gorin and Stewart, 1979), and water transport can only occur *via* coupled Na^+ active absorption (Matthay et al., 1982). One report showed that epithelial monolayers generally have an electrical resistance that is an order of magnitude higher than

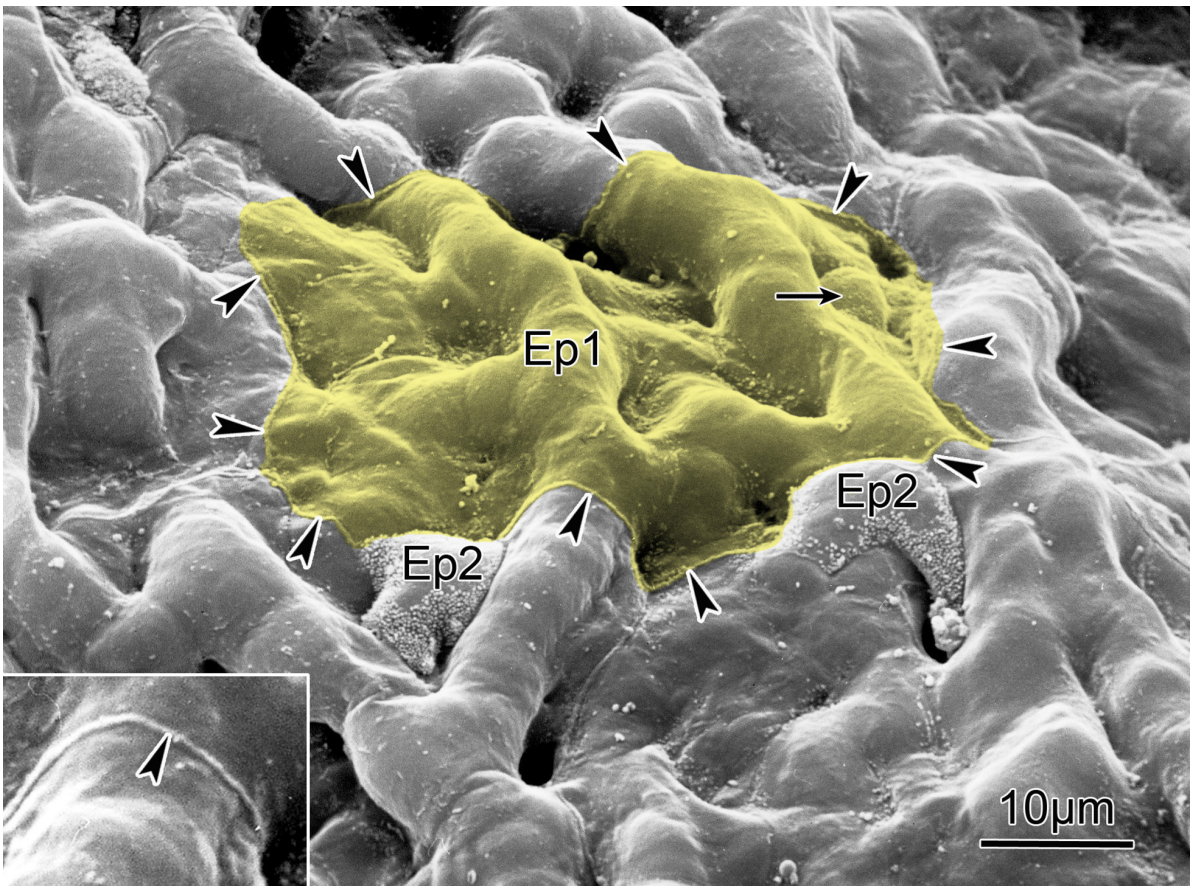

FIGURE 2 | Scanning electron micrograph of the alveolar surface of a human lung showing protruding capillaries and two type II cells (Ep2) sitting in niches and characterized by a rim of microvilli. Small arrow points to cell body of a type I cell (Ep1) that covers several capillary meshes (yellow); the boundary of its cytoplasmic leaflet is marked by arrowheads outlining a small lip of the cell junction between adjoining cells (inset, compare **Figure 1B**). The surface area covered by this cytoplasmic leaflet is 1,300 mm^2. Scale bar = 10 µm (from Weibel, 2015); reprinted with permission of the American Thoracic Society.

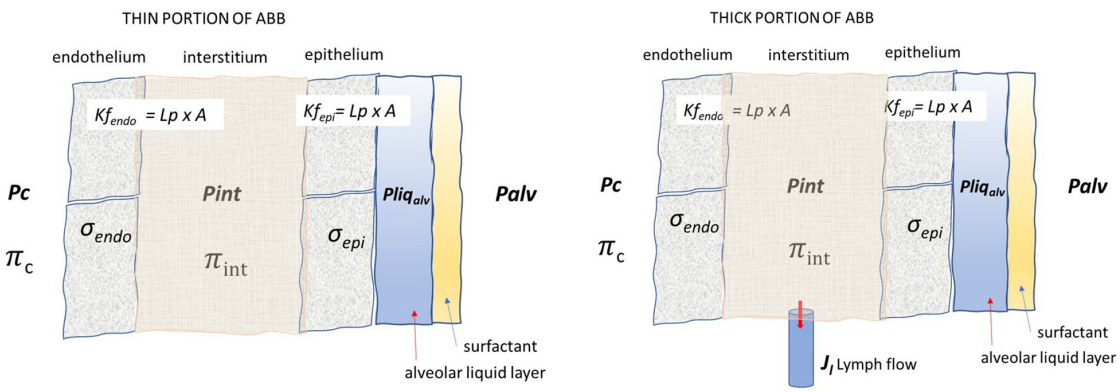

FIGURE 3 | Lung fluid compartments of the ABB and parameters governing fluid exchanges. Pc, Pint, and $Pliq_{alv}$ are capillary, interstitial, and alveolar liquid hydraulic pressure, respectively; Πc and Πint are capillary and interstitial oncotic pressure, respectively; σ_{endo} and σ_{epi} are the protein reflection coefficient of endothelium and epithelium, respectively; Palv is the alveolar pressure; Lp, A, Kf_{endo}, and Kf_{epi} are the hydraulic conductance, overall filtration surface, and endothelial and epithelial filtration coefficient, respectively. Lymphatic drainage (Jl) occurs from the thick portion of ABB where lymphatics are located.

TABLE 1 | Trans-endothelial and trans-epithelial Starling pressure gradients at end-expiration (functional residual capacity, FRC) and end-inspiration under physiological conditions (referring to Point A in **Figure 4**).

	Trans-endothelial			Trans-epithelial	
	End-expiration	**End-inspiration**		**End-expiration**	**End-inspiration**
Pcap*	9	9	Pint**	−10	−24
Pint**	−10	−24	Pliq alv#	∼ 0	∼ 0
σ end##	0.85	0.85	σ epi##	0.85	0.85
Πcap**	26.8	26.8	Πint**	13.8	13.8
Πint**	13.8	13.8	Πliq alv	0	0
			γ	1	1
ΔP	**19**	**33**	**ΔP**	**−10**	**−24**
σ·ΔΠ	**−11.0**	**−11.0**	**σ·ΔΠ**	**−11.7**	**−11.7**
Starling gradient	**8.0**	**22.0**	**Starling gradient**	**−21.7**	**−35.7**

Table reports the expected values for capillary, interstitial and alveolar liquid hydraulic pressure (Pcap, Pint and Pliq alv, respectively) as well as for capillary, interstitial and alveolar liquid oncotic pressure (Πcap, Πint and Πliq alv, respectively). Endothelial (σ endo) and epithelial (σ epi) protein reflection coefficients are also reported. In bold, hydraulic (ΔP) and oncotic (σΠP) pressure gradients and total Starling pressure gradient. Positive values of the Starling gradient at endothelial level indicate filtration into interstitium; negative value at epithelial level indicate alveolar reabsorption.

*Pressure values are expressed in cmH_2O; σ is a pure number. *From Hakim et al., 1993; **from Miserocchi et al., 1993; #calculated from Beck and Lai-Fook, 1983; ##from Parker et al., 2006. Surface tension γ = 1 dyne/cm.*

that of endothelial monolayers and much lower solute diffusion permeability (Parker et al., 2006).

Concerning the microvascular district, one should add the important notion that functional compartmentation has been described considering a "true" alveolar compartment and an extra-alveolar capillary compartment. The latter has higher Lp and lower surface area compared to the "true" alveolar district, which has a much lower Lp but an incredibly much higher surface. Definitely, the "true" alveolar compartment only contributes about 6% of total convective albumin flux recovered in the lung lymph (Parker, 2007). Therefore, under physiological conditions, despite the existence of trans-endothelial and trans-epithelial pressure gradients shown in **Table 1** (referring specifically to the "true" alveolar district), minimal fluxes occur thanks to low permeability coefficients. One shall consider that capillary surface area (A) might differ among humans, reflecting the individual extension of the alveolar capillary network that was found to vary by ∼3 fold based on the estimate of the capillary blood volume (Vc) (Miserocchi et al., 2008).

THE RESPONSE TO THE INCREASE IN MICROVASCULAR FILTRATION

Figure 4 shows how Pint varies with increasing water balance as expressed by the W/D ratio. Point A corresponds to the physiological condition, while Point B corresponds to the physiological response acting to buffer an increase in microvascular filtration in response to an edemagenic condition.

The latter may occur because of a variety of reasons, such as hyperventilation and increase in cardiac output on sustained exercise (e.g., marathon running) (Zavorsky et al., 2014). The lung is well-equipped to respond to increased microvascular filtration due to specific morpho-functional features of the interstitial tissue structure. Indeed, increased free water filtered in the interstitium is captured by hyaluronan to form gel, whose increase in steric hindrance causes a remarkable increase in Pint from −10 cmH_2O (**Figure 5**, point A) to ∼ +5 cmH_2O (point B) (Miserocchi et al., 1993). At point B, the W/D ratio does not exceed 5.5 (10% increase relative to the control value), reflecting low tissue compliance (∼0.5 $ml.mmHg^{-1}\cdot 100$ g of wet $weight^{-1}$) (Miserocchi et al., 1993). As long as the filtration coefficient and the protein reflection coefficient remain within physiological values, interstitial gel formation provides a "safety factor" against edema formation to face an increase in microvascular filtration. Indeed, the increase in interstitial pressure (see Eq. 1) prevents further filtration and may actually favor fluid re-absorption (see Paragraph 8 for estimate of actual Starling pressure gradients). The correlation between rigidity of the extracellular matrix and low microvascular permeability was confirmed in a micro-nano biomimetic system by growing endothelial and epithelial cell layers on opposite sides of a porous mesh mimicking the extracellular structure of the basal lamina in the lung. Varying the rigidity of the mesh, and using different concentrations of polycaprolactone with gelatine, it was found that effective barrier properties were actually increased by increasing the stiffness of the matrix (Higuita-Castro et al., 2017).

Respiratory mechanics was monitored by low-frequency forced oscillation technique (FOT) in closed-chest mechanically ventilated rats under conditions referring to points A and B (Dellacà et al., 2008). The results suggested that the changes in reactance and resistance when shifting from A to B could reflect the changes in viscoelastic properties of the lung tissue, in the absence of the accumulation of extravascular fluid into the alveoli. It was, therefore, proposed that the estimate of lung mechanics with FOT might represent a non-invasive, potentially clinically useful tool to detect the earliest stages of perturbation of lung fluid balance before the condition becomes life-threatening. The next paragraph describes how the loss of safety factor (**Figure 4**, point C), due to fragmentation of the interstitial matrix structure, leads to a decrease in Pint to 0 cmH_2O and to a critical increase in W/D ratio.

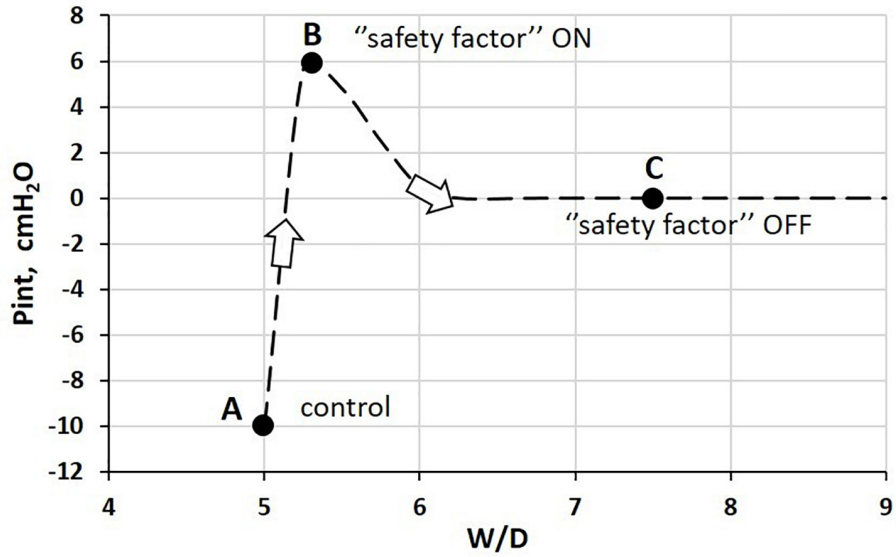

FIGURE 4 | Relationship between interstitial hydraulic pressure (Pint) and wet-to-dry weight (W/D) ratio at end-expiration. Point A corresponds to physiological condition; point B to the "safety factor" (see text for explanation); point C corresponds to the absence of the "safety factor" reflecting the remarkable loss of integrity of the interstitial matrix (Miserocchi et al., 1990, 1993).

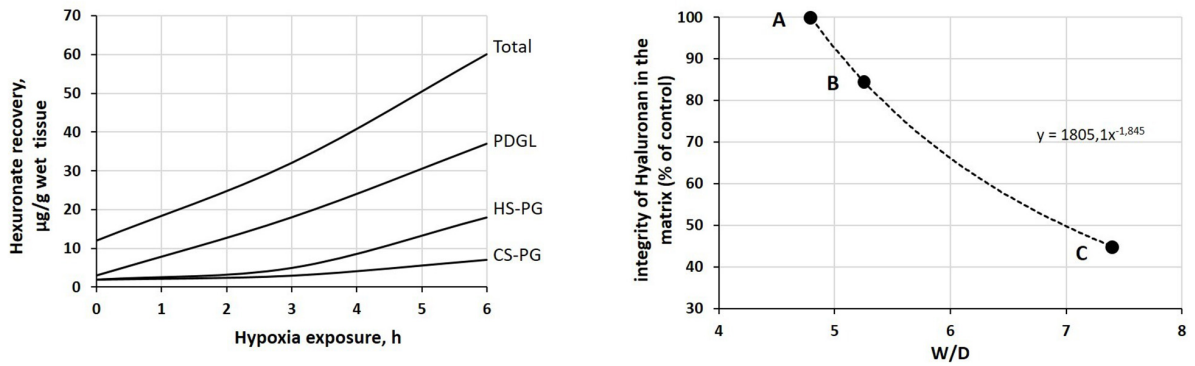

FIGURE 5 | Left: time course of the recovery of fragments of proteoglycan families upon 12% O_2 exposure (redrawn from Miserocchi et al., 2001). Peptidoglycans (PDGLs) control the permeability of the glycocalyx; heparin-sulfate proteoglycans (HS-PGs) control the permeability of endothelial and epithelial cells; chondroitin-sulfate proteoglycans (CS-PGs) provide rigidity to the interstitial matrix. **Right:** relationship between loss of integrity of interstitial hyaluronan (a hydrophilic non-sulfated glycosaminoglycan) and increase in W/D ratio. Points A, B, and C correspond to those reported in **Figure 4**.

THE TISSUE DAMAGE AND THE CRITICAL INCREASE IN W/D RATIO

The inflammation caused by a sterile (e.g., severe hypoxia, hyperoxia, surgery, excessive parenchymal stress/strain) or bacterial/viral mechanism may trigger the fragmentation of the macromolecular architecture of the interstitial compartment, involving the whole proteoglycan family. **Figure 5** (left) shows, as an example, the time course of the recovery of fragments of proteoglycan families on 12% O_2 exposure (Miserocchi et al., 2001). Over time, fragment recovery occurs at a greater rate for PDGL and HS-PG, compared to CS-PG. The loss of integrity of the native architecture of the proteoglycan family of the interstitial matrix leads to a combination of two effects: (i) increase in microvascular permeability, which weakens the restriction to fluid leak, and (ii) increase in tissue compliance that brings Pint back to 0 cmH_2O (**Figure 4**, point C, W/D ∼7.5). As a consequence, the "safety factor" is abolished and the corresponding increase in W/D ratio reflects the increase in filtration rate leading to the development of edema. The right panel of **Figure 5** shows the negative correlation between the loss of integrity of the interstitial matrix and the increase in W/D. With 50% fragmentation of native hyaluronan, W/D increases by up to ∼ 7.5, a frankly edematous state (Negrini et al., 1996; Miserocchi et al., 2001).

Differences in the sequence of a fragmentation of proteoglycans may be demonstrated among various models of experimental pulmonary edema (Negrini et al., 1996; Miserocchi et al., 1999). Thus, there may be a variable time-dependent contribution to edema formation due to the increase in microvascular permeability (due to the fragmentation of HS-PG and PGDL) and/or increase in tissue compliance (reflecting the fragmentation of CS-PG). The inflammatory-dependent activation of proteases (MMP-2 and MMP-9) contributes to proteoglycan fragmentation (Passi et al., 1999). Heparan-sulfate disassembly has been reported by recent findings of HA exudates in the alveolar spaces of the lungs of patients with coronavirus disease-2019 (COVID-19) (Hellman et al., 2020).

It is noteworthy to consider the difference in timing between the phase of fragmentation of the interstitial mesh (3–6 h) and the immunity response reported for patients with COVID-19 that develops with a much slower kinetics during the first week after infection (Hou et al., 2020; Long et al., 2020).

CAUSES OF LESION OF THE AIR-BLOOD BARRIER

Severe Acute Respiratory Syndrome Coronavirus-2

Sepsis is due to lung lesions (Bachofen and Weibel, 1977). Concerning the kinetics of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a tempting hypothesis is that the entrance of virus into the ABB might resemble that of engineered nanoparticles (NPs) delivered through the airways for therapy. Findings highlight the intimate connections between viruses and NP with host lipid interactions. Both viruses and NPs could exploit dual entry, namely, crossing the plasma membrane or uptake *via* endosomes. Human ACE2-binding interface tends to have a predominantly negative electrostatic potential, allowing interaction with the SARS-CoV-2 S protein (Hassanzadeh et al., 2020). It takes less than 1 h for NPs to move into the lung interstitial space and reach the circulatory system (Dal Magro et al., 2017). Recent data suggest that heparan-sulfate proteoglycan on cell surface facilitates the attachment of SARS-CoV-2 particles to the cell surface to promote cell entry (Zhang et al., 2020). In particular, it was shown that SARS-CoV-2 spike protein interacts with both heparan-sulfate and the binding domain of ACE2 (Clausen et al., 2020). Vascular and thrombotic complications, such as symptomatic acute pulmonary embolism, deep-vein thrombosis, ischaemic stroke, myocardial infarction, and systemic arterial embolism, have been reported in severe COVID-19 cases (Farhangrazi et al., 2020).

Reactive Oxygen Species

Molecular oxygen is activated in either hyperoxia or hypoxia into reactive oxygen species (ROS). Effective antioxidant defenses counteract the reactivity of ROS. However, the overwhelming production of ROS coupled with their insufficient scavenging by endogenous antioxidants will lead to tissue damage and cell death (Kulkarni et al., 2007). ROS are implicated in the increase in alveolar permeability (Waxman and Kolliputi, 2009; Kolliputi et al., 2010). It was found that exposure to 100% O_2 increased alveolar epithelium permeability similar to that caused by the alloxan model of lung edema, and that this occurred before the onset of interstitial or alveolar edema (Matalon and Egan, 1981, 1984).

Hyperoxia also causes damage to endothelial cells (Kistler et al., 1967) and tissue structure (Chow et al., 2003; Kallet and Matthay, 2013) because of increased production of reactive oxygen species (ROS) at mitochondrial level (Freeman and Crapo, 1981).

The risk of ROS-dependent lung injury is reported to occur at $FIO_2 > 0.7$, and may worsen at $FIO_2 > 0.8$ for prolonged exposure. To limit the exposure to high levels of FIO_2, there is a recommendation by ARDS Network to target PaO_2 levels between 55 and 80 mmHg in mechanically ventilated patients. Milder levels of hyperoxemia would be supported by data suggesting that $PaO_2 > 80$ mmHg is associated with worse clinical outcomes at all levels of acute respiratory distress syndrome severity (Aggarwal et al., 2018).

Type-1 epithelial (Epi1) cells could be completely destroyed by hyperoxia (Weibel, 1971), so, after a few days of hyperoxia exposure, the whole alveolar surface would be found to be lined only by cuboidal Epi2 cells (Kapanci et al., 1969; Kaplan et al., 1969), a finding also reported for oxygen poisoning in human lung (Nash et al., 1967). The role of Epi2 cells was interpreted as that of regeneration of new Epi1 cells (Adamson and Bowden, 1974; Bachofen and Weibel, 1974; Evans et al., 1975). Neo-formed Epi1 cells appear able to branch and form at least four or even more lining units on endothelial cells (so called "epithelial plate") (Weibel, 2015). Ischemia-reperfusion injury (a primary graft dysfunction) is a form of high-permeability pulmonary edema due to endothelial lesion (Liang et al., 2019).

Lung Overdistension

Lung overdistension is a major cause of tissue lesion. Going from low to high transpulmonary pressure causes an increase in fluid filtration rate (Bo et al., 1977). Inflating the lungs to total capacity is found to cause free solute movement across the lung epithelium because of larger pore radii. Furthermore, decreasing lung volume does not produce a smaller pore radius that either remains the same or becomes larger. This phenomenon is interpreted as depending on permanent lung lesion (Egan, 1980). Furthermore, data by Egan (1982) suggest that acute lung distension at Palv = 40 may cause epithelial albumin leak but not alveolar flooding: the interpretation of this result is that, upon short exposure to considerable lung distension, no time is given for remarkable matrix fragmentation. A 5-fold increase in Jv over 6 h with step change of Palv from 10 to 20 cmH_2O (Tarbell et al., 1999; Tarbell, 2010). W/D was found to increase from 6.63 to 7.45 over 2 h with Palv up at 48 cmH_2O (Yoshikawa et al., 2004); this same study reported an increase in albumin concentration in bronchoalveolar lavage fluid by up to 250 mg/ml.

Other data report a significant increase in endothelial Kf for alveolar pressure exceeding 42 cmH_2O (Parker et al., 1984). A remarkable increase in Lp was found over 8h due to increased size of pores, and thus increase in filtration surface area, for only 10% increase in strain (lung-on-a-chip microdevice model, Huh et al., 2012). Experimental models of an injurious ventilatory strategy were found to predispose to subsequent bacteremia and associated impaired host defense (Lin et al., 2003).

The Alveolar Folding/Unfolding Zone

The specific topology of Epi1 cells is such that they are far from being flat (see **Figure 2**; Weibel, 2015). Furthermore, these cells present numerous pleats on their surface, as deduced by following the profile of the basement membrane, that are subjected to the cyclic folding/unfolding process on changing lung volume (Bachofen et al., 1987). Based on data from electron microscopy, unfolding was found to be completed in healthy lung upon reaching a transpulmonary pressure ∼ 15 cmH_2O, corresponding to ∼75–80% Vital Capacity (VC), (with a surface tension not exceeding 5–7 dynes/cm, Bachofen et al., 1987). The folding-unfolding process was confirmed also for the human lung by estimating the increase in lung diffusion capacity on increasing lung volumes from FRC up to 100% VC. It was found that the increase in diffusion capacity could be modeled according to the increase of the ratio between the alveolar surface and thickness of the air-blood barrier (Salv/τ) (Miserocchi et al., 2008).

The result was that the increase in diffusion capacity was proportional to the increase of Salv up to a lung volume of ∼80% VC, at which the "reserve" surface of the unfolding pleats was completed. Above this volume, the diffusion capacity was proportional to the decrease in τ. It was, therefore, concluded that above ∼80% VC, thinning of septa occurred because of direct tissue stretching. Further data confirm that in the healthy lung, increasing volume might occur without much strain of the Epi1 cell surface thanks to the "reserve" surface of the pleats, and that direct stretching of the septa occurs with increasing strain above 75–80% VC (Knudsen and Ochs, 2018). **Figure 6** shows the volume-pressure curve of the lung, chest wall, and respiratory system in a healthy subject to show: (i) the FRC (red dot) and (ii) the percent of lung distention (65–80% of VC) and corresponding alveolar pressure (15–25 cmH_2O) upon approaching the saturation of the alveolar unfolding process (shaded area).

In the supine subject, FRC is decreased down to about 22% (Agostoni and Mead, 1964) so, assuming that the saturation of the unfolding process also occurs at about 80% of lung distension, the range of the folding/unfolding process is increased.

One may attempt to comment on the clinical outcome from mechanically ventilated patients with ARDS considering the ventilatory strategy with specific relation to the folding/unfolding zone. One can, therefore, pose the question of how a ventilatory strategy implying a cycling involvement of the folding/unfolding zone might have an impact on lung function. Protti et al. (2013) reported 50% increase of death when oscillating lung volume across 70% lung distension, compared to a strategy

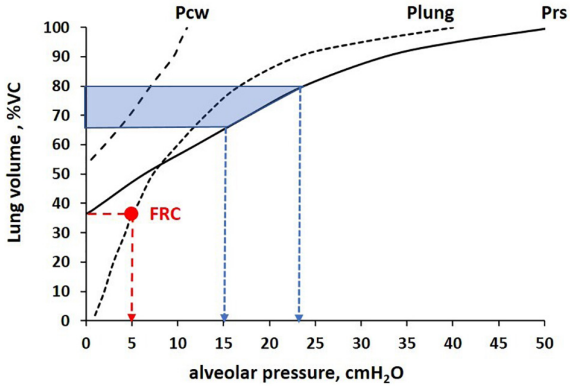

FIGURE 6 | Volume-pressure curve of the lung, chest wall, and total respiratory system (Plung, Pcw, Prs, respectively) to identify the relative lung distention at functional residual capacity (FRC) (Palv = 5 cmH_2O, red arrow) and completion of the alveolar unfolding process occurring in the range Palv 15–25 cmH_2O (blue arrows), corresponding to ∼65–80% Vital Capacity (VC, shaded area).

of ventilation set either above or kept below 70% VC. The occurrence of repetitive recruitment and de-recruitment during mechanical ventilation of a diseased lung (bleomycin treatment or surfactant deactivation by Tween 20 detergent) was shown to cause remarkable alveolar instability with atelectasis in some alveoli and overdistention of adjacent ones, referred to as atelectrauma and volutrauma, respectively (Perlman et al., 2011; Rausch et al., 2011; Cressoni et al., 2014; Makiyama et al., 2014; Bates and Smith, 2018; Knudsen and Ochs, 2018; Retamal et al., 2018; Bates et al., 2020; Gaver et al., 2020). Accordingly, it was hypothesized that these maneuvers set a vicious cycle by which the alveolar damage progresses within the lung from atelectatic to adjacent non-atelectatic lung regions through mechanical interdependence. Data from Hamlington et al. (2018) also prove that the sequence of cyclic folding/unfolding leads to a remarkable increase in microvascular permeability due to progressive increase in endothelial lesions (rich-get-richer scheme). It appears, therefore, that a ventilatory strategy implying a cyclic folding/unfolding process favors tissue damage.

The Rest Lung Approach (RLA) (Acute Respiratory Distress Syndrome Network et al., 2000) and Open Lung Approach (OLA) (Writing Group for the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial (ART) Investigators, 2017) are the two main protective ventilation strategies being used currently. The RLA assumes the two-compartment ARDS model with collapsed, edematous, and unstable tissue in the dependent portion of the lung and small volume of normal tissue in the non-dependent portion of the lung (Gattinoni and Pesenti, 2005). Using a low tidal volume and plateau pressure, the RLA is designed to "Rest" the dependent injured lung tissue by keeping it completely out of the ventilation cycle and "Protect" the small amount of normal lung tissue from overdistension. The OLA assumes that lung protection is better afforded by completely opening the

collapsed and edema-filled tissue using higher PEEP with and without recruitment maneuvers. Unfortunately, these protective ventilation strategies have not been effective in further lowering mortality over the last 20 years, suggesting the need for novel protective ventilation strategies to be tested (Caser et al., 2014; Fan et al., 2018).

Airway pressure release ventilation (APRV), set and adjusted using the Time-Controlled Adaptive Ventilation (TCAV^{TM}) method[1], sets ventilator pressure above the folding/unfolding zone and uses a very brief release phase, personalized to changes in lung collapse time constants, that does not give the tissue sufficient time to fold. The TCAV^{TM} method has been reported to be protective as compared to cyclic ventilation crossing the folding/unfolding zone (Roy et al., 2012, 2013a,b; Kollisch-Singule et al., 2014a,b, 2015). A reduced microstrain has been reported for a ventilation implying alveolar pressure above the folding/unfolding zone (PEEP 16–24 cmH_2O) and a short time at low pressure (Kollisch-Singule et al., 2014a) using the TCAV^{TM} method. Data from Yoshida et al. (2009) indicated that APRV with Palv 21 cmH_2O was more efficient than Pressure Support Ventilation (PSV) set at PEEP 10 with Ppeak 23 cmH_2O that implies cycling crossing of the folding/unfolding zone. In a bleomycin injury lung model, tissue damage could be moderated by ventilation at PEEP = 20 cmH_2O, where unfolding is almost completed (Knudsen and Ochs, 2018). Finally, a multilevel analysis of data from 3,562 patients with ARDS revealed that the least risk of death in the hospital correlated with a ventilatory strategy at alveolar pressure high enough to avoid a wide range of cycling crossing of the folding/unfolding zone (Amato et al., 2015). It has also been shown that in preterm infants ($\leq$32 weeks of gestation), alveolar recruitment is more efficient with continuous than with discontinuous CPAP ($\sim$5 cmH_2O), which normalizes the FRC volume (Lam et al., 2020).

The TCAV^{TM} method, which is a novel Stabilizing the Lung Approach (SLA), was developed based on the fact that cyclic recruitment-derecruitment in surfactant-deprived lungs leads to a significant degree of alveolar instability (Nieman et al., 2020b). The goals of the SLA are to very quickly stabilize alveoli and prevent folding/unfolding injury. Once stable, the collapsed tissue can be gradually reopened over an extended period of time (hours or days). Using a very brief release phase, directed by changes in lung collapse time constants, has been shown to very quickly stabilize alveoli. The extended inspiratory time, known as the continuous positive airway pressure (CPAP) phase, gradually opens the collapsed lung tissue over an extended period of time (Roy et al., 2012, 2013a,b; Kollisch-Singule et al., 2014a, 2015). The SLA may facilitate edema re-adsorption and reduce mechanical damage to endothelial and epithelial cells, as compared with the RLA and RLA methods (Nieman et al., 2020a,b). The possible mechanisms for the efficacy the TCAV^{TM}s methods in reducing edema accumulation and expediting edema removal are based on the following: (i) very quickly avoiding the cycling folding/unfolding of alveolar walls using a very brief release phase, (ii) the rapid lung inspiration at the end of the release phase gradually recruits alveoli *via* a "ratchet" mechanism similar to the way the newborn opens their collapsed fluid-filled lungs at birth (Tingay et al., 2021), (iii) stabilizing and then progressively opening alveoli will maintain normal surfactant protein concentration.

Patient-Self-Inflicted Lung Injury

The case of the patient-self-inflicted lung injury (*P-SILI*) deserves a particular consideration. Evidence has been provided for high tidal volumes developed by patients receiving non-invasive respiratory support based on increasing alveolar pressure. Furthermore, a correlation was found between diaphragmatic swings and risk for P-SILI due to lung overdistension (Carteaux et al., 2016; Brochard et al., 2017). The correlation between lung overdistension and lung lesion can be easily accepted (Cruces et al., 2020). However, the understanding of potential "exaggerated" diaphragm contractions has remained elusive. Perturbation in respiratory drive has been invoked by reviewing some possible reflex mechanisms of neural control of breathing (Brochard et al., 2017; Telias et al., 2018). We wish to consider a so far overlooked physiological feature of the diaphragm as a pressure generator. For a given neural drive, diaphragmatic contraction appears to be quite stable and cannot be modified once started, as the diaphragm is almost void of proprioceptors (Corda et al., 1965). From a mechanical standpoint, the question is: what is the outcome of diaphragmatic contraction when the diaphragm is facing a change in mechanical load? If the load is increased (e.g., adding external respiratory elastance), the inspired volume will decrease (Pengelly et al., 1971). In fact, no "load compensating reflex" is present, as no proprioceptor afferent input is present. The opposite case is now that of removing part of the elastance that the diaphragm faces during its contraction. This case occurs by increasing alveolar pressure; in fact, increasing alveolar pressure might remove part or all of lung elastance. The result is an increase in lung volume, since the first breath gives rise to over distension and lung injury (Pengelly et al., 1971). One could estimate that by removing the elastance roughly equivalent to that of the lung, the increase in inspiratory volume would amount to $\sim$150–200% of the control value. Such increase is expected to vary among patients, as lung elastance is related with the number of ventilated units, being higher with increasing alveolar flooding. Neuromuscular blockers obviously contrast this phenomenon (Forel et al., 2006; Papazian et al., 2010). External (inspiratory) intercostal muscles do exhibit proprioceptors innervation: accordingly, they might increase their contraction on adding external elastance ("load compensating reflex") but would be silent on increasing alveolar pressure (thus, the decrease in elastance remains unopposed). One could consider that a patient exposed to increased alveolar pressure has to perform active expiration that is normally passively relying on the elastic recoil of the respiratory system. If the above interpretation is valid, it would not be paradoxical to retain the P-SILI acronym, but the expansion should read Patient-Strategy Induced Lung Injury!

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FACTORS PREVENTING THE DEVELOPMENT OF EDEMA: INTERSTITIAL CAPACITANCE, LYMPHATIC FLOW, AND VASOMOTION

The first line of defense against increased microvascular filtration is fluid accumulation in the thick portion of the air blood barrier. Up to W/D ∼5.5, some fluid accumulates in the endothelial cells whose swelling may account for ∼45% of their control volume, while ∼85% of filtered fluid accumulates in the thick portion of the air-blood barrier (Conforti et al., 2002) where lymphatics are present. This morphological arrangement corresponds to a model of a relatively rigid initial compartment (the thin portion of the ABB) that communicates by a relatively high-resistance pathway to a larger downstream capacity (the thick portion of the ABB) (Unruh et al., 1984).

Human lymphatics extend deep inside the pulmonary lobule; indeed, a peripheral distribution of lymphatics (defined as interstitial lymphatics) was described down to the peri-microvascular district near the alveolar, the alveolar ducts, and the interalveolar septa (Schraufnagel, 1992; Hainis et al., 1994; Schraufnagel et al., 1994; Weber et al., 2018). These lymphatics converge into a central compartment, becoming large conducting vessels associated with the broncho-vascular bundle (Weber et al., 2018).

Under baseline conditions, lymph flow rate is 0.063 ml/min/100 g (Effros and Parker, 2009), and only about 4% of this value corresponds to alveolar fluid clearance. The latter corresponds to the transepithelial active sodium transport. If the "safety factor" is intact, most fluid clearance occurs *via* Starling-depended fluid re-absorption (Miserocchi, 2009) back into the capillaries, with only a minor fraction (∼18%) *via* lymphatics (Pearse et al., 1993).

Lymphatics can increase flow, and thus, provide a passive negative-feedback control loop to offset an increase in extravascular volume (Miserocchi, 2009). Interestingly, lymph flow was found to be proportional to the rate of increase in lung weight that is directly related to the microvascular filtration rate (Mitzner and Sylvester, 1986). Interstitial fluid ought to percolate through the interstitium down a pressure gradient generated by lymphatics themselves that were shown to generate a subatmospheric pressure compatible with the measured value of Pint (Miserocchi et al., 1989). Redistribution of fluid downstream from filtration sites was modeled by relating the increase in lymphatic flow in response to increase in filtration rate as a function of interstitial fluid resistance (Roselli et al., 1984). These authors found that, for interstitial compliance in the physiological range (intact matrix), a 3-fold increase in lymphatic flow could balance the increased filtration rate by holding interstitial pressure at about 2 cmH_2O. When interstitial hydration increases, matrix fragmentation would actually decrease tissue resistance for fluid flux toward lymphatics, in line with the model developed by Unruh et al. (1984). In case of experimental lung lesion, lung lymph flow was found to increase by ∼8- to 10-fold (Rutili et al., 1982).

A recent microfluidic study revealed considerable differences among lung regions concerning interstitial fluid dynamics from capillary filtration sites, through the porosity of the interstitium, and into the lymphatic network. This conclusion came about by combining specific lymphatic immunohistochemistry with high-resolution X-ray computed tomography and finite-element mathematical modeling that identified differences related to the functional interaction between tissue mechanical properties and the action of the lymphatic pump (Robinson et al., 2019). Lymphatic clearance of alveolar fluid can only occur with an epithelial barrier lesion, as confirmed by data from hyperoxia (Hainis et al., 1994; Schraufnagel et al., 1994) and ventilator-induced lung injury lesion models (Schraufnagel et al., 2003).

Mechanical ventilation and positive end-expiratory pressure (PEEP) impede lung lymph flow by increased intrathoracic pressure and increased central venous pressure. PEEP may, thus, enhance edema formation of the lung (Hedenstierna and Lattuada, 2008). Obviously, when lung W/D increases, the lymphatic flow cannot balance the flow of filtration. This occurs in case of severe endothelial and epithelial lesions. Alveolar flooding occurs when the increase in lung W/D exceeds 35% of baseline (Taylor and Parker, 1985), corresponding to ∼6.25.

Figure 7A shows the distribution of extravascular interstitial and alveolar water upon increasing W/D by up to 10, normalized to 1 kg lung (Cressoni et al., 2013) and assuming ∼35% increase in interstitial water (Negrini et al., 2001b). **Figure 7B** provides images of the morphological changes of the lung from control (left) to a transition phase implying fluid accumulation in the interstitial space (center) and alveolar flooding (right). On mechanical ground, alveolar flooding implies a progressive loss of alveolar expandable units, and correspondingly, a decrease in lung compliance.

Based on the pressure generating a trans-endothelial flow, a morphological-based model was developed to describe the interaction between control of extravascular lung water and capillary perfusion under edemagenic conditions (Mazzuca et al., 2016). The model focused in particular on the fact that the increase in Pint, besides buffering and even reversing the transcapillary Starling gradient, leads to capillary de-recruitment because of the compressive effect of positive interstitial pressure; the resultant decrease in exchange surface area is a potent factor to decrease Kf. Data on humans confirmed blood flow limitation in edemagenic lung regions, despite the administration of a vasodilator agent (Scherrer et al., 1996).

Precapillary vasoconstriction is a further strong anti-edemagenic mechanism (Negrini et al., 2001a; Mazzuca et al., 2019). In fact, redirection of blood flow from edematous to normal lung regions has been correlated with the increase in Pint in the former as well as with the corresponding precapillary vasoconstriction and vasodilation in the two regions, respectively (Rivolta et al., 2011). Interestingly, inter-individual differences have been documented concerning capillary recruitment/de-recruitment under edemagenic conditions, such as exercise or exposure to hypoxia; in particular, capillary de-recruitment has been interpreted as individual proneness to develop lung edema (Bartesaghi et al., 2014; Beretta et al., 2017a,b). In general, a greater density of pulmonary capillaries favors

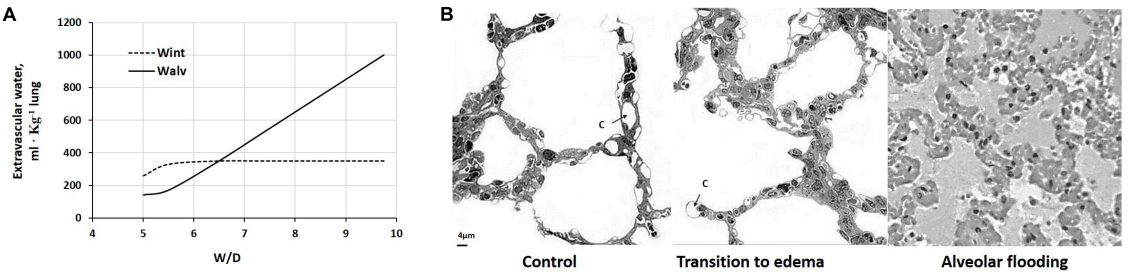

FIGURE 7 | (A) Distribution of extravascular lung water to the interstitial (Wint) and alveolar (Walv) compartment on increasing W/D. **(B)** Morphological changes of the lung from control (left) to a transition phase with fluid accumulation in the interstitial space (center) and alveolar flooding (right). c, pulmonary capillary (redrawn from Miserocchi, 2007).

oxygen diffusion-transport at the alveolar level (Beretta et al., 2017a,b); however, it involves a greater risk factor of developing lung edema because of greater capillary surface for microvascular filtration (Mazzuca et al., 2016). It is tempting to consider pulmonary hypertension, as observed under strong edemagenic conditions (hypoxia), as the consequence of precapillary vasoconstriction represents a powerful means to limit microvascular filtration (Negrini et al., 2001a; Mazzuca et al., 2019).

There is an interesting report concerning the preventive effect of gadolinium in models of lung lesions caused by Palv at ∼30 cmH_2O that induces a ∼2-fold increase in Kf. The data suggest that gadolinium prevents increased microvascular permeability caused by stretch-activated cation channel-induced increases in intracellular calcium concentration (Parker et al., 1998). Another potentially edema-preventive strategy considers the role of NADPH oxidase type 2 (NOX2) that is a major source of ROS in the lung. Pre-treatment with an inhibitor of NOX2 (Fisher et al., 2021) was hypothesized as preventive of the secondary inflammatory component. A further study suggests that dietary antioxidants could represent a potential treatment for oxidative stress lung injury in patients on mechanical ventilation (Patel et al., 2020).

MODELING THE STARLING GRADIENTS IN THE PROGRESSION TOWARD SEVERE LUNG EDEMA

Table 2 summarizes the database to compare the Starling gradients as edema progresses from point B ("safety factor") to point C. When the "safety factor" is on, trans-endothelial Starling gradients are decreased; in particular some fluid reabsorption may actually occur across the capillary wall at end-expiration. This last point is confirmed by Pearse et al. (1993) who, adopting an experimental model that limited the increase of W/D at ∼6, found that ∼42% of water filtered into the interstitium was cleared by reabsorption into the pulmonary capillaries. At point B, considering γ is still equal to 1 dynes/cm, negative values of Starling gradients across the epithelium prevent any risk of alveolar flooding.

We ignore the value of surface tension at point C (W/D ∼7.5), as well as the corresponding value of $Pliq_{alv}$. Assuming that γ value increased by up to 25 dyne/cm at point C (due to both surfactant dilution and de-activation), $Pliq_{alv}$ would decrease to −17 cmH_2O, so the resultant Starling trans-epithelial pressure gradient would potentially cause alveolar flooding. Modeling the increase in γ between 20 and 40 dynes/cm would only slightly change the result confirming that a W/D in the range 6.5–7 represents the critical threshold for alveolar flooding.

In **Figure 8**, we modeled the (A) trans-endothelial and (B) trans-epithelial Starling gradients on increasing W/D, in relation to the corresponding values of interstitial pressure (Pint), with Palv = 0 cmH_2O.

With the "safety factor" on (point B), both trans-endothelial and trans-epithelial Starling gradients promote fluid reabsorption, in line with data from Egan et al. (1976, 1977). The critical factor leading to alveolar flooding is the development of a remarkably increasing negativity of $Pliq_{alv}$ as a consequence of the progressive increase in surface tension (γ) due to surfactant de-activation. We assumed an increase in γ from 1 to 25 dynes/cm for W/D > 6.5 (see **Table 2**).

Changes in Starling gradients and permeability parameters may be combined differently within the whole lung. In fact, the available experimental evidence confirms the heterogeneity in both regional lung fluid accumulation and perfusion under edemagenic conditions (Bachofen et al., 1993). Basal lung regions are, in general, more exposed to edema because of greater blood perfusion, implying higher capillary Kf (Rivolta et al., 2011). Alveolar flooding was found to occur in a quite non-homogeneous fashion revealing considerable regional differences (Wu et al., 1995).

Figure 9 reports the estimated Starling pressure gradients at the (A) endothelial and (B) epithelial levels with increasing alveolar pressure of up to 25 cmH_2O, as a function of W/D. We modeled the Starling pressure gradients on increasing W/D by increasing surface tension values from 1 to 40 dynes/cm. For W/D in the physiological range (5–6.5), the increase in Palv shifts the trans-endothelial gradients toward filtration, and on the opposite, the trans-epithelial toward alveolar re-absorption. Both effects tend to wane as W/D increases. Thus, in the W/D range of 5–6.5, the increase in Palv causes capillary filtration and alveolar clearance; accordingly, lymphatics have to cope

TABLE 2 | Trans-endothelial and trans-epithelial Starling pressure gradients at end-expiration, with "safety factor" (point B in **Figure 4**) and after some degree of matrix fragmentation (point C in **Figure 4**).

	Trans-endothelial			Trans-epithelial	
	Point B (W/D = 5.5)	**Point C (W/D∼7.5)**		**Point B (W/D = 5.5)**	**Point C (W/D∼7.5)**
Pcap*	9	9	Pint**	5.7	0
Pint**	5.7	0	Pliq alv#	∼0	−17
σ end##	0.85	0.5	σ epi##	0.85	0.5
Πcap**	26.8	14.7	Πint**	13.8	9.3
Πint**	13.8	9.3	Πliq alv	0	5
			γ	1	25
ΔP	**4**	**9**	**ΔP**	**5.7**	**17**
σΔΠ	**−11.0**	**0.0**	**σΔΠ**	**−11.7**	**−2.15**
Starling gradient	**−7.0**	**6.3**	**Starling gradient**	**−6.0**	**14.85**

This table reports the expected values for capillary, interstitial, and alveolar liquid hydraulic pressures (Pcap, Pint, and Pliq alv, respectively) as well as for capillary, interstitial, and alveolar liquid oncotic pressures (Πcap, Πint, and Πliq alv, respectively). Endothelial (σ endo) and epithelial (σ epi) protein reflection coefficients are also reported. In bold, hydraulic (ΔP) and oncotic (σΠP) pressure gradients and total Starling pressure gradient. Positive values of the Starling gradient at endothelial level indicate filtration into interstitium; negative values at epithelial level indicate alveolar reabsorption. From B to C (the phase corresponding to progressive fragmentation of the matrix), Pint returned to zero, suggesting loss of the physiological alveolar mechanical tethering interaction (Mead et al., 1970). Accordingly, from point C on, we considered Pint = Palv, as suggested by Glucksberg and Bhattacharya (1991).

*Pressure values are expressed in cmH_2O; σ is a pure number. *From Hakim et al., 1993; **from Miserocchi et al., 1993; # calculated from Beck and Lai-Fook, 1983; ## from Parker et al., 2006. Surface tension (γ), dyne/cm.*

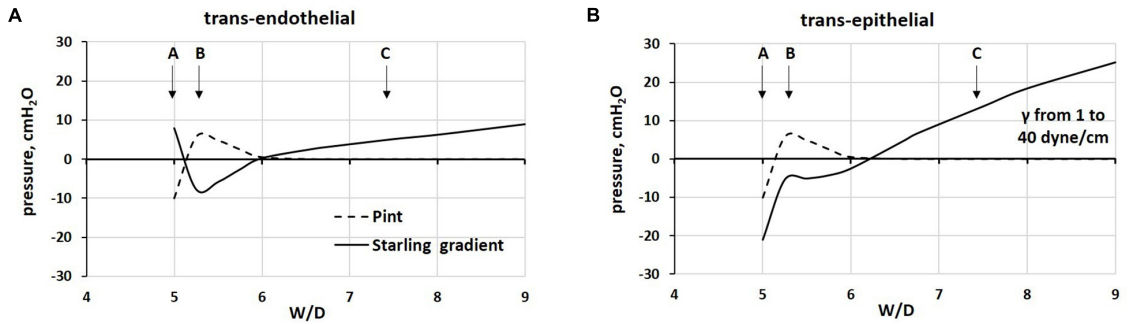

FIGURE 8 | Modeling of **(A)** trans-endothelial and **(B)** trans-epithelial Starling pressure gradients (solid line) on increasing W/D, based on experimentally measured values of interstitial pressure (Pint, dashed line), with Palv = 0 cmH_2O. Points A, B, and C as from **Figure 4**.

with both flows. For W/D > 6.5, both trans-endothelial and trans-epithelial gradients favor filtration, leading to alveolar flooding. Note that for W/D > 6.5, the Starling pressure gradients, referring to different Palv, overlap, reflecting the fact that when the proteoglycan matrix is disrupted, the increase in Palv impacts equally on the increase in capillary pressure, interstitial pressure (Glucksberg and Bhattacharya, 1991), and $Pliq_{alv}$ (Beck and Lai-Fook, 1983).

The graphs in **Figure 9** may be considered either as representing the Starling pressure-dependent course of developing edema, and as representing the regional differences in water balance within the lung. Indeed, novel CT imaging methods (Cereda et al., 2016, 2017) showed that aerated and non-aerated lung regions were diffusely intermingled. The erratic distribution of edema can be justified by the heterogeneous susceptibility to edema formation at alveolar level that was attributed to local differences in microvascular permeability possibly dependent upon alveolar morphology, as suggested by a novel lung imaging technique under experimental edemagenic conditions (Mazzuca et al., 2019). Heterogeneity in alveolar mechanics and fluid dynamics is confirmed by data from Smith et al. (2020) who generated VILI in mice ventilated with 50 cycles/min from FRC up to a plateau pressure of 37.5 cmH_2O. Studying the alveolar de-recruitability on decreasing Palv, three alveolar phenotypes were described as a consequence of VILI: (1) flooded alveoli that cannot be recruited at any pressure, (2) unstable alveoli that are open at high pressures but readily collapse as pressure is reduced, and (3) relatively normal alveoli that remain open at low pressures.

An important relationship exists during cyclic mechanical ventilation between peak airway pressure (Ppeak) and positive end-expiratory pressure (PEEP). The data in **Figure 9** suggest that the trans-endothelial filtration gradient increases with increasing Ppeak and PEEP. Conversely, no risk of alveolar flooding occurs for W/D < ∼6.5, as trans-epithelial gradients are in favor of fluid absorption. This may be partially balanced by a decrease in filtration surface as a consequence of a decrease in overall capillary blood volume (Miserocchi et al., 2008). Blood flow is also temporarily reduced, being proportional to the decrease in the difference between pulmonary artery and alveolar pressure.

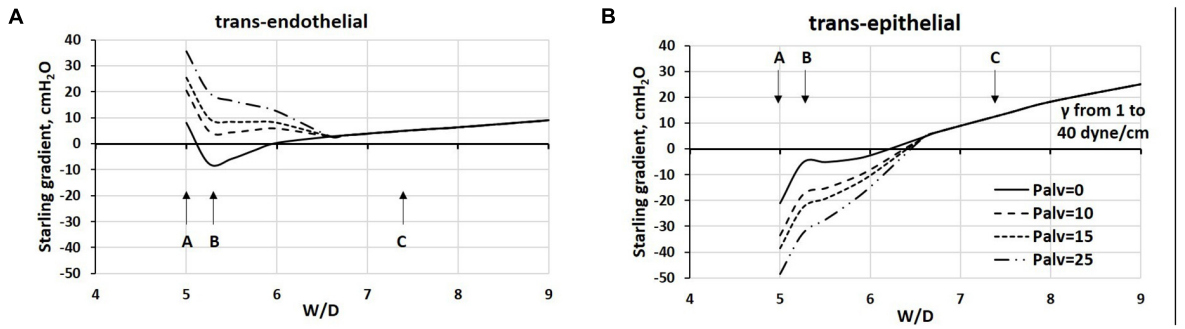

FIGURE 9 | Estimated Starling pressure gradients at **(A)** endothelial and **(B)** epithelial levels on increasing alveolar pressure by up to 25 cmH_2O, and on varying W/D from 5 to 9. Points A, B, and C refer to those in **Figure 4**.

Lymphatic drainage can increase in this situation to maintain lung fluid balance. However, the time evolution of this situation should be regarded in terms of increase in W/D ratio as related to the progression of the fragmentation of the interstitial matrix (**Figure 5**).

The TCAV^{TM} method, if applied pre-emptively before the development of severe lung injury, has been shown to prevent collapse of the acutely injury lung, maintaining a fully open lung, and if applied following the development of severe lung injury, will rapidly stabilize alveoli even with fast collapse time constants. If the ventilation strategy can put a "cast" within the acutely injured lung, much like putting a cast on a broken arm, the mechanisms of VILI-induced edema generation would be eliminated (Nieman et al., 2018).

One shall note that edema clearance in experimental models of ARDS can only occur in lung regions where W/D ratio does not exceed 6.5–7, as only in these regions the Starling gradients allow fluid reabsorption (see **Figure 9**). The presence of these regions can justify the observed average decrease in W/D ratio for the whole lung from about from ∼9 to ∼7; the latter value still corresponds to severe edema (Habashi, 2005; Matsuzawa et al., 2010; Roy et al., 2013a,b; Kollisch-Singule et al., 2014a,b, 2015).

TIME CONSTANT OF DEVELOPING SEVERE ALVEOLAR EDEMA

The development of lung edema depends upon various combination of an increase in Lp and in A as well as a decrease in σ, involving both the endothelium and the epithelium. Increase in capillary pressure and lung overdistension were shown to cause damage to the endothelial and epithelial barriers (Wu et al., 1995). The critical phase of edema pivots around a W/D of ∼ 6–6.25. We hypothesize that for W/D to exceed 6, capillary filtration should occur down this still low-pressure gradient *via* increase in Lp and decrease in σ acting over a progressively larger portion of the alveolar compartment surface area, which may cause matrix fragmentation. Consider that the Lp of the "true" alveolar compartment is physiologically 20 times lower than that of the extra-alveolar compartment (Parker et al., 2006) while the capillary surface area is in the order of 70 m^2 on a morphological basis. We suggest that a W/D ∼ 6.5 is the likely threshold, above which fluid will seep from capillary to alveoli down a small driving pressure gradient acting over a large surface of damaged air-blood barrier.

An exponential increase in lung weight (above a W/D of ∼ 6) has been modeled with a relatively short time constant (∼4 min) by moderate increase in either Lp or decrease in σ (Mazzuca et al., 2016). Similar time constants were found for the exponential increase in lung weight in response to a step increase in venous pressure (Parker and Townsley, 2004). The above hypothesis is strengthened by the finding that a >3-fold increase in microvascular filtration already causes an increase in trans-capillary convective transport of proteins compared to either diffusive or vesicular transport, suggesting lowering of the σ value (Egan et al., 1977). Other data predict that a 50% increase in lung water may occur within 45 min after a step increase in left atrial pressure (2–14 cmH_2O) and pulmonary artery pressure (13–22 cmH_2O) (Rutili et al., 1982).

THE WAY TO RECOVERY

A good model to explain the clearance of alveolar edema is the one proposed in full-term newborns (Miserocchi et al., 1994). Alveolar fluid clearance occurs down a two-step coupled mechanism: (1) epithelial water absorption *via* Na^+-dependent transport and (2) Starling-dependent capillary re-absorption. Note that fluid reabsorption into lung capillaries is a self-limited mechanism due to increase in the colloid osmotic pressure at the interstitial end of the glycocalyx generated by protein accumulation preventing further absorption (Curry, 2005; Levick and Michel, 2010; Mazzuca et al., 2019). A recent report (Tingay et al., 2021) shows that the transition from fluid-filled to aerated lungs in full-term newborns is accomplished using ventilation patterns characterized by a rapid high-peak inspiratory flow to "ratchet" open collapsed and flooded airway. The collapse of these newly recruited airways during expiration was minimized by partially closing the glottis to keep the lung pressurized. Thus, the inspiratory phase allows for rapid lung aeration extending from the central to distal lung portions; the expiratory phase maintains alveolar pressure allowing for fluid clearance.

Maintaining a pressurized lung during expiration in a lung with low permeability of the epithelium and low compliance of the interstitial matrix results in an increase in interstitial pressure (as at point B) generating a trans-endothelial absorption *via* the Starling gradient. These two features are incompletely developed in preterm lung, and therefore, hinder alveolar fluid reabsorption (Miserocchi et al., 1995). An intact epithelial barrier allows Na^+-dependent alveolar fluid reabsorption. Matthay and Wiener-Kronish (1990) provided the first evidence in humans to support the hypothesis that active ion transport across the alveolar epithelial barrier is the primary mechanism for the clearance of edema fluid. Na^+ transport across the distal airway epithelium is the main determinant of alveolar fluid clearance, as demonstrated in several different species, such as the human lung (Berthiaume and Matthay, 2007). With an intact epithelium, the clearance of alveolar fluid is rapid in patients with severe hydrostatic edema (Verghese et al., 1999). In contrast, in the majority of patients with acute lung injury, alveolar fluid clearance is impaired by reduced Na^+ absorption (Ware and Matthay, 2001).

Controversies regarding the optimal mechanical ventilation strategy in patients with severe edema centers around the maintenance of alveolar pressure that favors fluid clearance and avoids the complication of volutrauma and atelectrauma. To favor alveolar fluid re-absorption in experimental edema models, the ventilatory strategy should allow to decrease W/D below 6.5, a cut-off suggesting restoration of control of lung fluid balance (**Figures 8**, **9**).

The re-deposition of an interstitial macromolecular matrix assuring low microvascular permeability and low interstitial tissue compliance appears critical for the control of lung fluid balance, since this would re-establish a "safety factor." Most endothelial but not epithelial cells appear to be involved in the mechano-transduction signaling process in response to edemagenic conditions (Botto et al., 2006) that influence a specific cascade of cellular events aiming at remodeling of matrix macromolecules (Palestini et al., 2002, 2011; Daffara et al., 2004). There are indications that post-infection activation of fibroblast does not allow the re-establishment of a matrix composition similar to the native composition (Li et al., 2016). In particular, the activation of fibroblast impacts hyaluronan synthase that controls the deposition of bundles of hyaluronan that actually represents the fibrotic response to recovery. In the presence of hyaluronan fragments during the early phase of lung injury, the balance between hyaluronan synthase isoforms and hyaluronidases plays an important role in the pathogenesis of lung fibrosis (Li et al., 2000). Recent data suggest that hyaluronan synthesis occurs in a matter of days (Bell et al., 2019).

CONCLUSION

Although the sequence of events leading to severe lung edema have been delineated, at this point, there remains a wide gap in our knowledge of how to prevent the loss of lung fluid balance when W/D $>$ 6 and the critical role of properly set mechanical ventilation. The data from this analysis support the contention that in diseased portions of the lungs (W/D $>>$6.5) the mechanisms necessary to clear edema fluid have been lost. Since the distribution of edema is heterogeneous by nature, in the lung portions where W/D $<$ 6.5, the control of lung fluid balance is only possible when the built-in safety factors, intact matrix, interstitial capacity, and lymphatics not yet saturated, are still functional. Once both barriers (endothelium and epithelium) are breeched, and built-in safety components (capacity reservoirs and lymph flow) are saturated, massive flooding occurs. These considerations may help to "debug" the controversies concerning the outcome of various experimental models of lung lesion or clinical outcome in patients. Comparing data from different ventilatory strategies appears difficult, as these are highly influenced by the number of alveolar units allowing fluid clearance that cannot be directly measured. Reported values for W/D of the whole lung to validate ventilatory strategies aimed at facilitating edema re-adsorption, reducing mechanical damage, and preventing further increase in permeability never fell below 6.8 (see Paragraph 8). So far, neither CT scan nor ultrasound has been correlated to the corresponding regional values of W/D ratios. It would be useful to identify a non-invasive tool to detect the earliest stages of perturbation in lung fluid balance before the condition becomes life-threatening. Ventilatory support strategies, during either spontaneous breathing or mechanical ventilation, should carefully balance factors that can harm functioning alveolar units. Aiming at high oxygen saturation at the expense of alveolar hyperoxia should also be considered (see Paragraph 6.2). The clinical problem appears to be that of avoiding damage to the alveolar units that still retain gas diffusion-transport function. This certainly represents a critical question considering the severe complication of alveolar instability generated by cyclic recruitment-derecruitment in edematous lung regions (Bates and Smith, 2018; Bates et al., 2020; Gaver et al., 2020). The impact of positive airway pressure is complex for many reasons, considering in particular that an opposite flow may occur, depending on alveolar pressure, across the endothelium and the epithelium. A ventilation strategy that would keep the acutely injured lung open and stable would theoretically be optimal at minimizing pulmonary edema accumulation. The critical issue remains to be minimizing tissue damage caused by lung overdistension, and at the same time, avoiding tissue injury by cyclic folding/unfolding.

AUTHOR CONTRIBUTIONS

EB, JG, GN, and GM joined their competences to complete the study. GM and EB conceived the work. FR and GS contributed to the computational part. All authors contributed to the article and approved the submitted version.

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REVIEW
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Monitoring Expired CO_2 Kinetics to Individualize Lung-Protective Ventilation in Patients With the Acute Respiratory Distress Syndrome

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Mechanical ventilation (MV) is a lifesaving supportive intervention in the management of acute respiratory distress syndrome (ARDS), buying time while the primary precipitating cause is being corrected. However, MV can contribute to a worsening of the primary lung injury, known as ventilation-induced lung injury (VILI), which could have an important impact on outcome. The ARDS lung is characterized by diffuse and heterogeneous lung damage and is particularly prone to suffer the consequences of an excessive mechanical stress imposed by higher airway pressures and volumes during MV. Of major concern is cyclic overdistension, affecting those lung segments receiving a proportionally higher tidal volume in an overall reduced lung volume. Theoretically, healthier lung regions are submitted to a larger stress and cyclic deformation and thus at high risk for developing VILI. Clinicians have difficulties in detecting VILI, particularly cyclic overdistension at the bedside, since routine monitoring of gas exchange and lung mechanics are relatively insensitive to this mechanism of VILI. Expired CO_2 kinetics integrates relevant pathophysiological information of high interest for monitoring. CO_2 is produced by cell metabolism in large daily quantities. After diffusing to tissue capillaries, CO_2 is transported first by the venous and then by pulmonary circulation to the lung. Thereafter diffusing from capillaries to lung alveoli, it is finally convectively transported by lung ventilation for its elimination to the atmosphere. Modern readily clinically available sensor technology integrates information related to pulmonary ventilation, perfusion, and gas exchange from the single analysis of expired CO_2 kinetics measured at the airway opening. Current volumetric capnography (VCap), the representation of the volume of expired CO_2 in one single breath, informs about pulmonary perfusion, end-expiratory lung volume, dead space, and pulmonary ventilation inhomogeneities, all intimately related to cyclic overdistension during MV. Additionally, the recently

described capnodynamic method provides the possibility to continuously measure the end-expiratory lung volume and effective pulmonary blood flow. All this information is accessed non-invasively and breath-by-breath helping clinicians to personalize ventilatory settings at the bedside and minimize overdistension and cyclic deformation of lung tissue.

Keywords: volumetric capnography, dead space, acute respiratory distress syndrome, ventilator-induced lung injury, mechanical ventilation

INTRODUCTION

The acute respiratory distress syndrome (ARDS) is the most severe form of acute respiratory failure that affects the lungs in a heterogeneous way, profoundly impairing their mechanical properties and gas-exchange functions. Diffuse lung pan-endothelial inflammation, the hallmark of the syndrome, leads to the invasion of alveolar spaces by edema and inflammation reducing effective pulmonary lung volume (i.e., functional residual capacity, FRC). The lung becomes heavier exerting a superimposed pressure on the dependent parts of the lung, critically decreasing regional transpulmonary pressure. This further reduces FRC by promoting lung collapse, a common pathophysiological feature of ARDS (Gattinoni et al., 2006).

Mechanical ventilation (MV) is the principal life-support intervention in the management of patients with ARDS but at the risk to perpetuate or aggravate lung damage. Ventilation-induced lung injury (VILI) results from the need to use high transpulmonary pressures and frequently higher tidal volumes (VT) to oxygenate and ventilate the heterogeneously ARDS lung. The delivered VT is distributed in a much smaller lung volume with important regional differences, with three important consequences: (1) less diseased lung regions will receive a larger fraction of the VT causing an increased cyclic tissue deformation or strain, a mechanism that triggers lung inflammation, (2) more diseased but ventilated regions will be submitted to a higher transpulmonary pressure for any given delivered VT increasing the local mechanical stress also known as cyclic overdistension, and (3) collapsed regions eventually re-open and close with each tidal inflation and deflation causing cyclic recruitment–de-recruitment, which can be considered an extreme form of regional strain. These are three of the most important mechanisms by which MV damages lung parenchyma. Lung imaging techniques, such as CT or intravital microscopy, have confirmed the heterogeneity of lung injury in ARDS at different scales, revealing the coexistence of normal, collapsed, and overdistended alveoli in different lungs regions. The resulting non-uniform distribution of tidal ventilation can be visualized in real time at the bedside by electrical impedance tomography (EIT), whereas more sophisticated techniques, such as PET and single-photon emission tomography (SPECT) imaging, have located the main inflammatory response in normally ventilated areas but not in collapsed ones (Bellani et al., 2009, 2011). Thus, lung collapse acts as a stress-raiser since it contributes to lung heterogeneity creating areas receiving an excessive VT in relation to their regional volume.

The introduction of lung-protective ventilation strategies aimed at reducing the mechanical stress imposed by the ventilator has contributed to reducing morbidity and mortality of patients with ARDS (ARDS Network, 2000). Ideally, these strategies should be individualized, but this requires useful and directed bedside clinical monitoring. However, routine monitoring only includes basic lung mechanics, gas exchange and intermittently, lung imaging techniques and thus, the clinical detection of overdistension or lung strain, and VILI remains difficult. The analysis of expired gases, in particular CO_2, is a well-established, robust, and clinically accessible monitoring option. Volumetric capnography (VCap), representing the volume of CO_2 expired in one breath, has specific features regarding the analysis of body CO_2 kinetics that can be of great value in detecting lung overdistension by providing continuous non-invasive information on lung perfusion, convective gas transport, lung diffusion, and dead space ventilation (VD), all intimately related and sensitive to the effects of lung overdistension. However, this source of highly relevant biological information is still largely underused in clinical practice. A progressive better understanding of the complex behavior and physiology of CO_2 kinetics have led to recent new developments for advanced analysis of the volumetric capnogram and a derived capnodynamic method that hold great promise in overcoming the difficulties in adopting the expired CO_2 monitoring in routine clinical practice.

The aim of this manuscript is to review the principles, uses, and physiological basis of expired CO_2 kinetics monitoring. We will review new developments and value of VD monitoring using VCap in patients with ARDS and describe the new capnodynamic method that continuously monitors effective lung volume and perfusion in a non-invasive way.

VOLUMETRIC CAPNOGRAPHY

The integration of an infrared CO_2 sensor and a flow sensor in a mainstream configuration allows for the reconstruction of the volumetric capnogram at the airway opening (**Figure 1**). As the cardiorespiratory system has an open arrangement, VCap contains implicit information regarding these systems expressed not only in their derived variables and indexes but also in its shape that helps the interpretation of normality and diseases throughout its derived-parameters. As mentioned above, CO_2 kinetics is context-sensitive, which means that VCap parameters must be interpreted when changes in metabolism, pulmonary perfusion, or ventilation occur one at a time

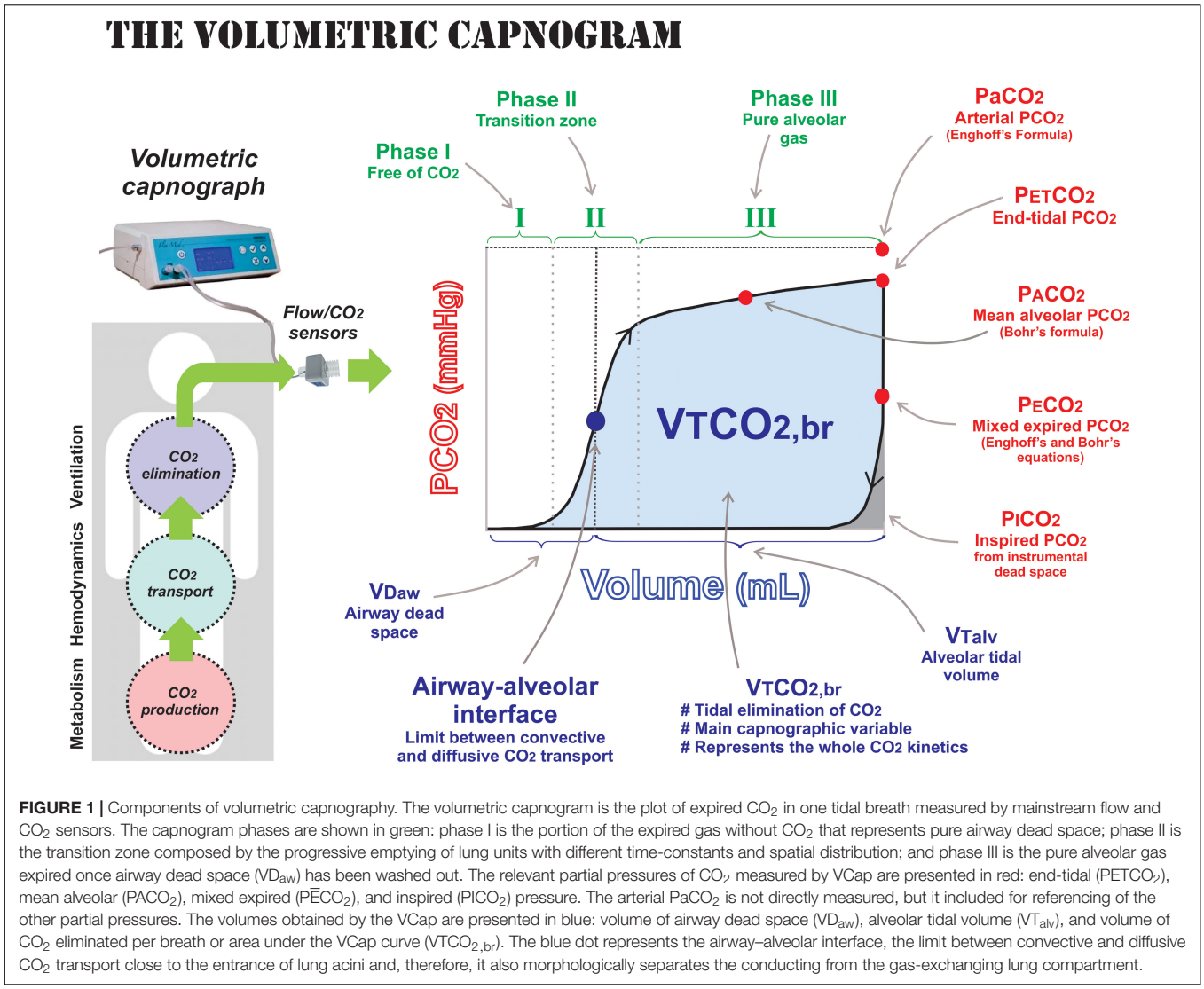

FIGURE 1 | Components of volumetric capnography. The volumetric capnogram is the plot of expired CO_2 in one tidal breath measured by mainstream flow and CO_2 sensors. The capnogram phases are shown in green: phase I is the portion of the expired gas without CO_2 that represents pure airway dead space; phase II is the transition zone composed by the progressive emptying of lung units with different time-constants and spatial distribution; and phase III is the pure alveolar gas expired once airway dead space (VD_{aw}) has been washed out. The relevant partial pressures of CO_2 measured by VCap are presented in red: end-tidal ($PETCO_2$), mean alveolar ($PACO_2$), mixed expired ($P\bar{E}CO_2$), and inspired ($PICO_2$) pressure. The arterial $PaCO_2$ is not directly measured, but it included for referencing of the other partial pressures. The volumes obtained by the VCap are presented in blue: volume of airway dead space (VD_{aw}), alveolar tidal volume (VT_{alv}), and volume of CO_2 eliminated per breath or area under the VCap curve ($VTCO_{2,br}$). The blue dot represents the airway–alveolar interface, the limit between convective and diffusive CO_2 transport close to the entrance of lung acini and, therefore, it also morphologically separates the conducting from the gas-exchanging lung compartment.

(Sipmann et al., 2014). For example, an increase in VT improves CO_2 elimination explained solely by a ventilatory change when metabolism and hemodynamics are constant.

Interpretation of CO_2 kinetics can be done during steady and non-steady state conditions, although the non-steady state is of particular interest in clinical monitoring as it allows to interpret and react to changes in pulmonary perfusion, end-expiratory lung volume, and/or alveolar ventilation after adjusting the ventilatory settings or to changing clinical conditions.

RATIONALE FOR THE MEASUREMENT OF DEAD SPACE AND ALVEOLAR CO_2 BY VOLUMETRIC CAPNOGRAPHY

Dead space ventilation is the wasted portion of ventilation not involved in gas exchange *per se*. It constitutes the evolutionary adaptive price paid by all mammals that depend on the bulk convective transport of ambient air to the gas–liquid gas-exchange interface, the alveolar–capillary membrane. By the end of the 19th century, Christian Bohr presented a method to estimate VD volume based on the mass balance of any gas breathed during the respiratory cycle (**Figure 2**) (Bohr, 1891). He brilliantly adapted his formula using CO_2 as the tracer gas, which still constitutes the basis of the clinical measurement of dead space. Aitken and Clark-Kennedy (1928) were the first to propose to represent CO_2 in relation to the expired volume, the VCap. Since then, the usefulness of this tool to calculate VD has been highlighted by many authors. Nowadays, major advances in CO_2 and flow sensing technology together with powerful hardware and computation capabilities have made real-time breath-by-breath VD calculations possible. The key feature to calculate VD using VCap relies on the precise measurement of mixed expired ($P\bar{E}CO_2$) and the mean alveolar ($PACO_2$) partial pressures of CO_2. The first necessary step was the simplification of the measurement of $P\bar{E}CO_2$, which represents the dilution of CO_2 within the lungs caused by the dead space effect. Initially, it could only be obtained by the cumbersome measurement using

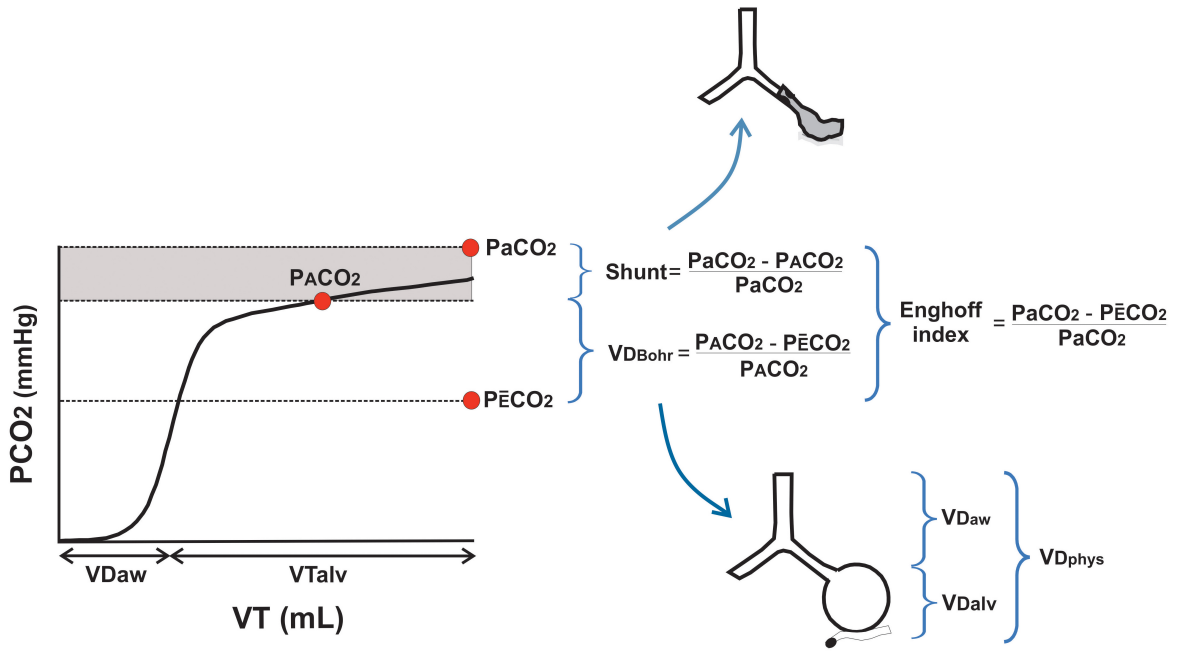

FIGURE 2 | Differences between Bohr's and Enghoff's approaches. The main physiological difference between Bohr's dead space (VD_{Bohr}) and Enghoff's index is visualized in one volumetric capnogram. While VD_{Bohr} represents true dead space in non-perfused alveoli and main conducted airways, the Enghoff's index includes also the effect of shunt and low V/Q in its calculation (gray area). $PaCO_2$, $PACO_2$, and $P\bar{E}CO_2$ are the arterial, alveolar, and mixed expired partial pressure of carbon dioxide, respectively. VD_{phys}, VD_{aw}, and VD_{alv} are physiological, airway, and alveolar dead spaces, respectively. VT is the tidal volume and VT_{alv} is the alveolar tidal volume.

the Douglas' bag. Currently, $P\bar{E}CO_2$ can be measured by VCap in one single breath by calculating the mixed expired fraction of CO_2 ($F\bar{E}CO_2$), which is then transformed to partial pressure and expressed in BTPS as follows:

$$F\bar{E}CO_2 = VTCO_{2,br}/VT$$

$$P\bar{E}CO_2 = F\bar{E}CO_2 \times (BP - PH_2O)$$

where $VTCO_{2,br}$ is the amount of CO_2 expired in one VT, P is barometric pressure, and PH_2O is water vapor pressure. The measurement of $P\bar{E}CO_2$ by VCap has been validated by different research groups, all showing good agreement with the Douglas bag method (Lum et al., 1998; Sinha and Soni, 2012) or a metabolic monitor (Kallet et al., 2005; Siobal et al., 2013) in children (Lum et al., 1998) and adults (Sinha and Soni, 2012) also with ARDS (Kallet et al., 2005; Siobal et al., 2013). More recently, using a more advanced analysis of VCap, Doorduin et al. (2016) compared $P\bar{E}CO_2$ measured by VCap versus the Douglas' bag in patients with ARDS and found a bias of 0.2 mmHg and limits of agreement of − 3.0 to 4.5 mmHg (Doorduin et al., 2016). Our data in an animal model of ARDS using the multiple inert gas elimination technique (MIGET), the gold standard method for gas-exchange analysis supported the above findings. Our group found a close correlation of $P\bar{E}CO_2$ measured by VCap with MIGET ($r = 0.92$; $p < 0.0001$), with a mean bias of −0.5 mmHg and limits of agreement between −2.5 and 1.5 mmHg (Tusman et al., 2011a).

The second, more recently introduced step, was the possibility to measure the mean alveolar partial pressure of CO_2 ($PACO_2$). This another essential component of the Bohr's formula is difficult to measure and is still controversial parameter because $PACO_2$ varies topographically and temporarily within inhomogeneous lungs along the respiratory cycle, even in healthy patients. This means that any single lung unit has its own $PACO_2$ according to its respective V/Q ratio. Therefore, many controversies arose about what the "alveolar gas" really means and what is the proper definition and representative value of $PACO_2$ (Rossier and Buhlmann, 1955). Two different approaches to describe the alveolar gas have been proposed in the past: the *ideal* and the *expired* alveolar gas. The *ideal alveolar gas* concept described by Riley and Cournand is based on the convenient and didactic assumption that the lung behaves as a perfect unit, where $PACO_2$ equals capillary PCO_2 (Riley and Cournand, 1949). However, this condition does not really exist even in healthy subjects due to the presence of airway dead space, anatomical shunt, stratified inhomogeneities, spatial and temporal V/Q mismatches, and incomplete gas mixing of inspired gases within the lungs (Fletcher et al., 1981; Crawford et al., 1985; Verbanck and Paiva, 2013). The impossibility to estimate $PACO_2$ in the past was solved by Henrik Enghoff who suggested to use PCO_2 in arterial blood ($PaCO_2$) in the Bohr's formula as a surrogate of $PACO_2$ (Enghoff, 1938). This solution to calculate VD is still used today although, strictly speaking, it calculates not only dead space but also all the spectrum of V/Q mismatch present in the lung (**Figure 2**). The *expired alveolar air* concept

offers a better and more realistic approximation to $PACO_2$. Alveolar CO_2 fluctuates during the respiratory cycle changing ∼4 mmHg between inspiration and expiration. DuBois described the "mean" $PACO_2$ as the absolute value representing the whole lung (Dubois et al., 1952). This concept was confirmed decades later using complex mathematical models that included other aspects that affect alveolar gas composition such as pulmonary capillary pulsatile blood flow, capillary recruitment, solubility of CO_2 in pulmonary tissue, and CO_2 chemical reactions in blood (Hlastala, 1972). The alveolar gas must necessarily be measured during expiration due to the location of the CO_2 sensor at the airway opening where inspiratory gases washes-out any remanent CO_2. Mean $PACO_2$ can conveniently be found at the midpoint of the phase III of the VCap. This phase is exclusively composed of alveolar gas where its slope represents the emptying of CO_2 from all alveoli at different rates during the expiratory time (Fletcher et al., 1981; Tusman et al., 2012). We tested this hypothesis in an animal model of ARDS by comparing the VCap-based $PACO_2$ with the one derived from the alveolar gas equation solved with data obtained from MIGET. We found a mean bias of − 0.1 mmHg with limits of agreement of −2.18 to 1.98 mmHg (Tusman et al., 2011a). This confirms the original DuBois' description of *mean* $PACO_2$, resolving the measurement of $PACO_2$ at the bedside and allowing the non-invasive calculation of Bohr's dead space breath by breath.

CURRENT INTERPRETATION OF BOHR'S AND ENGHOFF'S APPROACHES TO DEAD SPACE

According to the previous discussion, it is clear that Bohr's and Enghoff's approaches measure different but complementary aspects of gas exchange (**Figure 2**) (Tusman et al., 2012; Sipmann et al., 2014). The Bohr's equation measures "true" dead space because it uses parameters exclusively from the alveolar compartment, and thus is based on *the expired alveolar air concept*. The PA-$\bar{E}CO_2$ represents the degree of CO_2 dilution in naturally heterogeneous human lungs. This was confirmed in a model of ARDS where the Bohr's dead space calculated by VCap was in an excellent agreement (bias 0.01 and LoA −0.04 to 0.06) with the value obtained with the MIGET analysis (Tusman et al., 2011a). The Enghoff's approach measures not only dead space but also all the spectrum of V/Q mismatch present in the lung (**Figure 2**). This is because by using $PaCO_2$ as a surrogate of $PACO_2$ *(the ideal alveolar gas concept)*, the effects of low V/Q and shunt are included in its estimation. The true shunt and low V/Q zones let high venous blood PCO_2 pass through the alveoli increasing $PaCO_2$ much above $PACO_2$. Some authors called this effect "shunt" dead space (Fletcher et al., 1981), a misleading term as it mixes the two opposite extremes of V/Q mismatch. We observed a poor correlation between the Enghoff's approach with MIGET dead space ($r = 0.38$; $p = 0.0078$) but a good one with MIGET shunt ($r = 0.64$; $p < 0.0001$) (Tusman et al., 2011a). This is why we think that calling the result of the Enghoff's equation, a global index of gas exchange, as "dead space" is both physiologically and clinically incorrect!

DEAD SPACE SUBCOMPONENTS

The Bohr's equation calculates the whole *physiological* dead space. It can be expressed as an absolute volume in one breath (VD_{phys} in ml), as part of minute ventilation (VD in L) or, more commonly, as a fraction of VT (VD/VT) (Fletcher et al., 1981; Tusman et al., 2012). VCap is the only clinical monitoring tool that separates the volume of gas within conducting airways from the volume of gas in the alveolar compartment in one breath (**Figure 2**). The classical geometrical method to identify the midpoint of phase II of the capnogram, described by Fowler (1948), can be replaced by a more accurate mathematical analysis (Tusman et al., 2009). This is of great importance as the slope of phase II represents the emptying of lung units of different time constants and V/Q ratios and the midpoint the averaged interface between convective and diffusive intrapulmonary gas transport. We have named this point as the *airway–alveolar interface* (**Figure 1**), and it is needed to estimate airway dead space (VD_{aw}) and alveolar VT (VT_{alv}). Failure to correctly estimate this point can lead to interpretation errors of VCap and dead space components (Tusman et al., 2009). Finally, the alveolar dead space (VD_{alv}) is easily obtained by subtracting VD_{aw} from total VD_{phys} (Fletcher et al., 1981). VD_{aw} and VD_{alv} are commonly expressed as a fraction of VT to allow comparisons among different breaths and sizes of patients. The alveolar component is better expressed as a fraction of VT_{alv} (VD_{alv}/VT_{alv}) because it is an index that closely represents the inefficiency of gas exchange (Fletcher et al., 1981; Tusman et al., 2012).

VALUES OF DEAD SPACE IN PATIENTS WITH ACUTE RESPIRATORY DISTRESS SYNDROME

Table 1 shows a summary of published Bohr's and Enghoff's values from healthy volunteers to patients with ARDS (Nunn and Hill, 1960; Larsson and Severinghaus, 1962; Blanch et al., 1999; Åström et al., 2000; Beydon et al., 2002; Tusman et al., 2013; Doorduin et al., 2016; Gogniat et al., 2018). Mechanical ventilation *per se,* apart from pulmonary diseases, increases dead space. In patients with ARDS, VD is elevated by many other factors including instrumental dead space (HME, elbows, close-suction systems), using low VT ventilation, high respiratory rates, pulmonary vascular involvement, or high positive end-expiratory pressure (PEEP), among others. Instrumental dead space may be of major relevance in patients with ARDS because it is an easily modifiable factor and can sum up to 90 to 100 ml in some unfortunate configurations.

EVALUATION OF LUNG OVERDISTENSION BY VOLUMETRIC CAPNOGRAPHY

As discussed above, cyclic overdistension is one of the major mechanisms of VILI. It is referred to in different terms depending on whether it denotes functional (hyperdistension)

TABLE 1 | Published values of measured Bohr's and Enfhoff's approaches using capnography.

Kind of Patient	Authors	Ventilation	Enghoff's index	Bohr's VD/VT	VD_{aw}/VT	VD_{alv}/VT_{alv}
Healthy volunteers	Larsson and Severinghaus (1962) *n* = 11	Spontaneous mean VT ∼ 630 mL	0.23 to 0.31	–	0.18 to 0.24	–
	Åström et al. (2000) *n* = 38	Spontaneous mean VT ∼ 645 mL	Female = 0.23 Male = 0.31	Female = 0.20 Male = 0.26	Female = 0.16 Male = 0.21	–
	Tusman et al. (2013) *n* = 33	Spontaneous mean VT 546 mL	–	0.23 ± 0.08	0.17 ± 0.09	0.07 ± 0.06
Healthy anestetized	Nunn and Hill (1960) *n* = 12	Mandatory mean VT 474 mL	0.32	–	0.13	–
	Tusman et al. (2013) *n* = 33	Mandatory VT 6 mL/kg PEEP 6 cmH_2O	–	0.28 ± 0.07	0.18 ± 0.08	0.11 ± 0.05
Critically ill anestetized	Unpublished personal data *n* = 55	Mandatory VT 6 mL/kg PEEP 8 cmH_2O	–	0.41 ± 0.07	0.23 ± 0.07	0.23 ± 0.08
ARDS	Blanch et al. (1999) *n* = 17	Mandatory mean VT ∼ 510 mL PEEP 5–10 cmH_2O	–	0.53 to 0.63	–	–
	Beydon et al. (2002) *n* = 10	Mandatory mean VT ∼ 625 mL PEEP 5–10 cmH_2O	0.53 to 0.55	–	0.30 to 0.32	–
	Doorduin et al. (2016) *n* = 15	Mandatory VT 6.8 mL/kg PEEP 12 cmH_2O	0.68 ± 0.9	0.45 ± 0.7	–	–
	Gogniat et al. (2018) *n* = 14	Mandatory VT 6.5 mL/kg PEEP 10 cmH_2O	0.70 (0.58–0.74)	0.47 (0.45–0.56)	0.37 (0.31–0.45)	0.19 (0.15–0.23)

Data is presented as mean ± SD or median (1^{st}–3^{rd} quartiles). (–) data not available.

or morphological (hyperinflation) phenomena. Recently, a more conceptual and integrative framework adopted from bioengineering terminology has been introduced to describe the stress or lung deformation that the lung parenchyma suffers during inflation, especially at the end of inspiration when maximal airway pressures are reached. *Lung stress* is defined as the distribution of internal forces per area unit applied to an elastic material, and *lung strain* as the deformation or change in shape of an elastic material from a reference initial value when submitted to a force (Chiumello et al., 2008). Lung parenchyma is constituted by a network of elastic tissues that normally works within a certain range of normal stress and strain. The topographical distribution of stress and strain is heterogeneous due to the natural fractal configuration of the lungs and by the effects of gravity. Lung parenchyma is prone to damage if the normal limits of stress and strain for a particular lung region are exceeded (Protti et al., 2013; Hurtado et al., 2017).

Volumetric capnography informs about the occurrence of lung overdistension from a different but complementary perspective, as standard and transpulmonary lung mechanics. The Bohr's dead space increases with positive pressure ventilation, especially in the diseased lungs. The effects of PEEP on VD are well described in the literature (Blanch et al., 1999; Beydon et al., 2002). More importantly, PEEP and the resulting end-inspiratory pressure also increase VD_{alv} as it creates alveolar units with a high V/Q behavior. This is probably the most important parameter related to the risk of developing VILI as it reflects the phenomena occurring at the alveolar level, the most vulnerable part of the lung. Most reports describing the effects of PEEP or MV on VD and VD_{alv} use the Enghoff's approach, thereby introducing the confounder effect of low V/Q areas on its estimation. In their hallmark paper, Suter et al. (1975) defined the best PEEP as the one resulting in the highest compliance and the lowest VD_{alv}. This level also resulted in a reduced shunt, suggesting that the reduction in VD_{alv} could have been responsible in part to this effect. In an animal model of ARDS, we recently reproduced these results by applying 4 cmH_2O of PEEP below and above the "best" PEEP using the VCap analysis. The Bohr's derived VD_{alv}/VT_{alv} showed the lowest value at the open-lung PEEP and high values with 4 cmH_2O of PEEP below or above this value (**Figure 3**) (Tusman et al., 2011b). Of note, by measuring the Bohr's (i.e., true) dead space, we specifically analyzed the effects of PEEP on the high V/Q component, that is overdistension, eliminating the confounder of shunt or low V/Q regions, which is an important aspect to consider when aiming at monitoring overdistension.

Gogniat et al. (2018) analyzed the effects of PEEP on VD and VD_{alv} in 15 patients with ARDS. They found a similar behavior, i.e., increased overdistension when PEEP was not only above but also below the optimum level set according to the best lung compliance (**Table 2**) (Gogniat et al., 2018). The authors could identify two clear responses to PEEP when patients were split into two groups according to changes of driving pressure (DP) from baseline ventilation. Patients with DP < 15% (i.e., with better compliance in response to PEEP) presented the lowest dead space and Enghoff's index values at any PEEP, whereas those with DP > 15% responded exaggeratedly to an increased PEEP. **Table 2** shows that the Bohr's dead space increased proportional to PEEP, whereas Enghoff's index remained unchanged. As Enghoff's index includes all V/Q mismatches, it was affected by the opposite simultaneous effects of PEEP on true dead space (increased by overdistension) and shunt (decreased by alveolar recruitment), reducing the sensibility and specificity for detecting lung overdistension.

For examining the role of VD in detecting overdistension in more detail, the effects of increasing PEEP levels from 0 to

Variable		PEEP		
		<4-6 cmH_2O	"Best"	>4-6 cmH_2O
Suter et al., 1975	Shunt %	0.18 ± 0.03 *	0.15 ± 0.02	0.11 ± 0.02 *
	VD_{alv}/VT	0.27 ± 0.02 *	0.18 ± 0.01	0.22 ± 0.02*
Tusman et al., 2011	Shunt %	0.26 (0.20-0.36) *	0.08 (0.05-0.15)	0.06 (0.03-0.10) *
	VD_{alv}/VT_{alv}	0.28 (0.24-0.38) *	0.16 (0.12-0.18)	0.18 (0.16-0.25) *
	VD_{aw}/VT	0.43 (0.40-0.44) *	0.44 (0.42-0.47)	0.49 (0.45-0.50) *
	$VTCO_{2,br}$ (mL)	4.8 (4.1-5.9) *	5.1 (4.3-5.9)	4.8 (4.2-5.5) *

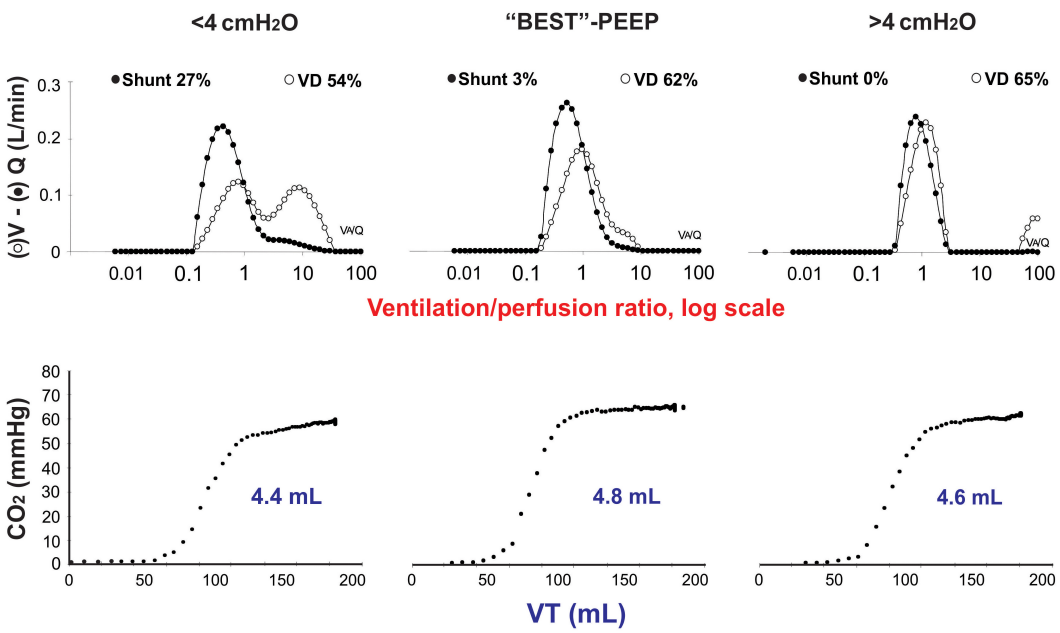

FIGURE 3 | Dead space at optimum PEEP. Comparison of the data obtained by Suter et al. (1975) using Enghoff's approach with our more recent data using VCap using Bohr's approach and multiple inert gas elimination technique (MIGET) (Tusman et al., 2011b). Physiological dead space measured by MIGET and VCap airway dead space increase proportional to PEEP, whereas shunt decreases with PEEP. An individualized level of PEEP ("Best" PEEP) corresponding to maximal respiratory system compliance, resulted in the lowest alveolar dead space and the highest elimination of CO_2 measured by VCap. PEEP above and below this optimum value results in an increased alveolar dead space and decreased the elimination of CO_2 per breath ($VTCO_{2,br}$). (*) $p < 0.05$ compared to best PEEP.

30 cmH_2O were analyzed in an experimental model of ARDS (Tusman et al., 2020). The Bohr's VD, with both its airway and alveolar components, increased in proportion when PEEP exceeded 15 cmH_2O, reflecting clear global lung overdistension that was confirmed by a parallel decrease in CO_2 elimination by the lungs, an increase in lung elastance, transpulmonary DP, and end-inspiratory transpulmonary pressure (**Figure 4**). However, at PEEP < 10 cmH_2O, VD_{aw} was minimal but VD_{alv} increased. This increase was associated to a low $VTCO_{2,br}$ and high lung elastance and transpulmonary DP.

TABLE 2 | Dead space and Enghoff's index in ARDS patients at different levels of PEEP.

Parameters	ΔP	Randomized PEEP (cmH_2O)			
		0	6	10	16
VDBohr/VT	All patients	0.44 (0.41–0.48)	0.45 (0.43–0.52)	0.47 (0.45–0.56)	0.51 (0.46–0.60)
	$\Delta P > 15\%$	0.50 (0.47–0.54)	0.55 (0.49–0.57)	0.59 (0.51–0.59)	0.61 (0.56–0.65)
	$\Delta P \leq 15\%$	0.41 (0.40–0.43)	0.44 (0.42–0.45)	0.45 (0.44–0.46)	0.47 (0.45–0.48)
		P = 0.012	*P = 0.008*	*P = 0.006*	*P = 0.001*
VDaw/VT	All patients	0.33 (0.29–0.36)	0.34 (0.30–0.40)	0.37 (0.31–0.45)	0.39 (0.34–0.47)
	$\Delta P > 15\%$	0.38 (0.31–0.40)	0.43 (0.33–0.45)	0.48 (0.36–0.50)	0.51 (0.41–0.55)
	$\Delta P \leq 15\%$	0.31 (0.29–0.33)	0.31 (0.29–0.34)	0.35 (0.29–0.37)	0.34 (0.30–0.38)
				P = 0.047	*P = 0.018*
VDalv/VTalv	All patients	0.18 (0.15–0.22)	0.19 (0.17–0.23)	0.19 (0.15–0.23)	0.22 (0.17–0.24)
	$\Delta P > 15\%$	0.20 (0.19–0.23)	0.22 (0.20–0.24)	0.21 (0.18–0.23)	0.25 (0.24–0.27)
	$\Delta P \leq 15\%$	0.16 (0.15–0.24)	0.17 (0.17–0.24)	0.16 (0.13–0.21)	0.16 (0.14–0.21)
			P = 0.047		*P = 0.008*
Enghoff's index	All patients	0.71 (0.60–0.73)	0.71 (0.58–0.74)	0.70 (0.63–0.75)	0.69 (0.59–0.77)
	$\Delta P > 15\%$	0.74 (0.73–0.74)	0.76 (0.74–0.77)	0.76 (0.75–0.76)	0.78 (0.77–0.79)
	$\Delta P \leq 15\%$	0.59 (0.56–0.70)	0.58 (0.55–0.69)	0.63 (0.54–0.69)	0.58 (0.53–0.63)
		P = 0.025	*P = 0.008*	*P = 0.006*	*P = 0.002*

VD_{Bohr}/VT = Bohr's dead space to tidal volume ratio, VD_{aw}/VT = airway dead space to tidal volume ratio, VD_{alv}/VT_{alv} = alveolar dead space to alveolar tidal volume ratio, and ΔP = driving pressure. Krustal-Wallis non-parametric test for ΔP inter-group comparison. Data is presented as median and 1^{st}–3^{rd} quartiles.

End-inspiratory transpulmonary pressure remained stable but high (∼20 cmH_2O). These findings could be interpreted as an increase in stress and strain in the alveolar compartment of the aerated lung, despite lower levels of PEEP, caused by the stressor-raiser role of atelectasis (**Figures 4B,C**).

CAPNODYNAMICS FOR MONITORING LUNG STRAIN

Recently, a new method for monitoring lung overdistension by directly measuring lung strain based on expired CO_2 kinetics, the capnodynamic method, has been described (Suarez-Sipmann et al., 2019). Based on the principles of mass balance for CO_2 in the lung and the differential Fick principle for CO_2, the capnodynamic equation provides two highly relevant parameters for monitoring purposes: effective pulmonary blood flow ($EPBFCO_2$) (i.e., the non-shunted portion of cardiac output) and end-expiratory lung volume ($EELVCO_2$):

$$EELVCO_2 \cdot (FACO_2^n - FACO_2^{n-1}) = EPBFCO_2 \cdot \Delta t^n \cdot (CvCO_2 - CcCO_2^n) - VTCO_2^n$$

where $FACO_2{}^n$ refers to the alveolar fraction of CO_2 at the *n*th breath and $FACO_2{}^{n-1}$ for the preceding breath. Δt^n is the duration of the *n*th respiratory cycle, $CvCO_2$ and $CcCO_2{}^n$ is the mixed venous and capillary CO_2 content, $VTCO_2{}^n$ is the volume of CO_2 eliminated by the *n*th breath.

The mass balance occurs between the CO_2 content in the lung (represented on the left side of the equation) equals the difference between the CO_2 supplied to the lung by perfusion and the amount of CO_2 eliminated by the lung (on the right side of the equation). To solve the equation for its three unknowns: $EELVCO_2$, $EPBFCO_2$, and $CvCO_2$, a small modification in CO_2 alveolar concentration must occur under the assumption that $CvCO_2$ remains constant during the measurement cycle. All other parameters can be obtained non-invasively by VCap. The change in $FACO_2$ is obtained by a minimal modification of the breathing pattern (three consecutive respiratory cycles in which a short expiratory hold is added are repeatedly interspersed between six normal cycles) in a passively breathing patient under MV. Applying a repetitive sequence of six normal and three prolonged breaths, iterative mathematics can solve the capnodynamic equation after a set of nine equations is obtained. From then on, any new breath is added to the sequence providing a new solution (i.e., a new value of $EELVCO_2$ and $EPBFCO_2$, which can be monitored continuously). A more in-depth description of the method is beyond the scope of this manuscript. The method has been submitted to extensive experimental validation in challenging pulmonary and circulatory conditions (Sander et al., 2014, 2015). Recently, the first validations of $EPBFCO_2$ in patients on general anesthesia (Sigmundsson et al., 2021) and of $EELVCO_2$ (Öhman et al., 2020) have been published. Both performed well in good agreement with clinical reference methods and excellent trending abilities.

The decrease in FRC is one of the major contributors to VILI during MV. A way to quantify this risk is to determine lung strain, a measure of lung tissue deformation during inflation. To calculate lung strain, VT needs to be normalized to lung volume (strain = VT/FRC) (Chiumello et al., 2008), which during MV is referred to as end-expiratory lung volume as resting volume is influenced by the level of PEEP applied. When a certain threshold of strain is exceeded, the potential for mechanical damage to the ARDS lung increases (Bellani et al., 2011; González-López et al., 2012). The capnodynamic method offers the unprecedented possibility not only to measure EELV at the bedside, which has

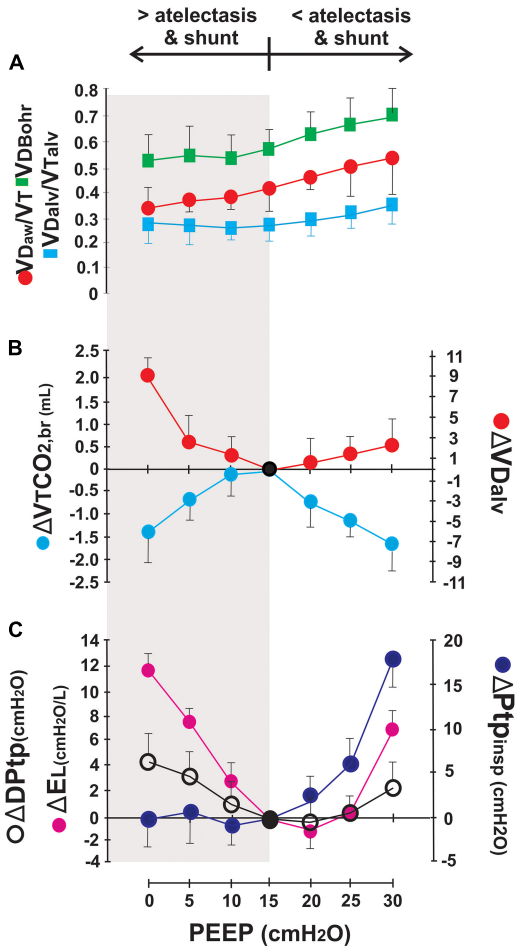

FIGURE 4 | Effects of PEEP in dead space and lung mechanics. Airway dead space increases in proportion to PEEP and has a direct impact on physiological Bohr's VD. Alveolar dead space (VDalv), on the contrary, changes according to the balance between recruitment and overdistension in the alveolar compartment. Panel **(A)** shows the effect of PEEP on absolute dead space values. Panels **(B,C)** present the differences in alveolar dead space (ΔVD_{alv}), elimination of CO_2 per breath ($\Delta VTCO_{2,br}$), transpulmonary driving pressure (ΔPtp), transpulmonary end-inspiratory pressure (ΔPtp_{insp}), and lung elastance (ΔE_L) between certain PEEP level with the reference15-PEEP considered as the "Best" PEEP according to findings in gas exchange, lung ultrasound, and respiratory mechanics adapted from Tusman et al. (2020).

been very difficult to date, but also to do it in a non-invasive continuous way, extending and complementing the possibilities of VCap to measure lung overdistension and strain.

LUNG PERFUSION ESTIMATED BASED ON CO_2 KINETICS AND LUNG OVERDISTENSION

The hemodynamic consequence of lung overdistension is crucial information in mechanically ventilated patients with ARDS. Volumetric capnography has the unique ability to describe lung overdistension both from the ventilatory and hemodynamics perspective. Volumetric capnography assesses pulmonary perfusion *qualitatively* through the parameters $PETCO_2$ and VCO_2 (Tusman et al., 2010) and *quantitatively* by calculating the effective capillary pulmonary blood flow ($EPBF_{CO2}$) using equations based on the differential Fick's formula and the above-described capnodynamic method (Sander et al., 2014, 2015). Lung overdistension induced by high alveolar pressure can collapse pulmonary capillaries, decrease pulmonary blood flow, and increase right ventricle afterload. The association of high dead space with low VCO_2 or $EPBF_{CO2}$ has been observed during high PEEP ventilation, resulting in a *functional* overdistension because CO_2 exchange and elimination are impaired.

VCO_2 or $EPBF_{CO2}$ are decreased not only by lung overdistension but also by other hemodynamical causes like hypovolemia, embolism, arrhythmias, or heart failure. Therefore, physicians involved in the care of ventilated patients should first rule out any other hemodynamic problem when evaluating the hemodynamic consequence of lung overdistension. Again, the context-sensitive nature of CO_2 kinetics is relevant to make differential diagnoses. Many questions arise when the operator observes changes in pulmonary perfusion in ventilated patients. Is any acute hemodynamic problem of extra-pulmonary origin responsible for the low elimination of CO_2? Is the patient with normovolemia or has a preload-dependency? Have alveolar ventilation or body metabolism changed?

PREDICTION OF ACUTE RESPIRATORY DISTRESS SYNDROME OUTCOME

The role of VCap to determine the prognosis of patients with ARDS has been well established. Enghoff's index has been found to be strongly associated with mortality in the early and late course of ARDS (Nuckton et al., 2002; Kallet et al., 2014). The same research group showed that the risk to death increased by 22% for every 0.05 increase in Enghoff's index (OR = 1.22, 95% CI 1.11–1.35, $p < 0.001$) in patients with moderate and severe ARDS (Kallet et al., 2017). The magnitude of changes in the Enghoff's index varied according to ARDS etiology although, in each ARDS subgroup, this variable was always higher in non-survivors than in survivors. Recently, surrogates of VD, such as the ventilatory ratio—an index calculated by the quotient between measured and predicted minute ventilation and $PaCO_2$—was independently associated with mortality in patients with ARDS (Sinha et al., 2019). Why are these CO_2-based indexes, such good predictors of survival, better than the usual oxygen-based index in ARDS? When $PaCO_2$ is used to calculate dead space (according to the ideal alveolar gas concept) what it is measured is a global index of gas exchange including low V/Q and shunt. Lung physiology explains that shunt is represented not only by the PA-aO_2 but also by the Pa-ACO_2 difference. Therefore, these indexes based on $PaCO_2$ reflect the severity of ARDS by assessing shunt in combination with dead space. The prognostic value of Bohr's "true" dead space in a patient with ARDS is still unknown, but it is very likely that it also has an important role as a direct measure of

lung ventilatory inefficiency and overdistension, both with likely strong influence on outcome.

CONCLUSION

Analysis of expired CO_2 kinetics by VCap provides important non-invasive cardiorespiratory information for clinical assessment, monitoring, and management of ARDS mechanically ventilated patients. Dead space and the Enghoff's index are calculated with high precision even in patients with very severe lung injury, as those with ARDS. The concept of VD is clinically useful not only to assess and adjust alveolar ventilation in the context of lung-protective MV but also to detect alveolar overdistension. Capnodynamic measurement of end-expiratory lung volume allows estimating strain that in combination with lung mechanics and lung perfusion can provide a more in-depth understanding of the lung condition and detect when this value exceeds safe limits. Real-time assessment of the elimination of CO_2 and effective pulmonary capillary blood flow provides information about the hemodynamic consequences of positive pressure ventilation. The combination of an increase in VD and a decrease in pulmonary capillary blood flow characterizes a situation of *functional* lung overdistension. Further studies are needed to explore the precise cutoff value to define harmful functional overdistension with VD and to determine its role as a screening tool to predict the evolution in patients with ARDS.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Circulating Skeletal Troponin During Weaning From Mechanical Ventilation and Their Association to Diaphragmatic Function: A Pilot Study

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Background: Patients with acute respiratory failure (ARF) may need mechanical ventilation (MV), which can lead to diaphragmatic dysfunction and muscle wasting, thus making difficult the weaning from the ventilator. Currently, there are no biomarkers specific for respiratory muscle and their function can only be assessed trough ultrasound or other invasive methods. Previously, the fast and slow isoform of the skeletal troponin I (fsTnI and ssTnI, respectively) have shown to be specific markers of muscle damage in healthy volunteers. We aimed therefore at describing the trend of skeletal troponin in mixed population of ICU patients undergoing weaning from mechanical ventilation and compared the value of fsTnI and ssTnI with diaphragmatic ultrasound derived parameters.

Methods: In this prospective observational study we enrolled consecutive patients recovering from acute hypoxemic respiratory failure (AHRF) within 24 h from the start of weaning. Every day an arterial blood sample was collected to measure fsTnI, ssTnI, and global markers of muscle damage, such as ALT, AST, and CPK. Moreover, thickening fraction (TF) and diaphragmatic displacement (DE) were assessed by diaphragmatic ultrasound. The trend of fsTnI and ssTnI was evaluated during the first 3 days of weaning.

Results: We enrolled 62 consecutive patients in the study, with a mean age of 67 ± 13 years and 43 of them (69%) were male. We did not find significant variations in the ssTnI trend ($p = 0.623$), but fsTnI significantly decreased over time by 30% from Day 1 to Day 2 and by 20% from Day 2 to Day 3 ($p < 0.05$). There was a significant interaction effect between baseline ssTnI and DE [$F_{(2)} = 4.396$, $p = 0.015$], with high basal levels of ssTnI being associated to a higher decrease in DE. On the contrary, the high basal levels of fsTnI at day 1 were characterized by significant higher DE at each time point.

Conclusions: Skeletal muscle proteins have a distinctive pattern of variation during weaning from mechanical ventilation. At day 1, a high basal value of ssTnI were associated to a higher decrease over time of diaphragmatic function while high values of fsTnI were associated to a higher displacement at each time point.

Keywords: acute hypoxemic respiratory failure, assisted mechanical ventilation, biomarker, diaphragm, diaphragmatic ultrasound, skeletal troponin, weaning

INTRODUCTION

Ventilatory support is an essential life-saving therapy for intensive care patients with acute respiratory failure (1). However, most patients under mechanical ventilation (MV) experience deleterious impact of mechanical ventilation on the diaphragm (2). The balance between lung and diaphragm protection remains challenging. As known, MV can trigger a sustained change in muscle fibers biochemistry (3), ultimately leading to diaphragmatic atrophy (4). Experimental evidence suggest that signs of increased oxidative stress and diaphragm fiber proteolysis may arise as early as 12 h from MV initiation (5). Ventilator induced diaphragmatic dysfunction (VIDD) (6) has a rapid onset, is related to the duration of ventilation support (7) and affects the clinical outcome (8–11). The diaphragm seems to be more susceptible to fast disuse atrophy, as compared to peripheral skeletal muscles (e.g., pectoralis muscle and latissimus dorsi) (12). In order to minimize VIDD, it has been suggested to implement assisted modes as soon as clinically feasible and safe (13), to minimize patient-ventilator asynchronies (14) and to avoid excessive expiratory braking (15, 16), despite definitive evidence are not available.

In critically ill patients, no bedside tools are available to monitor the muscular function except for ultrasound, which is non-invasive but highly operator-dependent (17). Phrenic nerve stimulation, which would be the "gold standard," is invasive, complex and thus limited in the routine clinical application.

Specific circulating biomarkers for skeletal muscles have been recently identified. In contrast with non-specific muscular markers, like creatine kinase (CPK), specific markers could potentially open the possibility to provide real time information on the integrity of different types of muscular fibers (18). Slow- and fast-twitch skeletal troponin I (ssTnI and fsTnI) have been shown to be promising markers of damage to slow oxidative (Type I) and fast glycolytic (Type II) muscular fibers (19, 20), respectively.

In a previous report in healthy subjects undergoing an inspiratory threshold loading trial (21), fsTnI was regarded as an early marker, more sensitive than CK, of subclinical diaphragmatic damage. Furthermore, recent data suggest the use of these markers for evaluating subclinical muscular damage (22), since their circulating level are influenced by both muscular mass and amount of muscle disruption.

It is not clear what happens to the circulating levels of skeletal troponin in patients with acute respiratory failure (ARF) undergoing mechanical ventilation, especially during the early phase of weaning. Therefore, the primary aim of the present study was to describe the trend of circulating skeletal troponins during the early part of weaning in a population of mechanically ventilated critically ill patients. Moreover, we hypothesized that values of skeletal troponin beyond normality could be associated to diaphragmatic dysfunction. To test this hypothesis, we compared ultrasound derived diaphragmatic parameters with the baseline levels and the trend of plasma skeletal troponins to determine if they could serve as early markers of diaphragmatic atrophy/ventilator over assistance.

METHODS

Study Population

This is a longitudinal, single-center, observational cohort study, conducted over a 24-months period (March 2017 to March 2019) in the ICU of the S. Anna University Hospital, Ferrara, Italy. The study was approved by the ethics committee of our institution (Azienda Ospedaliero-Universitaria Ferrara Ethic Committee, number of ethical approval 131084). Informed consent was obtained from each patient or next of kin. All consecutive patients recovering from acute hypoxemic respiratory failure (AHRF) with an expected length of mechanical ventilation of 72 h or more were screened for study inclusion. The inclusion criteria were: age 18 years or older, ventilation in assisted mode, Richmond Agitation Sedation Scale (RASS) between −1 and +1. Exclusion criteria were: history of neuromuscular disease, continuous infusion of muscle-paralyzing agents in the last 48 h, diaphragm atrophy or paralysis, abnormal values of myocardial and muscular damage markers at ICU admission, presence of moderate/severe acute kidney injury (23) at ICU admission, presence of thoracotomy, pneumothorax or pneumomediastinum, pregnancy.

Study Protocol

All patients were enrolled at the beginning of weaning from MV and therefore when able to trigger the ventilator. Specifically, the patients were studied from the 1st (defined Day 1) to the 3rd day (defined Day 3) since the switch from the beginning of assisted mode ventilation (PSV). The following data were collected: mode of mechanical ventilation, ventilator parameters [i.e., pressure support, positive end-expiratory pressure (PEEP), inspired oxygen fraction (FiO_2)], breathing pattern [i.e., tidal volume (V_T), respiratory rate (RR)] and the occlusion pressure at 100 ms (P0.1), defined as the negative pressure measured 100 ms after the initiation of an inspiratory effort. All included patients underwent a daily ultrasonographic evaluation of the diaphragmatic function. Arterial blood samples were collected

right after ultrasound measurements to evaluate arterial blood gasses and circulating markers. Creatinine was collected each day during the study period to evaluate the onset of moderate/severe acute kidney injury (23).

Mechanical Ventilation Setting

Patients with ARF were included in the study within 24 h after the initiation of assisted mechanical ventilation (i.e., pressure support ventilation, PSV) according to the attending physicians' judgment. The readiness to sustain PSV was based on the following criteria: (a) improvement of the condition leading to acute respiratory failure, (b) positive end-expiratory pressure (PEEP) lower than 10 cm H_2O and inspiratory oxygen fraction (FiO_2) lower than 0.5, (c) Richmond Agitation Sedation Scale (RASS) between −1 and +1, with no sedation or with low dose of continuous infusion of sedation (i.e., propofol 0.5–1.5 mg/kg/h and/or remifentanil 0.03–0.05 mg/kg/min or dexmedetomidine (0.3–1.0 μg/kg/h), (d) ability to trigger the ventilator, (e) hemodynamic stability (with norepinephrine ≤ 0.1 μg/kg/min or equivalent), (f) normothermia.

Pressure support ventilation was set to meet the following targets: V_T of 6–8 mL/kg/PBW, with RR 20–30 bpm. Pressure support (PS) was decreased if $V_T > 8$ mL/kg/PBW and/or RR < 20 while it was increases if $V_T < 6$ mL/kg PBW and/or RR > 30 and/or in the presence of respiratory distress (e.g., marked use of the accessory muscles). PEEP and then FiO_2 were increased if SpO_2 was < 90%, while FiO_2 and then PEEP were decreased if SpO_2 was > 96%. The PEEP and FiO_2 levels in use before the study were left unchanged. Patients returning into controlled mechanical ventilation due to deteriorating respiratory mechanics or general clinical conditions were excluded from the clinical study.

Ultrasonography

Ultrasonographic assessments were performed by a single well-trained physician (F.D.C.) by using the same ultrasonography machine (M-Turbo, SonoSite, Inc., USA). All measurements were performed in patients lying in the semi-recumbent position and on the right side. Diaphragmatic excursion (DE) was evaluated using a 3.5 to 5-MHz convex ultrasound probe using a subcostal approach (24–26).

Diaphragmatic thickness and thickening fraction were assessed using a 12-MHz linear ultrasound probe by using an intercostal approach, as previously described (27, 28). Diaphragm thickening fraction (TFdi) was measured in M-mode as $TFdi = [(T_{EI}\text{-}T_{EE})/T_{EE}] \times 100$, where T_{EE} and T_{EI} correspond to the thickness of the diaphragm at the end of expiration and inspiration, respectively. Normal diaphragmatic function was defined as the presence of a DE ≥ 10 mm (28) or a TFdi ≥ 30% (29).

Serum Sampling and Quantification of Skeletal Troponins

Serum samples were obtained from the arterial blood in anticoagulant-free tubes by centrifugation at 1,500 rpm for 10 min after clotting and stored in aliquots at −80°C until assay. To avoid possible loss of bioactivity, samples were analyzed within 3 months from the collection and thawed only once. Slow skeletal Troponin I (ssTnI, Mybiosource, Cat. No. MBS2510383) and fast skeletal Troponin I (fsTnI, Mybiosource, Cat. No. MBS927961) were assayed by commercially available ELISA kits according to manufacturer's instructions. Specific technical details can be found elsewhere (22).

Myoglobin, Creatine Kinase (CPK) and creatinine were determined by routine analysis from the hospital's clinical laboratory. The concentration of aldolase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were assayed on undiluted serum samples by coupled spectrophotometric enzymatic assays on a Tecan Infinite M200 (Tecan Group Ltd., Männedorf, Switzerland) as detailed elsewhere (22).

Demographic and Clinical Data Collection

Demographics, anthropometrics, comorbidities, information, and causes of hospitalization were recorded into study-specific case report forms and database. Simplified Acute Physiology Score II (SAPSII) values, etiology, diagnosis, and severity of AHRF, days on mechanical ventilation before study enrollment were collected for each patient. Sequential organ failure assessment (SOFA) was calculated daily throughout the study observation period. Finally, days of mechanical ventilation, ICU length of stay, hospital length of stay, ICU mortality, and 28-days mortality were recorded as outcome data.

Statistical Analysis

Given the observational nature of this pilot study we enrolled a convenience sample size of consecutive patients matching inclusion criteria over a 2-year period based on previous studies (30). Continue variables are expressed as mean ± standard deviation or medians [interquartile range] depending on their distribution, whereas categorical variables are presented as frequencies and percentages. The Shapiro-Wilk test was used to assess the assumption of normality. Categorical data were compared using the χ^2 test or Fisher exact test as appropriate. Unpaired Student's *t*-tests or Mann-Whitney *U*-tests for data with normal or non-normal distribution, respectively, were used to compare continuous variables.

Mixed ANOVA was used to test differences in breathing parameters, diaphragmatic ultrasound measurements and sTnI serum levels among different time points [24 h (Day 1), 48 h (Day 2) and 72 h (Day 3) from assisted mechanical ventilation initiation], after log transformation of variables. In this case, statin use and other variables like sex, age (centered to the mean of 67 years), and BMI (centered to the mean of 28.4 Kg/m^2) were included in the model as covariates to correct for possible confounding factors. The subjects were divided into two groups based on the presence of higher of lower values than the median of biomarkers at baseline (ssTnI = 66 pg/mL, fsTnI = 31 pg/mL, CK = 68 U/L, myoglobin = 151 ng/mL). Then, we performed a mixed ANOVA by using these groups as between-subject variable to observe the possible interaction between low/high levels of biomarkers at baseline on respiratory effort parameters.

Correlation between diaphragmatic ultrasound measurements and circulating muscle functionality biomarkers

TABLE 1 | Patients' clinical characteristics at ICU admission.

Variables	Patients ($N = 62$)
Age, years	67 ± 13
Male sex, *n* (%)	43 (69)
BMI, kg/m^2	28.4 ± 5.5
SAPS II score at admission	42 [35–49]
Smoker, *n* (%)	
Actual	18 (29)
Former	6 (10)
Comorbidities, *n* (%)	
Heart diseases	16 (26)
Hypertension	37 (60)
Chronic cardiac ischemia	7 (11)
COPD	9 (15)
Diabetes	13 (21)
CKD	5 (8)
Reason for MV initiation, *n* (%)	
AHRF	29 (47)
Sepsis	6 (10)
Septic shock	18 (29)
Hemorrhagic shock	4 (6)
Coma	4 (6)
Cardiogenic shock	1 (2)
Outcomes	
Days spent on MV	10 [6–16]
ICU LOS	13 [8–19]
Hospital LOS	31 [15–53]
28-days mortality	10 (16)

BMI, body mass index, SAPS, simplified acute physiology score, COPD, chronic pulmonary obstructive disease, CKD, chronic kidney disease, AHRF, acute hypoxemic respiratory failure, ICU, intensive care unit, LOS, length of stay.

TABLE 2 | Skeletal troponin and "traditional" muscle damage parameters during the study period.

Variables	Day 1	Day 2	Day 3	*p*-value
ssTnI, pg/mL	66 [15–164]	55 [18–140]	51 [18–154]	0.623
fsTnI, pg/mL	31 [5–90]	18 [5–76]	13 [2–67]	<0.05
CK, U/L	68 [26–243]	55 [21–176]	44 [15–104]	<0.0001
Myoglobin, ng/mL	151 [57–276]	85 [40–153]	79 [41–126]	<0.0001
AST, U/L	5.4 [1.6–10.9]	3.4 [1.3–9.7]	3.9 [1.3–10.6]	0.226
ALT, U/L	4.8 [2.1–20.8]	5.3 [2.2–13.8]	6.1 [2.2–13.2]	0.617
Aldolase, U/L	4.5 [3.2–5.7]	3.9 [3.3–5.6]	4.2 [3.4–5.7]	0.789

ssTnI, slow skeletal troponin I, fsTnI, fast skeletal troponin I, AST, aspartate aminotransferase, ALT, alanine aminotransferase, CK, creatine kinase.

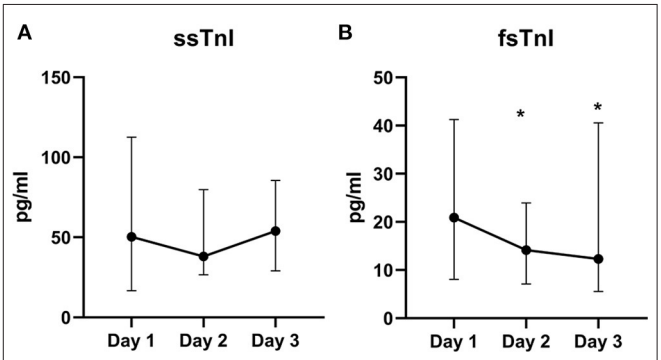

FIGURE 1 | Skeletal TnI serum levels over the study period. Value of ssTnI **(A)** were not different over time, whereas fsTnI **(B)** significantly decreased within the time frame of the study ($p < 0.05$ for within-subjects linear trend). The dots represent the median whereas the upper and lower bars the upper and lower 95% confidence interval, respectively. *$p < 0.05$ vs. T0.

were assessed by multivariate linear mixed-effects models, as stated elsewhere (31). sTnI values were tested as predictors of "normal diaphragmatic function" determined by diaphragmatic ultrasound through receiver operator characteristic (ROC) curves. For each ROC curve, sensitivity, specificity, accuracy, and optimal cut-off point using Youden's index were calculated. Statistical analyses were performed using SPSS 20.0 statistical software (SPSS Inc., Chicago, IL). In all statistical analyses, a 2-tailed test was performed and the $p \leq 0.05$ was considered statistically significant.

RESULTS

Patient Population

A total of 62 consecutive patients were included in the study after 1 [1–3] days from ICU admission. The main clinical characteristics of patients at admission are shown in **Table 1**. Their mean age was 67 ± 13 years old and 43 (69%) were male. Their median SAPSII score was 42 [35–49], resulting in a 29% predicted mortality. The most frequent causes for ICU admission were acute hypoxemic respiratory failure (AHRF) (43%), sepsis (37%), ARDS (24%) and hemorrhagic shock (9%). The median time spent on invasive mechanical ventilation was 10 [6−16] days, and their median ICU length of stay was 13 [8–19] days. No patient developed moderate/severe acute kidney injury during the study period.

Skeletal Troponin and "Classic" Muscle Damage Parameters

We did not find significant variations in the ssTnI trend ($p = 0.623$). On the contrary, fsTnI significantly decreased over time by 30% from Day 1 to Day 2 and by 20% from Day 2 to Day 3, $p < 0.05$, within-subjects linear contrast: $p < 0.05$ (**Table 2** and **Figure 1**). Of note, by correcting the values for the use of statin as a confounding factor, fsTnI still significantly decreased over time ($p < 0.05$). However, when considering the other confounding factors (sex, centered age, and centered BMI), the fsTnI trend was not significant.

A decreasing trend over time was also detected for myoglobin ($p < 0.0001$), with a 45% decrease from Day 1 to Day 2 and almost a 30% decrease from Day 2 to Day 3. Similar data were also observed for CPK, which showed a significant decrease over time (**Table 2**, $p < 0.0001$) with almost 20% lower values each following day. None of the other traditional markers (aldolase, AST, and ALT) showed significant variations within the study.

Of note, by correcting the values of both myoglobin and CPK for statin use the variables remained significant ($p = 0.011$ and $p < 0.001$, respectively), whereas they lose their significance in the fully corrected model.

Ventilation Parameters and Clinical Variables Over Time

Respiratory parameters and gas exchange remained stable throughout the study. The number of patients requiring the use of vasoactive drugs decreased over time (Day 1: 48%, Day 2: 37%, Day 3: 29%, **Table 3**).

TABLE 3 | Clinical and mechanical ventilation parameters during the study period.

	Day 1	Day 2	Day 3	*p*-value
Pressure support, cmH_2O	9 ± 4	9 ± 3	8 ± 4	0.036
PEEP applied, cmH_2O	8 ± 3	8 ± 3	8 ± 3	0.211
Respiratory rate, bpm	15 ± 5	16 ± 6	17 ± 5	0.548
V_E, L/min	7.5 ± 2.0	7.5 ± 2.0	8.1 ± 2.1	0.418
Cdyn, ml/cmH_2O	59 [40–70]	53 [39–64]	56 [40–74]	0.433
PaO_2/FiO_2	228 [156–293]	208 [153 −305]	230 [153 −280]	0.799
MAP, mmHg	83 [75–95]	85 [73–96]	83 [76–93]	0.740
SOFA score	6 [3–8]	5 [3–7]	4 [3–7]	0.852
Vasoactive drug use, *n* (%)	30 (48)	23 (37)	18 (29)	-
Steroid use, *n* (%)	26 (42)	29 (47)	26 (42)	-

PEEP, positive end-expiratory pressure, V_E, minute ventilation, Cdyn, dynamic compliance, PaO_2/FiO_2, oxygen partial arterial tension/inspired oxygen fraction, MAP, mean arterial pressure, SOFA, sequential organ failure assessment.

Skeletal Troponin and Diaphragmatic Ultrasound

We found a significant interaction effect between baseline ssTnI levels and DE [$F_{(2)} = 4.396$, $p = 0.015$], indicating that the observed decrease in DE over time was dependent on the baseline levels of ssTnI. In particular, subjects with high basal levels of ssTnI had a greater decrease in DE when compared to those with low ssTnI basal levels, identified by the steeper slope in **Figure 2A**. We did not find any significant interaction between baseline fsTnI levels and the DE trend [$F_{(2)} = 0.662$, $p = 0.518$, **Figure 2B**]. However, contrary to what observed for ssTnI, patients characterized by high basal levels of fsTnI had a higher DE at each time point than those with low fsTnI basal levels (log transformed variables: Day 1 mean difference: −0.356, $p = 0.006$, Day 2: −0.332, $p = 0.014$, Day 3: −0.102, $p = 0.102$). CK and myoglobin, did not any correlation with DE despite their decrease over time (data not shown). The TFdi trend was not influenced by the baseline levels of any of the measured muscular biomarkers.

Finally, we evaluated whether the frequency of patients developing diaphragmatic dysfunction (defined as DE < 1 cm at Day 3) was different between those demonstrating higher or lower levels of muscular biomarkers at Day 1 (i.e., higher or lower of the median serum concentration). The frequency of patients developing diaphragmatic dysfunction at Day 3 was independent from both ssTnI (40.6% of subjects each group, Fisher's exact test, $p = 1$), fsTnI (low fsTnI vs. high fsTnI: 47.1 vs. 35.3%, $p = 0.460$) (**Supplementary Figure 1**), CPK or myoglobin. When diaphragmatic dysfunction was evaluated by TFdi (i.e., <30%), the results were similar. We did not find any significant correlation between inspiratory effort parameters and muscular biomarkers within-subjects (e.g., at each time point,

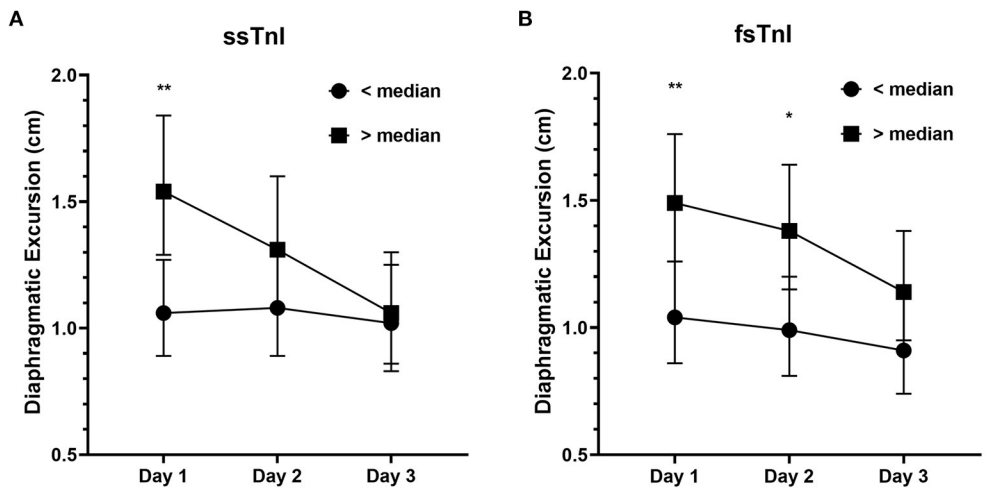

FIGURE 2 | Diaphragmatic excursion (cm) in patients with high or low median levels of skeletal troponins. **(A)** The decrease in diaphragmatic excursion was more prominent in patients with higher basal levels of ssTnI (i.e., >median) denoting the influence of ssTnI basal levels on DE. On the contrary, fsTnI basal levels **(B)** did not influence the decrease in DE. The dots represent the geometric means whereas the upper and lower bars the upper and lower 95% confidence interval, respectively. $^{**}p < 0.01$, $^{*}p < 0.05$.

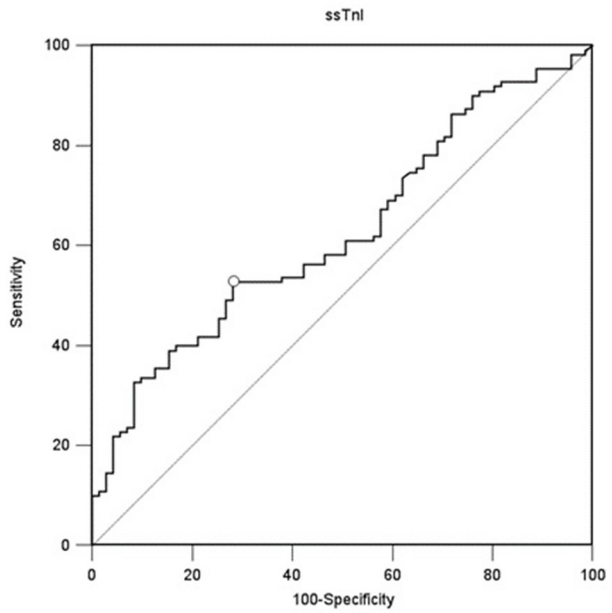

FIGURE 3 | ROC curve analysis for ssTnI as a predictor of normal diaphragmatic function as assessed by diaphragmatic ultrasound (diaphragmatic displacement ≥ 10 mm).

Supplementary Table 1). The only exception was for myoglobin, which showed a positive within-subjects relationship with both DE and TFdi ($r = 0.464$, $p = 0.001$ and $r = 0.462$, $p = 0.001$, respectively). Interestingly, ssTnI and fsTnI showed a significant between-subjects positive correlation with DE and TEE (for ssTnI $r = 0.332$, $p = 0.038$ for DE and $r = 0.346$, $p = 0.027$ for TEE and for fsTnI $r = 0.445$, $p = 0.005$ for DE and $r = 0.400$, $p = 0.009$ for TEE). As such, this indicates that subjects with higher serum levels of skeletal troponins had higher DE and TEE values. Instead, CK correlated only with TEE ($r = 0.381$, $p = 0.016$) and P0.1 ($r = 0.657$, $p = 0.002$).

Skeletal Troponins as Diaphragmatic Function Predictors

A value of ssTnI > 60.06 pg/mL was found to detect a DE ≥ 10 mm (AUC-ROC = 0.628, 95% CI = 0.551 to 0.696, $p = 0.003$) with sensibility 53 [43–62] % and specificity 72 [60–82] % (**Figure 3**). A value of fsTnI > 46.81 pg/mL was found to detect a DE ≥ 10 mm (AUC-ROC = 0.619, 95% CI = 0.545 to 0.689, $p = 0.004$) with sensibility 38 [29–48] % and specificity 83 [72–90] % (**Figure 4A**). A value of fsTnI > 18.29 pg/mL was found to detect a TFdi ≥ 30% (AUC-ROC = 0.617, 95% CI = 0.541 to 0.688, $p = 0.006$) with sensibility 59 [49–68] % and specificity 67 [55–76] % (**Figure 4B**).

DISCUSSION

In this study we described the trend of circulating skeletal troponin in a population of mechanical ventilated ICU patients during the early phase of weaning from mechanical ventilation. We found that (1) the fast but not the slow isoform of skeletal troponin decreased over time within the first 3 days of weaning, (2) patients with higher levels of ssTnI on day 1 had a higher decrease of diaphragmatic excursion while (3) patients with higher basal fsTnI had higher DE (4) both fsTnI and ssTnI showed a significant positive between-patients correlation with both DE and TFdi while no correlation was found between myoglobin and CPK levels and ultrasound-derived parameters.

Critical illness-associated diaphragm weakness can affect up to 64% of patients within 24 h after intubation (32) and up to 80% of patients requiring prolonged mechanical ventilation (33). However, to the best of our knowledge, no serum biomarker has been suggested to specifically diagnose and monitor the development of respiratory muscles damage. A major limitation of traditional serum biomarkers (i.e., CK, lactate dehydrogenase, myoglobin, AST) is that they have a large normal reference range for healthy subjects (19) and thus, low levels of injury in the individual patient may go undetected. In addition, although these markers are useful for the study of muscle-related diseases, they suffer from a low specificity in detecting solely the damage to skeletal muscles, since an increase of these proteins may also be related to myocardial injury (34).

We hereby describe for the first time the trend of circulating skeletal troponin during the early phase of weaning. We found that the fast skeletal troponin decreased over time during the 3 days of observation but not the slow isoform. The discrepancy between the circulating values of these two isoforms is not new and has been recently found in patients with neuromuscular disease (35). Elevations in both ssTnI and fsTnI have been recorded in severe trauma and ischemia (36), therefore suggesting that the increase of ssTnI into circulation may require muscle injury beyond the damage caused by defective contraction or, in our case, by mechanical ventilation. We might then speculate that an increase in fsTnI could be a marker of muscle overload, rather than a sign of loss of muscular mass caused by ischemia or direct muscular trauma. At the same time, the very decrease of ssTnI below the "normal threshold" could indicate a diaphragm dysfunctionality or muscular mass loss, given the found relationship between DE and ssTnI.

In fact, both fsTnI and ssTnI, but not the other classical markers of muscular damage, were positively correlated with ultrasonographic measurements of diaphragmatic displacement. In particular, subjects with higher levels of these two markers had both higher diaphragmatic excursion and diaphragmatic thickness at end-expiration.

Recently, Dres et al. (37) showed that the use of parasternal intercostal muscle ultrasound was responsive to respiratory load and a greater parasternal intercostal muscle thickening under pressure support ventilation was associated with diaphragm dysfunction. Accordingly, it could be interesting to investigate the relative contribution of parasternal intercostal muscle in relationship of skeletal troponin kinetics.

Our study population seemed to maintain clinical stability over time, with stable levels of mechanical ventilation assistance (i.e., pressure support and PEEP) and no substantial changes in respiratory mechanics and gas exchange. The values of P0.1 (ranging between 1.4 and 1.2) were relatively low and

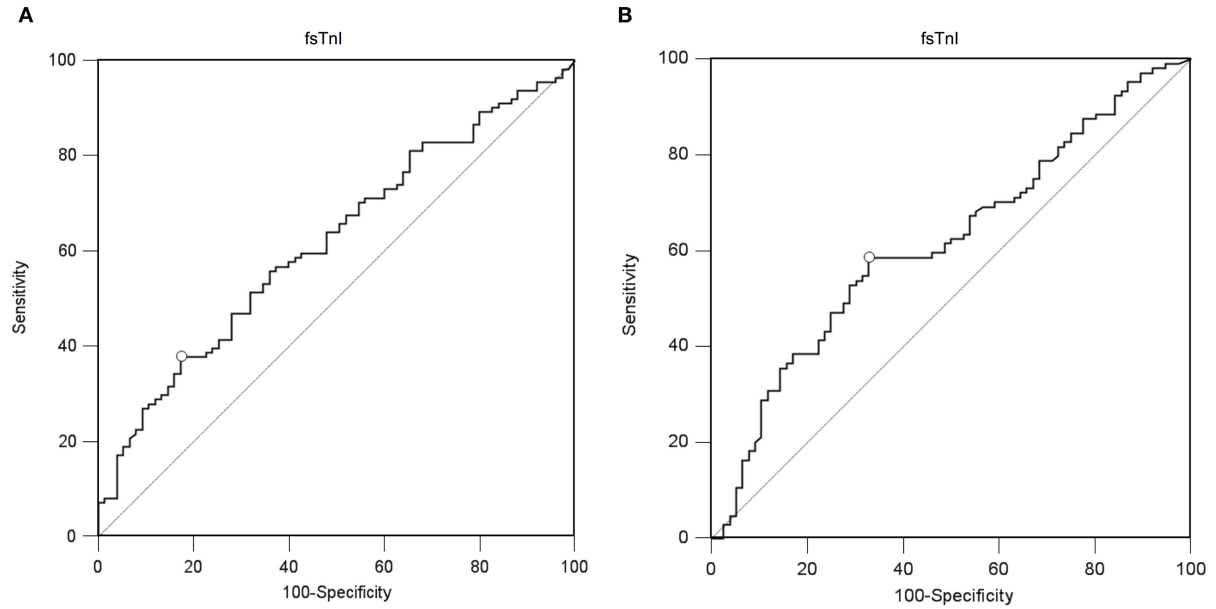

FIGURE 4 | ROC curve analysis for fsTnI as a predictor of normal diaphragmatic function as assessed by diaphragmatic ultrasound (**A**, diaphragmatic excursion ≥ 10 mm, **B**, diaphragmatic thickening fraction ≥ 30%).

both median values of diaphragmatic excursion and thickening fraction were below the thresholds for diaphragmatic weakness (9, 28, 38) indicating that a "diaphragm protective" mechanical ventilation was maintained (39). Nevertheless, we found an increasing trend of patients presenting a diaphragmatic dysfunction, as assessed by ultrasound, over time. These findings prompt the need of a continuous and accurate monitoring of respiratory muscle function during the ICU stay (40).

Diaphragmatic ultrasound is a non-invasive tool to assess the function of the diaphragm, but the technique is time consuming and highly operator dependent. Therefore, dosing fsTnI and ssTnI could potentially increase the number of patients screened for diaphragmatic dysfunction, improve the use of human resources, and allow this assessment also when and where an expert sonographer is not available.

Our study has relevant limitations. First, it is a single-center designed study with a small sample size. Nevertheless, this is a pilot study aiming at describing for the first time the trend of a novel serum marker in a general ICU population of patients recovering from AHRF and our study population might be used as a reference for normal values for a general ICU population during the early phases of their ICU stay. Second, we compared serum skeletal troponin levels only to diaphragmatic dysfunction, without taking into account global muscular mass or performing direct measurements of limb function. However, since the diaphragm is more sensitive to iatrogenic injury caused by MV (12), we decided to focus on this peculiar muscle after a relatively short period of controlled mechanical ventilation (i.e., 1 [1–3] days). Further studies are needed to assess whether the two isoforms of skeletal troponin are associated to systemic muscle wasting, sarcopenia and extradiaphragmatic wasting during ICU stay (41). Third, patients' population (i.e., patients recovering from AHRF from several causes) is representative of a general ICU population, but little is known on patients' diaphragmatic function before ICU admission. Fourth, we have followed patients for a relatively short time compared to their median ICU length of stay (i.e., 13 [8–19] days), the latter issue is related to the need to perform a time consuming, expensive and not readily available analysis for skeletal troponin serum levels. Finally, we have not studied patients both during controlled mechanical ventilation and during unassisted spontaneous breathing. Further studies are needed to assess skeletal troponin trend during the whole respiratory failure treatment, from complete assistance to complete weaning from mechanical ventilation.

CONCLUSIONS

Circulating fast and slow skeletal troponin have specific and different trends in the early phase of weaning from mechanical ventilation and they correlate with ultrasound derived diaphragmatic assessment parameters. The fsTnI decreased during the early phase of weaning while high initial values of ssTnI are associated to a higher decrease of diaphragmatic displacement over time. Further studies are needed to confirm the relationship between these novel biomarkers, protective mechanical ventilation and weaning outcome.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Ferrara. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SS, AT, FD, SG, TB, and CV were involved in the conception and the design of the study, analyzed the data, and wrote the paper. VC, VR, and VA collected the data. FD performed the statistical work. RD, GC, AT, and FD contributed to the analysis of the data, SS, FD, AT, SG, GC, TB, and CV contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.770408/full#supplementary-material

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Right Ventricular Function in Acute Respiratory Distress Syndrome: Impact on Outcome, Respiratory Strategy and Use of Veno-Venous Extracorporeal Membrane Oxygenation

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Acute respiratory distress syndrome (ARDS) is characterized by protein-rich alveolar edema, reduced lung compliance and severe hypoxemia. Despite some evidence of improvements in mortality over recent decades, ARDS remains a major public health problem with 30% 28-day mortality in recent cohorts. Pulmonary vascular dysfunction is one of the pivot points of the pathophysiology of ARDS, resulting in a certain degree of pulmonary hypertension, higher levels of which are associated with morbidity and mortality. Pulmonary hypertension develops as a result of endothelial dysfunction, pulmonary vascular occlusion, increased vascular tone, extrinsic vessel occlusion, and vascular remodeling. This increase in right ventricular (RV) afterload causes uncoupling between the pulmonary circulation and RV function. Without any contractile reserve, the right ventricle has no adaptive reserve mechanism other than dilatation, which is responsible for left ventricular compression, leading to circulatory failure and worsening of oxygen delivery. This state, also called severe acute cor pulmonale (ACP), is responsible for excess mortality. Strategies designed to protect the pulmonary circulation and the right ventricle in ARDS should be the cornerstones of the care and support of patients with the severest disease, in order to improve prognosis, pending stronger evidence. Acute cor pulmonale is associated with higher driving pressure ($\geq$18 cmH_2O), hypercapnia ($PaCO_2 \geq 48$ mmHg), and hypoxemia ($PaO_2/FiO_2 < 150$ mmHg). RV protection should focus on these three preventable factors identified in the last decade. Prone positioning, the setting of positive end-expiratory pressure, and inhaled nitric oxide (INO) can also unload the right ventricle, restore better coupling between the right ventricle and the pulmonary circulation, and correct circulatory failure. When all these strategies are insufficient, extracorporeal membrane oxygenation (ECMO), which improves decarboxylation and oxygenation and enables ultra-protective ventilation by decreasing driving pressure, should be discussed in seeking better control of RV afterload. This review reports the pathophysiology of

pulmonary hypertension in ARDS, describes right heart function, and proposes an RV protective approach, ranging from ventilatory settings and prone positioning to INO and selection of patients potentially eligible for veno-venous extracorporeal membrane oxygenation (VV ECMO).

Keywords: ARDS, right ventricle, VV ECMO, echocardiography, acute cor pulmonale (ACP)

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is characterized by protein-rich alveolar edema, reduced lung compliance and severe hypoxemia (Thompson et al., 2017). Despite some evidence of improvements in mortality over recent decades (Brun-Buisson et al., 2004; Phua et al., 2009) due to better understanding of its pathophysiology and routine application of protective mechanical ventilation, ARDS remains a major public health problem with an approximately 30% 28-day mortality in recent cohorts (Bellani et al., 2016; Combes et al., 2018; Constantin et al., 2019). Pulmonary vascular dysfunction (Snow et al., 1982; Price et al., 2012) is one of the pivot points of the pathophysiology, resulting in a certain degree of pulmonary hypertension, higher levels of which are associated with morbidity and mortality (Bull et al., 2010). The hemodynamic consequences of such remodeling of the pulmonary circulation has led clinicians to pay attention to the right ventricle as the deleterious impact of right ventricular (RV) failure on prognosis is well demonstrated (Mekontso Dessap et al., 2016).

This review reports the pathophysiology of pulmonary hypertension and RV injury, describes RV function, and explains the interest of proposing a RV protective approach to manage ARDS patients, ranging from ventilatory settings and prone positioning to nitric oxide (NO) inhalation and selection of patients potentially eligible for veno-venous extracorporeal membrane oxygenation (VV ECMO) in this context. A few specificities of ARDS-related COVID-19, if any, will be mentioned.

PATHOPHYSIOLOGY OF RIGHT VENTRICULAR INJURY IN ACUTE RESPIRATORY DISTRESS SYNDROME

Right Ventricular Physiology

The right ventricle is composed of the filling chamber and the outflow chamber. Under normal conditions, the right ventricle ejects the blood into the pulmonary circulation, a system of low resistance and high compliance. In contrast to the left ventricle, its isovolumetric contraction pressure is very low and its isovolumetric relaxation is insignificant (Redington et al., 1990): it acts as a passive conduit. This is why its systolic function is sensitive to any increase in pulmonary vascular resistance (PVR) with no adaptation reserve, leading to dysfunction and ultimately to failure. However, the right ventricle is able to adapt to a certain degree of pulmonary hypertension by dilating, due to its high diastolic compliance (Laks et al., 1967).

Pulmonary Vascular Dysfunction

ARDS is characterized by acute onset hypoxemia (ARDS Definition Task Force et al., 2012) with increased pulmonary vascular permeability, leading to non-cardiogenic pulmonary edema (Ashbaugh et al., 1967). Along with alveolar damage, ARDS directly causes injury to the pulmonary circulation, through several pathophysiological mechanisms, involving endothelial dysfunction, distal pulmonary vascular occlusion at the level of the capillaries, pulmonary vasoconstriction, extrinsic vessel occlusion by alveoli distension and ultimately vascular remodeling (Price et al., 2012). All of these phenomena lead to elevation of PVR, pre-capillary pulmonary hypertension and increased RV afterload.

In COVID-19, a certain "protection" of the pulmonary circulation could occur with first the development of pulmonary angiogenesis (Ackermann et al., 2020) and second the virtual absence of hypoxic pulmonary vasoconstriction (Archer et al., 2020). Conversely, proximal obstruction of the pulmonary circulation has been reported to be frequent.

Focus on the Effect of Mechanical Ventilation

Inadequate mechanical ventilation may have a deleterious effect on RV function. During spontaneous breathing in a healthy subject, RV function is optimal with adequate venous return due to negative pleural pressure (Guyton et al., 1957), and RV afterload is limited because of a low transpulmonary pressure (TPP) as lung compliance is normal. In ARDS, a situation where lung compliance is decreased, positive pressure ventilation induces increased TPP at least during tidal ventilation and sometimes during expiration in the case when too high a positive end-expiratory pressure (PEEP) is applied. As a consequence, the pulmonary capillaries are stretched and their caliber reduced, resulting in an increase in PVR (Whittenberger et al., 1960; West et al., 1964). Cyclic increase in PVR during tidal ventilation is responsible for cyclic changes in RV afterload, and then in RV outflow (Vieillard-Baron et al., 1999) eventually leading to pulse pressure variations (**Figure 1**). At the same time, ventilator settings may indirectly impact the pulmonary circulation through changes in PaO_2 and $PaCO_2$, both of which strongly mediate pulmonary vasoconstriction (Yamamoto et al., 2001).

It was suggested at least at the beginning of the COVID-19 pandemic that lung compliance was less decreased than in classical ARDS (Gattinoni et al., 2020), thus potentially inducing less interaction with the pulmonary circulation. This is, however, still questionable.

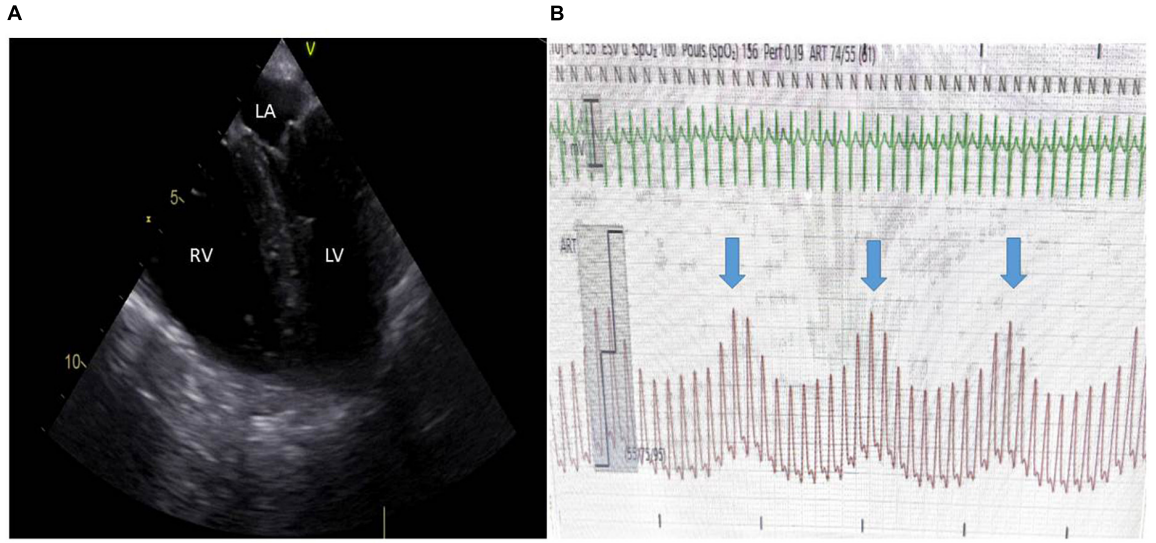

FIGURE 1 | Acute cor pulmonale in a patient ventilated for ARDS and in shock and completely adapted to the respirator. **(A)** A mid-esophageal 4-chamber view demonstrated severe RV dilatation with paradoxical septal motion. **(B)** Invasive low blood pressure with significant pulse pressure variation (blue arrows indicate insufflation) through a radial catheter. Central venous pressure was also elevated. LV, left ventricle; LA, left atrium; RV, right ventricle.

Right Ventricular Failure and Acute Cor Pulmonale

Acute cor pulmonale (ACP) is the last stage of the uncoupling between the right ventricle and the pulmonary circulation. It could be understood, especially in its most severe form, as an RV failure state. RV afterload is suddenly increased, and RV ejection is impaired. In consequence, the right ventricle increases in size. This RV dilatation participates in circulatory failure by compressing the left ventricle (LV) (Scharf et al., 1979). Moreover, in normal conditions, RV and LV systoles occur simultaneously, with the right and left ventricles starting and ending contraction almost at the same time. When RV systole is overloaded, RV contraction is prolonged, so that the right ventricle continues to push after the left ventricle has ended, and the pressure in the RV cavity is then higher than the pressure in the LV cavity during a short instant (Elzinga et al., 1980). This explains the paradoxical septal motion observed in ACP (**Figure 1A**).

ACUTE COR PULMONALE: INCIDENCE, RISK FACTORS, AND IMPACT ON OUTCOME

Prior to the widespread use of protective ventilation, ACP was reported in almost 60% of patients (Jardin et al., 1985). However, all patients were ventilated with high tidal volume and plateau pressure (Pplat) and all patients with severe RV dilatation finally died (Jardin et al., 1985). Since the era of protective ventilation, the incidence of ACP has declined to between 20 and 30% (Vieillard-Baron et al., 2001; Page et al., 2003; Mekontso Dessap et al., 2010, 2016), but may still be as high as 50% in the most severe ARDS (Vieillard-Baron et al., 2007). This leads physicians to take into consideration RV function in management strategies of patients with moderate to severe ARDS.

We still lack convincing data on the incidence of RV failure/ACP in ARDS related to COVID-19. One preliminary study in a very small series of patients reported an incidence of 17% (Evrard et al., 2020). Other studies not only including critically ill patients reported an RV dilatation in 35% of cases (Dweck et al., 2020) or an impact of RV dilatation on ICU transfer or death (Soulat-Dufour et al., 2021). In 90 COVID-19 patients, Bleakley et al. (2021) reported that radial RV dysfunction was common, while the longitudinal function was relatively spared. Micro-occlusive vasculopathy was also reported in COVID-19 by dual energy CT and was more clearly associated with RV dysfunction than the pulmonary embolism obstruction score (Ridge et al., 2020).

The largest study reporting risk factors for developing ACP was performed in 752 patients with moderate to severe ARDS submitted to protective ventilation (Mekontso Dessap et al., 2016). Driving pressure $\geq$ 18 cmH_2O, $PaCO_2 \geq 48$ mmHg, $PaO_2/FiO_2 < 150$ mmHg and pneumonia as causes of ARDS identified patients at risk of ACP. Incidence of ACP ranged from less than 10% when only one risk factor was present to close to 60% with 3–4 risk factors (Mekontso Dessap et al., 2016). Interestingly, neither Pplat nor PEEP was reported as a potential risk factor. An explanation could be that a low PEEP was homogenously applied (mean 8 cmH_2O) and Pplat was maintained below 27 cmH_2O in most patients. In other conditions, they both may affect pulmonary circulation and RV function. A high Pplat is associated with RV failure, especially when it reflects high TPP (Vieillard-Baron et al., 1999). The "safe Pplat" for the right ventricle was suggested to be below 27 cmH_2O (Jardin and Vieillard-Baron, 2007). Pplat is not always a surrogate of lung stress, because it reflects the compliance of

the respiratory system (Gattinoni et al., 2004; Chiumello et al., 2008) and chest wall compliance must be taken into account, especially in obese patients. Monitoring of pleural pressure with an esophageal balloon could be of value in these patients, while data are missing. This could also be a specificity of COVID-19 patients who could tolerate higher Pplat, as many patients are obese and the association between Pplat and outcome in this subpopulation is unclear (De Jong et al., 2018).

The potential effect of PEEP on RV function is more questionable. Because of the opposite effect of lung distension on intra- and extra-alveolar pulmonary blood vessels, the relationship between lung distension and PVR is U-shaped (Whittenberger et al., 1960). Thus, the choice of the level of PEEP set by the clinician can directly affect the RV afterload because poor lung aeration on one side and alveolar overdistension on the other side can both raise PVR. In an experimental study, RV function was impaired when the lung was de-recruited and normalized after re-aeration (Duggan et al., 2003). As a matter of fact, lung CT-scan has shown a low amount of potentially recruitable lung (and so a high potential for overdistension) in most ARDS patients (Gattinoni et al., 2006) and a high PEEP was shown to induce hemodynamic instability more frequently in a randomized controlled trial, while no information was given on RV function, which was associated with worse outcome [Writing Group for the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial (ART) Investigators, 2017]. Despite a strict limitation of Pplat, a PEEP of 15 cmH_2O produced a significant increase in PVR associated with a decrease in cardiac output (Schmitt et al., 2001). The reasonable goal of PEEP is then to reach a balance between enough recruitment and no or minimal overdistension. In other words, the goal is to set the best PEEP to recruit the zones of the collapsed lung, which typically characterize ARDS (Puybasset et al., 2000), without inducing alveolar dead space. Nowadays, no definitive manner to determine the best PEEP is available, but RV function evaluation can be used as a monitoring parameter to avoid PEEP resulting in too much overdistension.

As briefly discussed above, hypercapnia induces pulmonary vasoconstriction (Kiely et al., 1996). Hypercapnia is the consequence of respiratory strategy, i.e., protective ventilation designed to reduce ventilator-induced lung injury, but which also reflects the severity of ARDS (Nuckton et al., 2002).

Finally, one of the prognostic factors in ARDS is hemodynamic instability. And RV failure is one of its mechanisms. While still debatable, many arguments suggest that pulmonary vascular dysfunction and RV failure/ACP could thus have a negative impact on in-hospital mortality (Bull et al., 2010; Mekontso Dessap et al., 2016). This leads to discussion of the potential interest of an RV protective ventilation strategy.

EVALUATION OF RIGHT VENTRICULAR FUNCTION AT THE BEDSIDE

Historically, a pulmonary arterial catheter has been used to evaluate RV function at the bedside. Some of the key elements of monitoring proposed were as follows: low cardiac output, right atrial pressure (RAP) higher than pulmonary artery occlusion pressure (PAOP), and pulmonary hypertension. Recently, the so-called transpulmonary gradient, i.e., the difference between mean pulmonary artery pressure and PAOP, was reported to be frequently abnormally increased and associated with outcome (Bull et al., 2010). However, use of a pulmonary arterial catheter has progressively declined and critical care echocardiography has been progressively implemented and performed in ARDS (Dres et al., 2018). Echocardiographic definition of RV injury is still challenging but in ARDS ACP or severe RV dilatation accurately reflects RV failure, especially when RAP is elevated (Vieillard-Baron et al., 2018). It is recommended by experts in the field to monitor RAP and invasive blood pressure and to perform echocardiography (Vieillard-Baron et al., 2016).

RIGHT VENTRICULAR PROTECTIVE STRATEGY

The main "rules" for protecting the RV in ARDS, by means of avoiding or correcting RV failure, are reported in **Figure 2**. While in our usual practice, we apply systematic daily evaluation of RV function by echocardiography in ARDS patient, **Figure 2** also allows to reemphasize that when echocardiography is not so easily available, pulse pressure variation should be understood as a marker of a deleterious interaction between the RV and the ventilator and then requires further hemodynamic evaluation by echocardiography.

Ventilatory Strategy

As largely discussed above in the physiological rationale, Pplat should be maintained below 27 cmH_2O, and permissive hypercapnia should be limited by careful increase in respiratory rate and by replacing the heat and moisture exchanger by a heated humidifier. Oxygenation should also be increased without too much PEEP (Vieillard-Baron et al., 2013), with a view to optimizing arterial oxygen delivery rather than PaO_2/FiO_2. Indeed, it has long been known that increased PEEP may improve oxygenation but reduce oxygen delivery because of its potential negative hemodynamic effect (Kumar et al., 1970).

Prone Positioning

In the most severe ARDS, it is unlikely that all of the predefined goals of an RV protective approach will be reached. In this situation, prone positioning has been reported to efficiently unload the right ventricle (Vieillard-Baron et al., 2007). It improves oxygenation without increasing PEEP and decreases hypercapnia and Pplat due to lung recruitment of the dependent areas of the lung without overdistension of the non-dependent areas (Guérin et al., 2020), rendering lung ventilation more homogeneous.

To optimize hemodynamic improvement, prone positioning should be performed without chest support, which may be responsible for a decrease in systemic venous return and cardiac output due to excessive elevation of intra-thoracic pressure (Chiumello et al., 2006; Brown et al., 2013).

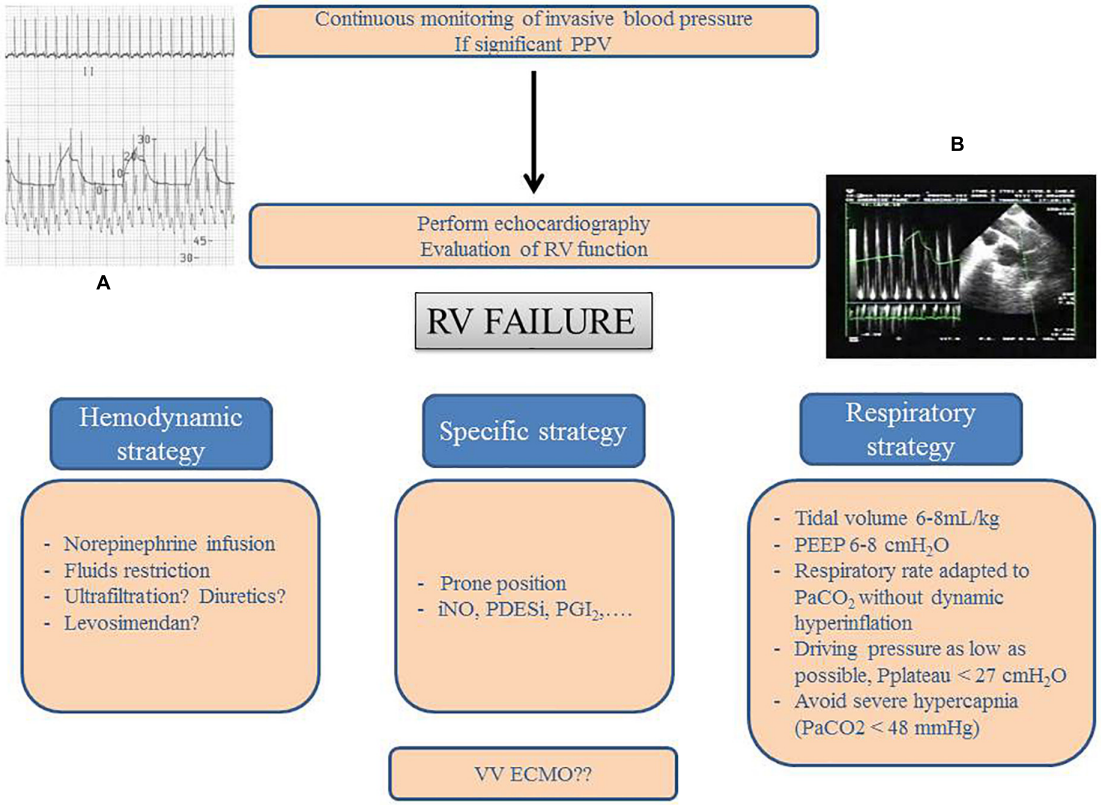

FIGURE 2 | Right ventricle protective strategy. Principles for detection and management of right ventricular (RV) failure in patients with ARDS. This should combine invasive blood pressure monitoring and echocardiography. In the case of significant pulse pressure variation (PPV, Panel **A**), critical care echocardiography must be performed. It usually demonstrates cyclic decrease in RV outflow at each insufflation (Panel **B** on an upper esophageal view with pulsed wave Doppler into the main pulmonary artery) with either isolated RV dilatation or acute cor pulmonale. Management is based on three different strategies: hemodynamic, respiratory, and specific. VV ECMO can be considered in the case of persistent RV failure. PEEP, positive end-expiratory pressure; iNO, inhaled nitric oxide; PDESi, phosphodiesterase type 5 inhibitor; PGI_2, prostaglandin I_2; VV ECMO, veno-venous extracorporeal membrane oxygenation.

Hemodynamic Support and Nitric Oxide Inhalation

When RV failure induces circulatory failure, hemodynamic support is based on two key principles: (i) strongly limit fluid expansion and (ii) restore blood pressure.

Fluid expansion may by itself induce RV failure (Patterson and Starling, 1914) and increase RAP and systemic congestion, leading to acute kidney injury (Chen et al., 2016, 2017). Moreover, it is very unlikely that fluid expansion increases cardiac output, even though significant pulse pressure variation, a marker of LV preload dependency, is observed (Vieillard-Baron et al., 2016; **Figure 1**). Correction of blood pressure by infusion of catecholamines helps improve RV function. In other experimental models of RV failure-related pulmonary circulation obstruction, norepinephrine decreases RV wall stress and RV end-diastolic pressure and improves RV stroke volume, unlike fluid expansion (Ghignone et al., 1984). One of supposed mechanisms is that norepinephrine corrects the functional RV ischemia induced by high RV wall stress combined with low blood pressure (Guyton et al., 1954; Vlahakes et al., 1981). The same observation was made in lung injury (Prewitt and Ghignone, 1983; Vieillard-Baron et al., 2003). In the case of associated LV systolic dysfunction, as observed in ARDS-related septic shock, dobutamine acting on both ventricles may be preferred, though there is no study supporting this approach.

Levosimendan is another inotropic drug called inodilator, acting *via* troponin C calcium binding. It was proposed when there is uncoupling between the right ventricle and the pulmonary circulation. This is strongly physiologically based in ARDS, but only one pilot study suggests an improvement in RV performance in ARDS patients (Morelli et al., 2006). Due to the potential side effects of levosimendan, more data are needed before making any recommendation.

Nitric oxide inhalation has nowadays been abandoned in ARDS after studies and meta-analyzes reported no beneficial effect on outcome (Gebistorf et al., 2016). However, the use of NO for a hemodynamic indication in a subgroup of patients with refractory RV failure despite respiratory optimization has never been evaluated. NO inhalation has been found to significantly decrease RV afterload in ARDS, especially in the case of hypercapnia (Puybasset et al., 2000).

In COVID-19-related ARDS, NO inhalation has been poorly studied, but the rationale is not strongly favored due to the virtual absence of hypoxic vasoconstriction. A few studies have reported an improvement in oxygenation (Longobardo et al., 2021; Robba et al., 2021), especially when cardiac biomarkers were elevated (Garfield et al., 2021), but no association was reported with RV function improvement (Bagate et al., 2020). However, the subgroup of patients with RV failure was not specially studied. In the absence of clear evidence, NO inhalation could be initiate when RV failure is persistent despite RV protective ventilator strategy or when prone position is contraindicated.

Veno-Venous Extracorporeal Membrane Oxygenation

The EOLIA trial suggested that ECMO could be effective in some of the most severe cases of ARDS, but failed to demonstrate a 20% increase in survival (Combes et al., 2018). One of the reasons, despite the non-negligible proportion of crossover between control patients and ECMO patients, could be that criteria for selecting eligible patients were mainly based on blood gas analysis, as proposed by the Berlin classification (Ferguson et al., 2012). By easily controlling blood oxygenation and decarboxylation (Schmidt et al., 2013), VV ECMO suppresses two of the major factors of raised PVR in ARDS and could then be sufficient to unload the right ventricle without the use of veno-arterial (VA) ECMO (Miranda et al., 2015). VV ECMO could also promote ultra-protective ventilation which could benefit the right ventricle by a more pronounced reduction of Pplat and driving pressure (Schmidt et al., 2019). Considering the inevitable complications of VV ECMO, including severe bleeding (Combes et al., 2018), better selection of patients is essential. How this subgroup of patients with severe ARDS and RV failure could be considered as the ideal target remains to be evaluated, while a recent pilot study showed in a non-selected echocardiographic cohort of severe ARDS patients fulfilling the EOLIA criteria that driving pressure and RV failure were the only two factors associated with ICU mortality, in contrast to classical severity markers in ARDS (Petit et al., 2021). Pre-ECMO implantation RV dysfunction is not rare and has an approximately 30% incidence of RV dilatation (Lazzeri et al., 2018).

Another potential technique to support the right ventricle is extracorporeal CO_2 removal. Data are too scarce for discussion of any recommendation (Papazian et al., 2019), but an experimental study in a porcine model of ARDS showed that CO_2 removal is able to decrease RV afterload and to improve coupling between the right ventricle and the pulmonary circulation (Morimont et al., 2015). Such a technique could be efficient and valuable in protecting the right ventricle in the case of severe ARDS with significant hypercapnia and RV failure despite application of an RV protective strategy, but probably does not promote ultraprotective ventilation in patients with moderate ARDS (McNamee et al., 2021).

CONCLUSION

Considering recent studies, RV failure in ARDS with its impact on outcome is now well recognized, as are its risk factors. Many studies suggest that to optimize respiratory settings it is essential to monitor RV function, while clinical impact of such a strategy on the outcome remains unclear. The RV protective approach should be prospectively evaluated in the future to improve the prognosis of the most seriously ill patients. ECMO could be part of this strategy in the most extreme situations.

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

MP, EJ, and AV-B wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Gene Therapy for Acute Respiratory Distress Syndrome

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Acute respiratory distress syndrome (ARDS) is a devastating clinical syndrome that leads to acute respiratory failure and accounts for over 70,000 deaths per year in the United States alone, even prior to the COVID-19 pandemic. While its molecular details have been teased apart and its pathophysiology largely established over the past 30 years, relatively few pharmacological advances in treatment have been made based on this knowledge. Indeed, mortality remains very close to what it was 30 years ago. As an alternative to traditional pharmacological approaches, gene therapy offers a highly controlled and targeted strategy to treat the disease at the molecular level. Although there is no single gene or combination of genes responsible for ARDS, there are a number of genes that can be targeted for upregulation or downregulation that could alleviate many of the symptoms and address the underlying mechanisms of this syndrome. This review will focus on the pathophysiology of ARDS and how gene therapy has been used for prevention and treatment. Strategies for gene delivery to the lung, such as barriers encountered during gene transfer, specific classes of genes that have been targeted, and the outcomes of these approaches on ARDS pathogenesis and resolution will be discussed.

Keywords: viral vectors, non-viral vectors, sepsis, acute lung injury, electroporation, alveolar fluid clearance, barrier function

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INTRODUCTION

Acute respiratory distress syndrome (ARDS) is a devastating clinical syndrome that leads to acute respiratory failure (Ware, 2006; Thompson et al., 2017; Matthay et al., 2019). ARDS can be directly caused by bacterial or viral infection of or chemical damage to the lung, or indirect due to injuries outside the lung or systemic inflammatory response, such as non-pulmonary sepsis, blood transfusions, and non-pulmonary injury (Pelosi et al., 2003; Rezoagli et al., 2017). In all cases, bacterial or viral infection is most commonly seen clinically. Most recently, the global pandemic of the coronavirus disease-2019 (COVID-19) has caused a high number of severe ARDS cases in the United States and around the world. As of October 24, 2021, there have been more than 45 million COVID 19 cases and more than 733,000 deaths in the United States (CDC, 2021). From multiple studies, approximately 33% of hospitalized COVID-19 patients develop ARDS, and there is a ∼70% mortality rate for COVID-19 patient-associated ARDS (Hasan et al., 2020; Tzotzos et al., 2020). The incidence of ARDS among non-survivors of COVID-19 is even higher, up to 90%, indicating that ARDS accounts for the majority of COVID-19 deaths (Tzotzos et al., 2020). Although the molecular mechanisms regarding the pathogenesis and progress of ARDS have been studied for decades,

the development of effective treatments has lagged, and clinical management strategies still rely on supportive care, broad activity pharmacological treatment, ventilation, prone positioning, and other supportive strategies (Matthay et al., 2020). This review will focus on the pathophysiological features of ARDS and summarize the state of gene therapy treatments for ARDS.

CELLULAR AND MOLECULAR MECHANISMS OF ACUTE RESPIRATORY DISTRESS SYNDROME

The key features of ARDS are pulmonary edema of noncardiogenic origin and pathologic diffuse alveolar damage (DAD; Thompson et al., 2017; Matthay et al., 2019), which is primarily caused by alveolar capillary barrier dysfunction and the resulting flooding of alveoli and lung interstitial space with protein-rich fluid (Matthay et al., 2019). The clinical hallmarks of ARDS include refractory hypoxemia due to insufficient gas exchange, fluffy bilateral infiltrates on X-ray radiographs, decreased lung compliance due to alveolar collapse and edema, and increased physiological dead space fraction due to lung microvascular destruction (Lewis and Jobe, 1993; Ware and Matthay, 2000; Nuckton et al., 2002; Matthay and Zemans, 2011). Although ARDS is defined by its pulmonary versus extrapulmonary origin, lung mechanical dysfunction is etiologically independent (Menezes et al., 2005). The observed edema and inflammation in both direct and indirect injured lungs indicate several significant events, namely, alveolar capillary barrier dysfunction, impaired alveolar fluid resolution, and uncontrolled neutrophil activation, sequestration, and their metabolite-mediated inflammatory responses (Ware and Matthay, 2001; Ware, 2006; Matthay and Zemans, 2011; Herrero et al., 2018). Perhaps the best illustration of these mechanisms remains the classic figure of the healthy and injured alveoli introduced by Lorraine Ware and Michael Matthay in 2000, although a relatively recent update incorporates several features now realized to be central to ARDS pathogenesis (**Figure 1**; Ware and Matthay, 2000). Understanding the mechanisms behind these events would provide insights to identify therapeutic targets for ARDS gene therapy.

Alveolar-Capillary Barrier Dysfunction

One early event during the exudative phase that defines ARDS is the accumulation of pulmonary edema, the major factor causing hypoxemia (**Box 1**; Bhattacharya and Matthay, 2013; Matthay et al., 2019). In the normal lung, fluid homeostasis is maintained by microvascular filtration, which provides the fluid source, and lymphatic clearance, which drains the filtrate flow away (Bhattacharya and Matthay, 2013). Vascular endothelial permeability is the determining factor for microvascular filtration (Staub, 1978; Mehta and Malik, 2006). It shows selectivity to sieve large protein molecules (e.g., albumin) in the plasma (Siflinger-Birnboim et al., 1987; Mehta and Malik, 2006; Sukriti et al., 2014), creating high osmotic pressure to encounter the pro-filtration force (hydrostatic pressure) and promote water retention in the circulation (Bhattacharya and Matthay, 2013). Besides the lymphatic system, the filtrate also flows into alveoli forming a protective liquid layer with surfactant (Lindert et al., 2007). Alveolar fluid balance is mainly determined by epithelial integrity, which provides a tight barrier preventing fluid influx and alveolar fluid clearance (AFC), which drives water out of the alveolar space based on the Na^+ osmotic gradient (Matthay et al., 2002; Johnson et al., 2006; Bhattacharya and Matthay, 2013; Herrero et al., 2018). The importance of the alveolar-capillary barrier and AFC can be reflected from the cardiogenic lung edema, which shows much less protein content (Sprung et al., 1981; Ware et al., 2010), and could be quickly resolved because of the relatively intact alveolar epithelial and capillary endothelial integrity and fluid clearance capacity (Matthay, 2014; Hamacher et al., 2018). However, in ARDS, accumulated pulmonary edema results from the loss of the alveolar-capillary barrier, hyperpermeability, and impaired AFC, allowing the influx of fluid and large amounts of proteins to accumulate in the interstitial and alveolar spaces (Ware and Matthay, 2000, 2001; Matthay et al., 2012; Matthay, 2014). In patients with ARDS, various endothelial injury markers, e.g., von Willebrand factor (VWF; Ware et al., 2004), can be detected in blood and epithelial apoptotic markers, e.g., cytokeratin-18 (Lee et al., 2008; Galani et al., 2010), and can be measured in bronchoalveolar lavage (BAL) fluid. Clinically, the decreased AFC capacity is associated with prolonged acute respiratory failure and higher mortality rate (Ware and Matthay, 2001); and the degree of injury to the alveolar epithelium appears to be a determinant of the severity of ARDS (Matthay et al., 2005; Yanagi et al., 2015). Multiple mechanisms are involved in alveolar-capillary barrier and AFC dysfunction, such as cell death, loss of cell-cell adhesion molecules and ion transporter activities, and activation of neutrophils and their products. Apoptosis induces alveolar epithelial cell death, and many studies show that apoptosis is the main route of cell death detectable immediately following lung injury (Albertine et al., 2002; Martin et al., 2003; Tang et al., 2008; Perl et al., 2010; Herrero et al., 2013; Jagrosse et al., 2019). In addition, cellular necrosis is also seen in patients with ARDS (Tomashefski, 2000; Cardinal-Fernández et al., 2017). Many studies have shown that Fas/Fas ligand (FasL) extrinsic pathway-mediated apoptosis contributes to ARDS (Herrero et al., 2013). The Fas/FasL system is significantly upregulated in the pulmonary edema fluid of patients with ARDS and is associated with increased mortality (Matute-Bello et al., 1999; Albertine et al., 2002). In animal models of ARDS, there is increased expression of Fas in epithelial cells and FasL in BAL (Fine et al., 1997; Hamann et al., 1998; Perl et al., 2007). A study using chimeric mice expressing Fas receptor exclusively on non-myeloid cells, including lung epithelial cells, demonstrated that lung injury is primarily via the activation of the pro-apoptotic pathway in alveolar epithelial cellsand is associated with increased alveolar permeability and edema formation (Matute-Bello et al., 2005a). Besides the Fas/FasL-mediated extrinsic apoptotic pathway, Bcl-2-mediated intrinsic apoptosis is also involved, which might be relevant to the mitochondrial dysfunction seen in this disease (Tang et al., 2008). During ARDS, cell death is greatly due to multiple cellular dysfunctions: elevated CO_2 levels and oxidative imbalance lead to mitochondrial DNA damage and cell toxicity (Herold et al., 2013), ventilator-induced

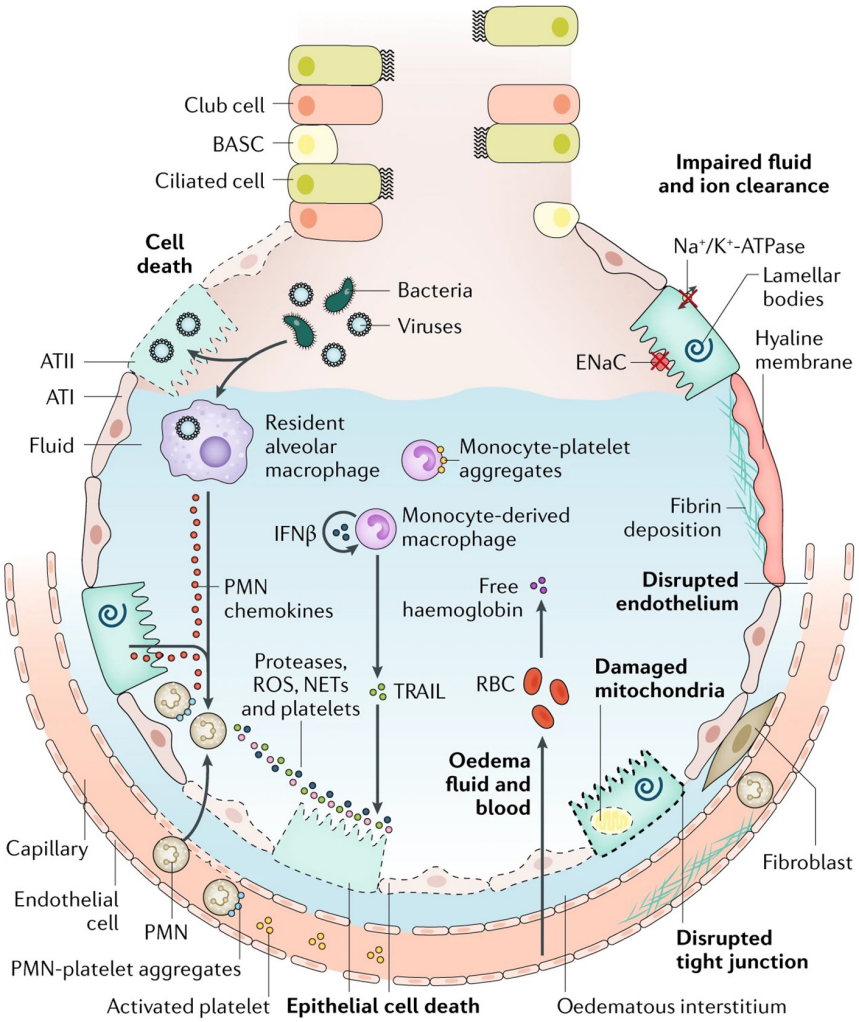

FIGURE 1 | Injured alveolus in the acute phase of lung injury and acute respiratory distress syndrome. A variety of insults (such as acid, viruses, ventilator-associated lung injury, hyperoxia, and bacteria) can injure the epithelium, either directly or by inducing inflammation, which in turn injures the epithelium. Direct injury is inevitably exacerbated by a secondary wave of inflammatory injury. Activation of toll-like receptors (not shown) on alveolar type II (ATII) cells and resident macrophages induces the secretion of chemokines, which recruit circulating immune cells into the airspaces. As neutrophils migrate across the epithelium, they release toxic mediators, such as proteases, reactive oxygen species (ROS), and neutrophil extracellular traps (NETs), which have an important role in host defense but cause endothelial and epithelial injury. Monocytes also migrate into the lungs and can cause an injury, such as epithelial cell apoptosis *via* IFNβ-dependent release of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), which activates death receptors. Activated platelets form aggregates with polymorphonuclear (PMN) leukocytes, which are involved in NET formation, and monocyte–platelet aggregates. Red blood cells (RBCs) release cell-free hemoglobin, which exacerbates injury *via* oxidant-dependent mechanisms. Angiopoietin 2 inhibits TIE2-stabilization of vascular endothelial cadherin (VE-cadherin); vascular endothelial growth factor and other permeability-promoting agonists also destabilize VE-cadherin *via* dissociation from p120-catenin, resulting in its internalization and enhanced paracellular permeability. Additionally, loss of cell–cell adhesion in the setting of actomyosin contraction results in the formation of occasional gaps between endothelial cells. Epithelial injury also includes wounding of the plasma membrane, which can be induced by bacterial pore-forming toxins or mechanical stretch, and mitochondrial dysfunction. Together, these effects result in endothelial and epithelial permeability, which further facilitates the transmigration of leukocytes and leads to the influx of edematous fluid and RBCs. Airspace filling with edematous fluid causes hypoxemia, resulting in the need for mechanical ventilation. Vascular injury and alveolar edema contribute to the decreased ability to excrete CO_2 (hypercapnia), accounting for the elevated pulmonary dead space in acute respiratory distress syndrome. In turn, hypoxemia and hypercapnia impair vectorial sodium transport, reducing alveolar edema clearance. ATI, alveolar type I cell; BASC, bronchioalveolar stem cell; ENaC, epithelial sodium channel. Reproduced with permission (Ware and Matthay, 2000).

mechanical stress (Syrkina et al., 2008), hypoxia (Tang et al., 2008), NO formation by iNOS (Rudkowski et al., 2004), and LPS-activated apoptotic signaling (Zeng et al., 2018). Since gene therapy requires living cells as targets in order for any gene expression to take place, the level of apoptotic or necrotic cells can have a profound effect on any such treatment. Thus, in order for gene therapy to be effective, the level of cell death needs to be relatively low or the treatment must be administered prior to significant cell loss.

The alveolar capillary barrier is composed of two physical barriers: a tight alveolar epithelial monolayer of flat ATI cells (95% of alveolar surface area) and cuboidal ATII cells (5%

BOX 1 | Mechanisms for edema formation.

I) **Alveolar-capillary barrier dysfunction**

- **Epithelial and endothelial cell injury markers:** Fas/FasL, RAGE (epithelial) VWF, Angiopoietin 2 (endothelial)
- **Cell death:** apoptosis; necrosis
- **Adhesion junction disruption and downregulation:** Proteolytic degradation: ZO-1; VE-cadherin; E-cadherin Claudin-4,5,18 Phosphorylation and internalization: occludin; VE-cadherin
- **Inducing factors:** oxidative stress; ROS; ventilation; mitochondrial damage NETs; cytokines; LPS; virus

II) **Alveolar fluid clearance dysfunction**

- **Decreased cell membrane abundance of Na, K, ENaC** hypoxia; ROS; IAV; IFNs; TRAIL; coagulation proteases; decreased adrenergic stimulation
- **Decreased mRNA and protein level of Na, k, ENaC, CFTR** hypoxia; IL-lb; TGFbl; TNFa; oxidant; INFr
- **Decreased transepithelial ion transport activities** loss of epithelial polarity reduced channel open probability

III) **PMN activation and inflammation mediators**

- **Increased PMN recruitment**
- **NETosis induced lung barrier disruption** DNA; histone; MPO; NE
- **extracellular matrix modeling** increased MM P-2,9
- **Increased cytokine level in plasma and BAL fluid** IL-lb, TNFa, IL-6 and IL-8

of alveolar surface area), and a relatively more permeable microvascular endothelial cell monolayer (Bhattacharya and Matthay, 2013; Knudsen and Ochs, 2018; Katz et al., 2019). A study measuring (Matthay and Zimmerman, 2005) I-labeled albumin flux in blood, surrounding interstitium, and alveolar space in sheep lungs indicates that more than 92% of resistance to albumin flux across the alveolar capillary barrier lies in the epithelial barrier (Gorin and Stewart, 1979). Adhesion molecules holding together neighboring cells in the monolayer are the major structural components regulating the paracellular permeability pathway, the major route for passage of large molecules, such as albumin, across both barriers (Mehta and Malik, 2006; Overgaard et al., 2012; Bhattacharya and Matthay, 2013). Tight junctions (TJs) are located in the most apical side of the alveolar epithelium and largely determine barrier tightness (Overgaard et al., 2012; Gunzel and Yu, 2013). Damage to TJs greatly contributes to epithelial barrier leakage, further increasing edema accumulation without necessary cell death (Hook et al., 2018; Matthay et al., 2019). Claudins, key tight junction proteins, are highly expressed in the alveolar epithelium with the predominant isoforms being claudin-3, 4, and 18 (Overgaard et al., 2012). Knockout (KO) of claudin18 in mice shows significant accumulation of FITC-albumin in the BAL 4 h after intraperitoneal instillation of labeled tracer, indicating increased alveolar epithelial permeability (LaFemina et al., 2014). Morphological disruption of this barrier is further confirmed by ultrastructural analysis of ATI and ATII cells (Bachofen and Weibel, 1977, 1982). In cultured alveolar epithelial cells, silencing of claudin 18 by siRNA shows decreased transepithelial electrical resistance (TEER), a measurement of epithelial tightness *in vitro*, and increased permeability to small size tracer markers (LaFemina et al., 2014; Srinivasan et al., 2015). Similarly, overexpression of claudin 4 increases TEER by nearly 50% (Mitchell et al., 2011); KO of claudin 4 in mice not only increases barrier permeability to solute but also, surprisingly, decreases AFC, which might be relevant to decrease in Na^+, K^+-ATPase activity (Wray et al., 2009; Overgaard et al., 2012). The promotion of AFC by claudin 4 in the alveolar epithelium is also indicated by the property of Cl^--selective paracellular permeability, since claudin4 limits paracellular Na flux but favors transepithelial Cl^- transport for electrical neutrality during Na^+ active transport for fluid clearance (Colegio et al., 2002, 2003). Occludin is another important transmembrane a TJ molecule expresses in both the epithelium and endothelium (Förster, 2008). The internalization and phosphorylation of occludin are associated with lung barrier dysfunction (Hirase et al., 2001; Förster, 2008). TJ proteins are targets of numerous factors during ARDS, namely, excessive ROS (Rao, 2008), pathogens (Lu et al., 2014), e.g., LPS, viruses and bacteria, ventilation (Liu et al., 2014), inflammatory mediators (Al-Sadi et al., 2009), hypoxia (Caraballo et al., 2011), hyperoxia (You et al., 2012), and inhaled particulate matter (PM; Wang et al., 2012).

Endothelial dysfunction is another important contributor to alveolar capillary barrier disruption, leading to uncontrolled extravascular fluid leakage. The microvascular endothelium is the first barrier encountered by fluid and neutrophils infiltrating from vessels into the alveoli. Under normal conditions, the endothelium is more permeable to large macromolecules than the alveolar epithelium. However, upon activation by pathogens, e.g., LPS-containing bacteria, the endothelial barrier properties are altered by a series of events, such as structural damage to the endothelial barrier, significant proinflammatory response, coagulation and micro-thrombosis formation, and vascular tone dysregulation (Vassiliou et al., 2020).

Endothelial hyperpermeability can directly result from structural damage to the endothelial barrier, through both endothelial cell apoptosis and inter-endothelial cell junctional complex disruption. For the former, mitochondrial DNA (mtDNA) damage may initiate endothelial cell death (Ruchko et al., 2005). Mitochondria are a major source of ROS in the endothelium (Ince et al., 2016). Under oxidative stress, mtDNA released from mitochondria, in turn, triggers mitochondrial dysfunction and induces apoptosis through cytochrome c and the intrinsic apoptosis pathway. Circulating mtDNA has been reported as a plasma biomarker for the severity of sepsis or sepsis-related ARDS with higher plasma cell-free mtDNA levels observed in ICU patients who died within 28 days of medical ICU admission, as well as in ICU patients with sepsis or ARDS (Nakahira et al., 2014). Receptor agonism-initiated extrinsic apoptosis also contributes to endothelial cell death. Tumor necrosis factor alpha (TNFα) receptor and Fas are also expressed in endothelial cells, and their activation has been shown to induce caspases 8 and 3 signaling, resulting in apoptosis (Hotchkiss et al., 2002). Recently, more types of cell death programs have been identified in dysfunctional endothelium during sepsis, such as necrosis and pyroptosis (Singla and Machado, 2018). Pyroptosis is of particular interest in sepsis, since it is triggered

by proinflammatory signals and is vital for endothelial injury when overactivated (Gao et al., 2018). Knockout of one of key pyroptosis mediators, such as caspase 1 or 11, displays resistance to endotoxic shock in mice and provides increased survival and protection against vascular injury, endothelial hyperpermeability, lung edema, and histological damage (Li et al., 1995; Cheng et al., 2017; Mitra et al., 2018).

The endothelial paracellular pathway is the major filtration route of the microvasculature, and disruption of inter-endothelial junction structures may account for another mechanism of barrier hyperpermeability (Bhattacharya and Matthay, 2013). Inter-endothelial junction molecules include tight junctions, adherens junctions, junctional adhesion molecules (JAMs), and other endothelial specific molecules, such as platelet endothelial cell adhesion molecules (PECAMs; Komarova and Malik, 2010). In contrast to the alveolar epithelium where tight junctions play a major role in the integrity of epithelial barrier function, in the capillary endothelium, tight junctions are secondary, while adherens junctions play a more significant role (Mehta and Malik, 2006; Aird, 2007). Vascular endothelial cadherin (VE-cadherin), a key component of the endothelial adherens junction, primarily maintains the architectural integrity of the endothelial barrier, rendering high permeability to plasma proteins, a key property in establishing protein (e.g., albumin) gradients for fluid balance in the lungs. Claudin-5 is the predominant tight junction molecule expressed in pulmonary endothelial cells (Kaarteenaho-Wiik and Soini, 2009) and has been found to be downregulated in various models of ALI, such as influenza infection (Armstrong et al., 2012). Pathogen-induced VE-cadherin phosphorylation, internalization, and lysosomal degradation are major forms of VE-cadherin disruption (Chan et al., 2020). All of these junctional molecules are also targets of oxidative stress, ROS, IL-1β and other stimuli (Xiong et al., 2020). In addition, some sepsis mediators, e.g., high-mobility group protein B1, have been shown to activate acto-myosin contraction, inducing endothelial cell retraction, which mechanically breaks apart adhesion junctions, leading to hyperpermeability (Wolfson et al., 2011). Such cytoskeletal contraction due to phosphorylation of myosin light chain (MLC) is a common cause of endothelial cell retraction, and signaling pathways involved in the activation of MLC have been extensively studied and shown to be mediated through a number of pathways, most notably RhoA/ROCK (Reutershan et al., 2007). Furthermore, influx of Ca2+ has been shown to increase vascular permeability and allow for migration of neutrophils across the alveolocapillary barrier, as well as overall vascular leakage (Alvarez et al., 2006).

Glycocalyx shedding has been recognized in recent years as another crucial mechanism undermining endothelial barrier integrity, leading to edema formation and sepsis-induced organ failure. The glycocalyx is a thin multicomponent fibrous matrix layer lining the luminal endothelial surface, which includes proteoglycans, glycoproteins, and glycosaminoglycans that protect the vascular endothelium from oxidants, hyperglycemia, cytokines, and bacterial endotoxins (Weinbaum et al., 2007; Ince et al., 2016). However, these toxins, in turn, often induce glycocalyx degradation, and that layer becomes thinner as a result of and during sepsis. Glycocalyx fragments, such as syndecan-1, shed into the blood and have been reported as potential clinical biomarkers for sepsis survival and respiratory failure (Smart et al., 2018). Bacteria, TNF-α, and ROS all induce degradation of the glycocalyx layer, making the endothelial lining more vulnerable to pathogens and leading to barrier disruption and protein-rich extravascular fluid leakage. That the glycocalyx provides such a protective layer to the endothelium is exemplified by the fact that Crocin, a chemical compound, has been shown to prevent LPS-induced ARDS by protecting against glycocalyx degradation (Zhang et al., 2020). Glycocalyx shedding also decreases the sensitivity of endothelial cell responses to sheer stress, leading to unbalanced release of nitric oxide and vascular tone dysregulation (Ince et al., 2016). In addition, glycocalyx shedding may exacerbate endothelial proinflammatory response by promoting neutrophil adhesion to endothelial cells.

Neutrophil activation and transmigration from the circulation into lung tissue are perhaps the most significant events of the proinflammatory response during the early stage of ALI. However, excessive neutrophil activation induces endothelial barrier damage and, ultimately, lung damage. Neutrophil transendothelial migration requires temporal and spatial increases in endothelial paracellular permeability, which is a process found in normal host defense. However, uncontrolled neutrophil transmigration results in the prolonged opening of intercellular junction structures and increased paracellular permeability, which leads to fluid accumulation and edema in the interstitial tissue, and, ultimately, ARDS (Tsushima et al., 2009). Moreover, toxic mediators (e.g., proteases and ROS, etc.) and cytokines released from activated neutrophils also damage endothelial cells, inducing vascular leakage. More details are addressed below in "Neutrophil activation and inflammatory mediators." In addition, endothelial homeostasis is disrupted during sepsis or ARDS, shifting to a pro-coagulant condition with massive production of thrombin, which directly affects the endothelial barrier, leading to hyperpermeability (Bogatcheva et al., 2002).

Alveolar Fluid Clearance Dysfunction

Alveolar fluid clearance, or AFC, is important for maintaining fluid homeostasis in the lungs and is regulated by osmotic pressure (Matthay et al., 2002; Huppert and Matthay, 2017). The active transport of Na^+ ions is the main contributor for the creation of the osmotic gradient: Na^+ is primarily transported through the amiloride-sensitive ENaC, as well as by the nonselective (NCC) or highly selective cation channels (SCC) and cyclic nucleotide-gated (CNG) channels on the apical epithelial surface and then extruded out of the cell by the Na^+, K^+ ATPase on the basolateral surface into the interstitium and the circulation (Matthay, 2014). The Na^+, K^+ ATPase is the primary determining factor for AFC since it is the major active Na^+ transporter expressed in the epithelial basolateral membrane, which continuously pumps Na^+ out of the alveoli by utilizing ATP (Mutlu and Sznajder, 2005). Instillation of ouabain, a cardiac aminoglycoside inhibitor of the Na^+, K^+ ATPase, into animal and *ex vivo* human lungs decreases AFC by more than 50% (Matthay et al., 2002). Conversely, adenovirus- or electroporation-mediated overexpression of the

Na^+, K^+ ATPase increased AFC by ∼100% in rat lungs (Machado-Aranda et al., 2005).

During lung injury, fluid clearance is impaired, ultimately resulting in hypoxemia (Huppert and Matthay, 2017). Majority of patients with ARDS show severe fluid clearance impairment, whereas 25% of all patients with hydrostatic lung edema have ARDS (Ware and Matthay, 2001). Multiple factors result in AFC impairment during ARDS (Vadasz et al., 2007). Loss of epithelial polarity due to disrupted epithelial TJs decreases AFC (Han et al., 2004; Zemans and Matthay, 2004). Hypoxia induces the downregulation of both ENaC and Na^+, K^+ ATPase at the mRNA level and membrane abundance of the Na^+, K^+ ATPase (Planès et al., 1997; Zhou G. et al., 2008). The Na^+, K^+ ATPase is most vulnerable to hypoxic effects, since it works by consuming ATP. Indeed, ∼40% of a cell's total energy is consumed by this transporter to maintain homeostasis, and during injury, reduced oxygenation limits ATP production (Milligan and McBride, 1985). Excessive resulting ROS triggers Na^+, K^+ ATPase endocytosis through α1 subunit phosphorylation (Dada et al., 2003). Pathogens, such as influenza A virus, induce degradation of membrane-localized Na^+, K^+ ATPase in nearby noninfected alveolar epithelial cells by activating pathways in the infected epithelium and resident macrophages that produce cytokines like type I IFN and IFN- related apoptosis-inducing ligand (TRAIL; Peteranderl et al., 2016). Proinflammatory cytokines, such as IL-1β, IL-8, and TGFβ1, are detected at high levels in the edema fluid of patients in the early stage of ARDS (Pugin et al., 1999; Lee et al., 2011). They downregulate the expression and function of ENaC and the Na^+, K^+ ATPase through activation of various pathways in the epithelium, causing decreased AFC (Pugin et al., 1999). Furthermore, mechanical ventilation also negatively affects Na^+ transport activity and AFC (Lecuona et al., 1999), although it is commonly used in the ICU to facilitate breathing and oxygenation. Indeed, in ATII cells isolated from rats after high tidal volume ventilation, the activity of Na^+, K^+ ATPase decreased by 50% compared to the control group (Lecuona et al., 1999).

Besides being critical for AFC, the expression and function of the Na^+, K^+ ATPase are closely involved in the regulation of epithelial barrier integrity (Rajasekaran et al., 2001b; Rajasekaran and Rajasekaran, 2003; Vadasz et al., 2007). Increasing evidence indicates that the role of the Na^+, K^+ ATPase in barrier junction formation is independent of its ion transport activity. For example, (1) the Na^+, K^+ ATPaseβ1 subunit may be necessary for membrane localization of TJs (Rajasekaran et al., 2007); silencing of β1 by siRNA disrupts the continuous staining pattern of ZO-1 and occludin, indicating that β1 might be directly or indirectly associated with TJs (Madan et al., 2007); (2) the Na^+, K^+ ATPase is required for establishing epithelial polarization in MDCK cells and co-expression of the Na^+, K^+ ATPaseβ1 subunit, and E-cadherin recovers the lost polarity and junctions seen in MSV-MDCK cells, a highly invasive cell line (Rajasekaran et al., 2001b); (3) the basolateral localized Na^+, K^+ ATPase also acts as an adhesion molecule, forming a trans-dimer junction structure mediated through the N-glycosylation of β1's extracellular domain (Vagin et al., 2012). The mechanisms by which the Na^+, K^+ ATPase regulates epithelial barrier function has not completely been characterized, but it appears related to stress fiber formation and actin assembly (Rajasekaran and Rajasekaran, 2003). RhoA GTPase, a small GTP-binding protein involved in stress fiber formation (Guasch et al., 1998), has been implicated as a downstream effector of Na^+, K^+ ATPase signaling for TJ assembly and function (Rajasekaran et al., 2001a). We recently identified MRCKα (CDC42-binding protein kinase alpha) by mass spectrometry as an interacting partner of the β1 subunit (Bai et al., 2021). These findings point to the interdependency of alveolar capillary barrier and AFC dysfunction in edema formation during ARDS. MRCKα is a Rho GTPase effector kinase that regulates diverse cell behaviors, such as actomyosin contraction-mediated junction formation (**Figure 2**; Unbekandt and Olson, 2014). Silencing of MRCKα by siRNA abolished the increased TEER seen in cultured AT1 cells following transfection with the β1 subunit, indicating that β1 signals through MRCKα to upregulate tight junction proteins and epithelial barrier function, at least in cells (Bai et al., 2021).

Neutrophil Activation, Inflammatory Mediators, and Coagulation

Recruitment of neutrophils is a hallmark of ARDS and is considered to play a key role in the progression of ARDS. An analysis of BAL fluid cellularity in patients with ARDS or animals in various injury models demonstrates elevated neutrophil infiltration into alveoli. This is correlated with ARDS outcomes and severity, with non-survivors having higher levels of chemotactic IL-8 than survivors of ARDS. Neutrophils are important components of the innate immune system. In response to stimuli, they are activated, recruited, and secrete various antimicrobial molecules, such as ROS, proteases, and cationic peptides, to destroy invading microorganisms. However, under some disease conditions, e.g., ARDS, excessive neutrophil activation and imbalanced inflammatory responses can cause additional tissue damage. NETosis is an important mechanism of neutrophil defense against invading pathogens, in which neutrophils release DNA combined with histones, myeloperoxidase, neutrophil elastase, and extracellular fibers into the extracellular environment to form a network for microorganism trapping (Thiam et al., 2020). This increased extracellular NET production is correlated with ARDS severity and alveolar capillary barrier dysfunction in experimental models. Elastase in NETs degrades alveolar capillary barrier integrity, contributing to edema formation. It proteolytically degrades junctional molecules, such as ZO-1, E-cadherin, and VE-cadherin, to induce intercellular adhesion disruption in cultured epithelial and endothelial monolayers. Histones are the most abundant proteins in NETs, and it has been shown that incubation of endothelial cells or ATII cells with histones induces cell death, suggesting a role in damage to alveolar capillary barrier integrity. Finally, matrix metalloproteinases (MMPs) are produced by a variety of cell types, such as neutrophils, and the levels of MMP-2 and 9 are increased in BAL of patients and are correlated with ARDS severity.

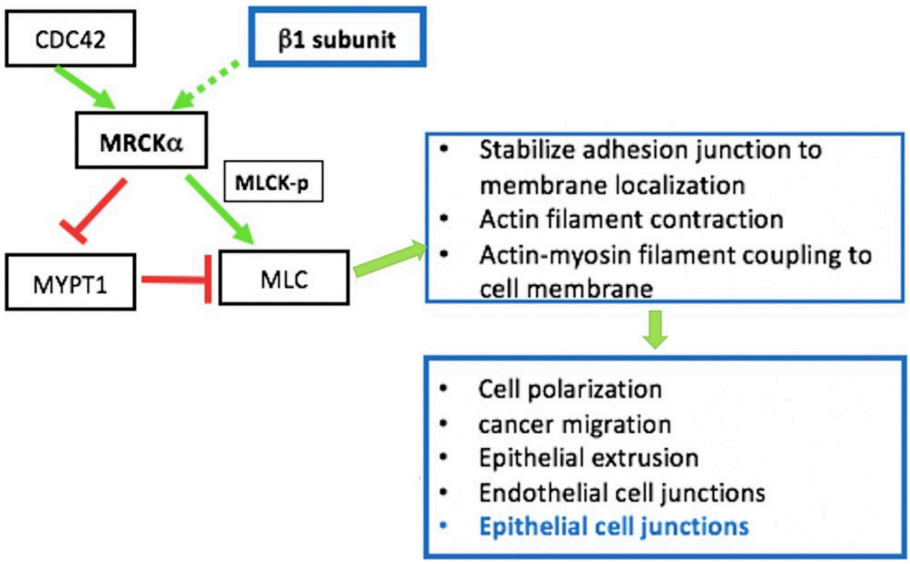

FIGURE 2 | MRCKα signaling pathway is involved in epithelial intercellular junction regulation. MRCKα is activated (solid green arrow) by cdc42, a Rho family of small GTPases. The MRCKα kinase activates myosin light chain (MLC) either by directly phosphorylating/activating (green arrow) myosin light chain kinase (MLCK) or by inactivating (red arrow) myosin phosphatase target subunit 1 (MYPT1), which dephosphorylates/ inactivates (red arrow) MLC. The two consecutive negative activities (red arrows) result in the indirect activation of MLC by reducing MLC dephosphorylation/ inactivation. These events regulate multiple cellular functions such as actin-myosin contraction, which is involved in epithelial junction regulation. In addition, our previous data indicate that overexpression of β1- Na^+, K^+-ATPase increases activation/phosphorylation of MLC (Bai et al., 2021).

Uncontrolled inflammatory response is another hallmark of the early stage of ARDS that contributes to lung barrier disruption and AFC impairment, leading to edema formation (Ware and Matthay, 2000; Matthay and Zimmerman, 2005). Inflammatory response is initiated, amplified, and regulated by a network of various cytokines and other inflammatory molecules (Park et al., 2001). High concentrations of cytokines, such as IL-1β, TNFα, IL-6, and IL-8, are detected in plasma and BAL fluids and are associated with poor clinical outcomes of ARDS, such as mortality rate (Pugin et al., 1996; Parsons et al., 2005; McClintock et al., 2008). In several experimental models, highly expressed TNFα in BAL fluid triggers caspase-8-mediated apoptotic signaling in the epithelium, inducing cell death, and consequently, alveolar epithelial barrier dysfunction (Patel B. V. et al., 2013).

Coagulation is a critical host response to infection. However, it is also involved in ARDS pathogenesis with markedly increased release of soluble tissue factor and microthrombi formation in the pulmonary microvasculature and decreased fibrinolytic activities with diffuse alveolar and interstitial fibrin deposition (Grinnell et al., 1980; Nagy et al., 1995). Some studies have shown that pro-coagulant activities observed during ARDS can increase alveolar capillary barrier permeability (Abraham, 2000; Mosnier et al., 2007). As seen in both patients and experimental animal models, upregulated levels of soluble tissue factor, thrombin, and fibrinolysis inhibitors are present in BAL fluid (Idell et al., 1991; Fuchs-Buder et al., 1996). In cultured epithelial cells, thrombin induces F-actin polymerization and stress fiber formation, which further increases cell contraction, stiffness, and epithelial barrier permeability (Hayashi et al., 2006). Platelets are another important component of the host defense system and may contribute to the neutrophil-dependent lung injury (Rossaint et al., 2018). Activated platelets have been shown to directly interact with neutrophils, facilitating their extravasation and recruitment to the lungs (Zarbock and Ley, 2009; Rossaint et al., 2018). In acid-induced lung injury, platelet-neutrophil interactions in the vascular endothelium could be visualized by electron microscopy within 30 min of injury (Zarbock et al., 2006). These studies showed that platelet depletion significantly reduces neutrophil rolling and adherence to the endothelium, thereby markedly reducing lung edema and increasing mouse survival.

Current Pharmacological Treatments for Acute Respiratory Distress Syndrome

Over the past several decades, considerable research efforts have made ARDS well-understood in terms of pathogenesis, risk factors, genetic predispositions, and various signaling pathways and molecules involved. Although large efforts have been committed to developing pharmacological therapies for ARDS, the results have been discouraging. A review from 2018 summarized that large-scale clinical trials with positive results only account for 5% of the 20 most recent large pharmacological studies on both sepsis and ARDS (Laffey and Kavanagh, 2018). The incidence and overall hospital mortality of ARDS have not changed considerably in the past 10 years (Villar et al., 2016). In the 2016 cross-country study LUNG SAFE, the mortality rate remained at 40% for moderate ARDS and even higher at 46% for severe ARDS (Bellani et al., 2016). In the

evolving COVID-19 pandemic, the mortality rate for patients with COVID-19 associated ARDS is even higher, which further highlights the importance of developing novel treatments or therapies for ARDS.

β_2 Adrenergic agonists have been demonstrated to enhance AFC *in vivo* through the activation of the cAMP pathway, which increases transepithelial ion transport by upregulating the activity and membrane abundance of ENaC, Na^+, K^+-ATPases and chloride channels (Bertorello et al., 1999; Matalon and O'Brodovich, 1999; Fang et al., 2002; Mutlu et al., 2004; Mutlu and Sznajder, 2005). While treatment of mice with existing acute lung injury with several different β_2 agonists, such as albuterol and salmeterol, can treat the disease and give positive outcomes in lung function, inflammation, edema clearance, and survival, none of these treatments have been proven effective in patients. Indeed, it has even been suggested to avoid using these drugs in patients with ARDS (Boyle et al., 2013). In several clinical studies, β_2 adrenergic therapy showed no significance in the primary outcome of ventilator-free days and even worsened the outcome of increased mortality, although there was some amelioration in pulmonary fluid accumulation (Perkins et al., 2006; National Heart Lung Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network et al., 2011; Gao Smith et al., 2012; Matthay et al., 2017; Laffey and Kavanagh, 2018). Several possibilities might account for the failure of β_2 receptor agonist therapy. For example, prolonged β_2 adrenergic agonism by endogenous catecholamines could desensitize β_2 receptors, which would prevent further receptor stimulation with exogenous catecholamines (Berthiaume et al., 2002). In some patients with no response to β adrenergic therapy, the alveolar epithelium might be too injured to benefit from any transporter upregulation (Hamacher et al., 2018). In addition, some circulating factors could limit the action of β-adrenergic agonists (Berthiaume et al., 2002).

Corticosteroids are commonly used for both prevention and treatment of ARDS, given their anti-inflammation properties (Khilnani and Hadda, 2011). Although nearly 20% of patients with ARDS receive systemic steroids, there is no clear-cut efficacy of steroids in attenuating lung injury (Boyle et al., 2013; Bellani et al., 2016). Similarly, non-steroidal anti-inflammatory agents ketoconazole and lisofylline also failed in clinical trials for the early treatment of ARDS (The ARDS Network, 2000; The ARDS Clinical Trials Network, 2002). However, the recent identification of ARDS sub-phenotypes (hypo- vs. hyper-inflammatory phenotypes) may help to specify ARDS cohorts and show promise for anti-inflammation therapy to ARDS. For example, statins have been proposed for use in ARDS because of their anti-inflammatory function beyond lowering cholesterol, and they have shown to significantly improve the 28-day survival of patients compared to placebo in a "hyper-inflammatory" subgroup (Calfee et al., 2018). However, no difference was detected in a "hypo-inflammatory" subgroup in terms of the same outcome. Nitric oxide (NO) is crucial in regulating vascular tone and blood flow. Inhaled NO has also been shown to improve pulmonary gas exchange and oxygenation in animal models of ARDS, but as for most other drugs, in patients, inhaled NO has shown no long-term survival benefits (Putensen et al., 1994; Rossaint et al., 1995; Adhikari et al., 2007).

N-Acetylcysteine (NAC) is a common antioxidant widely used for treating conditions characterized by the generation of free oxygen radicals (Shahin et al., 2009). It has been tested in multiple trials on sepsis and lung-injury related ARDS, as well as COVID-19-induced ARDS. However, the benefit of antioxidant therapy with NAC is not consistent among studies, including early mortality rate, duration of ICU stay, and oxygenation (Adhikari et al., 2004; Shahin et al., 2009; Lu et al., 2019). A recent clinical study using NAC for COVID-19 treatment, a high dose of NAC showed no significant benefit in terms of mortality, ICU admission, or time of invasive mechanical ventilation compared to the placebo group (de Alencar et al., 2020).

Apart from these examples, a number of potential therapies have shown promising results in preclinical studies but have been proven ineffective or even harmful in clinical trials. These include surfactant replacement, neutrophil elastase (NE) inhibitors, aspirin, heparin, and angiotensin-converting enzyme (ACE) inhibitors. The use of ACE inhibitors has been limited in preclinical studies because of their side effects on systemic hypotension (Arndt et al., 2006). However, ACE2, which counteracts the activity of ACE, was demonstrated to have a treatment effect on patients with ARDS in a pilot study (Imai et al., 2005; Khan et al., 2017). In addition, ACE2 is a functional receptor for SARS-CoV-2, the coronavirus that caused the COVID-19 pandemic in 2020, and recombinant ACE2 has entered clinical trials (NCT04335136) for COVID-19 (Bao et al., 2020).

PULMONARY STRUCTURAL BARRIERS FOR GENE DELIVERY

The lung is a highly specialized and delicate organ that has evolved to maximumly expose blood to air for gas exchange (Hsia et al., 2016). Functionally, conducting airways connecting the inner and outer pulmonary environments dominate the airflow during inhalation and exhalation. Thus, easy access to both the airways and vascular network makes the lung attractive for gene delivery (Pouton and Seymour, 2001; Gautam et al., 2002). Genes can be easily administered through intranasal or oral inhalation using nebulizers or by bronchoscopy-mediated intratracheal administration (Gautam et al., 2002; Katz et al., 2019). For small rodents, oropharyngeal aspiration is a straightforward and simple method for gene delivery into the lung (Zhang S. et al., 2013; Bale et al., 2016; Lin et al., 2016). Compared to intravascular injection, airway delivery shows lower DNA and RNA degradation by nuclease activities (Liu et al., 2007; Henning et al., 2010). Normally, the human lung has approximately 480 million alveoli, which compose a large surface area (∼140 m; Guggino and Cebotaru, 2017) for any gene delivery and account for more than 99% of total lung internal surface area (Crapo et al., 1983; Ochs et al., 2004). Furthermore, there is a massive pulmonary micro-capillary network surrounding alveoli for gas exchange. The thin alveolar epithelial monolayer (0.1–0.5 μm; Weibel, 1973) and its formed massive lung surface area, together

with the high permeability of the alveolar-capillary membrane, provide superior conditions for gene and/or particle deposition and uptake into the lung (Labiris and Dolovich, 2003).

Although the lung is unique and has advantages for gene delivery, low transfection efficiency has hindered the progress of gene therapy for lung diseases because of multiple barriers, such as complex branching of the conducting airways, alveolar-capillary barrier, surfactant, lining fluid and mucus, basement membrane, and host immunological defenses. The conducting airways defend the lungs from exogenous particles and bacterial and viral insults through mucociliary clearance (Nicod, 2005). However, they also trap therapeutic genes and decrease the efficiency of delivery (Duncan et al., 2016; Bustamante-Marin and Ostrowski, 2017). For patients with cystic fibrosis, their airways are progressively filled with thickened mucus that forms a solid barrier hindering viral vector or nonviral liposomal vector penetration into the lungs (Sanders et al., 2009; Schuster et al., 2014). Sufficient delivery of gene materials into the parenchymal session of the lungs is also challenging since it heavily relies on particle deposition (Paranjpe and Müller-Goymann, 2014). The respiratory airway branches multiple times to the distal terminal bronchioles and ends in the alveolar sacs (Patwa and Shah, 2015; Hsia et al., 2016; Sondhi et al., 2017). Once delivered cargos, e.g., plasmids, viral vectors, peptides, and siRNA, reach the alveoli, they face the risk of phagocytosis by resident alveolar macrophages (Patton, 1996; Patton et al., 2004; Katz et al., 2019). Localization of a Cy3-labeled adenoviral vector by fluorescence microscopy shows that adenoviral vectors are rapidly internalized (∼1 min) by alveolar macrophages after reaching the alveolar surface (Zsengellér et al., 2000). In a mouse model, within 24 h following administration, 70–90% of adenovirus genomes were cleared, indicating the significance of macrophages in transfection efficiency (Worgall et al., 1997). Surfactant proteins, SP-A and SP-D, can interact with gene delivery agents containing carbohydrate domains, decreasing their transfection efficiency (Vadolas et al., 2002). In addition, the thin alveolar epithelial monolayer provides a large surface area for particle deposition (Labiris and Dolovich, 2003); meanwhile, the tightness formed by junction molecules between cells increases difficulties for gene transfer to subepithelial cells (Patton and Byron, 2007; Murgia et al., 2014).

In addition, the pathological conditions of several diseases would complicate those barrier mechanisms, and metabolic products could also be potential barriers hindering gene delivery (Weiss, 2002). For example, sputum from patients with CF could slow down the adeno-associated virus (AAV) vector diffusion rate by >1,000 fold compared to water (Schuster et al., 2014), and some patients' sputum also contains adenovirus-specific antibodies that neutralize the Ad vectors for further inhibition (Perricone et al., 2000). During acute lung injury and ARDS, pulmonary edema influx, which results from the loss of the alveolar epithelium and alveolar capillary barrier dysfunction, collapsed alveoli, excessive mucus secretion, and the proinflammatory environment make it hard to transduce the injured lung (Zhang et al., 1998; Weiss, 2002; Matthay et al., 2019).

GENE DELIVERY SYSTEMS

Because both the cell membrane and nucleic acids are highly negatively charged and, thus, repel each other, delivering exogenous genetic materials into cells requires either a carrier or vector to mask the nucleic acid's charge or a physical method to circumvent the membrane. Vectors usually refer to virus particles, and carriers typically imply nonviral chemical agents. Ideally, the vector/carrier should show high transduction efficiency with a sufficient number of cells transduced, cell or tissue specificity, enough stability to protect the transgene from extracellular and intracellular degradation, and, perhaps most importantly, minimal immune and inflammatory responses (Mehier-Humbert and Guy, 2005; Katz et al., 2013, 2019). Gene delivery vectors can be classified into two broad categories: viral vectors and nonviral physical or chemical methods (Nishikawa and Huang, 2001; Nayerossadat et al., 2012; Ginn et al., 2018). In either case, high-quality, clinical-grade vectors (viral or nonviral) could be used for any clinical trial. Currently, a number of biotechnology and pharmaceutical companies are focusing on the development of virus- and nonvirus-based systems for gene therapy with the goal of providing single-dose medications available for use in the ICU or even in the outpatient setting (depending on the indication). Thus, like the mRNA-based Covid19 vaccine, any nucleic acid-based gene therapy approach would be available from hospital pharmacies for direct use in patients.

Viral Vectors

Viral vectors are widely used for gene therapy because of their high transduction efficiency and oftentimes long duration of transgene expression (Bouard et al., 2009). For use as a gene transfer vector, the viral genome is modified to limit viral replication by removing critical viral genes, for example, Gag, Pol, and Env genes in retroviruses, and replacing them with the desired transgene for the target protein (Verma and Weitzman, 2005). Both RNA and DNA viruses have been employed extensively for lung gene delivery (Driskell and Engelhardt, 2003; Sondhi et al., 2017). In the case of RNA viruses, the retrovirus was first used in *ex vivo* lung gene therapy for α1AT deficiency (Garver et al., 1987). Because the RNA genome requires conversion into double-stranded DNA, which then can integrate into the host chromosomal genome (Chiu and Davies, 2004; Yi et al., 2011; Sondhi et al., 2017), it was seen as an ideal vector for long-term durable expression. However, retroviruses are only able to transduce proliferating cells (Hu and Pathak, 2000; Schambach and Morgan, 2016) and have been of limited use for *in vivo* lung gene delivery, since most of the cells in the lungs are not dividing at any given time (Driskell and Engelhardt, 2003; Sondhi et al., 2017). Compared to retroviruses, lentiviral vectors are able to infect nondividing cells (Naldini et al., 1996)and have been proven to be more useful for lung gene delivery (Driskell and Engelhardt, 2003; Patel M. et al., 2013; Marquez Loza et al., 2019). Two types of lentivirus, human immunodeficiency virus (HIV; Goldman et al., 1997) and feline immunodeficiency virus (FIV; Wang et al., 1999), have been shown to infect the airway epithelium for cystic fibrosis gene therapy. However, their

application is hindered by the lack of suitable receptors expressed on the epithelial apical surface for viral approach (Walters et al., 1999). In the face of this disadvantage, envelope glycoprotein-pseudotyped lentiviruses were developed to widen their host range (James et al., 2005). For example, pseudotyped lentiviral vectors from filoviruses (Kobinger et al., 2001; Sinn et al., 2017a), baculovirus (Sinn et al., 2012, 2017b), Ebolavirus (Kobinger et al., 2001), influenza virus (Patel M. et al., 2013), and Sendai virus (Mitomo et al., 2010; Griesenbach et al., 2012) all confer access to receptors on the apical side of airway epithelial cells, allowing gene transfer to these cells. In contrast, vesicular stomatitis virus (VSV) glycoprotein-pseudotyped vectors predominantly enter from the epithelial basolateral surface (Goldman et al., 1997; Johnson et al., 2000; Kremer et al., 2007), which limits their direct translation into clinical use. Since RNA viruses integrate the transgene into the host genome in a random fashion, there exists the possibility of oncogene activation or induced mutagenesis for these vectors (Anson, 2004; Bushman, 2007; Milone and O'Doherty, 2018). So far, most application of lentivirus to the lung has focused on chronic diseases, e.g., cystic fibrosis, rather than ARDS, and it is being used for RNA interference-mediated gene knockdown (Tiscornia et al., 2003; Copreni et al., 2004). One study using lentivirus delivered shRNA to silence CD36, which is required for latent TGF-β1 activation, and showed antifibrotic effects after injury to the lung in a silicosis model (Wang et al., 2009). However, since short-term gene expression is more desirable for acute indications like ARDS, such integrating vectors are not appropriate.

The most studied and widely used DNA viral vectors include replication-deficient adenovirus (Ad) and adeno-associated virus (AAV) derived vectors (Crystal, 2014; Katz et al., 2019). Ad contains a large, double-stranded linear DNA genome (∼36 kb), whereas AAV contains a single-stranded DNA genome that is relatively small (∼4.7 kb) (Verma and Weitzman, 2005). Since the Ad genome can be transcribed and replicated episomally (Samulski and Muzyczka, 2014; Sondhi et al., 2017), without necessary integration into the host genome, Ad vectors can confer moderate duration expression and show high transduction efficiency in non-dividing cells of the airway (Crystal, 2014). Unfortunately, Ad vectors are highly immunogenic and induce strong host inflammatory and immune responses specifically against products of these viral genes (Schiedner et al., 1998; Ahi et al., 2011; Lundstrom, 2018), which hinder repetitive administration of the vector and limit gene expression to 2–3 weeks (Crystal, 2014). Several generations of Ad vectors have been developed in order to minimize the host inflammatory and immune responses (Capasso et al., 2014). Compared to the first and second generations of Ad vectors with partial viral genome deletion (Verma and Weitzman, 2005; Capasso et al., 2014), the third generation, called "gutted/helper dependent" Ad vector (Kochanek et al., 2001; Capasso et al., 2014), has had the whole viral coding region deleted to minimize viral antigen expression (Alba et al., 2005; Verma and Weitzman, 2005). Although these advanced Ad vectors enable *in vivo* gene delivery by maximally reducing initial inflammatory responses to administration (Koehler et al., 2006), their capsid proteins can still induce cytotoxic T-cell destruction of the infected cells by antigen presentation and induce antibody production, again preventing subsequent vector administration (Alba et al., 2005; Katz et al., 2019). Of note, a common drawback to *in vivo* viral delivery is immune responses that might confront animals or patients with preexisting lung injuries to a higher risk of inflammatory toxicities (Lin and Dean, 2011). For example, adenovirus transfected rat lungs with high efficiency and uniform distribution of marker genes; however, all end point measurements, such as AFC, were taken 7 days after animal recovery because of viral infection-induced inflammatory responses (Factor et al., 1998). In comparison, electroporation is an effective method to deliver plasmid DNA to living animal lungs with no extra damage and high-level gene expression (Somiari et al., 2000; Dean et al., 2003).

Compared to Ad vectors, AAV vectors show less immunogenicity but have high transduction efficiency, persistent transgene expression, and broad host range (Mingozzi and High, 2013; Samulski and Muzyczka, 2014). Several serotypes of AAV vectors, for example, 2, 5, 6, and 9, have been shown to effectively transduce the airway and alveolar epithelium, and have allowed limited re-administration in experimental animals (Limberis et al., 2009; Li et al., 2011). In a rat model of ARDS, delivery of aerosolized AAV serotypes 2 and 6 to rat lung showed high transduction efficiency of transgene expression, which significantly prevented subsequent LPS-induced lung injury in a protection model (MacLoughlin et al., 2015). AAV-mediated gene therapy for CF has even moved into phase I/II clinical trials (Guggino and Cebotaru, 2017). However, the application of AAV for clinical trials is still limited because of its small packaging capacity, difficulty producing large quantities, issues with re-dosing, and various immune responses in different organs (Mingozzi and High, 2011; Sondhi et al., 2017; Wang et al., 2019).

Nonviral Gene Delivery: Chemical Vectors

Although viral vectors remain the major delivery method for gene therapy, accounting for approximately two-thirds of total vectors used in clinical trials in 2017 (Ginn et al., 2018), nonviral vectors have been increasingly used in clinical trials since 2004 (∼23%) (Ginn et al., 2018). Compared to viral vectors, nonviral vectors possess some inherent advantages for gene delivery (Nayerossadat et al., 2012; Yin et al., 2014; Ginn et al., 2018; Patil et al., 2019): (1) much larger transgene packaging capacity; (2) much better safety profile; (3) ability to carry and deliver DNA or RNA by chemical carriers; (4) ability for repeat administration; (5) low immunogenicity due to lack of antigen presentation to adaptive immune system; and (6) ease of synthesis and production in large quantities. Indeed, DNA- and RNA-based vectors (plasmids, minicircles, mRNA, and siRNA) are simple and relatively inexpensive to produce on a large scale, especially when compared to their viral counterparts. Although nonviral gene transfer has been widely performed in research in laboratories, its applications in clinical trials have been hindered by several obstacles, such as lower transfection efficiency, lack of specific cell targeting,

and lack of stability compared to viral vectors (Glover et al., 2005; Ramamoorth and Narvekar, 2015; Ginn et al., 2018). For example, naked plasmid DNA delivered systemically is degraded quickly, and its half-life is estimated to be only 10 min following intravenous (IV) injection in mice (Kawabata et al., 1995). Thus, various physical and chemical methods have been developed to enhance gene transfection efficiency *in vivo* (Nayerossadat et al., 2012). Cationic lipid- (lipoplexes) and cationic polymer- (polyplexes) based vectors are the most commonly used chemical transfection reagents and are extensively used in gene transfer to the lungs (Davis and Cooper, 2007; Aneja et al., 2009; Jones et al., 2013). Both types of delivery agent interact electrostatically with the negatively charged DNA, forming a net positively charged lipoplex- or polyplex-DNA complex for further interaction with the cell membrane (Zuidam and Barenholz, 1998; Zhu and Mahato, 2010; Jones et al., 2013). Consistent with fundamental purposes of using vectors for gene delivery, namely to overcome multiple extracellular and intracellular barriers and facilitate therapeutic nucleic acids reaching target cells or tissues, chemical vectors are designed to increase the stability and transfection efficiency of DNA complexes and decrease their biodegradability (Zhu and Mahato, 2010). Following intravenous administration, DNA complexes face multiple barriers, such as endonuclease degradation, traversing the vascular wall, intercellular junctions, the cytoplasmic membrane of target cells, and avoiding entrapment in endosomal vesicles (Song et al., 1997; Pack et al., 2005; Hill et al., 2016). Thus, it is important to investigate vector structure-activity relationships and their optimization for lung gene delivery.

Cationic lipids are diverse in structure, but there are three basic structural components, a cationic head group, a hydrophobic tail, and a linker connecting both the head and tail groups (Zhu and Mahato, 2010; Jones et al., 2013). The positively charged hydrophilic head group is the major domain that interacts with negatively charged DNA molecules, leading to plasmid condensation, enhanced cellular uptake, and endosomal escape (Miller et al., 1998; Tseng et al., 2009; Zhu and Mahato, 2010). The hydrophobic tail group is usually composed of saturated or monounsaturated fatty acid chains (aliphatic chains) with various lengths (Niculescu-Duvaz et al., 2003; Kou et al., 2011). It is widely accepted that gene transfection efficiency is inversely correlated with chain length, which means the shorter the chain length (e.g., C14), the higher the transfection efficiency. This is presumably due to increased lipoplex stability (Felgner et al., 1994; Adir et al., 2008; Jones et al., 2013). Cholesterol is a commonly used alternative for the hydrophobic tail domain and shows enhanced fusion with the host cell membrane (Mahato et al., 1997; Jones et al., 2013; Monteiro et al., 2014). The linker that connects the cationic head to the hydrophobic tail domain can greatly impact the stability and biodegradability of the lipoplex (Mahato et al., 1999). Ether bonds, such as those in DOTMA, are indicated to render good gene delivery efficiency because of their stable and nondegradable properties, but their cytotoxicity is higher than that of other linker chemicals, such as esters and amides (Singhal and Huang, 1994; Freedland et al., 1996; Zhu and Mahato, 2010; Jones et al., 2013). Cleavable linkers are also used as an alternative. Additionally, the cationic nature of the head group can be another source of cytotoxicity, since it can interact non-specifically with negatively charged serum proteins (albumin, lipoproteins, and IgG), resulting in charge neutralization, reduced cellular uptake, hemolysis, and decreased transfection efficiency (Escriou et al., 1998; Zelphati et al., 1998; Lv et al., 2006; Jones et al., 2013). It has been shown that intravenous injection of DNA lipoplexes actually induces embolization in the lungs because of large complex size (>5 μm) by interaction with blood components, leading to failure to pass through capillaries (Litzinger et al., 1996; Nishikawa and Huang, 2001).

Cationic polymers also form complexes with DNA through electrostatic interactions and coat the complex with a net positive charge (Nishikawa and Huang, 2001; Eliyahu et al., 2005). Polyethylenimine (PEI) is one of the most commonly used synthetic polymers with a highly positive charge (Eliyahu et al., 2005; Jin L. et al., 2014). The different amine groups in PEI's structure affect the polyplex's endosomal escape after uptake by displaying buffering capacities over a wide range of pH (Zhu and Mahato, 2010). It has been proposed that different types of amines work as a "proton sponge", which can be protonated to different levels as the endosomal environment acidifies, leading to ultimate endosomal breakup and release of their contents (i.e., DNA) (Boussif et al., 1995; Eliyahu et al., 2005; Pack et al., 2005; Jin L. et al., 2014). Compared to linear PEI, which contains almost all secondary amines in its backbone, branched PEI contains primary, secondary, and tertiary amino groups (Fischer et al., 1999; Zhu and Mahato, 2010), which confers PEI with larger buffering capacity (Boussif et al., 1995), leading to early endosomal escape of plasmids, and offers protection of the DNA from lysosomal degradation (Zhu and Mahato, 2010). In PEI, the nitrogen to DNA phosphate (N/P) ratio, one indicator of the properties of DNA polyplex, along with complex size, net surface charge, and stability, is associated with transfection efficiency (Nimesh et al., 2007; Vu et al., 2012; Gary et al., 2013). With an N/P ratio < 1, the PEI /DNA complex is characterized by incomplete DNA condensation, whereas when the ratio > 3, the free PEI is thought to enhance endosomal escape, contributing to DNA intracellular release (Mislick and Baldeschwieler, 1996; Boeckle et al., 2004; Perevyazko et al., 2012; Jin L. et al., 2014). The molecular weight of PEI is another determinant of transfection efficiency (Jones et al., 2013; Jin L. et al., 2014).

Physical Methods for Gene Delivery

Gene transfer of naked or plasmid DNA by physical means is an attractive delivery system for gene therapy because it is simple and has low cytotoxicity (Gao et al., 2007). Physical delivery approaches are popular in clinical trials, accounting for 14% in 2004, 18% in 2007, 18.3% in 2012, and 16.6% in 2017, of total gene therapy clinical trials (Edelstein et al., 2004, 2007; Ginn et al., 2013, 2018). Physical methods to introduce exogenous genes into cells have been explored both *in vitro* and *in vivo* (Dean et al., 2003; Gehl, 2003; Matsuda and Cepko, 2004; Dean, 2005). Basically, a physical force, produced by mechanical force, electrical pulses, ultrasound, laser irradiation, or magnetic fields, is employed to transiently disrupt the cell membrane and create small pores, so that DNA can diffuse into

cells (Schneckenburger et al., 2002; Gehl, 2003; Mehier-Humbert and Guy, 2005; Li et al., 2008; Liu et al., 2012; Nayerossadat et al., 2012). Although naked DNA can be directly injected into local tissues, e.g., skeletal muscles or liver, and into the systemic circulation *via* tail vein, this "simplest" delivery method shows low transfection efficiency due to rapid *in vivo* degradation by nucleases and clearance by tissue-resident macrophage (e.g., Kupffer cells in the liver), limited extravasation from the circulation, and high interindividual variability (Kawabata et al., 1995; Mahato et al., 1995; Mir et al., 1999; Heller et al., 2000; Zhang et al., 2012; Yin et al., 2014). Plasmid DNA incubated in isolated rat plasma can degrade quickly with a half-life of 1.2 min for the supercoiled form, 21 min for the open circular plasmid DNA, and 11 min for the linear form (Houk et al., 1999). Cytoplasmic nucleases are another barrier impeding the efficient expression of plasmid DNA (Lechardeur et al., 1999; Bai et al., 2017). In contrast to direct injection of naked DNA into tissues, the "gene gun" or gene-mediated particle bombardment takes advantage of the high velocity of a particle carrier (e.g., gold beads) to deliver DNA into target tissues, such as skin, liver, and muscle (Wolff et al., 1992; Nishikawa and Huang, 2001). This method demonstrates increased transfection efficiency, for example, for epidermal tissues with 10–20% transfection of cells in the bombarded area, but is still limited in clinical trials because of concerns of poor penetration (<0.5 mm depth) into organs (Yang et al., 1990; Williams et al., 1991; Zelenin et al., 1997). The major application of gene gun in human trials is for DNA vaccination or suicide gene therapy to treat cancers (Trimble et al., 2003; Fuller et al., 2006). To date, this approach has not been used successfully in the lungs.

Electroporation, or EP, has been widely used in clinical settings to treat cancer (electrochemotherapy) and deliver drugs or vaccines to target cells. It was first used for DNA transfection of cultured mouse lyoma cells in 1982 (Neumann et al., 1982). It is a fast and reproducible approach, and requires a relatively low dose of DNA (Dean et al., 2003). In principle, when the transmembrane potential applied by the external electric field exceeds the cell resting potential, EP transiently disrupts the cell membrane and forms hydrophilic pores so that various molecules surrounding cell surface, such as DNA, RNA, oligonucleotides, ions, drugs and antibodies, can pass into the cells (Weaver and Chizmadzhev, 1996; Somiari et al., 2000). This delivery approach is not limited to small DNAs like AAV or other viruses. Indeed, delivery of plasmids with large loading capacity (e.g., 100 kb) and co-transfection of several plasmids to cells can be achieved through EP (Magin-Lachmann et al., 2004). For *in vivo* applications, DNA is delivered to the tissue, usually by injection, and then the electric field is applied with penetrating needles or surface electrodes. Several advantages are highlighted for EP in gene transfer *in vivo*. First, EP shows high transfection efficiency with relatively little interindividual variability and increases tissue transgene expression by 100–1,000 folds compared to direct injection of naked DNA. For example, EP of plasmid-encoding IL-5 into mouse tibialis muscle produced 20 ng/ml of IL-5, while direct delivery of plasmids without EP generated only 0.2 ng/ml of IL-5 in the blood (Aihara and Miyazaki, 1998; Mir et al., 1999; Wells, 2004). One critical step for gene transfer by EP is that EP should be applied immediately after DNA administration (Gao et al., 2007). For one thing, the short time interval between these two procedures would minimize DNA degradation by extracellular nucleases (Gao et al., 2007). For another, several studies indicate that there is almost no gene transfection, comparable to direct injection of plasmids, if naked DNA is injected into tissue (e.g., skeletal muscle) after EP application, suggesting that DNA must be present while the electric pulse is being applied (Mir et al., 1999; Satkauskas et al., 2002). Second, EP can be used to deliver genes locally to tissues, rather than by systemic delivery, which avoids the unnecessary exposure of other tissues to electric fields and also reduces the DNA dose needed (Mir et al., 1991; Gilbert et al., 1997; Gehl, 2003; Mir, 2014). Any solid tissue, for example, skin, liver, skeletal muscle, lung, kidney, cornea, and retina, prone to exposure to electric fields could be subject to EP-mediated gene delivery (Mir et al., 1999; Blair-Parks et al., 2002; Dean, 2003; Dean et al., 2003; Franquesa et al., 2005; Jaichandran et al., 2006; Zhou and Dean, 2007; Matsuda and Cepko, 2008; Medi and Singh, 2008). The electrodes delineate the area for gene transfer, which increases gene targeting specificity (Gehl, 2003). Third, EP can be applied to all cell types and cells in the dividing and non-dividing stages, since the mechanism of transfection does not depend on the uptake function of cells, but rather the transiently formed pores on the plasma membrane and the electrophoretic force during EP (Wells, 2004; Hirao et al., 2008; Escobar-Chávez et al., 2009). Additionally, EP does not induce any immune response, which is a significant safety concern in viral vector delivery system. However, a recent report indicated that a small transient increase in neutrophils could be detected in the lungs of mice within the first hour of electroporation, but that this returned to normal within 24 h; whether this has any lasting effects is unknown at this point, but, given that EP has been used by multiple groups to treat ARDS in mouse and pig models, suggests that this is not of a great concern (Eliseeva et al., 2021). Some inflammatory responses can be potentially provoked by any unmethylated CpG motifs in plasmids, but this can be reduced by plasmid modification (Krieg et al., 1995; Nishikawa and Huang, 2001).

Electroporation has been developed to deliver plasmid DNA into the lungs to treat diseases (Dean et al., 2003; Hasson et al., 2005; Jones et al., 2005). Traditionally, solid tissues (e.g., skeletal muscle, heart, and liver) have been directly injected with plasmids, and then the electric field is applied for *in vivo* gene delivery (Dean, 2005). However, lungs are not completely amenable to this approach (Dean, 2003; Young et al., 2014). For one thing, plasmid solution usually needs to be injected through some syringe or needle into solid tissues (Wolff et al., 1990), whereas the structure of the lungs is not appropriate for direct injection. For another, the electrodes designed for solid tissues, such as penetrating electrodes and caliper plate electrodes (Somiari et al., 2000), are not suitable for lungs. The lung is a delicate organ, which directly contacts the external environment through the airway and has a large epithelial surface area for gas exchange (Katz et al., 2019). This easy access *via* the airways makes the lung amenable to plasmid delivery through intratracheal administration (Zhou R. et al., 2008). Our laboratory and others have developed protocols for DNA delivery

into the lungs (i.e., aspiration or inhalation) followed by EP, which show high transfection efficiency (Dean et al., 2003; Jones et al., 2005; Machado-Aranda et al., 2005; Gazdhar et al., 2006). Specifically, a plasmid solution containing 140 mM NaCl is administered to the lungs by aspiration in anesthetized mice, and then a pair of pre-gelled pediatric pacemaker surface electrodes is placed on either side of the chest under the armpits to deliver electric pulses that would travel through multiple tissue layers, e.g., skin, fat, and muscle, to reach the lungs (Dean, 2003). The parameters for optimal field strength have been determined to be 200 V/cm, using eight continuous 10 ms square wave pulses with 1 s interval (Dean et al., 2003). These parameters are also optimal in rats for lung delivery (Machado-Aranda et al., 2005). For larger animals such as 50-kg pigs, the DNA is delivered to anesthetized animals by bronchoscope to the desired lobe(s), and surface electrodes (in this case defibrillation pads) are used to deliver a train of eight pulses of ∼150 V/cm but with a shorter duration (∼100 to 150 μs each) (Dean et al., 2011; Emr et al., 2015). The distribution of transgene expression in the lungs has been evaluated by transferring reporter genes such as lacZ and GFP (Dean et al., 1999, 2003; Dean, 2003; Gottfried et al., 2016; Lin et al., 2016). Histological, immunohistochemical, and immunofluorescent analyses of mouse lung sections indicate that most cells receive and express transgenes throughout the lungs and in all cell types, such as the airway epithelium, alveolar epithelium (both ATI and ATII cells), endothelial cells, smooth muscle cells (both airway and vascular), and fibroblasts (Dean et al., 2003). Although plasmids could be administered intravenously to target the lungs, DNA nucleases are much higher in the serum than in the airway, and the injected DNA would be quickly degraded in the blood, resulting in low transfection efficiency (Song et al., 1997; Barron et al., 1999).

Choice of Gene Therapy Approach

Apart from their various properties, the choice of which type of gene therapy vector to use depends in great part on the disease being treated and its presentation. For example, in the case of a monogenetic disease such as sickle cell disease or cystic fibrosis, replacement of the defective genomic copy of the gene may be desired. If this is the case, homologous recombination methods such as CRISPR/Cas9 may be used, delivered either virally or by plasmid. Alternatively, the long-term expression of wild type copies of these genes may be sufficient to overcome the phenotype of the disease (e.g., overexpression of fetal hemoglobin in sickle cell patients), in which case using a viral vector that integrates into the genome such as a retrovirus or lentivirus would be desired. However, in the case of ARDS, three main issues should be considered. First, this is an acute disease that requires limited term expression of transferred genes. For example, if treatment involves overexpressing a Na^+ transporter such as ENaC or the Na^+, K^+-ATPase to reduce pulmonary edema, overexpression should be only for a short time so that disease-associated edema is cleared. If the gene was expressed long-term (e.g., by integration of the vector) in healthy individuals after resolution, increased fluid clearance from the lungs could result in mucus-rich, dehydrated lungs. Second, since ARDS is an acute-onset disease, any gene that is transferred to the lungs should be turned on quickly so that it would have maximal time to elicit benefit. In this case, mRNA could be a great choice, since it leads to almost immediate translation of proteins upon entry into the cell. However, the only drawback to mRNA approaches is that they are transient (perhaps too transient in this case), and unstable since mRNA is rapidly degraded by host nucleases. Plasmid DNA can also elicit rapid gene expression following entry into cells and tissues, with significant levels of expression seen in skeletal muscle in mice being detected within minutes of injection (Doh et al., 1997). Finally, in inflammatory diseases such as ARDS, the last thing that is wanted is using a gene delivery system that exacerbates the injury by causing more inflammation. Thus, viral vectors are not the best choice; nonviral vectors, with their greater safety profile, would be a more appropriate choice.

Current Gene Therapy for Acute Respiratory Distress Syndrome

A number of gene-based therapies have been developed over the past few decades for ARDS treatment (**Table 1**). In contrast to hereditary diseases, which require permanent alteration of the defective genome, ARDS is an acute disease of lung dysfunction, so short-lived or transiently altered gene expression is sufficient to treat the disease (Devaney et al., 2011). Gene delivery systems, such as viral vectors and conventional nonviral vectors, and physical delivery approaches have been well- developed to target the lungs for overexpression or silencing and make pulmonary gene transfer clinically possible (Jin L. et al., 2014; Ginn et al., 2018). Unfortunately, ARDS is not caused by a single gene, making gene therapy more difficult. However, a number of obvious target genes exist to promote AFC and edema resolution, repair the alveolar capillary barrier function, and relieve inflammation (**Figure 3**).

Gene Therapy to Improve Alveolar Fluid Clearance

In the setting of lung injury or ARDS, fluid clearance is impaired, and reduction in AFC rate correlates with increased mortality (Ware and Matthay, 2001; Huppert and Matthay, 2017). Thus, enhancing AFC has been considered one of the primary therapeutic goals for gene therapy for ARDS. Fluid clearance is based on the osmotic pressure created by transepithelial ion transport. Thus, similar to the principle of using β_2 agonists to enhance fluid clearance, gene delivery to potentiate AFC is based on the rationale that by introducing genes encoding various ion channels or transporters into the lungs, ion transport activities can be directly or indirectly upregulated. However, underlying the complexity of the disease, many factors need to be considered to develop any ARDS gene therapy. First, are the mechanisms known to stimulate AFC in normal lungs also effective in acutely injured lungs? One possible reason accounting for the failure of β_2 agonist therapy is the desensitization and internalization of β_2 receptors by long-term endogenous catecholamine stimulation, leading to decreased response during disease. Adenovirus-mediated gene transfer of the β_2-adrenergic receptor into healthy rat lungs increased receptor sensitivity to endogenous catecholamines and consequently upregulated Na^+,

TABLE 1 | Gene therapy approaches for acute respiratory distress syndrome (ARDS).

Target gene	Vector	Results	Clinical significance	Lung injury model	References
Gene therapy to enhance AFC					
ATP1 b1	Ad	Basal AFC, 100%	–	Normal rat without injury	Factor et al., 1998
ATP1 a2	Ad	+Na, K-ATPase activity; AFC	–	Normal rat without injury	Ridge et al., 2003
ATP1 b1	Ad	+AFC +Survival	Prevention	Hyperoxia and rat	Factor et al., 1999, 2000
ATP1 b1	Ad	+AFC, 100%	Prevention	Acutely elevated left atrial pressure	Azzam et al., 2002
b2 Adrenergic receptor	Ad	+AFC, 100% +Receptor function	–	Normal rat without injury	Dumasius et al., 2001
ATP1 a1 and ATP1b1	Cationic lipoplex	-W/D ratio +Na pump activity	Prevention	Thiourea, I.P injection, and mouse	Stern et al., 2000
ATP1 b1	Ad	+AFC	Prevention	High tidal volume ventilation and *ex vivo* rat	Adir et al., 2003
ATP1 a2	Ad	+AFC +Na pump activity -Barrier permeability	Prevention	High tidal volume ventilation and *ex vivo* rat	Adir et al., 2008
ATP1 b1	EP	+AFC, 100%	–	Normal rat without injury	Machado-Aranda et al., 2005
ATP1 b1	EP	+AFC, -W/D ratio -Cellularity, -protein in BAL, -barrier permeability	Prevention; treatment	I.t. delivery of LPS and mouse	Mutlu et al., 2007; Lin et al., 2016
ATP1 b1 ENaC-α1	EP	-W/D ratio, +survival, +Compliance, +PaO2/FiO2 ratio	Treatment	PS + I/R in gut, pig	Emr et al., 2015
CFTR	Ad	AFC, 100%	–	Normal rat without injury	Mutlu et al., 2005
Gene therapy to target pulmonary inflammation					
IL-10	Ad	-TNFα, IL-1, MPO in BAL -Inflammation -Histological injury +IL-10 level	Prevention	I.t. delivery of bacteria, pig	Morrison et al., 2000
IL-10	AAV	-Proinflammatory cytokines (IL-1β, TNFα, MIP-1α, and KC) -Histological injury	Prevention	I.t. delivery of bacteria, mouse	Buff et al., 2010
IL-10	Cationic lipoplex	-Lung, liver, and kidney injury (PMNs, MPO, and MDA) -TNFα	Prevention	Cecal ligation and puncture model of sepsis, mouse	Kabay et al., 2007
IL-10	Ad	Dose of adv vector inversely correlates to survival rate	Prevention	I.p. injection of zymosan, mouse	McAuliffe et al., 2006
IL-12	Ad	+Survival +IL-12 level in tissue and BAL	Prevention	I.t. delivery of bacteria, mouse	Greenberger et al., 1996
TGFb1	Cationic lipoplex	-Histologic score of rejection +Oxygenation	Prevention	Acute lung allograft rejection by left rat lung transplantation	Mora et al., 2000
Manganese SOD	Ad	+AFC +Na pump activity	Prevention	Hypoxia and rat	Litvan et al., 2006
CuZn-SOD; catalase	Ad	-Histologic injury score, inflammation +SOD, catalase activity	Prevention	100% oxygen, rat	Danel et al., 1998
Extracellular SOD	AAV	+Oxygen saturation +Lung compliance -PMNs, protein in BAL	Prevention	I.t. delivery of LPS, rat	Hassett et al., 2011
HO-1	Ad	+Survival -PMNs in BAL -Histologic injury	Prevention	Hyperoxia and rat	Otterbein et al., 1999
HO-1	Ad	-Histologic injury -Cytokine (KC, TNFα, and IL-10) in BAL	Prevention	Inhalation of aerolized LPS, mouse	Inoue et al., 2001
HO-1	Ad	-PMNs, KC, and TNFα in BAL -Epithelial cell death	Prevention	I.t. delivery of bacteria, mouse	Tsuburai et al., 2004
HO-1	Ad	+Survival -PMNs, total cell, cytokine in BAL -Histologic injury	Prevention	I.n. delivery of influenza A virus, mouse	Hashiba et al., 2001

(Continued)

TABLE 1 | (Continued)

Target gene	Vector	Results	Clinical significance	Lung injury model	References
HO-1	Cationic polyplex	PMNs, total cell, cytokine in BAL -Histologic injury	Treatment 2 h post	I.t. delivery of LPS, rat, mouse	Kim et al., 2019; Park et al., 2012
Ang-1	Ad	+Survival -Lung barrier permeability	Prevention	Septic shock model by i.p. injection of LPS, mouse	Huang et al., 2008
Ang-1	EP mediated transfection of MSC, *ex vivo*	-Lung barrier permeability (protein, Albumin, and IgM in BAL) -Proinflammatory cytokine, chemokine -Histologic injury	Treatment 30 mins post	I.t. delivery of LPS, mouse	Mei et al., 2007
HSP-70	Ad	-PMN infiltration -Mortality rate -Histologic injury	Prevention	Cecal ligation and double puncture model of sepsis, rat	Weiss et al., 2002
Adiponectin	Cationic polyplex	-TNFα, IL-1β in BAL and tissue -Histologic injury	Prevention	I.t. delivery of LPS, mouse	Piao et al., 2017
PGD synthase	Retroviral infection of fibroblast *ex vivo*	-BAL total cells -Edema +Survival	Prevention	I.t. delivery of bleomycin, mouse	Ando et al., 2003
NF-κB p65	siRNA	-NF-κB, MMP9 -W/D, histology -total cell, IL-6,17 in BAL	Prevention	Cecal ligation and puncture model of sepsis, mouse	Jin L.-Y. et al., 2014
NF-κB	siRNA	-NF-κB, TNFa level -W/D, white blood cell +Rectal temperature	Prevention	I.p. injection of LPS, rat	Li et al., 2016
KC, MIP-1	siRNA	-IL-6, MPO, and MIP-2 KC and PMNs	Prevention	Cecal ligation and puncture after hemorrhage induction, mouse	Lomas-Neira et al., 2005
FER	EP	+Survival, lung compliance, anti-bacterial immune response, and bacterial clearance -Total cells in BAL -Histological injury	Prevention	Lung contusion with secondary bacterial pneumonia, mouse	Dolgachev et al., 2016
FER	EP	+Survival, anti-bacterial immune response (IFN-γ, TNF-α, and KC), bacterial clearance	Prevention Treatment	Bacterial pneumonia and mouse	Dolgachev et al., 2018
Gene delivery to restore alveolar capillary barrier function					
ATP1 b1	EP	+AFC, -W/D ratio -Cellularity, -protein in BAL, -barrier permeability	Treatment	I.t. delivery of LPS, mouse	Lin et al., 2016
ATP1 b1	EP	+AFC, -W/D ratio -Cellularity, protein in BAL	Treatment	I.t. delivery of LPS, mouse	Mutlu et al., 2007
ATP1 b1 and ATP1 a2	EP	+Lung compliance -Lung barrier permeability -Histologic injury	Treatment (immediately after trauma)	Lung contusion by blunt chest injury, mouse	Machado-Aranda et al., 2012
MRCKα	EP	-W/D ratio -Cellularity, -protein in BAL, -barrier permeability	Treatment	I.t. delivery of LPS, mouse	Liu and Dean, 2021
FER	EP	+Survival, lung compliance -Lung permeability -Total cells in BAL -Histological injury	Prevention	Lung contusion with secondary bacterial pneumonia, mouse	Dolgachev et al., 2016
Ang-1	Ad	+Survival -Lung barrier permeability -W/D, MPO	Prevention	Septic shock model by i.p. injection of LPS, mouse	Witzenbichler et al., 2005; Huang et al., 2008

+, increase; –, decrease. ATP1, Na^+, K^+-ATPase; W/D, wet to dry ratio; CFTR, cystic fibrosis transmembrane conductance regulator; KC, murine chemokine CXCL1; MIP2, murine chemokine CXCL2; MIP-1a, macrophage inflammatory protein-1 alpha; PMN, neutrophil; MPO, myeloperoxidase; MMP9, matrix metallopeptidase 9; MDA, malondialdehyde; SOD, superoxide dismutase; NOS-2, nitric oxide synthase; PGD synthase, prostaglandin D synthase; HSP-70, heat shock protein 70; HO-1, heme oxygenase-1; MRCKa, CDC42-binding protein kinase alpha; AFC, alveolar fluid clearance; BAL, bronchoalveolar lavage fluid; TEER, transepithelial/trans-endothelial electrical resistance; EP, electroporation.

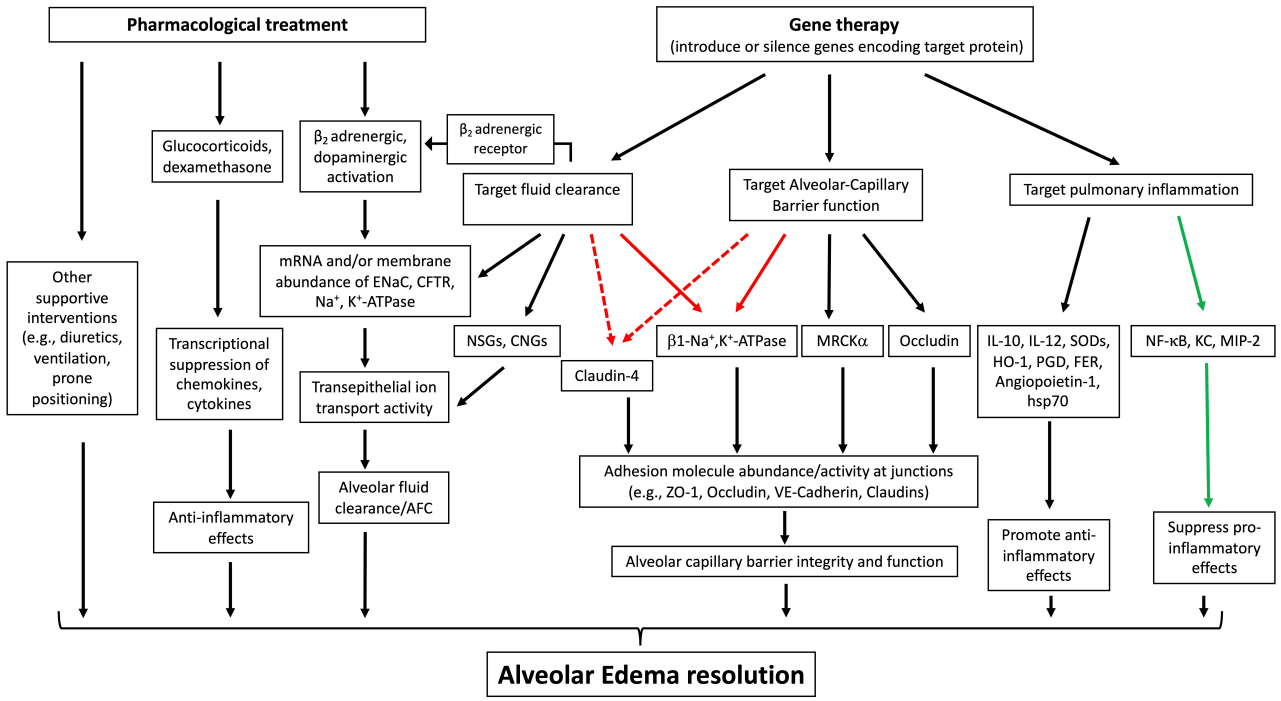

FIGURE 3 | Therapeutic approaches to treat acute lung injury (ALI)/ARDS. Pharmacological interventions primarily focus on enhancing fluid clearance through b2 adrenergic or dopaminergic activation, dampening inflammation with glucocorticoids, and use of supportive strategies, all of which have led to limited therapeutic effects. Gene therapies to overexpress or silence target genes have targeted alveolar fluid clearance, alveolar capillary barrier function, and pulmonary inflammation. The protein targets of gene therapy for ARDS/ALI have been validated in various animal or *ex vivo* organ models, indicating their therapeutic potential. Black solid arrow: increase, upregulate or target downstream effects; green solid arrow: decrease or inhibit target proteins; red solid arrow: has been validated in various animal models to have a dual role in both upregulating fluid clearance and lung barrier function; red dotted arrow: has the potential for dually upregulating fluid clearance and lung barrier integrity. ENaC, epithelial sodium channel; CFTR, cystic fibrosis transmembrane conductance regulator; NSGs, non-selective cation channels; CNGs, cyclic nucleotide-gated channels; MRCKa, CDC42 binding protein kinase alpha; ZO-1, zonula occludens-1; VE-cadherin, vascular endothelial-cadherin; HSP-70, heat shock protein 70; HO-1, heme oxygenase-1; KC, murine chemokine CXCL1; MIP2, murine chemokine CXCL2; PGDsyn, prostaglandin D synthase.

K^+-ATPase activity and ENaC expression in the lungs, leading to improved AFC (Dumasius et al., 2001, 2003). In a hyperoxia-induced injury model, gene transfer of the β_2 receptor protected animal lungs from subsequent injury, showing decreased lung edema and increased survival (Mutlu et al., 2004). However, most studies showing beneficial effects by upregulating β_2 receptors in lung injury have been limited to animal models with gene delivery prior to inducing injury, and such protection studies have limited clinical significance.

Second, what aspects really matter for the upregulation of fluid clearance by delivered genes encoding different ion transporters or channels? The underlying mechanisms of catecholamine-activated Na^+ transport include increased protein synthesis and membrane recruitment of the Na^+, K^+-ATPase and ENaC, as well as increased open probability of the channels (Yue et al., 1995; Minakata et al., 1998; Bertorello et al., 1999; Saldías et al., 1999). Although catecholamine activation significantly increases edema clearance in various animal models and in isolated human lungs (Sakuma et al., 1994; Lecuona et al., 1999; Frank et al., 2000), the experimental design of these studies is not entirely reflective of the pathophysiology of ARDS in which the alveolar-capillary barrier is at least partially disrupted. In all these experimental cases, there is an intact alveolar epithelial barrier, allowing for effective AFC by upregulating Na^+ transport (Mutlu and Sznajder, 2004). This brings up the fact that effective net AFC depends on an intact epithelial barrier that can transport ions across the alveolar epithelium (Huppert and Matthay, 2017). Overexpression of Na^+, K^+-ATPase, ENaC, cystic fibrosis transmembrane conductance regulator (CFTR), or other ion channels through gene delivery directly increases ion transport activity, accelerating fluid clearance. For example, overexpression of the Na^+, K^+-ATPase α2, or β1 subunit through adenoviral transfer or electroporation of β1 subunit plasmids into healthy rat lungs increased fluid clearance rates by >100 (Factor et al., 1998), 250 (Ridge et al., 2003), and 74% (Machado-Aranda et al., 2005), respectively. Adenoviral delivery of the Na^+, K^+-ATPase into rat lungs before induction of lung injury (e.g., by hypoxic injury or VILI) prevented and even somewhat reversed the decrease in AFC, compared to injury alone (Factor et al., 2000; Adir et al., 2003, 2008). However, in most *in vivo* studies, adenoviral vectors were delivered either to healthy lungs or prior to induction of lung injury by various insults, such as ventilation, acute elevation of lung pressure, or 100% oxygen, demonstrating that gene therapy to enhance AFC could protect against experimental lung injury. These studies could be implied to suggest that gene delivery to an intact alveolar epithelium was

required for upregulating transepithelial ion transport and net AFC. In this respect, the β1 subunit of the Na^+, K^+-ATPase has been identified as a unique target for gene delivery to treat acute lung injury, since the Na^+, K^+-ATPase is not only the major driver of AFC but is also closely involved in the regulation of epithelial barrier integrity (Rajasekaran and Rajasekaran, 2003; Vadasz et al., 2007). Our laboratory has focused on β1 subunit gene therapy using different animal models, such as mice (Mutlu et al., 2007), rats (Machado-Aranda et al., 2005), and pigs (Emr et al., 2015), and our previously published data have shown that electroporation-mediated gene delivery of the β1 subunit of the Na^+, K^+-ATPase can rescue endotoxin pre-injured mouse lungs by both increasing AFC and, more importantly, restoring lung barrier function. These studies indicate that lung barrier integrity is important for gene therapy targeted at increasing net AFC (Lin et al., 2016).

Apart from ion channel transporters, some paracellular tight junction molecules, for example, claudin-4, play roles as regulators of ion transport across the epithelium, showing selective permeability to ions (Brune et al., 2015). For one thing, they are required for maximal epithelial barrier function and are upregulated during epithelial repair (Wray et al., 2009). For another, they are not ion transporters but are closely involved in paracellular ion conductance and have an impact on fluid clearance (Rokkam et al., 2011). Because of this, claudin-4 could be a potential target for gene therapy to increase AFC and barrier integrity. Claudin-4 is highly expressed in both ATI and ATII cells (LaFemina et al., 2010), and staining of human lungs shows a positive correlation of claudin-4 with AFC, indicating that higher claudin-4 expression is associated with higher rates of fluid clearance (Rokkam et al., 2011). It is still not clear whether claudin-4 levels are altered from baseline during injury in human lungs, although its expression was increased during early experimental lung injury.

Nonselective cation channels (NSCs), cyclic nucleotide-gated channels (CNGs), and the CFTR Cl channel are also expressed apically and contribute to the creation of the transepithelial osmotic gradient (Mutlu and Sznajder, 2005). In fluid-filled lungs of mice with genetic KO of acid-sensing ion channel 1 (ASIC1), a nonselective cation channel, fluid clearance was reduced by more than 50% compared to wild type, which is comparable to the AFC reduction seen in mice with ENaC inhibition, indicating that ASIC1 could also be a potential target for gene delivery for AFC (Trac et al., 2017). CFTR is important for maintaining alveolar fluid homeostasis and has been identified contributing to lung fluid reabsorption (Fang et al., 2002). Indeed, overexpression of CFTR by adenoviral infection of mouse lungs increases AFC 90% over that seen following infection with empty vector control (Mutlu et al., 2005).

Gene Therapy to Target Pulmonary Inflammation

In the early stage of ARDS, the uncontrolled production of cytokines and chemokines initiate proinflammatory responses, which further exacerbates lung injury. Therefore, gene delivery of target proteins to suppress or dampen proinflammatory effects or enhance anti-inflammatory effects has been studied on various experimental models of ARDS and proven successful *in vivo*. One strategy has been to deliver genes to overexpress anti-inflammatory cytokines, anti-oxidant enzymes, antiproteases, and other protective proteins to attenuate inflammation-associated lung injury during ARDS (Liu and Slutsky, 1997). However, most experiments delivered target genes before inducing injury and primarily demonstrated the protection effect with less clinical significance. IL-10 is an anti-inflammatory cytokine and is produced to suppress proinflammatory responses by inhibiting proinflammatory cytokine release, thus limiting the excessive injury induced by inflammation (Couper et al., 2008). Ad delivered IL-10 into animal lungs prior to bacterial infection significantly prevented histological lung injury, release of proinflammatory TNα in BAL, and overall inflammation (Morrison et al., 2000; Buff et al., 2010). Furthermore, in a systematic sepsis model in mice, lipoplex-delivered IL-10 significantly prevented lung and other organ injury (Kabay et al., 2007). However, it was reported that adenoviral-delivered IL-10 protected lungs with improved outcome only when a relatively low dose of vector was administered and that survival rates actually worsened at higher doses (McAuliffe et al., 2006). This highlights the inherent problems associated with using a proinflammatory viral vector to treat an inflammatory disease. Other cytokine therapeutic targets for gene delivery have included IL-12, which has shown protection from subsequent lethal doses of Klebsiella in a pneumonia model (Greenberger et al., 1996).

Superoxide dismutases (SODs) are a group of metalloenzymes that form the front line of defense against oxidative stress, e.g., ROS, in the body (Landis and Tower, 2005). During ARDS, the overwhelmed inflammatory response leads to excessive ROS, contributing to disease progression (Ware and Matthay, 2000). Overexpression of SOD in transgenic mice has been shown to have a protective effect on lung injury induced by hyperoxia (Ahmed et al., 2003), hypoxia (Litvan et al., 2006), LPS (Bowler et al., 2004), and influenza virus (Suliman et al., 2001). However, the pharmacological administration of SODs for clinical treatment of ARDS has shown limited success due to the enzyme's short half-life in circulation, rapid renal excretion, and inability to penetrate cells to remove ROS, leading to low accumulation within injured lungs (Danel et al., 1998). Adenoviral-mediated gene transfer of SODs prevented hypoxia-induced lung injury, counteracting ROS-induced endocytosis of the Na^+,K^+-ATPase and subsequent decrease in alveolar fluid reabsorption (Litvan et al., 2006). Another study further demonstrated the protection effects of SODs on LPS-induced injury, in terms of physiological oxygen saturation and lung compliance, lung barrier function, and inflammation (Hassett et al., 2011). However, again, all studies have used protection, not treatment protocols, thus calling into question the utility to treat ARDS clinically.

Heme oxygenase-1 (HO-1) is another candidate for gene delivery to fight against excessive oxidative stress during lung injury. It is a critical enzyme for catabolizing heme and shows antioxidant, anti-inflammatory, and anti-apoptotic properties for vascular protection (Araujo et al., 2012). Chemical

inhibition or genetic silencing of HO-1 significantly increases proinflammatory cytokine release, immune cell infiltration, and apoptosis, indicating that HO-1 has a protective role in LPS-induced ALI (Wiesel et al., 2000; Gong et al., 2008; Zhang Y. et al., 2013). Ad transfer of HO-1 has been shown to confer protection against hyperoxia, aerosolized endotoxin, and bacteria- or virus-induced lung injury (Otterbein et al., 1999; Hashiba et al., 2001; Inoue et al., 2001; Tsuburai et al., 2004). In a more recent study, the treatment effect of HO-1 nonviral gene delivery was tested, in which HO-1 plasmids were co-delivered *via* a polymeric complex with an anti-inflammatory compound after LPS had been administered intratracheally to induce injury. Thus, gene delivery was carried out in lungs that were already injured. From the results, the histological lung injury and proinflammatory cytokine release were significantly rescued (Kim et al., 2019). Other gene therapeutic targets for anti-inflammatory strategy include angiopoietin-1 (see below), heat shock protein 70, and adiponectin, but while all have shown some promise in limiting inflammation, improving edema resolution, and/or improving survival, all have either carried out protection studies only, or have failed to have an effect when tested in a treatment strategy to decrease damage and disease in lungs with pre-existing injury (Weiss et al., 2002; Huang et al., 2008; Piao et al., 2017).

Prostaglandins (PGs) are a group of lipid compounds that mediate inflammatory responses (Ricciotti and FitzGerald, 2011). They are converted from arachidonic acid through various PG synthases, the rate-limiting enzymes in the cyclooxygenase pathway of arachidonic acid metabolism (DeWitt, 1991). Multiple observations have indicated that PGs, such as PG E2 (PGE_2) and PG D2 (PGD_2), have anti-inflammatory and protective effects on ARDS (Birukova et al., 2007; Scher and Pillinger, 2009; Murata et al., 2013). Thus, gene transfer of PG synthase has been evaluated to upregulate endogenous PGs for treating lung injury. This approach worked in protection studies to reduce bleomycin-induced lung injury, but more importantly, retrovirally introduced PGD2 synthase into murine lungs that were pre-injured by bleomycin remarkedly increased animal survival rate and reduced edema accumulation, leukocyte infiltration, and plasma extravasation (Ando et al., 2003).

Another interesting approach has been to mine high-throughput sequencing analyses to identify potential therapeutic targets for ARDS. A recent genome-wide association study (GWAS) identified the non-receptor cytosolic tyrosine kinase FER as being associated with increased survival in patients with pneumonia and sepsis (Rautanen et al., 2015). In a mouse model of combined lung contusion and pneumonia, the expression of FER was down compared to controls. When FER was overexpressed in the lungs by electroporation-mediated gene transfer, survival of the animals was improved and antibacterial response genes were activated (Dolgachev et al., 2016). In follow-up studies, it was shown that in a Klebsiella pneumonia model in mice, electroporation-mediated delivery of FER plasmids to the lungs of mice previously infected with Klebsiella not only improved survival but also reduced bacterial counts in the lungs. Further experiments suggested that FER gene transfer activates the STAT pathway to enhance innate immunity and accelerate bacterial clearance in the lung (Dolgachev et al., 2018).

Another strategy to fight against the overwhelming inflammation in early ARDS is to suppress the proinflammatory effects by silencing genes that directly regulate the production of proinflammatory cytokines and chemokines or indirectly promote inflammation through RNAi (Lomas-Neira et al., 2008). Intratracheal delivery of siRNA could target the local lungs with high efficiency (Lomas-Neira et al., 2005). NF-κB is an important inflammation inducer that regulates the transcription of a number of downstream proinflammatory cytokines or mediators, such as IL-1b, IL-6, and TNF-α (Jha and Das, 2017). In recent studies, siRNA-mediated local or systematical silencing of NF-κB significantly protected animals against further increased proinflammatory cytokine (TNF-α, IL-6) release, histological and other lung injury scores in animals subsequently injured with LPS compared with injury and scramble control, indicating that NF-κB could be a potential target for developing RNAi gene therapy for ARDS (Jin L.-Y. et al., 2014; Li et al., 2016). A decoy strategy has also been employed to target the NF-κB inflammatory pathway, to block the binding of NF-κB to promoter regions of its targeted genes, resulting in the inhibition of proinflammatory gene transcription. In this approach, multiple copies of oligonucleotides encoding the NF-κB consensus binding site are delivered to cells or tissues to compete for activated NF-κB binding, thereby reducing normal signaling. In a sepsis model of cecal ligation and puncture (CLP), tail-vein injection of synthetic double stranded oligodeoxynucleotides (ODNs) to decoy NF-κB showed protective effects on septic lung injury, such as decreased transcription of sepsis-induced proinflammatory genes (iNOS, COX-2), decreased histological damage in terms of alveolar wall thickening, immune cell infiltration and hemorrhage, decreased vascular permeability, and improved blood gas exchange capacity (Matsuda et al., 2005). Other proteins that have a potential for siRNA therapeutics include neutrophil chemo-attractant Keratinocyte derived-chemokine (KC) and macrophage inflammatory protein-2 (MIP-2), which alleviate neutrophil activation-induced excessive inflammation and injury (Lomas-Neira et al., 2005). Similarly, in a hemorrhage induced sepsis mouse model, intratracheal delivery of siRNA to locally silence chemoattractant cytokines KC and MIP-2 significantly suppressed neutrophil influx into the lungs and decreased tissue or plasma level of IL-6, MIP-2 and MPO, activity (Lomas-Neira et al., 2005).

Gene Delivery to Restore Alveolar Capillary Barrier Function

Alveolar capillary barrier disruption is a primary cause of ARDS leading to the influx of protein-rich fluid into alveoli and accumulation of pulmonary edema. Damage to junctional structures between epithelial cells and/or between endothelial cells, and cell death both result in alveolar capillary barrier dysfunction.

In recent years, gene delivery of target proteins to repair lung barrier function has been investigated. The Na^+, K^+-ATPase has been primarily studied for its function in promoting AFC, although increasing evidence demonstrates that the expression

and function of the Na^+, K^+-ATPase are also closely involved in the regulation of epithelial barrier integrity (Rajasekaran et al., 2001b; Rajasekaran and Rajasekaran, 2003; Vadasz et al., 2007). For example, the Na^+, K^+-ATPase β1 subunit has been shown to be necessary for membrane localization of TJs, e.g., ZO-1, occluding. Furthermore, silencing of the β1subunit by siRNA disrupts the continuous staining pattern of ZO-1, indicating that the β1subunit might be directly or indirectly associated with TJ proteins (Madan et al., 2007; Rajasekaran et al., 2007). The Na^+, K^+-ATPase is required for establishing epithelial polarization and co-expression of the Na^+, K^+-ATPase β1 subunit, and E-cadherin has been shown to recover the lost polarity and junctions in MSV-MDCK cells, a highly invasive cell line (Rajasekaran et al., 2001b). In addition, the basolateral membrane-localized Na^+, K^+-ATPase acts as an adhesion molecule, forming a trans-dimer junction structure between cells. This structure appears dependent on the N-glycosylation of the β1 subunit's extracellular domain (Vagin et al., 2012). Because of the dual role of Na^+, K^+-ATPase in enhancing both AFC and epithelial barrier function, this transporter has been targeted for gene delivery to treat ALI in various experimental animal models. Recent data from our laboratory showed that overexpression of the rat or human Na^+, K^+-ATPase β1 subunit upregulated the protein expression and membrane localization of TJ proteins occludin and ZO-1 (Lin et al., 2016; Bai et al., 2021). Furthermore, in cultured monolayers of rat alveolar epithelial ATI cells, overexpression of the β1 subunit increased transmembrane epithelial electrical resistance (TEER) by 30% compared to untransfected cells or those transfected with empty plasmids (Bai et al., 2021). More importantly, electroporation-mediated gene delivery of the rat or the human β1-subunit of the Na^+, K^+-ATPase to the lungs of mice with existing LPS-induced lung injury rescued experimental ALI by upregulating both AFC and TJ protein abundance, and pulmonary barrier function, as demonstrated by decreased lung permeability, total protein, and cellularity in BAL fluid, and improved overall histological injury outcomes (Mutlu et al., 2007; Lin et al., 2016).

Based on these findings, our laboratory pursued two avenues for further treatment: evaluation of the ability of gene transfer of individual tight junction protein genes to protect and treat ARDS in animal models, and identification of the pathway(s) by which the β1-subunit of the Na^+, K^+-ATPase upregulates barrier function. Occludin plays a central role in the formation, maintenance, regulation, and structure of tight junctions. In unpublished studies, when an occludin-expressing plasmid was delivered to the lungs of mice that had been injured 24 h previously with intratracheal LPS, occludin was overexpressed, which resulted in decreased pulmonary edema, improved histology, and reduced inflammation (numbers of infiltrating neutrophils and reduced levels of proinflammatory cytokines), compared to animals that received empty plasmids or no treatment at all (Lin et al., submitted for publication). However, it was not determined whether expression was localized to the alveolar epithelium or the capillary endothelium. Either way, these results suggest that gene therapy to increase levels of tight junction proteins directly may be a viable approach to treat ARDS.

Claudin family TJ proteins are critical components that are required to form apical junction complexes for alveolar epithelial barrier function. The major claudins expressed in ATI and ATII cells include claudins 3 and 4 (Frank, 2012). Although claudins 3 and 4 are highly homologous in a peptide sequence, they have opposite effects on the regulation of alveolar barrier function: overexpression of claudin 4 leads to decreased epithelial permeability and increased TEER, while opposite responses are observed for claudin 3 (Mitchell et al., 2011). In response to various injury-inducing stimuli (e.g., hyperoxia, ventilation, and septic shock), claudin 4 expression has been shown to be downregulated in mice, although an early upregulation of claudin 4 mRNA level was observed at 4 h in a ventilation-induced ALI mouse model (Wray et al., 2009; Cohen et al., 2010; Herrero et al., 2018; Vyas-Read et al., 2018). So far, there is no direct *in vivo* evidence indicating that overexpression of claudin 4 could benefit or protect from injury in living lungs, but claudin 4 could be a potential target for ARDS by gene therapy. Taken with the fact that levels of claudins show a positive correlation with AFC in human lungs, it suggests that this approach may have merit (Rokkam et al., 2011). In addition, claudin 5 is a primary tight junction component expressed in pulmonary endothelial cells (Kaarteenaho-Wiik and Soini, 2009) and has been shown to be downregulated in the lungs during influenza infection and other models of ALI (Armstrong et al., 2012). In several cell culture experiments, the overexpression of claudin 5 significantly protected endothelial cells from LPS-induced decreased permeability to molecules and restored TEER in these cells, indicating a role for claudin 5 in barrier protection and pulmonary leakage (Soma et al., 2004; Armstrong et al., 2012). The therapeutic potential of claudin 5 was further confirmed in a study investigating the beneficial effects of simvastatin on ALI, which concluded that claudin 5 is an important mediator of ALI protection by simvastatin (Chen et al., 2014). These studies indicate that claudin 5 might be a promising target for gene therapy for lung barrier protection.

Claudin-18 is another major tight junction protein expressed specifically in alveolar epithelial cells. So far, there has been no study directly reporting the effects, of claudin-18, by gene delivery, on ALI in an animal model. This is probably due to the complex role of claudin-18 in edema resolution. Claudin-18-knocked out mice did not exhibit apparent respiratory dysfunction and showed unchanged wet-to-dry weight ratios from baseline, although claudin-18 was downregulated at the protein and transcriptional levels during injury (Ohta et al., 2012; Li et al., 2014). However, the claudin-18-knocked out mice showed increased lung permeability to ions and solutes of various sizes, significantly enhanced fluid clearance rates with increased ion transport activities, and altered expression of claudins 3 and 4 (LaFemina et al., 2014; Li et al., 2014). These results suggest that the overexpression of claudin-18 may have actually exacerbated the injury, although because of its ability to regulate other claudins, cytoskeletal organization, b_2 adrenergic signaling, and CFTR activity, it is difficult to say what ultimate effects its overexpression could have.

Fas/FasL apoptosis signaling has been identified as a main form of lung epithelial cell death, leading to alveolar capillary

barrier damage. The Fas/FasL system is significantly upregulated in the BAL fluid of ARDS patients (Albertine et al., 2002). Using a FasL analog to compete with FasL for Fas binding improved pneumococcal bacterial clearance from the lungs of mice, indicating that the Fas/FasL axis might have a therapeutic potential (Matute-Bello et al., 2005b). In another study on hemorrhage-induced septic ALI, intratracheal instillation of siRNA 4 h after hemorrhagic shock and sepsis induction to silence local Fas in the lung showed markedly decreased levels of cytokines, such as TNFα, IL-6, and IL-10, and caspase 3 activity, indicating a protective effect by blocking Fas/FasL (Perl et al., 2005). Although this study primarily targeted local alveolar epithelial cells, the Fas receptor is also expressed in vascular endothelial cells, resulting in endothelial apoptosis (Hotchkiss et al., 2002). Thus, targeting Fas expression in the vascular endothelium for ARSD or sepsis endothelial injury might be a possible direction for future study.

Lung microvascular endothelial dysfunction plays an important role in the pathogenesis of ARDS and sepsis, and numerous approaches to modulate endothelial cell activation and decrease vascular leakage have been and are being investigated. For example, the bioactive sphingolipid metabolite sphingosine-1-phosphate (S1P) and its receptor, S1PR, have been reported to have protective effects on the endothelial barrier. S1P-mediated cellular events induce MLC phosphorylation, activation of Rho GTPase, and recruitment and assembly of adhesion junction molecules. Furthermore, genetic variants of the S1P receptor, such as S1PR3, have been shown to be positively associated with risk of ARDS (Natarajan et al., 2013). A recent publication reported that S1P receptor 1 (S1PR1) deletion resulted in increased endothelial permeability, and that S1PR1-expressing endothelial cells are required for barrier repair, suggesting a therapeutic potential of the S1P pathway in endothelial barrier dysfunction (Akhter et al., 2021).

Angiopoietin-1 (Ang-1) is an agonist for the endothelial-specific receptor tyrosine kinase Tie2 and has been shown to protect from microvascular endothelial permeability and plasma leakage both *in vitro* and *in vivo*. The Ang-1-Tie2 axis signals to downstream effector proteins and regulates intercellular junction complexes, like VE-cadherin or other adhesion molecules (e.g., PECAM-1, ICAM-1, VCAM-1, and E-selectin) (Gamble et al., 2000; Parikh, 2017). The anti-inflammatory role of Ang-1 has also been shown to be due to its suppression of neutrophils' adherence to the endothelium during transmigration in ALI (Gamble et al., 2000). In mice with LPS-induced lung injury, Ang-1 expression was decreased in the lungs (Karmpaliotis et al., 2002). In mouse models of endotoxin-induced septic shock, animals pre-treated with adenoviral-mediated delivery of Ang-1 showed more resistance to subsequent injury, demonstrating significantly decreased lung edema, vascular permeability, histological damage, and mortality rate (Witzenbichler et al., 2005; Huang et al., 2008).

Mesenchymal stem cell (MSC)-mediated gene therapy has been reported to have a promising therapeutic potential for treating diseases such as sepsis. There are several advantages in using MSC therapy to treat ALI and sepsis. MSCs can be stably expanded *in vitro* to produce sufficient quantities for use while maintaining an undifferentiated state (Laffey and Matthay, 2017). This means that from another perspective, delivering target genes to manipulate endothelial damage, inflammation, or vascular injury could be more easily achieved through plasmid transfection of MSCs *in vitro*. In recent studies, systemic delivery of MSCs that overexpress an Ang-1 transgene to animals before LPS injury showed protective effects such as decreased lung leakage of plasma protein and decreased neutrophil infiltration (McCarter et al., 2007; Xu et al., 2008). Although these are promising results, there are fewer studies showing that Ang-1 overexpression could benefit the pre-injured lungs in animals. In addition, potential side effects of Ang-1 treatment were also reported (Sullivan et al., 2003; Long et al., 2008). Second, MSCs can easily access and traverse the vascular endothelium and incorporate into injured lungs *via* intravenous infusion, all while maintaining the ability to divide and self-renew. In addition, MSCs have good safety records with minimal immunogenicity, which helps avoid immune responses, a common adverse effect seen during viral vector-mediated gene delivery (Laffey and Matthay, 2017).

Several studies have reported that MSC-mediated gene therapy could repair alveolar capillary barrier disruption by targeting various genes. Ang-1 is the most studied one. Administration of MSCs overexpressing Ang-1 decreased IgM and albumin levels in BAL by more than 50% compared to LPS-induced lung hyperpermeability alone in mice, indicating significant restoration of lung barrier function (Mei et al., 2007). Growth factor genes, e.g., fibroblast growth factor (FGF), keratinocyte growth factor (KGF), and vascular endothelial growth factor (VEGF), also show a therapeutic potential for MSC-mediated gene therapy (Lee et al., 2011). FGF-activated signaling is required for the maintenance of interendothelial adhesion, the inhibition of which results in dissociation of the VE-cadherin/p120-catenin complex and disassembly of adherens and tight junctions, and eventually leads to endothelial barrier disruption and vascular leakage (Murakami et al., 2008). Moreover, FGF signaling is necessary for glycocalyx reconstitution, protecting against glycocalyx shedding during sepsis (Yang et al., 2017). Tail vein injection of MSCs overexpressing FGF2 into mice in an LPS-induced sepsis model significantly decreased the level of total protein and cytokines in BAL, lung edema, and histological lung damage, indicating the treatment potential of MSC-mediated FGF in lung injury (Zhao et al., 2015). KGF signaling is critical for pulmonary epithelial repair and proliferation. In animal lungs injured by intratracheal LPS administration, KGF gene-modified MSCs protected lung permeability, significantly decreased total protein levels in BAL, decreased edema (wet to dry ratio), attenuated inflammation with decreased expression of cytokines (IL-1β and TNF-α) and myeloperoxidase (MPO) activity in BAL fluid, and increased animal survival (Chen et al., 2013). Finally, VEGF is notable as a vascular permeability factor that has been shown to induce endothelial barrier disruption. In addition, paracrine factors secreted by MSCs, such as hepatocyte growth factor (HGF) and VEGF, were also investigated as having protective effects on endothelial barrier integrity in experimental LPS-induced lung injury models (Yang et al., 2015, 2016).

As a second approach to repair barrier function in ARDS, our lab has worked to identify the pathway(s) by which the β1 subunit of the Na^+, K^+-ATPase upregulates barrier function in cells and mouse models. Using a proteomics approach, we recently demonstrated that the novel CDC42-related kinase MRCKα is a specific interacting partner of the Na^+, K^+-ATPase β1-subunit, and that it is responsible for the upregulation of tight junction protein abundance in the membrane and activity seen following β1 overexpression (**Figure 2**; Bai et al., 2021). Interestingly, MRCKα expression appears down in the lungs of patients with ARDS compared to control lungs (Bai et al., 2021). In cultured rat ATI cells, we demonstrated that MRCKα was both necessary for β1's ability to upregulate tight junction expression and TEER in cells using siRNA to knock down MRCKα expression and using specific inhibitors of the kinase (Bai et al., 2021). Furthermore, we also showed that the overexpression of MRCKα itself was sufficient to increase TEER seen in the monolayers. More recently, we have asked whether MRCKα overexpression in the lungs of mice in which lung injury had been previously induced by administration of LPS and have found that the overexpression of MRCKα leads to the same upregulation of tight junction proteins as does the transfer of the β1 subunit of the Na^+, K^+-ATPase, and provides the same degree of treatment of the disease, reducing pulmonary edema, lung permeability, measures of inflammation, and histological health of the lungs (Liu and Dean, 2021). As expected, the overexpression of MRCKα had no effect on the rates of AFC in mice. Taken together, these results suggest that repair of the epithelial and endothelial barrier activities is more important than upregulation of AFC alone.

While mouse models are often the first test of a given gene therapy, or for any therapeutic, approach for utility *in vivo*, they do not always reflect what will actually be of use therapeutically in patients. Indeed, this has been a major problem in developing effective treatments for ARDS in the past. An animal model that accurately duplicates the complex inflammatory and hemodynamic response that occurs in humans over several days during the development of sepsis-induced ARDS is critical to the understanding and treatment of lung injury. It has been shown that the success of clinical trials testing sepsis therapies depends on sound preclinical data using appropriate animal models (Piper et al., 1996). The best model, termed a "good evidence" model, is one in which the results of the study would be similar in a clinical trial (Piper et al., 1996). For example, while studies testing anti-endotoxin HA-1A demonstrated improved survival in a murine model of sepsis (Teng et al., 1985), a phase III clinical trial did not show survival benefit (Ziegler et al., 1991). However, when a clinically relevant "good evidence" large animal model of chronic septic shock was utilized to test the efficacy of HA-1A, the results were very similar to those of the clinical trial (Quezado et al., 1993). Similarly, a provocative article in PNAS several years ago generated great interest and press since it suggested that findings from mouse models alone do not always translate to larger species or humans (Seok et al., 2013).

There is a consensus that chronic, insidious-onset large animal models that mimic the pathogenesis of sepsis-induced ARDS are superior to small animal models as predictors of clinical efficacy (Parker and Watkins, 2001). To this end, Nieman et al. developed a chronic (48 h) two-hit sepsis and gut ischemia/reperfusion porcine model that accurately resembles the pathologic progression from injury to systemic inflammatory response syndrome, to septic shock, and finally to ARDS seen in human patients (Steinberg et al., 2005). Following injury, the animals are maintained and sedated according to the ARDSnet treatment paradigm (Network, 2000), making comparisons to existing human clinical trial data, more relevant and clear. Since sepsis is the leading cause of indirect ARDS, this model is highly germane developing any ARDS treatment (Sigurdsson et al., 2013). This model contains all of the key components of a "good evidence" animal model, such as a randomized, controlled study with supportive therapy (fluids, antibiotics) in a large animal, insidious-onset, chronic model of septic shock and ARDS (Piper et al., 1996). Moreover, the injury induced in this model yields all the features of ALI identified in the ATS consensus report on ALI in animals (Matute-Bello et al., 2011). The data generated from this model, which includes inflammatory mediator response, hemodynamic measures, lung function, and blood chemistry have allowed us to demonstrate that electroporation-mediated gene delivery of ENaC and Na^+, K^+-ATPase subunits 4 h after injury provides effective treatment of lung injury and statistically significant survival benefit in the pig.

Emr et al. (2015) transferred a mixture of two plasmids expressing GFP-tagged rat Na^+, K^+-ATPase β1 subunit and DDK-tagged α1 subunit of ENaC, or a non-coding, empty plasmid as control, to pigs in this two-hit model of lung injury (**Figure 4**). Four h after injury (to mimic when a patient would present in the ER after traumatic injury leading to ALI), a bronchoscope was used to deliver 50 ml of DNA in saline (50 mg endotoxin-free plasmid), each into the left lower and right lobes, within a 2-min period. The bronchoscope was removed, and the animals were electroporated using external defibrillator pads placed on either side of the chest ("external electroporation"). Alternatively, in several experiments, the bronchoscope was left in the lung and used as an internal electrode coupled to one external electrode ("Internal electroporation"). Eight pulses (2,000 V at 150 μs each; ∼150 V/cm) were then applied to the animals. The anesthetized animals were maintained on mechanical ventilation and administered vasopressors according to the human ARDSnet and Early Goal Directed Therapy protocols (Network, 2000; Rivers et al., 2001). Initially, the animals were ventilated at 10 cc/kg tidal volume (V_T) with a PEEP of 5 cmH_2O, respiratory rate of 12, FiO_2 30%, and inspiratory to expiratory Ratio 1:2. The animals were transitioned to low V_T (6 cc/kg) when they met ARDSnet clinical criteria of $PaO_2/FiO_2 < 300$ (mild ARDS) the per ARDSnet protocol (Network, 2000). Appropriate adjustments were made to maintain adequate minute volume. PEEP and FiO_2 were adjusted in response to changes in SaO_2 along the "High PEEP, Low FiO_2" scale (Network, 2000). If airway plateau pressure (P_{plat}) rose above 30 cm H_2O, V_T was further reduced by 1 cc/kg increments to 4 cc/kg with appropriate adjustments in respiratory rate to maintain equivalent minute volume per ARDSnet guidelines. The upper limit for respiratory rate was 35 BPM, with titrations made in V_T if respiratory acidosis

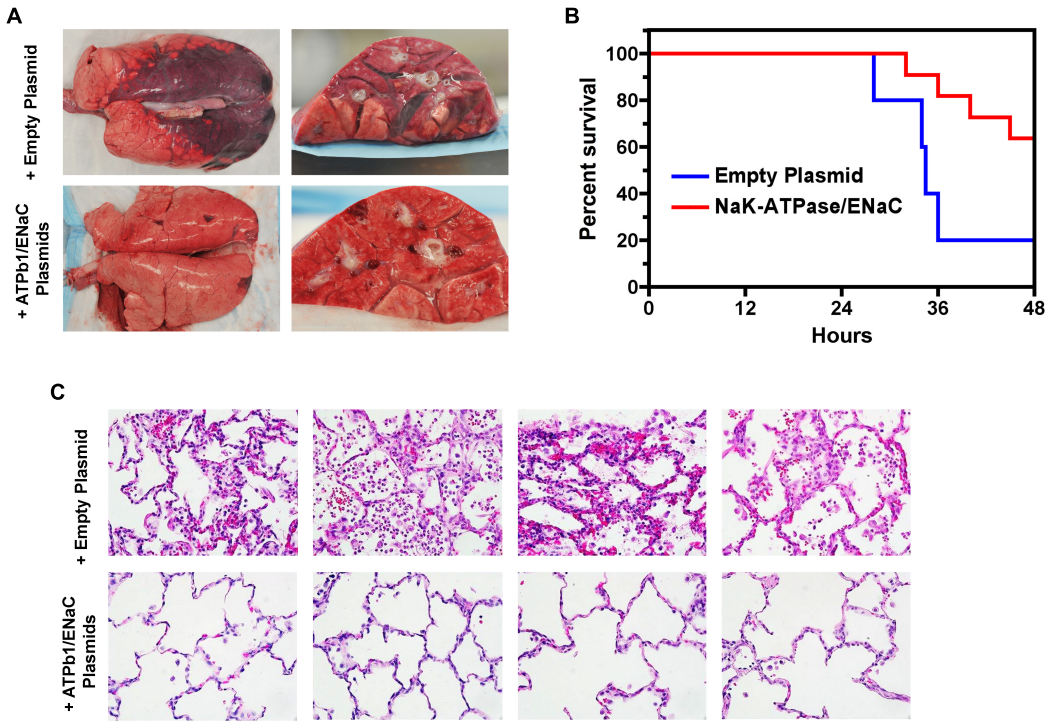

FIGURE 4 | Electroporation-mediated gene transfer of Na^+,K^+-ATPase β1 subunit and α-ENaC plasmids can treat lung injury in a severe septic pig model of ARDS. Lung injury was induced in pigs (40 kg) at $t = 0$ by inducement of a fecal clot into the abdomen and ischemia-reperfusion injury of the superior mesenteric artery for 30 min and 4 h later, either empty plasmids or plasmids expressing α-ENaC and the Na^+, K^+-ATPase β1 subunit (ATP1b1) were delivered to the right and left lower lobes by electroporation. ARDSnet ventilation and vasopressor protocols (Network, 2000) were followed until death or the 48-h pre-set endpoint. **(A)** Gross histology of lungs from both groups of animals. Note the high degree of atelectasis and glossy presence of excess fluid in lungs from animals receiving empty plasmids. **(B)** Histology of lungs shows a much lower degree of injury in animals receiving the plasmid treatment. **(C)** Survival curves show that gene transfer of Na^+, K^+-ATPase β1 and α-ENaC plasmids increases survival (Dean, unpublished).

was detected (pH < 7.15) according to the ARDSnet protocol. Broad-spectrum antibiotics (ampicillin 2 g IV and metronidazole 500 mg IV) were given following abdominal closure and every 12 h until the end of the study. The results seen following electroporation-mediated gene transfer of the Na^+, K^+-ATPase and ENaC genes were remarkable (**Figure 4**).

First, gene transfer of an empty plasmid (pCDNA3) did not change the course of the injury compared to animals with no intervention. In contrast, the transfer of Na^+, K^+-ATPase/α-ENaC plasmids lead to improved lung function, improved kidney function, less injured lungs upon gross and microscopic histological analysis, and greater survival. In terms of lung function, electroporation of the treatment plasmids increased P/F ratios and compliance in the pigs, while the amount of PEEP needed to keep the lungs open was less in these animals, as was plateau pressure. As in mice, gene transfer of Na^+,K^+- ATPase/α-ENaC plasmids increased the abundance of tight junction proteins ZO-1 and occludin in the septic pigs (Emr et al., 2015; Lin et al., 2016). This upregulation may indeed result in improved barrier function and may account in part for the improved outcome. Gene transfer also had an effect on cellular death with 3.5 ± 1.4% (mean ± SD) of alveolar cells staining tunnel positive for apoptosis in empty plasmid-treated pigs compared to 1.9 ± 0.9% in Na^+,K^+-ATPase/α-ENaC pigs ($p < 0.01$). The health of the lungs was evident upon visual inspection of lungs removed from the euthanized animals (Emr et al., 2015). Histologically, the animals receiving either no intervention or empty plasmid showed all the signs of ALI/ARDS, namely, thickened alveolar septa, cell infiltrates, hemorrhage, edema, fibrin deposits, hyaline membranes, and vascular congestion, while animals receiving the Na^+,K^+-ATPase/α-ENaC plasmids presented much more like healthy lungs (**Figure 4**). Quantitative histological analysis revealed statistically significant differences between these groups (Emr et al., 2015). Wet to dry ratios showed significantly drier lungs in the animals receiving the Na^+,K^+-ATPase/α-ENaC plasmids. Finally, survival was also improved. The success of this electroporation-mediated gene therapy in such a stringent model demonstrates the robust nature and promise of this approach for possible use in critically ill patients.

CONCLUSION

Despite the fact that there has been no single gene or combination of genes identified that are responsible for ARDS, a number of genes have been effectively targeted for up- or down-regulation

in multiple animal models that have shown varied degrees of alleviation of many of the symptoms and severity of ARDS. While in the past, most focus has been aimed at increasing the expression of ion channels and transporters to aid in alveolar fluid removal, these have not been as successful in treatment studies. More promising strategies are targeted toward dampening inflammatory responses and repairing or strengthening the alveolar-capillary barrier. In both cases, a number of genes have shown promise.

Perhaps the major problem with developing treatments for ARDS at the gene therapy level has been that most studies have relied on prevention approaches as opposed to treatment. In these instances, genes are transferred to healthy lungs before any lung injury is induced. While this allows for maximal gene delivery, since healthy lungs allow for greater distribution of any viral or nonviral gene delivery agent and maximal transcription from delivered transgenes, it is not reflective of how any gene therapy or other treatment would be given clinically (i.e., after development of lung injury). Furthermore, a myriad of studies have clearly shown that mice are not humans, and that ALI and ARDS in mice do not show all of the same pathological hallmarks as the human disease. Larger animals that more closely reflect human pathophysiology are needed to make any advancement toward the clinic. Thus, greater emphasis should be placed on those studies that (1) use experimental designs aimed at treating existing disease and (2) use larger, clinically relevant animal models of ARDS.

Using a number of different animal models and humans, many of the molecular mechanisms that contribute directly and indirectly to the pathogenesis of ARDS have been identified over the past 30 years. Developing strategies to treat this and any disease at the genetic level using gene therapy is perhaps the most direct way to change the pathophysiology and resolve injury, if concerns for safety and appropriate gene expression are kept in balance. While a number of different families or types of targets have been studied (inflammatory, edema clearance, and barrier, etc.), perhaps the major limitation for effective gene therapy remains optimizing gene delivery itself. Gene delivery systems that are nontoxic and non-inflammatory allow for repeat dosing and produce therapeutic levels of gene product, all the while being targeted to desired cell types is needed. Although each different viral vector or nonviral chemical or physical technology may address one or more of these problems, none has yet solved all of them. However, advances are rapid in both the areas of gene delivery and our understanding of the disease itself, so it is highly likely that safe and efficacious treatments for ARDS are within reach.

AUTHOR CONTRIBUTIONS

JL and DD contributed to, wrote, revised the manuscript, and approved the submitted version.

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Conflict of Interest: DD has applied for patent protection for the use of MRCKa in the gene therapy treatment of ARDS. DD was also a consultant for several non-viral gene therapy companies providing guidance in the area of vector design, but not for any aspect of using gene therapy to treat lung diseases, including ARDS.

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.

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Static and Dynamic Measurements of Compliance and Driving Pressure: A Pilot Study

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Rationale: Monitoring tidal cycle mechanics is key to lung protection. For this purpose, compliance and driving pressure of the respiratory system are often measured clinically using the plateau pressure, obtained after imposing an extended end-inspiratory pause, which allows for relaxation of the respiratory system and redistribution of inflation volume (method A). Alternative methods for estimating compliance and driving pressure utilize the measured pressure at the earliest instance of zero flow (method B), the inspiratory slope of the pressure-time tracing during inflation with constant flow (method C), and the expiratory time constant (method D).

Methods: Ten passive mechanically ventilated subjects, at a large tertiary referral center, underwent measurements of compliance and driving pressure using the four different methods. The inspiratory tidal volume, inspiratory to expiratory ratio, and positive end expiratory pressures were then adjusted from baseline and the measurements re-obtained.

Results: Method A yielded consistently higher compliance and lower driving pressure calculations compared to methods B and C. Methods B and C most closely approximated one another. Method D did not yield a consistent reliable pattern.

Conclusion: Static measurements of compliance and driving pressure using the plateau pressure may underestimate the maximum pressure experienced by the most vulnerable lung units during dynamic inflation. Utilizing the pressure at zero flow as a static measurement, or the inspiratory slope as a dynamic measurement, may calculate a truer estimate of the maximum alveolar pressure that generates stress upon compromised lung units.

Keywords: compliance, static compliance, dynamic compliance, driving pressure, plateau pressure, respiratory mechanics, ventilator induced lung injury, mechanical ventilation

INTRODUCTION

Measuring compliance of the respiratory system provides critical insight into the mechanics of the lungs and chest wall and the dynamics of breathing. Apart from characterizing the ease or difficulty of chest inflation, tidal compliance tracks the severity and progress of acute lung injury (ARDS). Moreover, compliance determines the driving pressure necessary to inflate the lungs with a specific tidal volume, a key indicator of the risk for ventilator-induced lung injury (VILI) (Amato et al., 2015; Goligher et al., 2021). In current clinical practice, compliance and driving pressure are commonly measured under passive conditions after an extended end-inspiratory pause is performed to obtain the stable plateau pressure ($P_{plateau}$). While this average value for driving pressure measured under "stop flow" conditions has definite worth, is simple to obtain, and is universally measured, it may underestimate the maximum stress to which some lung units are exposed under the dynamic conditions of tidally inflating the mechanically heterogeneous lungs of ARDS.

When VILI prevention is the focus of attention, $P_{plateau}$ (Method A) may not precisely reflect the maximum alveolar pressure experienced by the most vulnerable alveoli (Milic-Emili et al., 1990). The end inspiratory breath hold allows for relaxation of the respiratory system and redistribution of inflation volume and distending pressures. These processes usually decrease the measured pressure. An alternative method of measuring compliance uses the pressure measured at the first point of zero flow during the end-inspiratory pause (P_{zf}), which usually occurs about 2 s earlier than the $P_{plateau}$ (Method B) (**Figure 1**) (Milic-Emili et al., 1990). This latter method excludes the airflow resistance component from the recorded pressure measurement without allowing full relaxation and gas redistribution within the respiratory system. Measuring compliance using $P_{plateau}$ and P_{zf} may still not accurately characterize the maximum pressure experienced by some alveoli, especially in a mechanically complex environment; both are measurements made under quasi-static conditions that may or may not perfectly characterize the non-static (dynamic) tidal inflation of the lungs.

Compliance can also be measured using dynamic values obtained during inflation or deflation. One such method calculates compliance during constant flow, based on the slope of the pressure-time tracing (Method C). During inflation under constant flow conditions, time is a direct linear analog of volume. Two pressure values during inflation and their corresponding volumes can therefore be used to measure compliance using the following equation: Compliance = (V2-V1)/(P2-P1) where P1 and P2 are two pressure measurements during inflation while V1 and V2 are the corresponding tidal volumes occurring at those same time points. Another dynamic method of assessing compliance (Method D) assumes uniexponential decay of the tidal volume and computes the deflation time constant, the product of resistance and compliance (R × C) (Al-Rawas et al., 2013). Under the assumptions of uniexponential decay and unchanging resistance, the time constant is a quotient of volume at any point on the deflation curve and its corresponding instantaneous flow rate. The calculated time constant is then divided by resistance measured at end-inspiration under constant flow conditions to obtain compliance, i.e., Compliance = expiratory time constant/resistance. For each of these four methods, Driving Pressure = inspiratory volume/compliance.

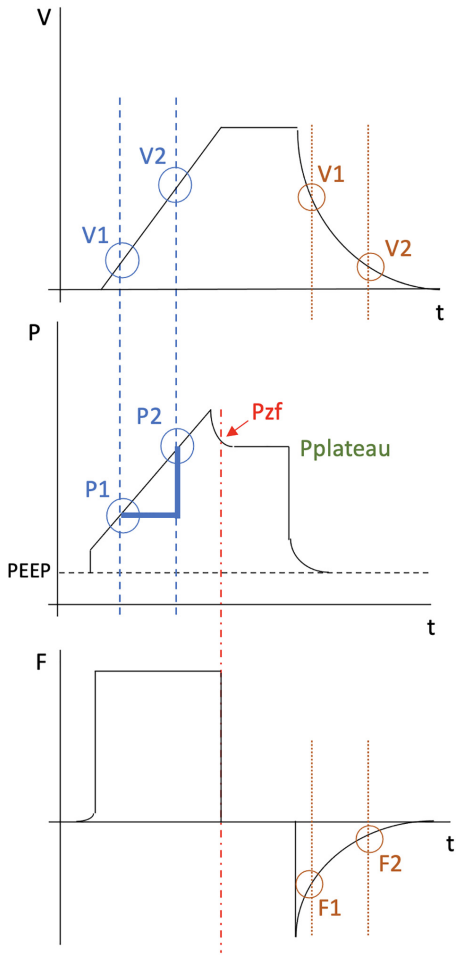

FIGURE 1 | Illustration of the inspiratory and expiratory phase of a typical breath under constant flow and the various methods for calculating compliance and driving pressure. The top waveform plots volume (V) over time (t). The middle waveform plots pressure (P) over time. The bottom waveform plots flow (F) over time. Compliance calculation in method A (green) is performed by dividing the change in volume by the change in pressure (Pplateau minus PEEP). Compliance calculation in method B (red) is performed by dividing the change in volume by the change in pressure utilizing instead the pressure at zero flow (Pzf minus PEEP). Compliance calculation in method C (blue) is performed using the following equation: [V2-V1] / [P2-P1]. Compliance calculation in method D (orange) requires measurement of multiple expiratory time constants. For example, time constant #1 equals V1/F1. Time constant #2 equals V2/F2. Compliance is then calculated by dividing the average of the different time constants by the resistance. For each of these four methods, Driving Pressure = inspiratory volume / compliance.

Although a signature property of bedside lung mechanics used in decision making, various questions arise regarding compliance and driving pressure measurements. It is unclear how these four alternative methods compare to one another in

the same patient. It is also unknown how these relationships are altered by varying the ventilator settings of tidal volume and inspiratory flow. Our purpose, therefore, was to compare such methods of measuring compliance and driving pressure in the clinical setting and to explore the impact of ventilator parameters on these methods. We hypothesized that differences would arise between the inspiratory versus expiratory methods of compliance and driving pressure estimation as well as between these values obtained by the dynamic versus static methods.

MATERIALS AND METHODS

Study Design

This study was performed at Regions Hospital in St. Paul, Minnesota, and was approved by the Regions Hospital Institutional Review Board. We conducted a prospective cohort study of passive mechanically ventilated subjects. All subjects receiving mechanical ventilation in the medical intensive care unit were evaluated for inclusion. Subjects 18 years and older who were deemed to be passively ventilated, either by receiving neuromuscular blockade, or who had a Richmond Agitation–Sedation Scale (RAAS) of −3 or −4 and demonstrated no evidence of active breathing at the bedside (i.e., over-breathing, dyssynchrony), were included. Subjects who were pregnant, had undergone prior lung, chest wall or abdominal surgeries, had known deformity of the chest wall, or had known parenchymal or obstructive lung disease were excluded. Family members of subjects meeting the study criteria were approached for consent.

Study Procedures

Since deflation may not occur uniexponentially (Hamahata et al., 2020), there are potentially multiple time constants throughout expiration. For the purposes of this study, two time constants were measured, and their mean was used to obtain an average for the compliance equation.

After consent was obtained, subjects were re-evaluated at the bedside to ensure ongoing passive ventilation and stable hemodynamics. Prior to data collection, all subjects were ventilated using pressure regulated volume control. Each subject's flow profile was changed to an equivalent constant flow, maintaining the subject's same inspiratory to expiratory ratio (I:E), tidal volume (TV), respiratory rate (RR), oxygen concentration (FIO2) and positive end expiratory pressure (PEEP). At these "baseline" settings, an inspiratory breath hold was performed, followed by a breath without a hold maneuver, followed by a breath with an end expiratory hold. The ventilator screen was then frozen, and the cursor dial maneuvered to obtain the necessary measurements from the time-based tracings of pressure, volume, and flow. On the inspiratory limb of a pressure-volume display, two pressures and corresponding volumes were measured (for method C calculations). These points were chosen from the middle 80% of the inspiratory limb to avoid variability from inspiratory flow initiation and cessation. For the paused breaths, the P_{zf} was recorded, followed by the $P_{plateau}$, measured 2 s after the breath hold was initiated (for methods A and B calculations). From the second breath (without a breath hold maneuver), two expiratory flow measurements and corresponding volume measurements were obtained from the middle 50% of the generated graph (method D). For method D, the initial 25% and latter 25% of the expiratory limb of the loop were excluded to best approximate an average time constant. Compliance and driving pressure were calculated for each method.

The following ventilator setting adjustments were then performed unless they violated the prespecified protocol safety parameters. Breath holds and data gathering steps were performed as detailed above for the following variations. Tidal volume was first increased from baseline and then decreased from baseline by 2 ml/kg of ideal body weight (IBW). Next, I:E ratio was increased from the baseline ratio by 1.0 and then decreased from baseline by 1.0. Finally, PEEP was increased and then decreased from its baseline value by 2 cmH2O. After each ventilator adjustment, we allowed for a 1-min equilibration period. The ventilator was not adjusted if the following pre-specified safety parameters were surpassed: tidal volume $<$ 4 ml/kg of IBW or $>$ 10 ml/kg of IBW; baseline $P_{plateau}$ greater than 35 cmH2O; baseline PEEP $<$ 5 cmH2O or PEEP $>$ 12 cmH2O in a subject with BMI less than 30 kg/m^2; baseline PEEP $<$ 5 cmH2O or PEEP $>$ 16 cmH2O in a subject with BMI greater than 30 kg/m^2.

Data Collection

Study data were collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools, a secure, web-based software platform, hosted at Regions Hospital (Harris et al., 2009).

Statistical Analysis

Participants' demographic and clinical characteristics were summarized using counts and rates or medians and ranges. Radar plots were used to visualize each participant's compliance and driving pressure measurements at each ventilator setting for each of four calculation methods (**Supplementary Materials 1, 2**). To examine the average effect of ventilator setting changes on compliance and driving pressure for each calculation method, linear mixed-effects models were fit with fixed effects terms for tidal volume, I:E ratio, and total PEEP, and a random effect term for participant to account for repeated measurements within participant. To compare compliance and driving pressure between calculation methods at each ventilator setting combination, similar mixed-effects models were fit with the differences between each pair of calculation methods as the outcomes. Results are summarized using mean effects with 95% confidence intervals. Analyses were conducted using R version 4.1.1 (R Core Team, 2020).

RESULTS

The study was conducted over 8 months from October 2020 through June 2021. The study was stopped by the investigators

due to low enrollment, primarily owing to a majority of considered subjects having a degree of active breathing on the ventilator (**Figure 2**). Ten subjects completed the study (**Table 1**). Five subjects underwent all ventilator settings adjustments. The remainder completed some, but not all, of the adjustments due to protocol safety limits (**Figure 2**). The subjects were primarily male (7/10, 70%) and Caucasian (8/10, 80%). The median number of days undergoing mechanical ventilation at the time of data collection was three (range 1–10 days). Reasons for intubation were COVID ARDS (5/10, 50%), encephalopathy (2/10, 20%), cardiac arrest (1/10, 10%), cardiogenic shock (1/10, 10%), and aspiration pneumonia (1/10, 10%). P/F calculations indicated a range from normal oxygenation to all degrees of ARDS severity: median P/F of 134.5 (range 75–623). Of the five subjects who completed all ventilator setting adjustments, four subjects had moderate to severe ARDS by P/F criteria. A minority of subjects received epoprostenol (2/10, 20%) and a minority were prone at the time of data collection (3/10, 30%). These two interventions did not appear to create a discernable deviation or change in the compliance or driving pressure calculations. Only two subjects were paralyzed (2/10, 20%); lack of paralysis did not appear to create a pattern of deviation or inconsistency in the calculations.

TABLE 1 | Demographics and clinical characteristics of the tested subjects.

Age (median, years)	**61.5 (range 37–81)**
Sex	
Male	7/10 (70%)
Female	3/10 (30%)
Race	
Caucasian	8/10 (80%)
Hispanic	1/10 (10%)
Southeast Asian	1/10 (10%)
Height (median, cm)	173.9 (range 154.9–188.0)
BMI (median, kg/m2)	32.3 (range 24.6–40.2)
Ventilator days at time of data collection (median, days)	3.0 (range 1–10)
Primary diagnosis requiring intubation	
Encephalopathy	2/10 (20%)
Aspiration pneumonia	1/10 (10%)
Cardiac arrest	1/10 (10%)
Cardiogenic shock	1/10 (10%)
COVID ARDS	5/10 (50%)
RAAS	
−3	2/10 (20%)
−4	4/10 (40%)
−5	4/10 (40%)
Position at time of data collection	
Supine, flat	1/10 (10%)
Supine, upright at 30–40 degrees	6/10 (60%)
Prone	3/10 (30%)
Baseline volume (median, mL)	444.5 (range 304–579)
Baseline I:E (median)	1:2.31 (range 1:1.47–1:3.29)
Baseline total PEEP (median, cmH2O)	8.57 (range 5–15)
pH (median)	7.29 (range 7.24–7.45)
PaO2/FIO2 (median, mmHg)	134.5 (range 75–623)
Hgb (median, g/dL)	9.0 (range 7.2–14.7)
WBC [median, ×10(9)/L]	11.3 (range 6.1–18.3)
Number of subjects receiving each sedative	
Hydromorphone	6/10 (60%)
Fentanyl	3/10 (30%)
Propofol	2/10 (10%)
Lorazepam	5/10 (50%)
Midazolam	4/10 (40%)
Dexmedetomidine	1/10 (10%)
Number of subjects receiving paralytics	2/10 (20%)
Number of subjects receiving inhaled epoprostenol	2/10 (20%)

The study aimed to compare compliance and driving pressure measurement methods. Radar plots of the four methods for each subject, under baseline ventilator settings and with each ventilator adjustment, are available in **Supplementary Materials 1, 2**. A demonstrative plot for one subject is shown in **Figure 3**. Average compliance and driving pressure measurements, under baseline ventilator settings and with each adjustment, were compared. Average differences between each method were calculated along with 95% confidence intervals and Bland-Altman limits of agreements of these differences; with statistical significance indicated by confidence intervals that do not intersect the zero line (**Supplementary Material 3**). Method A consistently produced statistically significant higher values of compliance, and lower values of driving pressure, compared to method B and C. Compliance and driving pressure measurements from methods B and C most closely approximated each other; there was no statistically significant difference in method B compared to method C under baseline, volume decrease, I:E increase or decrease, PEEP increase or decrease conditions. There was a statistically significant difference between method B and C under the volume increase condition, with method C on average calculating greater compliance by 2.5 cmH2O (95% CI: −4.7 to −0.3) than Method B and lower driving pressure by 2.04 cmH2O (95% CI: 0.49 to 3.59). Method D did not show a consistent pattern, demonstrating higher measurements of compliance and lower measurements in driving pressure in eight subjects and the reverse findings in two subjects, compared to the other methods.

Linear mixed-effects regression models were used to assess the impact of each ventilator change on each measurement method (**Supplementary Material 4**). Model summaries show the average linear effect of each ventilator setting change on the compliance or driving pressure outcome; effects greater than 0 indicate that increasing that setting is associated with an increase in the outcome, and decreasing that setting with a decrease in the outcome; and, vice versa for effects less than 0. An increase in I:E resulted in a statistically significant increase in compliance and decrease in driving pressure for methods B and D, with reciprocal changes for a decrease in I:E. An increase in PEEP resulted in a statistically significant decrease in compliance and increase in driving pressure for methods A and B, with reciprocal changes for a decrease in PEEP. However, these values are averages and the patterns did not consistently hold for each individual subject. Tidal volume adjustments had

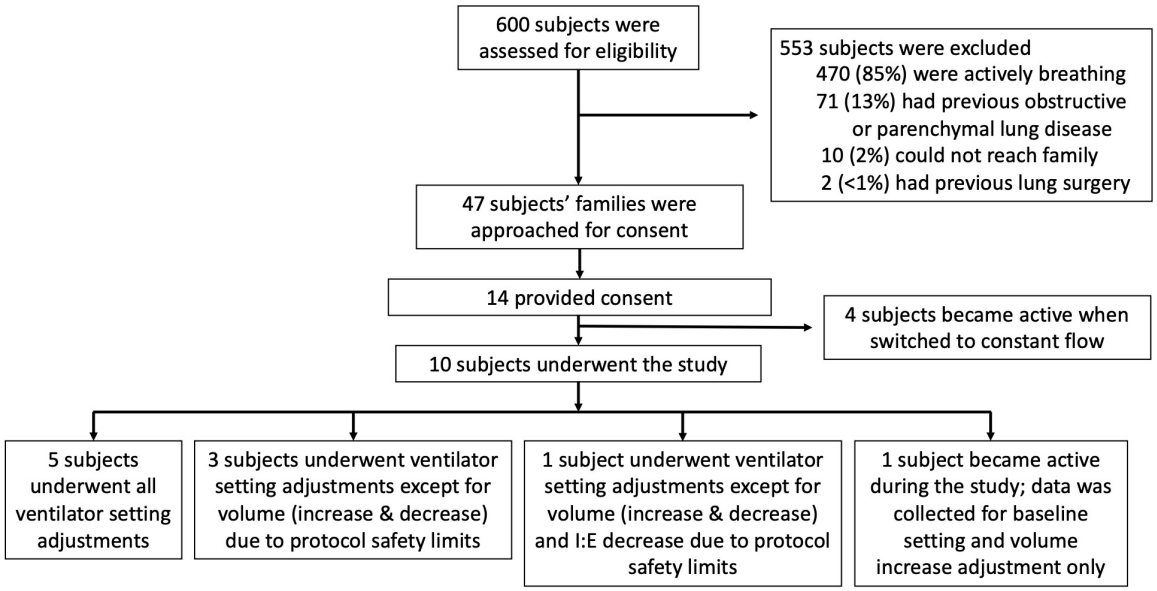

FIGURE 2 | Flow diagram outlining subjects assessed for eligibility, reasons of exclusion or inability to enroll, and breakdown of the ventilator setting adjustments that were tested.

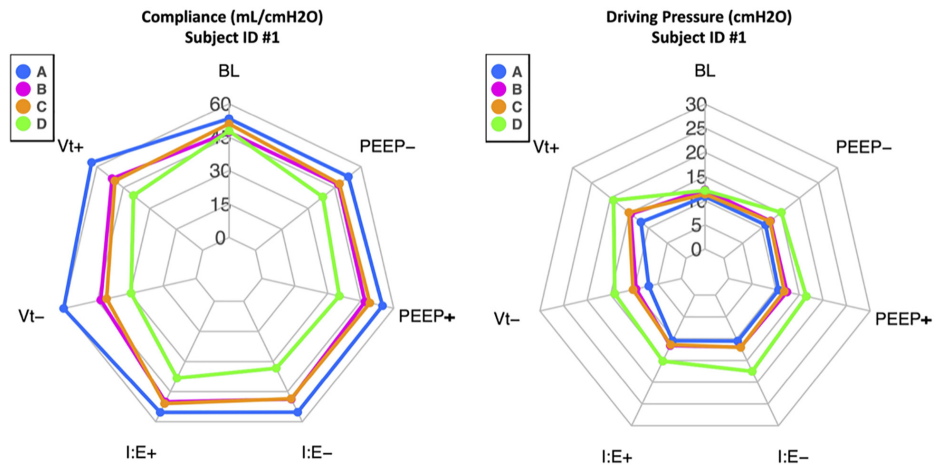

FIGURE 3 | Example radar plots for one subject (ID #1) showing compliance (mL/cmH20) and driving pressure (cmH2O) calculations. The blue line denotes calculations by method A, the pink line by method B, the orange line by method C, and the green line by method D. The baseline (BL) calculations by each of the four methods are in the top center axis. One ventilator adjustment was then performed, and the data recalculated. The changes were performed and are listed in counter-clockwise fashion: Vt+ is tidal volume increase by 2 ml/kg of ideal body weight from baseline, Vt- is tidal volume decrease by 2 ml/kg of ideal body weight from baseline, I:E+ is the ratio increase by 1.0 from baseline, I:E- is the ratio decrease by 1.0 from baseline, PEEP+ is PEEP increase by 2 cmH2O from baseline, and PEEP- is PEEP decrease by 2 cmH2O from baseline. Among the ten subjects, methods B and C best approximated one another, and Method A consistently calculated higher compliance and lower driving pressure. Method D did not demonstrate a consistent pattern.

the smallest sample size (n = 5) owing to safety limitations. An increase in tidal volume resulted in a statistically significant increase in compliance measurement for methods B, C and D and vice versa. An increase in tidal volume resulted in a statistically significant increase in driving pressure as well in all. Once again, these values were averages and the patterns of tidal volume's impact on compliance and driving pressure did not consistently hold for each individual subject. This discrepancy where tidal volume has a positive relationship with both compliance and driving pressure is due to the averaging of numerical values with different magnitudes.

Lung severity as assessed by P/F ratio, BMI, paralysis, and use of epoprostenol did not demonstrate any statistically significant impact on the measurements although the small sample size precludes any definitive statement regarding the impact of these variables.

DISCUSSION

The aim of this study was to evaluate four ways—two quasi-static methods and two dynamic methods—of measuring compliance and driving pressure of the respiratory system. In comparison to the standard method utilizing the $P_{plateau}$ minus PEEP (method A), measurements using the inspiratory slope of a constant flow pressure tracing (method C) and using the P_{zf} minus PEEP (method B) both demonstrated consistently lower compliance and higher driving pressure calculations. This would suggest that the standard method of measuring compliance and driving pressure, calculated after stress relaxation and gas re-distribution facilitated by the inspiratory hold, may overestimate the compliance and underestimate the driving pressure. Our data, therefore, suggest that utilizing the inspiratory hold may underestimate the risk to the most vulnerable lung units. Current numerical guidance for mechanical ventilation (i.e., peak pressure less than 30 cmH2O, driving pressure less than 15 cmH2O) utilizes the end-inspiratory breath hold and the resulting plateau pressure (Brower et al., 2000; Esteban et al., 2002; Amato et al., 2015). Although the numerical difference in calculations between methods A, B and C may not be dramatic, and the threshold for damage likely differs throughout regions of the heterogenous ARDS lung, these values assume significance if one aims to not surpass such numerical boundaries for safe ventilation. There was no statistically significant difference between compliance and driving pressure averages for methods B and C, except when tidal volume was increased by 2 ml/kg above baseline. Overall, method B and C most closely approximated each other compared to the other methods. P_{zf} may therefore be a better quasi-static marker for the maximum pressure experienced by the most vulnerable lung units than $P_{plateau}$. Furthermore, for purposes of lung protection, measuring compliance dynamically, utilizing the inspiratory slope during inflation by constant flow ventilation, may be a neglected but more relevant indicator than the static measure we customarily use.

Despite the small total number of subjects, the number of data points collected and the use of each subject's baseline as a self-control allow for reliable comparisons between methods. The subjects had heterogenous pathology. However, the patterns held true across this heterogenous group. Consequently, our investigation would appear to serve as a valid pilot study for larger future comparisons among these various methods. Both P_{zf} and inspiratory slopes are not commonly displayed ventilator outputs. Ventilator manufacturers may find utility incorporating P_{zf} outputs during an inspiratory hold. Likewise, although the contour-defining stress index has been incorporated into some ventilator monitoring during constant inspiratory flow, dynamic compliance and driving pressure algorithms based on the inspiratory pressure tracing are not yet available and should rather easily be programmed into any modern ventilator so as to provide relevant, intervention-free, and dynamic breath-by-breath information of additional interest.

The expiratory time constant (method D) did not provide a consistent pattern of compliance and driving pressure measurements, at times greater and at times less than the other methods. Various explanations account for this lack of precision. First, lung deflation typically is not uniexponential (Hamahata et al., 2020). There are therefore multiple time constants that apply during different phases of the expiratory cycle. Second, the speed of lung deflation is impacted by the opening of the expiratory valve and the decompression of the ventilator circuit, which further skews the data. Finally, energy can be lost between inspiration and expiration, known as lung hysteresis (Marini, 2020). As such, measures of the mechanics of lung inflation do not necessarily parallel those of deflation. Our data therefore suggest that expiratory time constant, using an average of two data points in the exhalation phase, is unlikely to be a reliable surrogate for the pressures, stress or strain experienced by the respiratory system during inflation. This method may perform better, however, if conducted using the reportedly successful Al-Rawas et al. protocol, where point-by-point values within the 0.10–0.50 s of the expiratory loop, are recorded (Al-Rawas et al., 2013). This, however, would require either a cumbersome recording process or a programmed data acquisition software that records and transfers data from the ventilator on the order of each millisecond—more frequently than currently reported or displayed.

Ventilator settings were changed (tidal volume, I:E ratio and PEEP) within a standard range of typical clinical adjustments. Some changes of settings appeared to cause statistically significant differences among methods regarding the average compliance and driving pressure measurements. It is difficult, however, to draw firm conclusions regarding predictions of machine adjustments for any given subject; the mentioned patterns of averages for machine setting adjustments did not consistently apply to each individual subject, who varied with regard to underlying lung disorder and severity.

There are various limitations to this study. First, due to judicious use of paralytic agents at our institution, most subjects were not paralyzed. However, subjects and ventilator waveforms were evaluated at the bedside by multiple clinicians to reduce the likelihood of actively initiated ventilation. Additionally, our sample size was diverse and small, owing to light sedation of many intensive care patients and difficulty consenting families of the most critically ill. The modest sample size of the study precludes more detailed conclusions from the data that might apply to disease and severity targeted populations. However, the trends discussed provide a compelling need to reevaluate $P_{plateau}$ as the standard base for compliance and driving pressure calculations that currently influence bedside decision-making. Furthermore, this study does not directly address which technique for measuring lung mechanics correlates best with VILI risk, which almost certainly varies with lung vulnerability and with the local environment of the alveoli in question. Yet, as all lung protective techniques attempt to reduce the maximum pressure applied to the alveoli, a technique that reveals higher pressure measurements does suggest that some alveoli are subjected to somewhat higher stresses and strains than previously appreciated.

In conclusion, this study serves as a pilot investigation into various methods for measuring tidal compliance and driving pressure. The standard approach utilizing $P_{plateau}$ may overestimate the compliance and underestimate the driving pressure during lung inflation. Further studies are needed to assess the impact of titrating driving pressure and compliance using these alternative methods, the influence of ventilator and patient parameters on these methods, and the potential correlation of these methods with clinical outcomes.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regions Hospital Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PT prepared the manuscript, participated in the study design, performed the data collection, and assisted in the data analysis. MS, DD, and JM designed the study and obtained the institutional review board approval for the project. FE participated in the study design, data collection, and data interpretation. ME performed statistical analysis of the data. JM was the principal investigator involved in all components of the project. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.773010/full#supplementary-material

Supplementary Material 1 | Radar plots showing compliance calculations (in mL/cmH20) for each subject. The blue line denotes calculations by method A, the pink line by method B, the orange line by method C, and the green line by method D. For each subject, the baseline (BL) calculations by each of the four methods are in the top center axis. One ventilator adjustment was then performed, and the data recalculated. The changes were performed and are listed in counter-clockwise fashion: Vt+ is tidal volume increase by 2 ml/kg of ideal body weight from baseline, Vt- is tidal volume decrease by 2 ml/kg of ideal body weight from baseline, I:E+ is the ratio increase by 1.0 from baseline, I:E- is the ratio decrease by 1.0 from baseline, PEEP+ is PEEP increase by 2 cmH2O from baseline, and PEEP- is PEEP decrease by 2 cmH2O from baseline. For subject ID #14, a visual representation could not be displayed as data points were collected only for baseline and Vt+. Among the ten subjects, methods B and C best approximated one another, and Method A consistently calculated higher compliance. Method D did not demonstrate a consistent pattern.

Supplementary Material 2 | Radar plots showing driving pressure calculations (in cmH2O) for each subject. The blue line denotes calculations by method A, the pink line by method B, the orange line by method C, and the green line by method D. For each subject, the baseline (BL) calculations by each of the four methods are in the top center axis. One ventilator adjustment was then performed, and the data recalculated. The changes were performed and are listed in counter-clockwise fashion: Vt+ is tidal volume increase by 2 ml/kg of ideal body weight from baseline, Vt- is tidal volume decrease by 2 ml/kg of ideal body weight from baseline, I:E+ is the ratio increase by 1.0 from baseline, I:E- is the ratio decrease by 1.0 from baseline, PEEP+ is PEEP increase by 2 cmH2O from baseline, and PEEP- is PEEP decrease by 2 cmH2O from baseline. For subject ID #14, a visual representation could not be displayed as data points were collected only for baseline and Vt+. Among the ten subjects, methods B and C best approximated one another, and Method A consistently calculated lower driving pressure. Method D did not demonstrate a consistent pattern.

Supplementary Material 3 | Mean compliance and driving pressure measurements for methods A, B, C, and D under each ventilator setting and the differences between these means for each method under each setting. Ninety five percent confidence intervals for the differences and Bland-Altman limits of agreement (LOA) are calculated. A statistically significant difference is denoted by confidence intervals that do not intersect the "zero line."

Supplementary Material 4 | Linear mixed-effects regression models showing the average linear effect of each ventilator setting change on the compliance or driving pressure outcome; effects greater than 0 indicate that increasing that setting is associated with an increase in the outcome, and decreasing that setting with a decrease in the outcome; and, vice versa for effects less than 0. Although statistically significant patterns were sometimes noted, the values are averages and the patterns typically did not hold for each individual subject.

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Mechanical Ventilation in Pediatric and Neonatal Patients

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Pediatric acute respiratory distress syndrome (PARDS) remains a significant cause of morbidity and mortality, with mortality rates as high as 50% in children with severe PARDS. Despite this, pediatric lung injury and mechanical ventilation has been poorly studied, with the majority of investigations being observational or retrospective and with only a few randomized controlled trials to guide intensivists. The most recent and universally accepted guidelines for pediatric lung injury are based on consensus opinion rather than objective data. Therefore, most neonatal and pediatric mechanical ventilation practices have been arbitrarily adapted from adult protocols, neglecting the differences in lung pathophysiology, response to injury, and co-morbidities among the three groups. Low tidal volume ventilation has been generally accepted for pediatric patients, even in the absence of supporting evidence. No target tidal volume range has consistently been associated with outcomes, and compliance with delivering specific tidal volume ranges has been poor. Similarly, optimal PEEP has not been well-studied, with a general acceptance of higher levels of F_iO_2 and less aggressive PEEP titration as compared with adults. Other modes of ventilation including airway pressure release ventilation and high frequency ventilation have not been studied in a systematic fashion and there is too little evidence to recommend supporting or refraining from their use. There have been no consistent outcomes among studies in determining optimal modes or methods of setting them. In this review, the studies performed to date on mechanical ventilation strategies in neonatal and pediatric populations will be analyzed. There may not be a single optimal mechanical ventilation approach, where the best method may simply be one that allows for a personalized approach with settings adapted to the individual patient and disease pathophysiology. The challenges and barriers to conducting well-powered and robust multi-institutional studies will also be addressed, as well as reconsidering outcome measures and study design.

Keywords: PARDS, lung injury, neonatal and pediatric mechanical ventilation, high frequency percussive oscillation, high frequency oscillatory ventilation, airway pressure release ventilation

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INTRODUCTION

Pediatric acute respiratory distress syndrome (PARDS) remains a significant cause of morbidity and mortality, with mortality rates as high as 50% in children with severe PARDS (Schouten et al., 2016). Despite this, pediatric lung injury and mechanical ventilation has been poorly studied, with the majority of investigations being observational or retrospective and with only a few randomized controlled trials (RCTs) (Kneyber et al., 2017). The most recent and universally accepted guidelines for pediatric lung injury are based on consensus opinion rather than objective data (Pediatric Acute Lung Injury Consensus Conference Group, 2015). Therefore, most neonatal and pediatric mechanical ventilation practices have been arbitrarily adapted from adult protocols, neglecting the differences in lung pathophysiology, response to injury, and co-morbidities among the groups.

Neonates, in particular, have a complex set of diseases that require mechanical ventilation but have no adult mimic, such as prematurity, congenital diaphragmatic hernia, meconium aspiration syndrome, persistent pulmonary hypertension, and congenital heart disease (Langham et al., 2003). As a reflection of this unique lung pathophysiology, there are a greater number of pulmonary-related ECMO runs in neonates as compared with adults and pediatric patients (Ecmo, 2021). Babies can be born as early as in the canalicular stages of lung development, where the bronchioles are still not fully developed (Iliodromiti et al., 2013). Applying adult ventilation strategies to neonatal lungs that have yet to form alveoli or cells to produce surfactant is erroneous. Even in children, more similar to adults in terms of their lung disease and co-morbidities, the process of alveolarization is thought to continue even up to 8 years of age (Weibel and Gomez, 1962) and possibly even through adolescence (Narayanan et al., 2012). Additionally, the chest walls of infants are more compliant than the chest walls of adults, with stiffening occurring through the first 2 years of age, at which time the lung and chest wall compliance are nearly equal, as they are in adults (Papastamelos et al., 1995). Thus the pleural pressure and response to mechanical ventilation would be expected to be different in infants (Gattinoni et al., 1998; Kollisch-Singule et al., 2015).

One of the challenges to investigating mechanical ventilation in the pediatric population is that mortality is frequently used as a therapeutic endpoint, but the majority of deaths in pediatric patients on mechanical ventilation are due to neurologic causes rather than refractory hypoxemia (Dowell et al., 2018). Up to 44% of children with no previous respiratory co-morbidities have long-term outcomes of pulmonary dysfunction after a pediatric intensive care unit (ICU) stay for acute respiratory failure including persistent need for adjunct therapies such as oxygen supplementation, bronchodilators, and corticosteroids, or persistent asthma or recurrent pneumonia (Keim et al., 2020). These secondary markers of lung injury may therefore make for a useful alternative marker mortality (Keim et al., 2018).

Further challenges associated with pediatric mechanical ventilation trials includes the lower incidence and mortality as compared with adult patients, shorter duration of mechanical ventilation, and diverse population with an estimated need for 60 participating centers to achieve an adequately powered study (Santschi et al., 2010). This has resulted in a lower number of and less recruited RCTs in children with one of the largest recruited studies having only 153 children (Willson et al., 2005).

Mechanical ventilation modes are grouped similarly as in adults: "conventional" ventilation (CV, including both volume- and pressure- regulated modes), airway pressure release ventilation (APRV), and high frequency ventilation (HFV) which can be subdivided into high frequency oscillatory ventilation (HFOV), high frequency jet ventilation (HFJV), and high frequency percussive ventilation (HFPV). CV is the most commonly used ventilation mode in pediatric patients (Pediatric Acute Lung Injury Consensus Conference Group, 2015; Koopman et al., 2019), with the remaining modes often being used as a rescue for refractory hypoxemia. This is in part because many of the non-CV modes (APRV, HFOV, and HFJV) are able to achieve a higher mean airway pressure without increasing peak airway pressures, and all have unique methods of achieving ventilation. There are few well-powered, recent RCTs in both conventional and non-conventional modes: HFOV (Arnold et al., 1994; Samransamruajkit et al., 2016; Snoek et al., 2016), HFJV (Carlo et al., 1990; Keszler et al., 1991; Keszler, 1997), and APRV (Lalgudi Ganesan et al., 2018). Studies that evaluate non-CV as a rescue mode may be providing an unfair evaluation, as many patients have often been on conventional mechanical ventilation for more than 24 h and have refractory hypoxemia. These studies must therefore be interpreted with caution as they are not necessarily comparing one mode vs. another but whether a non-CV mode can rescue a patient from CV. Although matched cohorts and regression can reduce this bias, they may not fully account for it. There can be no recommendations made for a "best mechanical ventilation" strategy, but it is important to understand the benefits and limitations of the ventilation modes available for clinical use. Thus, the focus of this review is to analyze the studies performed to date on mechanical ventilation strategies in neonatal and pediatric populations.

DEFINING LUNG INJURY

Because of the challenges with conducting well-powered pediatric mechanical ventilation trials, the Pediatric Acute Lung Injury Consensus Conference (PALICC) was held over 2 years with 27 experts voting on 151 recommendations in nine different topics varying from definition of lung injury to treatment and adjunctive therapies (Pediatric Acute Lung Injury Consensus Conference Group, 2015). Although the central premise of developing consensus guidelines is laudable, one must be cautious when using these consensus guidelines and applying them to the individual patient. Of the experts, 22 (81%) were located in the United States and Canada with the remaining five from Spain, Netherlands, England, Switzerland, and Australia, with certain experts hailing from the same institution, suggesting some degree of in-group bias (Pediatric Acute Lung Injury Consensus Conference Group, 2015).

Diagnosing PARDS according to standard criteria (Force et al., 2012) with strict reliance on the P_aO_2/F_iO_2 ratio or oxygenation

index (OI) is more challenging as children are less likely to have routine arterial blood gases performed. In order to study and compare outcomes in pediatric and neonatal patients, the PALIC Conference determined a definition for PARDS using non-invasive oxygenation criteria. They proposed using an S_pO_2/F_iO_2 ratio or oxygen saturation index (OSI) when P_aO_2 ratio is not available (Pediatric Acute Lung Injury Consensus Conference Group, 2015), as these have been validated in pediatrics (Khemani et al., 2009b). Broadening this inclusion criteria may also help capture children who would otherwise have been excluded due to lack of invasive oxygenation criteria from blood gases (Santschi et al., 2010), also leading to an under-recognition of lung injury (Khemani and Newth, 2010). Embracing non-invasive oxygenation criteria can increase patient eligibility by an estimated 25% (Khemani et al., 2009b). Using this more inclusive definition, the follow-up observational PARDIE study revealed that the PALICC definition of severe PARDS was associated with higher mortality (32.7%) but mortality rates were similar between mild (12.4%) and moderate (10.3%) PARDS (Khemani et al., 2019).

In a meta-analysis of observational and randomized controlled trials investigating PARDS, the overall pooled mortality was determined to be 24% with a marked reduction in mortality over two decades from 40% mortality to an 18% mortality (**Figure 1**; Wong et al., 2019). This improvement in survival is likely due to an increased recognition of milder forms of PARDS, improvement in mechanical ventilators and strategies, and also suggests that children overall fare better than adults, likely due to a decrease in co-morbidities. It is important to consider the results of this meta-analysis (Wong et al., 2019) when comparing studies because trials spanning several years may not accurately reflect differences between ventilator modes but rather with patient inclusion, and advancement in ventilator practices and adjunctive therapies.

CONVENTIONAL MECHANICAL VENTILATION

Conventional ventilation is generally the application of a tidal volume or set inspiratory pressure to a baseline positive-end expiratory pressure (PEEP) with an inspiratory time set shorter than the expiratory time (**Figure 2A**). The majority of pediatric patients (75.2%) are placed on CV with 26.6% of those patients ventilated with a volume control mode and the remainder ventilated with a pressure control or regulated mode (Santschi et al., 2010). There is marked variability in the management of pediatric patients placed on conventional ventilation (Santschi et al., 2010), suggesting the term "conventional" may be a misnomer as there is not yet a convention in terms of mode (pressure- vs. volume-), tidal volume, or PEEP strategy.

Low tidal volume ventilation has become standard-of-care after the ARDSnet trial in adults demonstrated that 6 mL/kg significantly reduced mortality as compared with 12 mL/kg (ARDSnet, 2000). For lack of a similar comparison trial, low tidal volume ventilation has been mostly adopted into the mechanical ventilation strategy of pediatric patients (Pediatric Acute Lung Injury Consensus Conference Group, 2015; Koopman et al., 2019). In neonates, there are similarly no trials supporting one tidal volume goal over another, but tidal volume targets of 5–6 mL/kg are generally well-accepted (Keszler et al., 2009; Patel et al., 2009).

Although translating the practice of low tidal volume ventilation to pediatric patients has been advocated (Hanson and Flori, 2006; Dellinger et al., 2008), and is generally the standard to which other ventilator modes are compared, there are no large well-powered studies on it (Santschi et al., 2010; Koopman et al., 2019). One observational study demonstrated that higher tidal volumes were affiliated with decreased mortality (Erickson et al., 2007) and neonates were less susceptible to lung injury and inflammation with supraphysiologic tidal volumes (Copland et al., 2004; Kornecki et al., 2005; Smith et al., 2010). In a meta-analysis of eight randomized clinical trials and observational studies, there was no association between tidal volumes (7, 8, 10, 12 mL/kg) and mortality both in patients with and without ARDS (de Jager et al., 2014).

Tidal volumes in children tend to average around 8 mL/kg [8.0 mL/kg (Erickson et al., 2007), 8.1 mL/kg (Albuali et al., 2007), and 8.3 mL/kg (Santschi et al., 2010) but with marked variability (with a range from < 4 to > 15 mL/kg], highlighting the absence of a consensus (Santschi et al., 2010). Even PEEP guidelines have not been standardized (Villar, 2005) with more than half of pediatric patients being placed on PEEP < 5 cm H_2O with a range of 0–15 cm H_2O (Santschi et al., 2010). Pediatric intensivists are also more likely to adopt a low PEEP-high F_iO_2 strategy as compared with adults (Khemani et al., 2009a; Koopman et al., 2019). This is particularly relevant because exposure to high F_iO_2 concentrations leads to increased alveolar surface tension, in part due to increased surfactant frangibility (Smallwood, 2017). In addition to an unintended consequence of decreased pulmonary compliance and potential exacerbation of an underlying lung injury, higher F_iO_2 concentrations have been associated with bronchopulmonary dysplasia and longer supplemental oxygen requirements in neonates (No Authors List, 2000; Askie et al., 2003). Caution is deserved when comparing another ventilator mode with "conventional" ventilation since the comparisons will be between one (and possibly two) modes that do not have standardized methods of setting ventilator parameters and a wide variability in practice.

AIRWAY PRESSURE RELEASE VENTILATION

Airway Pressure Release Ventilation (APRV) is a pressure-regulated, time-dependent mode where the upper pressure (P_{High}) is sustained for a prolonged time (T_{High}), creating a continuous positive airway pressure (CPAP) phase to allow for alveolar stabilization and recruitment. The CPAP Phase is interrupted with a lower pressure (P_{Low}) for a brief [millisecond] period of time (T_{Low}) creating a Release Phase to allow for ventilation (**Figure 2B**; Habashi, 2005). A primary advantage to APRV is that it can achieve higher mean airway pressure, promoting recruitment and oxygenation, while limiting peak inspiratory pressures (Anderson and Speicher, 2006). It also

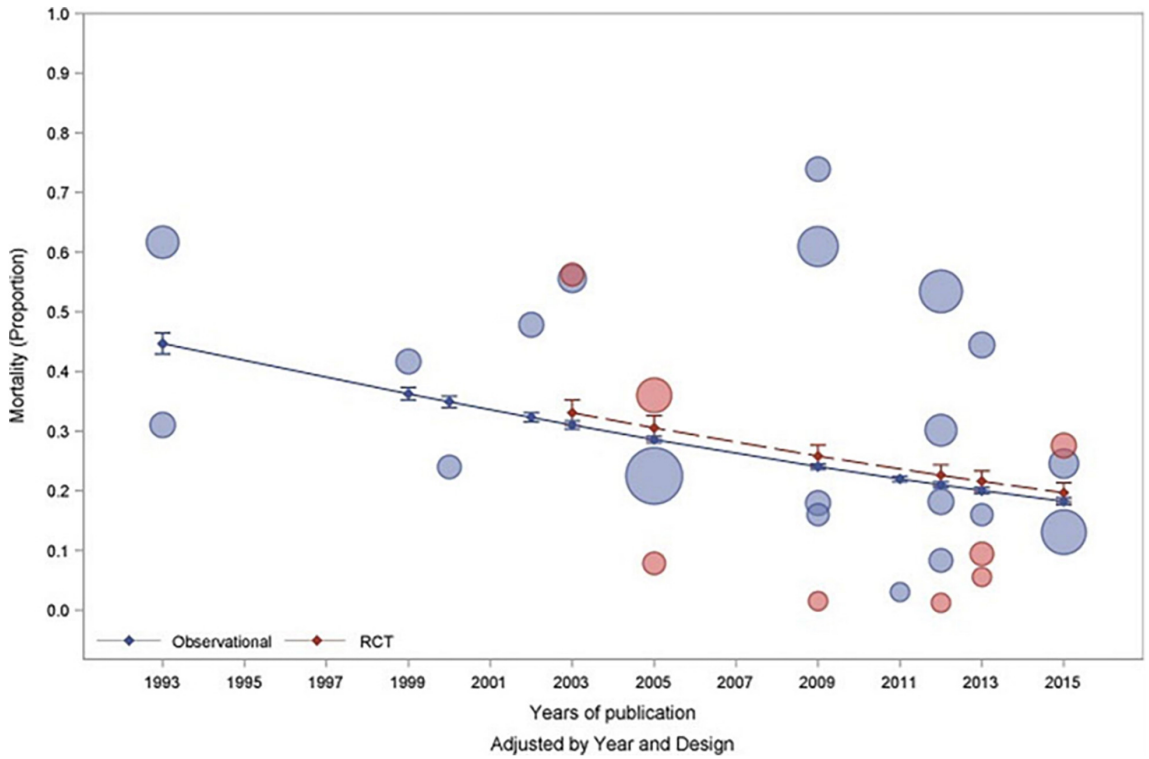

FIGURE 1 | Published with permission from Wong et al. (2019) Bubble plot demonstrating mortality rates associated with pediatric acute respiratory distress syndrome by year of study and study design (observational—blue and RCT—red). The size of the bubbles are proportional to the total number of patients recruited into the individual study.

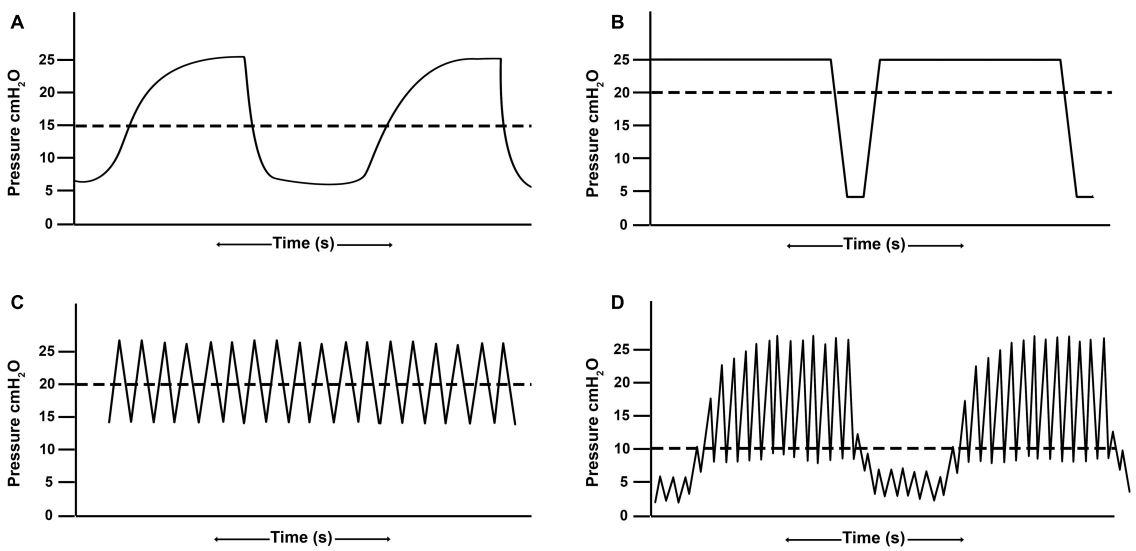

FIGURE 2 | Demonstrative waveforms of **(A)** conventional ventilation **(B)** airway pressure release ventilation, **(C)** high frequency oscillatory ventilation, and **(D)** high-frequency percussive ventilation. Airway pressure release ventilation and high frequency oscillatory ventilation maintain alveolar recruitment by achieving a higher mean airway pressure (horizontal dashed line) as compared with conventional ventilation without raising peak inspiratory pressures.

allows for spontaneous breathing (Krishnan and Morrison, 2007), thereby increasing patient comfort and limiting need for neuromuscular blockade (Frawley and Habashi, 2004; Krishnan and Morrison, 2007). Spontaneous breathing additionally allows for distribution of gas to the dependent regions of the lung, promoting further alveolar recruitment

(Habashi, 2005). Although there is a wide variability in APRV settings, as with any mode (Jain et al., 2016), the most frequently cited is the Time-Controlled Adaptive Ventilation (TCAV^{TM}) method which precisely sets the time at expiration (T_{Low}) based on the expiratory flow curve to optimize ventilation while limiting alveolar derecruitment (Frawley and Habashi, 2004; Habashi, 2005; Jain et al., 2016; Nieman et al., 2020).

Although APRV has been an available ventilator mode since 1987 (Stock et al., 1987), it is not widely used in pediatric or neonatal populations (Krishnan and Morrison, 2007). APRV is largely considered a rescue ventilator mode (Gupta et al., 2013) and the incidence of use in pediatric patients in the literature is just 1.6% as compared with 11.3% in adults (Gupta et al., 2013). This lack of clinical experience suggests that patient management can be quite variable from one intensivist to another (Anderson and Speicher, 2006). The sparse APRV studies must therefore be interpreted with caution with close attention to the method by which the mode was set.

PEDIATRIC AIRWAY PRESSURE RELEASE VENTILATION

In pediatric patients, APRV is set similar to adult patients with a P_{High} set at the plateau pressure achieved in the CV mode or at the mean airway pressure on HFOV plus 2–4 cm H_2O (Habashi, 2005). Pediatric patients naturally have a higher respiratory rate and minute ventilation as compared with adults and therefore the T_{High} is often set shorter. As compared with adults with healthy lungs (where the T_{High} is set at 4–6 s), pediatric patients with healthy lungs will have the T_{High} set at 3–5 s. In pediatric patients with injured, derecruited lungs, the T_{High} may be set at a shorter duration of 1–3 s to allow for bulk ventilation. Generally, the longest possible T_{High} is selected that maintains adequate CO_2 clearance (Frawley and Habashi, 2004; Habashi, 2005). The T_{Low} is adjusted as in adults to terminate the expiratory flow at 75% of the peak expiratory flow (Habashi, 2005).

In a prospective, randomized, crossover clinical trial of 15 pediatric patients with mild to moderate lung disease, APRV was found to have similar oxygenation, ventilation, hemodynamics, and patient comfort as compared with SIMV but with a lower peak inspiratory and plateau pressures. The shortcoming of this study was the absence of reported ventilator settings for either group (Schultz et al., 2001). One of the cited concerns for APRV is whether there are hemodynamic consequences given the higher mean airway pressure. In a small case series of pediatric patients, transition to APRV was associated with no alteration of hemodynamics and no need for neuromuscular blockade, while improving oxygenation (Krishnan and Morrison, 2007). Similarly, in patients with refractory hypoxemia transitioned from CV, APRV reduced neuromuscular blockade requirements as compared with HFOV, however, the HFOV group was represented by a younger cohort with a higher OI (Yehya et al., 2014a).

The only published RCT applying APRV to pediatric patients was performed by Lalgudi Ganesan et al. (2018) This was a single-center study conducted over a 2.5-year period but was terminated after 50% enrollment (52 children) due to higher mortality in the APRV arm. This study must be interpreted thoughtfully because it does not necessarily suggest that the APRV mode is harmful but rather that the method of setting it may have been. The authors adjusted the P_{High} to maintain a release tidal volume of 6–7 mL/kg ideal body weight (Lalgudi Ganesan et al., 2018). In doing so, the patients were placed on an open lung technique designed to recruit the lung and number of alveoli available to accommodate a larger tidal volume, but restricted the tidal volumes (Frawley and Habashi, 2004). By comparison, the TCAV^{TM} method does not restrict tidal volumes as the lung opens. Rather, larger tidal volumes are viewed as evidence of increasing lung recruitment and improved compliance (Kollisch-Singule et al., 2014).

NEONATAL AIRWAY PRESSURE RELEASE VENTILATION

In neonatal patients transitioning to APRV, the P_{High} is similarly set at the plateau pressure achieved in the CV mode or at the mean airway pressure on HFOV plus 0–2 cm H_2O. Neonates have a further decrease in their set T_{High} to 1–2 s with the T_{Low} adjusted to terminate the expiratory flow at 75% of the peak expiratory flow (Habashi, 2005). No large studies of APRV have been performed in neonates to date. Gupta et al. (2013) reported a case series of 5 infants ranging in gestational age from 24 to 28 weeks and found that the infants tolerated APRV well with no adverse events.

In a neonatal lamb model with oleic acid induced lung injury, APRV demonstrated improved oxygenation and ventilation as compared with CPAP. APRV and CV had similar ventilation and oxygenation but APRV achieved this with a lower peak airway pressure and with no hemodynamic instability (Martin et al., 1991). In a 24-h model of respiratory distress syndrome, piglets were birthed at the equivalent of a 28-week human gestation. APRV set and adjusted by the TCAV^{TM} method led to increased lung recruitment, improved ventilation, and decreased oxygen requirements without altering hemodynamics (Arrindell et al., 2015). In a subsequent 48-h porcine model of respiratory distress syndrome where piglets were birthed at the equivalent of a 25-week human gestation, APRV set according to the TCAV^{TM} method led to a significant increase in lung compliance with a trend toward improved oxygenation with lower oxygen requirements (Kollisch-Singule et al., 2017).

HIGH-FREQUENCY OSCILLATORY VENTILATION

High frequency oscillatory ventilation operates using a push-pull application of pressure to the airway opening by either piston/diaphragm or microprocessor gas controllers. Fresh gas is supplied within the ventilator circuit as a bias flow, and mean airway pressure is adjusted according to the relationship between fresh gas inflow and any positive or negative pressure placed on the gas outflow from the bias flow circuit. The clinician has the ability to set the oscillatory frequency, pressure amplitude (Δ), oscillator displacement (volume), inspiratory/expiratory

ratio, and bias flow. The mean airway pressure or continuous distending pressure (CDP) is generally set at higher value to improve alveolar recruitment and oxygenation (**Figure 2C**). Carbon dioxide elimination is correlated with the coefficient of gas transport (DCO_2), which is the product of frequency (f) and tidal volume-squared ($f * Vt^2$). Thus, a high DCO_2 leads to an improvement in ventilation, as modified by frequency and especially the tidal volume. Increases in frequency can, however, lead to decreases in DCO_2 by secondarily decreasing tidal volume unless the pressure amplitude is simultaneously increased (Sanchez Luna et al., 2013).

High frequency oscillatory ventilation is the most commonly used HFV mode with 16.4% of patients receiving HFOV in a cross-sectional observational study, but with the majority of patients placed on CV (Santschi et al., 2010). This is higher than the 2.9% reported by Arnold et al. (2000) 10 years earlier, which could reflect institutional bias or an increased acceptance of the mode. Intensivists are more apt to start HFOV on infants as an early therapy as compared with their pediatric counterparts, but it is still generally considered a rescue therapy in pediatric populations (Ben Jaballah et al., 2006). Patients are on CV between 2.2 to 11.4 days before being switched to HFOV and with an OI ranging from 27.1 to 36.7 (Arnold et al., 2000). HFOV is also the most studied of the HFV modes, but indications, timing, and strategy of HFOV remain poorly defined (Kneyber et al., 2012). Also, there was a significant difference in performance among earlier generation high-frequency oscillators such that similar settings may have generated opposing results, especially in terms of gas exchange, barotrauma, and intraventricular hemorrhage/periventricular leukomalacia (Jouvet et al., 1997). This is likely contributing to disparate results across earlier studies, particularly when applied to patients who have been transitioned from CV due to refractory hypoxemia. Technological advances have markedly improved the high-frequency oscillators that are available for clinical use such that individual ventilator differences may be less critical. However, even newer generation oscillators have substantive differences ranging from volume delivery and frequency range to required ancillary equipment and available features (Pillow, 2015). Performance among high-frequency oscillators therefore also varies, with discrepancies identified between the set and delivered ΔP in certain machines and disparate tidal volume delivery generation, especially at higher frequencies (Pillow et al., 2001; Tingay et al., 2015). It is therefore important for clinicians to recognize these nuances and understand the high-frequency oscillator that is being applied to the patient in order to improve performance (Pillow, 2015).

PEDIATRIC HIGH FREQUENCY OSCILLATORY VENTILATION

In a RCT of 70 patients, Arnold et al. (1994) demonstrated that HFOV led to an improvement in oxygenation with an increased mean airway pressure but decreased peak airway pressures, however, the study was underpowered to detect significant differences (Arnold et al., 1994). In that study, up to 66% of patients in the CV group crossed over to the HFOV group but only 38% of patients in the HFOV group crossed over to the CV group with the patients not analyzed in the initially randomized group (Arnold et al., 1994). In a smaller RCT, HFOV combined with recruitment maneuvers led to superior oxygenation as compared with CV without a marked change in hemodynamics (Samransamruajkit et al., 2016).

HFOV has been associated with both a shorter (Wong et al., 2020) and longer (Gupta et al., 2014; Curley et al., 2015) intensive care unit length of stay, which may partly be explained by the timing of HFOV application and disease severity. One retrospective study found a shorter duration ICU stay but increased mortality, suggesting that HFOV does not improve outcomes in patients with fatal lung injury, but patients with recoverable lung disease may benefit from improved oxygenation and a decrease in lung injury (Yehya et al., 2014a,b; Wong et al., 2020). In a larger retrospective review of over 9,000 patients from 98 hospitals, the use of HFOV was associated with longer duration of ventilation and ICU length of stay, as well as a higher mortality (Gupta et al., 2014). These results might be explained by the study design in which patients were matched according to propensity score matching rather than pulmonary disease type or ventilator parameters (Gupta et al., 2014). In the patients who were placed on HFOV and survived, earlier application of HFOV (within 24 h) was associated with a shorter ventilation course and length of stay as compared with patients in whom HFOV was applied later (Gupta et al., 2014).

In contrast, the results of a secondary analysis of the Randomized Evaluation of Sedation Titration for Respiratory Failure (RESTORE) trial (Curley et al., 2015) found that earlier application of HFOV was correlated with a greater length of mechanical ventilation as compared with later application of HFOV and conventional mechanical ventilation, however, there was no association with mortality (Bateman et al., 2016). The combination of these results is confusing and the discrepancies may suggest that different methods of setting HFOV were used among patients and between studies. It is important to recognize that the HFOV settings that are applied initially or early will be different from those applied later. The goal for both is to achieve a homogeneously aerated lung, but the acutely injured lung must be nudged open slowly, particularly if it has been subject to a prolonged time on non-open-lung strategies (Froese and Kinsella, 2005).

Optimal settings of HFOV have not been thoroughly illuminated (Kneyber et al., 2012). Most protocols do not involve recruitment maneuvers (Kneyber et al., 2012) and set frequency somewhat arbitrarily based on patient age and weight in the range of 5–8 Hz (Froese and Kinsella, 2005; Kneyber et al., 2012; de Jager et al., 2019), where frequency should rather be optimized to minimize the pressure cost of ventilation to reduce lung injury (Venegas and Fredberg, 1994). As an example of the challenges of standardizing HFOV, the power or amplitude is adjusted according to the chest wiggle factor, or the level to which the chest wiggles on HFOV (Meyers et al., 2019). This is hardly precise and difficult to standardize across intensivists, and especially so among institutions. Animal models have been designed to assist in determining optimal means of setting HFOV. In a saline lavage

injury in lambs, stepwise escalation in mean airway pressure modified lung volume and optimized lung recruitment (Pellicano et al., 2009). The ideal method of setting HFOV would be with a mean airway pressure sufficient to stabilize alveoli but as low as reasonable possible, with the smallest superimposed oscillations to minimize alveolar strain (Kneyber and Markhorst, 2016).

NEONATAL HIGH FREQUENCY OSCILLATORY VENTILATION

One of the earliest randomized studies in 1989 comparing CV vs. HFOV was the HiFi study (Group, 1989) in preterm infants (750–2,000 g), in which HFOV did not reduce mortality or BPD rates (Group, 1989; No Authors List, 1990a). They found an increased rate of intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL) (Group, 1989). In a follow-up study at 16–24 months post-term age, respiratory status was similar between the two groups but neurodevelopmental outcome were worse in the HFOV group with a correlation between cognitive defects and hydrocephalus with IVH (No Authors List, 1990b).

Although HFOV is often used as a rescue therapy with success, it has not yet become a primary mode to use early on ventilated patients. To study this, in 1996, the multicenter Provo trial reported that early application of HFOV to premature newborns born less than 35 weeks gestation with moderate to severe respiratory distress resulted in decreased lung injury, improved oxygenation, and even a lower incidence of necrotizing enterocolitis (Gerstmann et al., 1996). A follow-up study published 5 years later determined that there was no difference in childhood neurodevelopmental outcomes, however, the CV group had some markers of obstructive lung pathology with a decrease in peak expiratory flow but an increase in residual volume (Gerstmann et al., 2001). This study was especially important to illustrate that reducing pulmonary morbidity in neonates can decrease subsequent pulmonary dysfunction into childhood (Gerstmann et al., 2001).

To summarize, in three longer-term observational studies evaluating the neurodevelopmental outcomes of preterm infants with respiratory distress syndrome, one demonstrated worse neurodevelopmental outcome in the HFOV group (No Authors List, 1990b), one showed similar neurodevelopmental outcome in the HFOV group but improved respiratory function (Gerstmann et al., 2001), and another demonstrated similar neurodevelopmental and respiratory outcomes (Marlow et al., 2006). These changes can be attributed to improvement in adjunctive strategies, comfort level with HFOV, and improvement in methods of setting HFOV. For instance, surfactant therapy was not a routine treatment during the HiFi study (Courtney et al., 2002). There was also a higher cross-over rate from HFOV to conventional ventilation which could be interpreted as less comfort with the ventilator given that the methods reported that the oscillator was selected after bench testing available machines (Group, 1989). The oscillator chosen (Senko Medical Instrument Manufacturing, Tokyo, Japan) only has the ability to deliver an I:E ratio of 1:1 leading to higher CDP, and contributing to a higher incidence of barotrauma, and IVH (Bryan and Froese, 1991). The HiFi study also used a low volume HFOV strategy, which is now thought to be sub-optimal for infants (Bryan and Froese, 1991). Nevertheless, with conflicting results from these three RCTs, it is not surprising that there is a lack of consensus on ventilating neonatal patients.

In a later multicenter clinical trial, Courtney et al. (2002) randomized very low birth weight infants (601–1,200 g) to HFOV or synchronized intermittent mandatory ventilation and revealed that infants were more likely to be extubated early with HFOV and with a decreased rate of supplemental oxygen requirements by 36 weeks postmenstrual age. This study did not reveal a difference in IVH or PVL (Courtney et al., 2002). In a separate randomized comparison of pre-term infants born < 30 weeks of age, HFOV led to decreased surfactant requirements but no improvement in pulmonary outcomes (Moriette et al., 2001).

Other studies have revealed that HFOV is successful in preventing ECMO (Erdeve et al., 2019) and with similar (Snoek et al., 2016) or decreased (Erdeve et al., 2019) mortality rates. It has been affiliated with a lower (Courtney et al., 2002) and similar (Snoek et al., 2016) incidence of chronic lung disease and similar rates of death and IVH (Clark et al., 1994). A Cochrane review of elective HFOV RCTs spanning as early as the HiFi trial determined that the 28-and 30-day mortality between HFOV and CV was similar. They also found an increase in pulmonary air leaks in the HFOV group, a decrease in severe retinopathy of prematurity, and a decrease, albeit inconsistent, in chronic lung disease (Cools et al., 2015).

To further illustrate the importance of fully understanding studies, a meta-analysis of 17 randomized trials comparing HFV to CV determined that changes in outcomes between HFV and CV are more likely due to the method by which the mode was set as compared with the mode itself (Thome et al., 2005). Adjunctive therapies in pediatric and neonatal ICU patients have been rapidly progressing, including surfactant therapy and nitric oxide application. Studies must therefore also be taken in context with the year they were performed (Courtney et al., 2002). Recognizing that with the arsenal of critical care techniques available, the limits of what can be done has also expanded, where studies were previously reporting infants born < 35 weeks of age and weighing < 1.751 kg in 1992 (Clark et al., 1994) to infants weighing as little as 601 g in 2002 (Courtney et al., 2002).

HIGH-FREQUENCY PERCUSSIVE VENTILATION

High-frequency percussive ventilation (HFPV) is delivered by a pneumatically powered, flow-regulated, time-cycled, pressure-controlled ventilator. HFPV delivers a small tidal volume (or sub-tidal volume) at a high frequency, in combination with a low frequency bulk distribution of gas similar to that of a pressure limited CV breath (**Figure 2D**). The most unique feature of HFPV is the breathing circuit and the patient airway interface. It uses a sliding venturi, operating simultaneously as inhalation and exhalation valves. It is permanently open to ambient, through which sub-tidal breaths are delivered into the lungs. By venturi effect, flow volume delivery is always inversely proportional to the

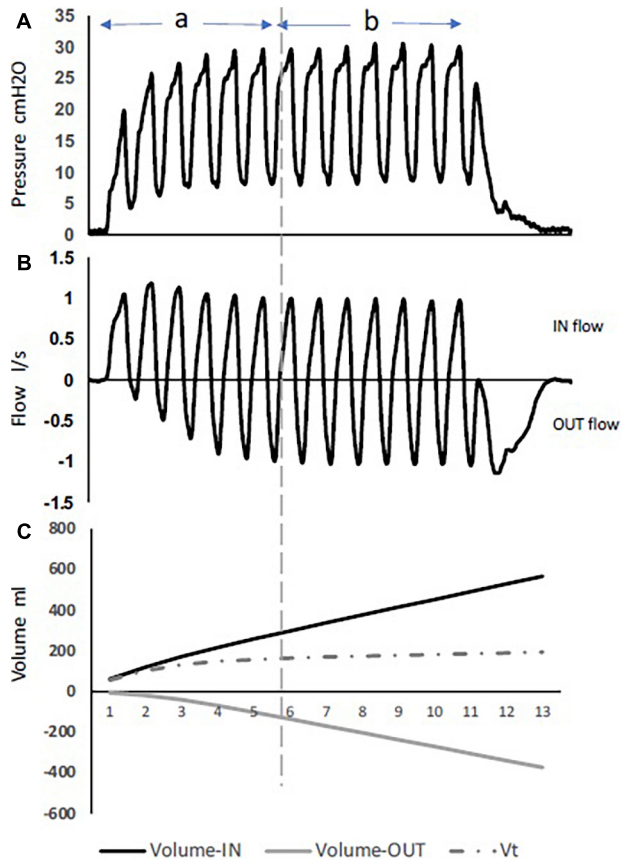

FIGURE 3 | Pressure, flow, and volumes during HFPV inspiratory interval, using a lung model. During phase **(A)** full flow acceleration with an increase in volume-in delivery, while phase **(B)** is characterized with more volume-out. **(C)** The difference between volume-in and volume-out is the tidal volume. Note the tidal volume value remains stable during the whole plateau equilibrium.

pressure reached at the level of the airway. The high frequency flow interrupter generator of the ventilator allows the sliding venturi to be regulated in such a manner that there is a stepwise increase in airway pressure during inspiration to the scheduled peak inspiratory pressure (**Figure 3**; Bougatef et al., 2007).

Two phases describe the inspiration period during HFPV. The first is an initial phase characterized by an inspiratory flow acceleration with a progressive increase in airway pressure. As the inspiratory interval progresses, an expiratory flow component develops. The second phase is where the pressure and flow reach an oscillating plateau equilibrium at the scheduled peak inspiratory pressure, and where the flow signal is characterized by an equilibrium between the inspiratory flow and the expiratory flow components (**Figure 3**). While the second phase is user-controlled (pressure, amplitude, time), the initial phase is dependent on the patient's thoraco-pulmonary mechanics. Unlike the other high-frequency modes (HFOV, HFJV), HFPV does not rely on establishing a higher mean airway pressure to maintain alveolar recruitment. Instead, HFPV maintains gas distribution by synergizing with the time constants of the lung compartments, maintaining open alveoli while preventing overinflation (Lucangelo et al., 2010).

During inspiration, the flow is interrupted between two consecutive pulses, resulting in a pressure drop to a value that depends on the thoraco-pulmonary mechanics. The gradual stacking of the successive pulsed volumes results in a progressive increase in lung volume. Like flow and pressure, sub-tidal volume deliveries during the inspiratory interval follows two phases. At the beginning of inspiration, pulsed volume-in are larger in size, while the exhaled volumes (volume-out) are small. As the inspiratory interval progress, pulsed volume-in decreases and volume-out increases in size to reach an equilibrium characterizing the second phase (**Figure 3**).

After each volume-in, the pulsed flow is interrupted, allowing the exhalation port to vent the proximal airway to ambient, and a volume-out is exhaled by the patient. This represents an important beneficial mechanism of HFPV in the improvement of gas exchange. The tidal volume is calculated by the difference between the cumulative volume-in and the cumulative volume-out (**Figure 3**).

PEDIATRIC HIGH-FREQUENCY PERCUSSIVE VENTILATION

In a retrospective observational study of 31 patients who failed conventional ventilation, application of HFPV led to improved oxygenation and ventilation while decreasing the peak inspiratory pressure from 38 to 26 cm H_2O (Rizkalla et al., 2014). HFPV has found particular application to patients with acute burn injury and respiratory failure, likely because it has been effective in clearing secretions due to percussive bursts (Allan et al., 2010). Three RCTs (Carman et al., 2002; Mlcak et al., 2002; Reper et al., 2002) and one case-controlled series (Cortiella et al., 1999) cumulatively demonstrated improved oxygenation (Cortiella et al., 1999; Carman et al., 2002; Reper et al., 2002) a decrease in peak inspiratory pressures (Cortiella et al., 1999; Carman et al., 2002), and decreased rates of pneumonia (Cortiella et al., 1999; Mlcak et al., 2002) in mechanically ventilated pediatric patients with burns and/or inhalation injury ventilated with HFPV as compared with CV.

NEONATAL HIGH-FREQUENCY PERCUSSIVE VENTILATION

High-frequency percussive ventilation is less well-reported in neonatal studies, often being lumped together with other HFV modes. Only small case series exist in neonates ventilated with HFPV (Pfenninger and Gerber, 1987; Pfenninger and Minder, 1988; Paviotti et al., 2014), but have demonstrated improvements in oxygenation without increasing airway pressures (Pfenninger and Gerber, 1987; Paviotti et al., 2014), and improved static compliance (Pfenninger and Minder, 1988). Nasal high-frequency percussive ventilation has recently been popularized (De La Roque et al., 2011; Moresco et al., 2020; Renesme et al., 2020) and compared with nasal continuous positive airway

pressure, demonstrated non-inferiority (Renesme et al., 2013), possible benefit, and that it is well-tolerated (De La Roque et al., 2011). In a piglet model of meconium aspiration syndrome, HFPV and CV were associated with lower mean airway pressures and OI as compared with HFOV, but there was no apparent difference in histologic lung injury (Renesme et al., 2013).

High-Frequency Jet Ventilation

High frequency jet ventilation is seldom used in adults and most often reported in neonates, particularly in preterm infants (Miller et al., 2021a). With HFJV, a high velocity gas jet is delivered *via* an adapter or jet injector inserted into the endotracheal tube. Similar to HFOV, it delivers small tidal volumes at a rapid rate of up to 660 cycles/min with an inspiratory time set as short as 0.02 s (Smith et al., 1993; Miller et al., 2021a). Unlike HFOV, HFJV is set on top of a conventional ventilation mode which regulates the mean airway pressure (Miller et al., 2021a). Additionally, exhalation is passive and dependent on the rate and inspiratory to expiratory ratio, but allows for marked CO_2 elimination (Miller et al., 2021a). The jet pulses and continuous air stream are also hypothesized to improve mucous clearance (Miller et al., 2021a). Like the other HFV modes, HFJV is largely used as a rescue mode when CV has failed (Smith et al., 1993).

PEDIATRIC HIGH FREQUENCY JET VENTILATION

No RCTs exist in the pediatric population and the majority of studies are case series (Miller et al., 2021a), but it has been deemed safe for use as evident in a case series of eleven pediatric patients with respiratory syncytial virus (Valentine et al., 2016). In a physiologic comparison of HFJV vs. CV, HFJV was found to generate a higher intrinsic PEEP leading to an increase in end-expiratory lung volume and improved oxygenation, while also achieving a higher minute ventilation (Berner et al., 1993). In support of this, a retrospective review of 35 pediatric critical care patients (including neonates) transitioned from CV to HFJV were found to have a decrease in acidosis and oxygen requirements (Miller et al., 2021b). HFJV has also been shown to resolve clinically relevant pulmonary barotrauma and air leaks caused by CV at lower mean airway pressures (Smith et al., 1993).

NEONATAL HIGH FREQUENCY JET VENTILATION

In two neonatal RCTs, HFJV was found to improve ventilation at lower peak airway pressures (Keszler et al., 1991; Keszler, 1997). Cross-over was allowed in one of the trials, and 84% of patients who crossed over from CV to HFJV responded well to HFJV, whereas only 9% of those who crossed over from HFJV to CV were successful (Keszler et al., 1991). The incidence of chronic lung disease was similar between CV and HFJV in that study (Keszler et al., 1991), whereas in the other RCT of preterm infants <36 weeks, HFJV was found to reduce the incidence of BPD as well as the need for future supplemental oxygenation (Keszler, 1997). Two different methods of setting HFJV were used in that study: a low airway pressure (Low P_{aw}) strategy and an optimal volume (OV) strategy. In a subgroup analysis, the HFJV-OV strategy was found to have an improvement in oxygenation whereas the HFJV-Low P_{aw} had an increase in ventilation (Keszler, 1997). Other observational studies comparing HFJV with CV have revealed similar oxygenation, ventilation, and hospital days (Wiswell et al., 1996) and no difference in mortality, bronchopulmonary dysplasia, or cross-overs (Carlo et al., 1990), but similar rates of air leaks has been consistent across studies (Carlo et al., 1990; Keszler et al., 1991; Keszler, 1997).

Neurologic outcomes with HFJV have been conflicting. In one of the RCTs, the HFJV-OV strategy was affiliated with a decreased incidence in IVH and PVL as compared with the CV and HFJV-Low P_{aw} groups (Keszler, 1997), whereas the other found no difference (Keszler et al., 1991). In another study of 73 premature infants born less than 33 weeks, HFJV had a higher incidence of PVL or poor neurologic outcome (Wiswell et al., 1996). In another study of 42 infants with severe respiratory distress syndrome there was no difference in IVH incidence between HFJV and CV (Carlo et al., 1990).

Other special considerations that have been studied with HFJV include congenital heart disease, congenital diaphragmatic hernias, and persistent pulmonary hypertension. HFJV in infants with congenital heart disease were found to have decreased acidosis and improved ventilation but no significant difference in oxygenation (Miller et al., 2021c). HFJV has demonstrated improved ventilation in infants with congenital diaphragmatic hernias (Zhang et al., 2013) and a trend toward improved survival as compared with CV (Kuluz et al., 2010). HFJV has been compared against HFOV in infants with persistent pulmonary hypertension (Coates, 2008), but no randomized controlled trials exist (Ethawi et al., 2016). In infants with persistent pulmonary hypertension, HFJV and HFOV led to similar outcomes once adjusted for differences in comparison groups (Coates, 2008). Other special considerations for neonates include meconium aspiration syndrome and pneumonia, in which HFJV may benefit from being combined with surfactant (Davis et al., 1992; Calkovska et al., 2005).

DISCUSSION

Although the results of this review are seemingly bleak, with no consistent outcomes among studies or determination of optimal modes or methods of setting them, a few conclusions may be drawn. The first is that caution must be taken when interpreting studies and comparing modes against one other, particularly when a protocol for setting a mode has not been established, and when comparing studies spanning large periods of time. The second is that there may not be a single optimal mechanical ventilation approach. The best method may simply be one that the intensivist is comfortable with and one that allows for an adaptive approach so the settings are personalized to the individual patient and disease pathophysiology (Gattinoni et al., 2016). Finally, when considering study design, not only does the

number of enrolled centers and patients need to be considered, but also patient phenotype. A well-designed multi-center study would ideally also have a centralized method of providing continuous oversight of ventilator settings and waveforms of patients across participating institutions to standardize ventilator adjustments and maximize internal validity.

There has been a call for an increased number and quality of RCTs in pediatric and neonatal mechanical ventilation trials. With the lower incidence of lung injury in pediatric and neonatal patients, it is well-accepted that a properly powered study would require involvement of several institutions over a few year period, not only creating difficulty maintaining inter-institutional protocols and compliance but also over an extended period of time (Khemani and Newth, 2010). Future pediatric mechanical ventilation studies must be carefully designed, not only to ensure adequate power, but also with appropriate age stratification and inclusion/exclusion criteria. Even a perfectly controlled multi-institutional RCT can prompt the question of whether the average of a population with varying clinical characteristics and disease phenotypes can be applied to the individual patient (Goligher et al., 2015). The most prominent example of this is in patients with extrapulmonary vs. pulmonary lung injury. These two quite distinct phenotypes are both lumped together under the umbrella of ARDS yet patients with one vs. the other will respond disparately to ventilator setting adjustments (Kollisch-Singule et al., 2018). Not only should patients be partitioned into pediatric and neonatal categories, but also specific age groups to account for changes in chest wall stiffness and alveolarization. Patients should also be analyzed according to lung injury phenotype in pediatric patients (pulmonary vs. extrapulmonary) and disease type in neonates (persistent pulmonary hypertension, respiratory distress syndrome, congenital heart disease, meconium aspiration syndrome) and whether or not they are on ECMO.

One of the great challenges with RCTs is that a clinician may be expected to set and adjust a mechanical ventilator mode that they are not accustomed to. All clinicians are hostage to their experience, both in training and with previous patients. It is unrealistic to expect improved outcomes in a mode that a clinician is uncomfortable with, particularly if the methods are not well-protocolized. The "best" mechanical ventilation mode is not only dependent on the patient and disease pathophysiology, but also on the experience of the individual making the ventilator adjustments. Therefore, to make an RCT successful requires teaching modules, clinician humility, and consideration toward making ventilator adjustments in a coordinated team fashion.

CONCLUSION

In summary, there are a variety of mechanical ventilation modes available for neonatal and pediatric use, each with benefits and drawbacks but with no definitive indications or protocols for use. In part, this is due to the lack of definitive evidence from trials, however, Froese (Froese and Kinsella, 2005) well-articulated that "*a premature trial can kill a good technique (almost)*." More trials may not provide the answers we are searching for and may provide more conflicting data, particularly without thoughtful study design. There may never be one universalized mechanical ventilation protocol that can be safely and effectively applied to all pediatric and neonatal patients, but it is important to be open to additional strategies and understand the fundamentals of each so they may be titrated to the individual patient. We will likely find that the best mechanical ventilation strategy is that which is personalized and adaptive to the patient.

AUTHOR CONTRIBUTIONS

MK-S: manuscript drafting. GN, PA, JS, HR, SB, LG, NH, and AB: critical revisions. All authors contributed to the article and approved the submitted version.

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Airway Pressure Release Ventilation With Time-Controlled Adaptive Ventilation (TCAV^{TM}) in COVID-19: A Community Hospital's Experience

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Santa Cabrini Ospedale, a community hospital in Montreal, Canada, used the airway pressure release ventilation following a time-controlled adaptive ventilation (APRV-TCAV^{TM}) approach for several patients in the first wave of the coronavirus disease 2019 (COVID-19) outbreak in the spring of 2021. Based on favorable patient responses, it became the primary mode of invasive mechanical ventilation—from initiation through extubation—during the second and third waves of COVID-19. In this article, we describe our success with APRV-TCAV^{TM} over more conventional modes and protocols and look at three cases that aptly demonstrate our experience. We then outline several risks with our approach and the lessons learned from our experience. While we generally saw improvement in patients' clinical course with APRV-TCAV^{TM}, there are inherent risks with this approach that others must prepare for if they attempt to implement it in their practice.

Keywords: ARDS, COVID-19, APRV, TCAV, respiratory failure, critical care

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic hit the world in January of 2020 and reached Montreal, Canada, in March of that year. The harrowing reports of COVID-19's proclivity to develop acute respiratory failure with exceedingly high mortality were concerning to our critical care team. Of particular concern was the atypical nature of the COVID-19-associated acute respiratory distress syndrome (C-ARDS). Many patients displayed profound hypoxia with relatively little dyspnea—"happy hypoxics"—raising numerous questions about pathophysiology and treatment implications (Ferguson et al., 2020; Goligher et al., 2021). There were also early reports of dichotomized L and H phenotypes, which perhaps necessitated differing ventilatory strategies (Gattinoni et al., 2020; Marini and Gattinoni, 2020). While the optimum ventilator strategy for C-ARDS was unclear, a low tidal volume (LTV) or Acute Respiratory Distress Syndrome Network (ARDSnet) strategy was endorsed by most guidelines and widely applied (Alhazzani et al., 2020; Alqahtani et al., 2020; ANZICS, 2020). But the rapid multiorgan failure following intubation and high mortality rates belied confidence that this ventilatory approach was best suited to C-ARDS. The LTV approach utilizes a tidal volume based on the ideal body weight, but lung volume correlates poorly with this; furthermore, the pathologies causing C-ARDS do not affect the lung parenchyma according to a weight-based pattern.

One of the silver linings of the COVID-19 pandemic was the increased level of collaboration that occurred among medical professionals across the globe. While the online medical community has

been gaining momentum in recent years through blogs, podcasts, Twitter, etc., under the rubric of Free Open-Access "Meducation" (FOAMed), the pandemic greatly accelerated this community's growth and impact. Through this collaboration, our critical care team at Santa Cabrini Ospedale explored the literature around airway pressure release ventilation (APRV) and posited that APRV following a time-controlled adaptive ventilation (TCAV) strategy (i.e., APRV-TCAV^{TM}) might provide ventilation better tailored to the diverse and unique pathologies of C-ARDS. And since our center did not have extracorporeal membrane oxygenation (ECMO) capabilities, we thought it all the more important to utilize a ventilatory strategy that could adequately support oxygenation.

Our hospital trialed the mode on select patients during the first wave and, in certain cases, achieved remarkable success. Given this success, we then transitioned to utilizing APRV-TCAV^{TM} as the primary mode for all intubated COVID-19 patients during the second and third waves and found our care markedly improved from what was expected using more traditional LTV approaches. This success came about through trial and error, however, and many lessons were learned that now inform a more thoughtful, safe, and effective way to use the mode and protocol.

THE AIRWAY PRESSURE RELEASE VENTILATION WITH TIME-CONTROLLED ADAPTIVE VENTILATION^{TM} APPROACH: A CURSORY REVIEW

In brief, APRV is a mode of ventilation that alternates between two levels of continuous positive airway pressure (CPAP) and allows for spontaneous breathing throughout the entirety of the respiratory cycle. It requires setting four parameters, namely, the pressure at each level of CPAP and the time spent at each—pressure high (P_{High}), pressure low (P_{Low}), time high (T_{High}), and time low (T_{Low}). These parameters are guided by a protocol referred to as time controlled adaptive ventilation (TCAV). TCAV^{TM} starts by setting the P_{High} at the prior mode's plateau pressure for a T_{High} that is based on the prior respiratory rate. A faster respiratory rate will result in a shorter T_{High} and a slower rate will result in a longer T_{High}. In general, most patients with severe ARDS require a P_{High} of 25–30 cm H_2O, and a T_{High} of 2.5–6 s. This time at a high level of CPAP is punctuated by infrequent, brief drops in pressure to zero, P_{Low}, and referred to as a "release." The duration of the releases, T_{Low}, is usually very short and depends upon the expiratory flow curves as described below. Put as succinctly as possible, TCAV^{TM} is a high CPAP with brief releases.

The extended time at a higher CPAP results in markedly increased mean airway pressures without concomitant increases in peak or plateau pressures. Over time, a lung subjected to these higher mean airway pressures is able to recruit and "open" (assuming the lung has not entered a fibroproliferative phase and all other clinical parameters being equal). The functional residual capacity can be regained, and more normal, homogenous lung architecture can be restored. In fact, APRV is sometimes described as simply a prolonged and judicious recruitment maneuver.

To maintain sufficient minute ventilation and to offload some of the ventilatory burden on the patient, releases are employed. While the release pressure, P_{Low}, is set to zero, some level of intrinsic positive end-expiratory pressure (iPEEP) is retained by terminating the release breath while there is still significant expiratory flow. This is performed by measuring the peak expiratory flow and adjusting the T_{Low} such that it cuts off expiration at or above 75% of peak flow (**Figure 1**). In some ventilators, it is carried out automatically, and once the expiratory flow decays by 25%, the T_{Low} terminates and pressure reverts back to P_{High}. In other words, the T_{Low} is set according to the time it takes the expiratory flow to decay to 75% of the peak expiratory flow. "Trapping" of air based on a time is the key element of this ventilatory strategy and results in an auto-peep that stabilizes the lung by minimizing the alveolar size variation during expiration. Initial *in vivo* animal experiments showed that with a cutoff of 75%, most of the exhaled air was from the conducting airways with a minimal change in the alveolar size (Nieman et al., 2018; Kollisch-Singule et al., 2019) thereby "splinting" the alveoli open. This air splint, in theory, minimizes atelectrauma and maximizes the maintenance of recruitment. While many misinterpret the settings as indicative of a driving pressure of P_{High}—P_{Low} (which would be excessively high in most cases) the driving pressure to the alveoli is in fact P_{High}—intrinsic PEEP. This intrinsic PEEP is easily confirmed with a brief expiratory hold, which always demonstrates a marked discrepancy between the set P_{Low} of zero and the measured airway pressure during a hold (**Figure 2**). Setting the P_{Low} and T_{Low} in this manner has been demonstrated in numerous animal studies to keep the alveoli stable, i.e., they do not collapse with each breath (Nieman et al., 2018).

As the lung recruits, the alveoli get a chance to heal, and if the patient's overall condition improves, the higher pressures can be gradually reduced and the number of releases spread out, a process referred to as "drop and stretch." With this, the patient takes on a larger share of the ventilatory burden, and support is slowly withdrawn until the patient reaches a point of breathing almost entirely on a lower level of CPAP.

Several points bear highlighting. First, the entirety of ventilation can be accounted for by the releases so that APRV can be used on paralyzed patients. In this case, any benefit from spontaneous breathing will be lost, but given the potential risks of patient self-inflicted lung injury (P-SILI), this may not necessarily be a bad thing. Second, nothing prevents APRV-TCAV from being used in a prone position. Third, restoration of lung volume and improvement in V/Q matching often result in improved oxygenation, an "apparent cure," before the previously injured alveoli have had a chance to heal. As a result, many clinicians often prematurely reduce the pressures, and as a result, alveoli quickly collapse again. Finally, tidal volume and traditional PEEP are not directly set. While P_{High}, P_{Low}, T_{High}, and T_{Low} can be adjusted to account for preferred tidal volumes and PEEP levels, their lack of direct control often results in clinicians adjusting the mode in ways that might be deleterious in the context of TCAV^{TM}, e.g., extending the T_{Low} to achieve more minute ventilation. Finally, not all APRV is TCAV^{TM}. While

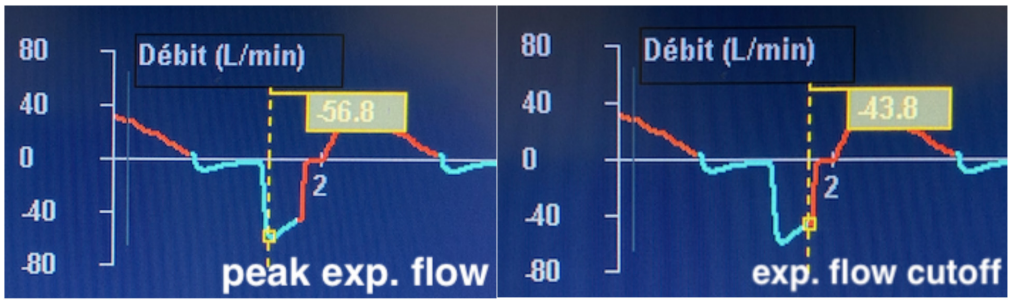

FIGURE 1 | Manual adjustment of TLow: peak expiratory flow is measured (left) and the TLow is set to obtain a cutoff at or above 75% of peak flow, in this case 77%.

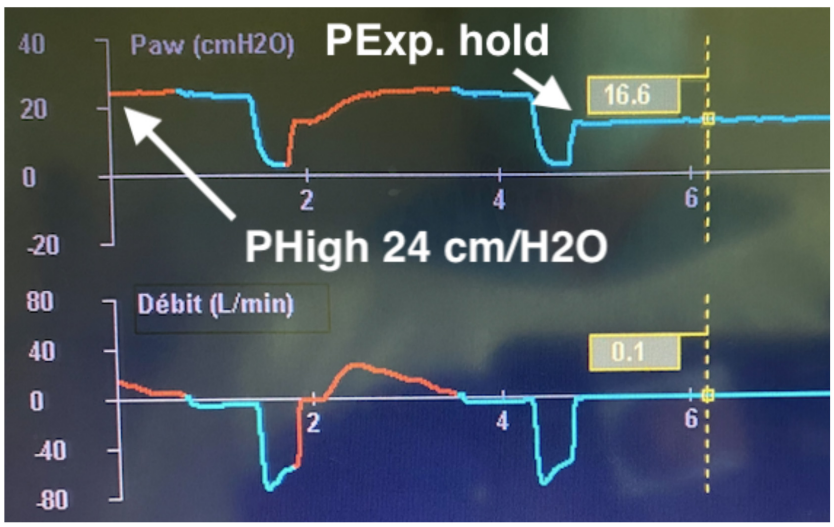

FIGURE 2 | Calculation of Effective PEEP/Driving pressure: an expiratory hold is performed and equilibration of pressure occurs. This is the effective PEEP. Driving pressure is PHigh – PExpiratory hold, in this case approximately 7–8 cmH2O.

many practitioners claim to practice a TCAV^{TM} approach, the settings outlined above are precise and interlinked. Deviation from them in even small ways can result in profound harm. For a more extensive discourse on the mode and protocol, we refer readers to Kollisch-Singule et al. (2019) and Habashi et al. (2021).

OVERVIEW OF OUR EXPERIENCE THROUGH THE DIFFERENT WAVES

As one of the designated centers for COVID-19, our center was rapidly overwhelmed with deteriorating patients. Profound hypoxia was the rule, and we liberally used non-invasive ventilation and awake proning on the wards. Despite this, many deteriorated further, and our expanded-26-bed intensive care unit (ICU) had over 90% of its patients intubated, many requiring frequent proning cycles.

In the first wave, we saw a preponderance of elderly patients and an overall mortality rate of 67%, which is consistent with reports in the literature (Hyman et al., 2020; Luo et al., 2020). APRV-TCAV^{TM} was mostly used as a rescue therapy since the medical and respiratory therapy teams were not familiar or comfortable with the APRV-TCAV^{TM} approach. Despite this lack of experience, APRV-TCAV^{TM} still rescued a few seemingly intractable cases, and we decided to leave those patients on the mode for the remainder of their course. They were among the few survivors of the ventilated cohort. While the numbers are too small to draw meaningful conclusions, they piqued our interest and established the mode as a viable alternative.

After reviewing our first-wave experience, delving into the literature, and discussing with colleagues at other institutions, we proceeded with APRV-TCAV^{TM} as the primary mode of ventilation from intubation to liberation for the majority of cases during the second and third waves. Refractory hypoxia was limited to a few cases unresponsive to TCAV^{TM}, which usually ended up requiring transfer for ECMO. With a decreased incidence of refractory hypoxemia came a concurrent decrease in proning—a welcome relief from the nursing work intensity that was needed on other modes of ventilation during the first wave.

In most cases, the following would occur in, approximately, the first 24 h:

(a) Oxygen requirements would significantly decrease.
(b) Driving pressures would markedly decrease, often to less than 10 cm/20 h.
(c) Chest imaging would improve substantially.

All this would occur without substantial deterioration in other organ functions, e.g., there was no escalation of vasopressors, need to initiate continuous renal replacement therapy (CRRT), or worsening sedation needs. If anything, the patients' overall condition would improve in concert with improvement on the ventilator.

CHALLENGES AND CONSIDERATIONS WHEN USING AIRWAY PRESSURE RELEASE VENTILATION WITH TIME-CONTROLLED ADAPTIVE VENTILATION^{TM}

False Reassurance by Improved Oxygenation

These improvements were often misleading, however. Several consultants unfamiliar with APRV-TCAV^{TM} were surprised that the patients were not being more aggressively weaned since they "clearly no longer had ARDS" based on their FiO_2 and chest X-ray (CXR). But while the lung may have been recruited and traditional parameters were improved, there was still an underlying pathology that required additional time to heal. If the P_{High} was decreased before the true alveolar stability was achieved, then the CXR would abruptly worsen and gas exchange would quickly regress. This phenomenon of needing to "stabilize" the lung after recruitment on APRV-TCAV^{TM} has been reported elsewhere and likened to needing a cast for a period of time after setting a fracture (Nieman et al., 2018). Hence, clinicians will have to carefully observe patients' breathing patterns, ventilator curves, and gas exchange during weaning.

Risks With Changing Modes

The clinical improvement associated with the use of TCAV^{TM} must be taken into account when considering a change of modes. While changing from a traditional pressure-control mode to an assist-control mode (given similar tidal volumes and PEEP) would not likely result in large swings in oxygenation, this does not always hold true for TCAV^{TM}. The clinician should clearly understand that there are several elements that will impact oxygenation in a potentially drastic way, especially if the change occurs before the alveolar stability is achieved as described above. First, TCAV^{TM} is similar to the inverse ratio in that more time is spent at a higher pressure. Hence, switching to alternative modes with roughly the same high and low pressures but a substantially different time at those pressures can result in a significant drop in mean airway pressure. Second, if alveolar stability is not achieved, patients will likely derecruit. The rapidity and severity of deterioration are difficult to predict but can occur in minutes and likely correlate to the required P_{High} and the time spent there previously.

In our experience, these premature and/or inappropriate transitions occurred most often after hand-offs between treating physicians and during patient transport. When a physician is unfamiliar with TCAV^{TM} and the clinical state requires a change to the settings, the clinician often changes to a mode that is more common and familiar. Transitioning to an alternative mode from APRV-TCAV^{TM} before a lung has stabilized is fraught with complexities and nuance that is usually more difficult than making an adjustment to the APRV-TCAV^{TM} settings. It is in this back-and-forth where patients are at most risk for harm and is one of the main reasons why a team buy-in is necessary. This holds true for patient transport as well where portable ventilators are often incapable of providing APRV-TCAV^{TM}. Hence, when deciding on, say, a CT scan, the risks of transporting with an alternative ventilatory mode must be weighed against the benefits of the scan. In our center, bedside ultrasound is used extensively, yet trips to CT scan were still required from time to time. This issue also applies to interhospital transfers.

One potential solution for transfers is to approximate TCAV^{TM} by using inverse ratio ventilation with pressure control. The inspiratory pressure should match the P_{High}, and the PEEP should be zero. The inspiratory/expiratory (I:E) ratio will need to reflect the T_{High}:T_{Low}, often being set at the maximum ratio, and patients may need additional sedation and/or paralysis. APRV utilizes floating valves that allow patients to breathe *ad lib* as they would on CPAP. This is not possible on inverse ratio pressure control and will likely be too uncomfortable for spontaneously breathing patients. Utilizing this approach can often create a respiratory rate around 25 with a T_{Low} equivalent of 0.4 and may come close to matching the termination of expiratory flow at 75% (60/25 = 2.4 s cycle. At an I:E of 5:1 that is a "T_{High}" of 2 s and a "T_{Low}" of 0.4 s).

Bagging the Time-Controlled Adaptive Ventilation Patient

Personnel must understand that bagging is unlikely to help the hypoxic patient on TCAV^{TM} and 100% FiO_2. Although a time-honored practice, hand-bagging will likely result in a much lower mean airway pressure—even when a PEEP valve is used—and may result in deterioration. Rapidly passing a suction catheter to rule out occlusion, analyzing the ventilator waveform and end-tidal CO_2 ($ETCO_2$), carefully observing the patient, and quickly scanning with point-of-care ultrasound (POCUS) or obtaining a CXR to rule out pneumothorax should be the initial reflexes. Bagging should only be considered after serious deliberation with the clear understanding that this may result in massive derecruitment.

Timing of Interhospital Transfer

In the escalation of ventilatory therapy, APRV usually sits just below veno-venous ECMO (VV-ECMO). In some cases, it may avoid the need for such escalation (Lim et al., 2016); however, when it is being used in a center that does not have VV-ECMO in-house, it is important for the treating team to realize that the inherent risks of transfers will be heightened if the patient is on maximal support with 100% FiO_2 and very high APRV-TCAV^{TM}

settings. Hence, the transfer should be considered when there is still some margin of safety as transferring may require coming off TCAV, which carries risks as described earlier. For those transfers we carried out, we kept the ventilator on for as much of the physical transfer to the ambulance stretcher and only switched to the transport ventilator when the whole team was ready to roll off with the patient.

CLINICAL VIGNETTES

In the following cases, we have illustrated some of these issues as well as showed the different ways in which APRV-TCAV^{TM} was used.

Case 1—First Wave

A 55-year-old woman was admitted with severe COVID pneumonia initially managed with non-invasive ventilation but, on day 2, was intubated following severe desaturation to 21% SpO_2. She was initially put on pressure-controlled ventilation (PCV) with an inspired pressure (P_{insp}) of 24 cm H_2O, PEEP of 12 cm H_2O, and an FiO_2 of 80% (**Figure 3A**). On the ensuing days, there was little improvement, and she required serial proning. She continued needing an FiO_2 over 80% and her PCV settings increased to P_{insp} of 29 cm H_2O and PEEP of 15 cm H_2O with a driving pressure of 19 cm H_2O. She developed acute kidney injury (AKI) requiring renal replacement therapy. On day 9, PCV was set to inverse ratio ventilation of 2.7:1 with a slight improvement in oxygenation. Her oxygen requirements progressively worsened, and on day 14, while proned and paralyzed on CMV with a plateau pressure of 38 cm H_2O and FiO_2 100% for a PaO_2/FiO_2 (P/F) ratio of 58, APRV-TCAV^{TM} was initiated at 34/2.5/0/0.4 (all APRV settings are referred to as $P_{High}/T_{High}/P_{Low}/T_{Low}$). In the next morning, her FiO_2 was down to 70% on 36/2.3/0/0.4 and later that day was further decreased to 55% with a P/F ratio of 100. Paralytics were discontinued. Over the next 5 days, her P/F ratio rose to 156, FiO_2 dropped to 45%, and a percutaneous tracheostomy was carried out on day 17 without complication (**Figure 3B**). There was no hemodynamic instability, and renal replacement therapy was ongoing. Following a changeover of the medical team, on day 23, while on APRV-TCAV with FiO_2 30%, a decision was made to switch to CMV. In the next few hours, her oxygen requirements rose progressively with concomitant hemodynamic instability requiring reinstitution of vasopressors. On the next day, with a P/F ratio of 83 on a PEEP of 10 cm H_2O, she would intermittently desaturate to 30–35% SpO_2. She was proned, but her SpO_2 remained in the 60's with a P/F ratio of 42. At this point, TCAV was reinstated at 42/2.4/0/0.3 in hopes to rerecruit the lungs. In the next 3 h, her SpO_2 climbed to 92%; however, her P/F ratios never rose above 100 thereafter, and she died suddenly on day 28, without any evidence of pneumothorax.

This case tragically illustrates the dangers of switching modes from APRV-TCAV^{TM} without ensuring that alveoli have had sufficient time to heal and stabilize. It also demonstrates the need for teamwork and buy-in when using a mode with which all team members may not be comfortable. It also shows how excellent gas exchange can occur due to TCAV^{TM} prior to achieving inherent alveolar stability.

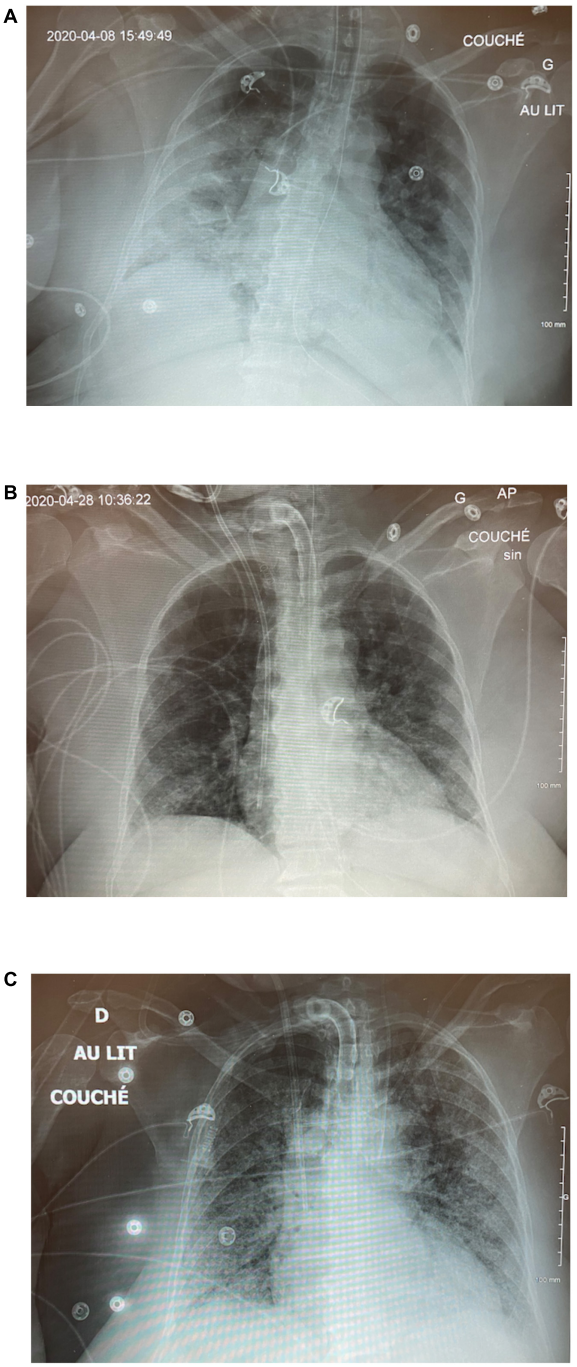

FIGURE 3 | Case 1: **(A)** CXR on ICU day 2 on PCV. **(B)** CXR on day 17 post-tracheostomy. **(C)** CXR following switch to CMV.

Case 2—Second Wave

A 32-year-old man presented to the ED with dyspnea and an SpO_2 of 60%. He was morbidly obese (150 kg) but with no other past medical history. His CXR showed severe volume loss

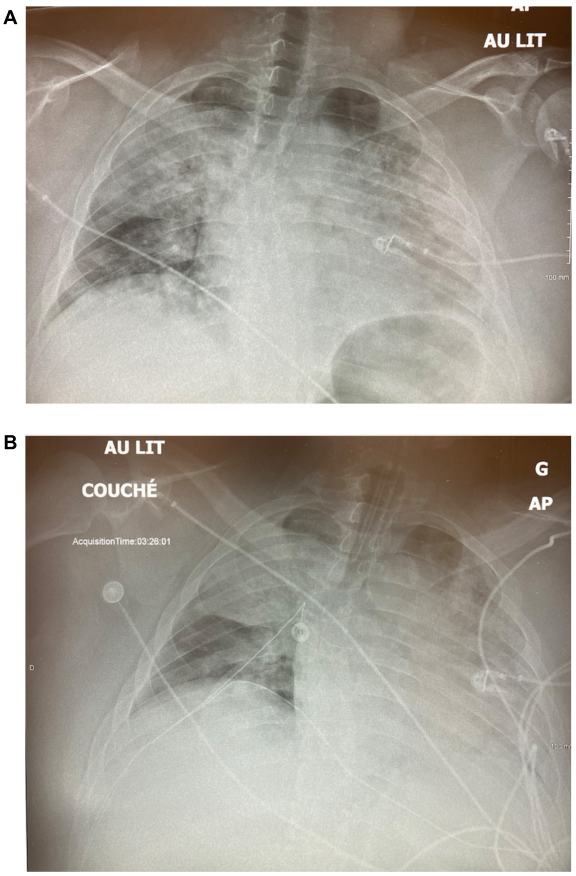

FIGURE 4 | Case 2: **(A)** Day 1 pre-intubation. **(B)** Day 1, 2 h post-intubation, on CMV.

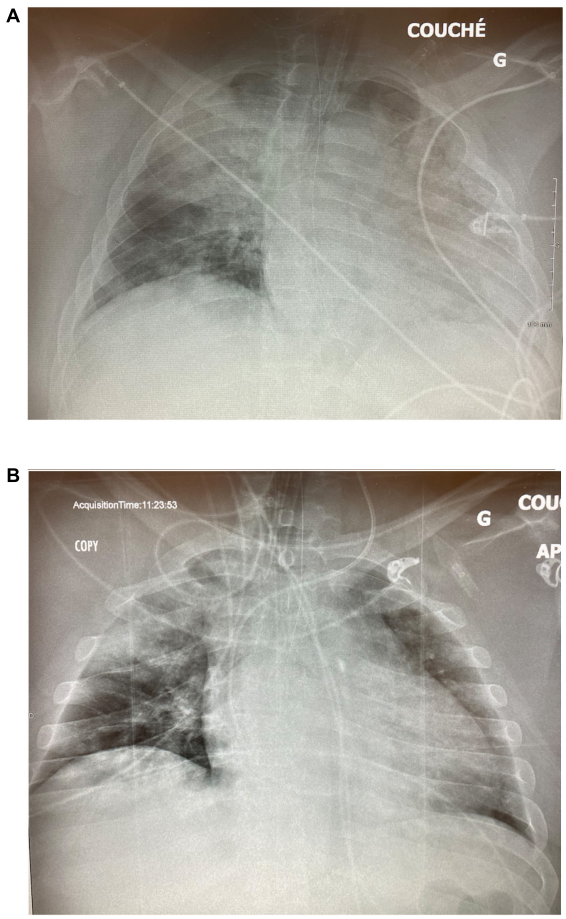

FIGURE 5 | Case 2: **(A)** Day 1, 1 h post APRV-TCAV. **(B)** Day 2, approximately 30 h after initiaition of TCAV(tm).

and bilateral infiltrates (**Figure 4**). Oxygenation only improved to 84% with high-flow nasal cannula (HFNC) and proning, and the decision was made to intubate him after he displayed respiratory fatigue. Following intubation, the patient suffered from profound desaturation to 20% with severe bradycardia to 18 bpm necessitating atropine, epinephrine, and 2 min of chest compressions. He was placed on CMV, and upon arrival of the critical care team, saturations had increased to 92% on FiO_2 of 100% and a PEEP of 14 cm H_2O, as well as an infusion of norepinephrine. With tidal volumes of 400 ml (approx 5 ml/kg ideal body weight), he had a driving pressure of 18 cm H_2O. His initial arterial blood gas (ABG) showed a pH of 7.28, a PCO_2 of 59 mmHg, and a PO_2 of 63 mmHg for a P/F ratio of 63. The CXR 2 hours post-intubation showed almost complete opacification of the left lung and significant infiltrate in the right upper lobe (**Figure 4B**). He was then put on APRV-TCAV^{TM} 35/1.8/0/0.3 and admitted to the ICU. Approximately, 1 hour after initiation of TCAV^{TM}, a second CXR showed slight recruitment of the lower left lobe with some appearance of the diaphragm (**Figure 5A**). To further augment recruitment, the P_{High} was increased by 2 cm H_2O to 37 cm H_2O. Mild hemodynamic instability persisted requiring continued norepinephrine infusion. POCUS revealed a moderately dilated right ventricle, relatively preserved left ventricular function with an ejection fraction of 50%, and a type 2 right ventricular outflow tract (RVOT) pattern indicative of mild pulmonary hypertension (López-Candales and Edelman, 2012). A pulmonary artery (PA) catheter was then inserted to monitor PA pressures, which were 35/25 cm H_2O at that time.

About 14 hours following TCAV^{TM} initiation, his FiO_2 was decreased from 100 to 35% and the P/F ratio rose to 300, while the driving pressure decreased from 18 to 11 cm H_2O. By the next morning, his CXR showed significant recruitment of the left lung (**Figure 5B**), and norepinephrine was weaned off. He developed some AKI, but given adequate cardiac output, moderately elevated CVP, and grade 1 venous excess ultrasound (VExUS) score, fluids were not deemed beneficial (Beaubien-Souligny et al., 2020). On day 5, the driving pressure had further decreased to 6 cm H_2O and the CXR showed further recruitment (**Figure 6A**). A small, probable pneumopericardium was noted and followed carefully but never required intervention. Over the next few days, his FiO_2 remained between 25 and 35% with driving pressures below 10 cm H_2O. His sedation was lightened, he was allowed to breathe more, and his AKI resolved, but his P_{High} could not be decreased below 33 cm H_2O without desaturation. On day 13, he was dropped to a P_{High} of

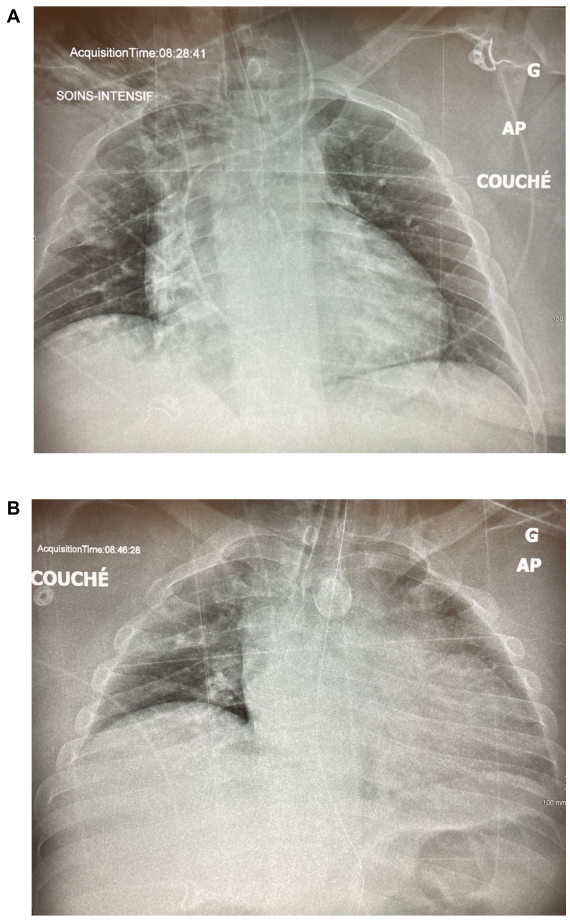

FIGURE 6 | Case 2: **(A)** ICU Day 5. **(B)** ICU Day 13.

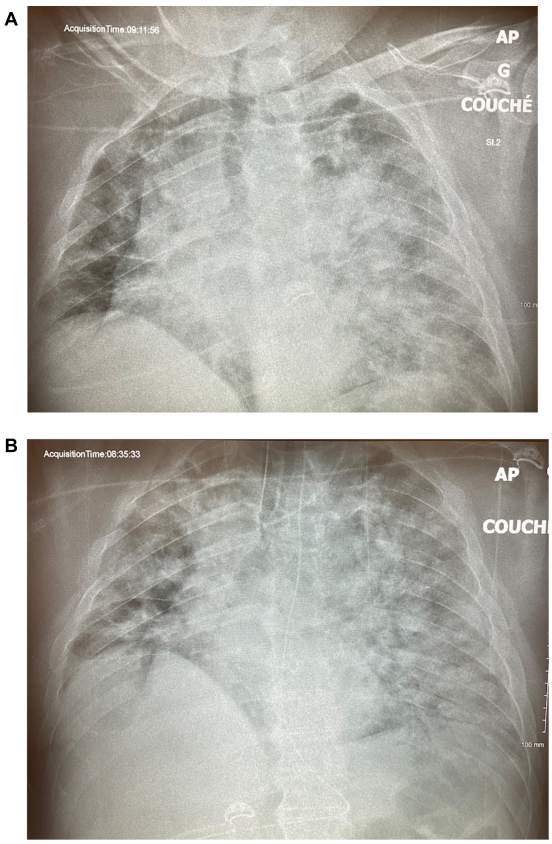

FIGURE 7 | Case 3: **(A)** CXR on Day 1 of admission to ICU, pre-intubation. **(B)** CXR on ICU Day 2, after 20 h of ventilation on PEEP 16cm/H_2O.

28 cm H_2O, but there was a clear loss of volume/derecruitment on CXR (**Figure 6B**) over 24 hours, despite PaO_2 remaining reasonable at 67 mmhg on 25% FiO_2. At this point, the P_{High} was increased to 33 cm H_2O as it was felt that alveolar stability had not been reached. Over the next few days, he remained stable and eventually tolerated dropping the P_{High}, and he finally was extubated on ICU day 18. This patient never required paralysis, nor proning, partly because of his body habitus, but also because once recruited, his P/F ratios never required it.

This case illustrates the concept of alveolar stability, which is not typically part of the daily assessment of most critically ill ventilated patients but is an inherent part of the TCAV^{TM} approach. It is likely that had the patient been rapidly weaned and extubated after his initial improvement in oxygenation, he would have required reintubation and regressed to a condition worse than after his initial intubation. His reassuring CXRs and persistent P/F ratios >200 nicely demonstrate the concept of "apparent cure" before the alveolar stability is achieved. It should also be noted that the patient had a profound drop in driving pressure, 18 to 6 cm H_2O, suggesting a significantly reduced level of mechanical power or energy delivered to the lung parenchyma (Costa et al., 2021).

Case 3—Third Wave

A 58-year-old man with obesity, hypertension, and dyslipidemia presented with a 1 week history of dyspnea and presented to a community hospital emergency room where he was put on high flow nasal cannulae and then transferred to our center for further management. On arrival, he was saturating 88% on 70% FiO_2, and his initial CXR showed extensive bilateral infiltrates and volume loss (**Figure 7A**). He developed agitation, and his saturation deteriorated to the low 80's despite increasing his FiO_2 to 100%. He was intubated and put on CMV with a tidal volume of 500 ml, respiratory rate of 26, and a PEEP of 16 cm H_2O with a resulting plateau pressure of 27 cm H_2O and a driving pressure of 11 cm H_20. Paralytics were not required. He required a norepinephrine infusion to maintain sufficient perfusion pressures. The FiO_2 was gradually reduced to 50%, but on day 2, his CXR showed no significant improvement (**Figure 7B**), and a transesophageal echocardiogram ruled out significant ventricular dysfunction and valvular disease. The decision was made to put him on APRV-TCAV^{TM} for recruitment purposes, and his initial settings were 29/1.5/0/0.4. An expiratory hold on these settings showed a driving pressure of 10. On the next day, his CXR showed significant recruitment (**Figure 8A**), and on day 4, his FiO_2 was down to 35% on settings of 29/1.5/0/0.4. An ABG showed pH of 7.48, PCO_2 of 51 mmHg, and PO_2 of 80 mmHg for a P/F ratio of 228. At this point, his norepinephrine drip

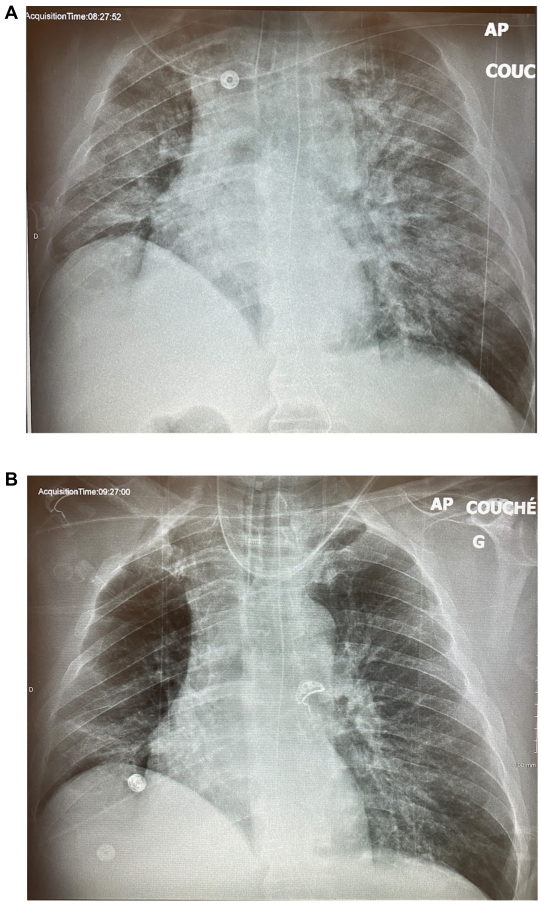

FIGURE 8 | Case 3: **(A)** CXR on ICU Day 3, after 24 h of APRV-TCAV(tm). **(B)** CXR on ICU Day 6.

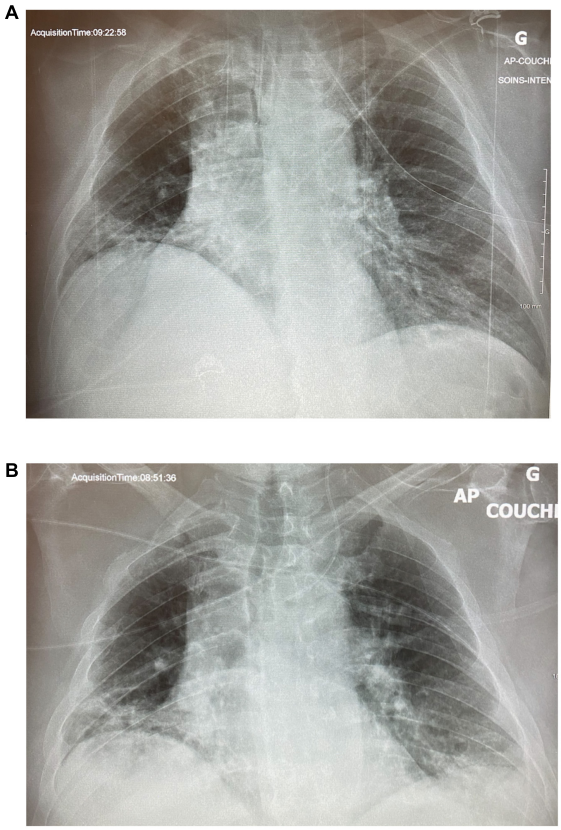

FIGURE 9 | Case 3: **(A)** ICU Day 11. **(B)** CXR on day 14, 24 h post-extubation.

had been weaned off, and the patient appeared stable. Several consultants suggested weaning, as the patient no longer met ARDS criteria; however, dropping the P_{High} transiently at the bedside resulted in significantly decreased release volumes with associated desaturations and an increase in the $EtCO_2$, suggesting derecruitment. Consequently, the treating team judged that alveolar stability had not been reached, and his settings were reverted back to their previous values. On day 10, he was successfully "dropped and stretched" to 18/10/0/0.4, which was essentially equivalent to a CPAP 18 cm H_2O. He was then extubated to HFNC and initially did reasonably well but 4 h later, desaturated and required reintubation. He was placed back on APRV-TCAV^{TM} with the setting of 28/2.5/0/0.4. On day 11, norepinephrine, which had been required post reintubation, was weaned off, and again his ventilatory settings were adjusted to 22/2.5/0/0.4, and FiO_2 was decreased to 25%. The CXR appeared well recruited (**Figure 8B**), and he was extubated on day 13 after several hours on a CPAP of 12 CmH_2O (**Figure 9A**) and subsequently remained stable (**Figure 9B**). This patient never required paralysis and subsequently did well and was eventually discharged.

This case illustrates several key elements of APRV-TCAV^{TM}. First, one can see how little recruitment happened in the first 24 h of CMV, whereas an impressive amount occurred in the first 24 h of TCAV^{TM}. Second, the time factor in alveolar stability was clear, as it took several hours before desaturation was noted, despite the patient having been on only 25% FiO_2 prior to the failed first extubation. While gas exchange was excellent, alveolar stability had not truly been achieved yet, which again illustrates the phenomenon of "apparent cure" when well-recruited patients may no longer fit the definition of ARDS but quickly decompensate with the desolation of ventilators support and pressures.

Time-Controlled Adaptive Ventilation in Clinical Practice Today

In general, different ventilatory modes are used in different phases or severity of illness. It is difficult to estimate the frequency of APRV-TCAV^{TM} use in the critical care community, but in our experience, while APRV as a mode is relatively well known, the TCAV^{TM} approach seems to be less so. However, recent studies and even meta-analyses have been published, indicating that, at least in some centers, experience with it is growing (Zhou et al., 2017; Ibarra-Estrada et al., 2021). Strategically, one may use CMV as a primary mode during acute illness and pressure support during weaning. Several modes or strategies may be used as rescues, such as inverse

ratio ventilation, high-frequency oscillation, or APRV. APRV-TCAV™ can be used as a primary or as a rescue mode, but the authors would caution against inexperienced users attempting it without experienced supervision particularly in the most fragile rescue cases.

CONCLUSION

In the second wave onward, we had several patients who had an uneventful course with APRV-TCAV™ for the vast majority of their ventilation and were successfully extubated, a few who required ECMO, and some who passed away of non-pulmonary COVID complications, such as intracerebral hemorrhage and arterial thrombosis. Our experience is heterogeneous and uncontrolled, not one from which outcome data can be inferred, although it was clear in several cases that APRV-TCAV™ was able to oxygenate and rescue patients where a traditional lung-protective strategy had failed. Challenges abound with implementing TCAV™, the most critical ones being education and team buy-in. In inexperienced hands and minds, APRV-TCAV™ certainly has more challenges than a traditional LTV/ARDSnet approach, but in the authors' experience, APRV-TCAV offers substantial physiological advantages that are worth the investment to understand and implement. What is needed is well-designed comparative trials to see if the promising cases in our experience can translate into a survival benefit if APRV-TCAV™ is used as a primary mode.

ETHICS STATEMENT

Written, informed consent was obtained from the participant/s or next of kin for the publication of any patient-related data or information.

AUTHOR CONTRIBUTIONS

PR was the clinician who managed and reported cases described. BD contributed extensive writing of the main text and review of the document. Both authors contributed to the article and approved the submitted version.

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REVIEW
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The Renin-Angiotensin System as a Component of Biotrauma in Acute Respiratory Distress Syndrome

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Acute respiratory distress syndrome (ARDS) is a major concern in critical care medicine with a high mortality of over 30%. Injury to the lungs is caused not only by underlying pathological conditions such as pneumonia, sepsis, or trauma, but also by ventilator-induced lung injury (VILI) resulting from high positive pressure levels and a high inspiratory oxygen fraction. Apart from mechanical factors that stress the lungs with a specific physical power and cause volutrauma and barotrauma, it is increasingly recognized that lung injury is further aggravated by biological mediators. The COVID-19 pandemic has led to increased interest in the role of the renin-angiotensin system (RAS) in the context of ARDS, as the RAS enzyme angiotensin-converting enzyme 2 serves as the primary cell entry receptor for severe acute respiratory syndrome (SARS) coronavirus (CoV)-2. Even before this pandemic, studies have documented the involvement of the RAS in VILI and its dysregulation in clinical ARDS. In recent years, analytical tools for RAS investigation have made major advances based on the optimized precision and detail of mass spectrometry. Given that many clinical trials with pharmacological interventions in ARDS were negative, RAS-modifying drugs may represent an interesting starting point for novel therapeutic approaches. Results from animal models have highlighted the potential of RAS-modifying drugs to prevent VILI or treat ARDS. While these drugs have beneficial pulmonary effects, the best targets and application forms for intervention still have to be determined to avoid negative effects on the circulation in clinical settings.

Keywords: acute respiratory distress syndrome, ventilator-induced lung injury, renin-angiotensin system, biotrauma, mass spectrometry

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INTRODUCTION

The renin-angiotensin system (RAS) plays a role in many cardiovascular, renal, and pulmonary processes. It is a network of peptides (**Figure 1**) that are enzymatically cleaved from the precursor protein angiotensinogen (56.8 kDa) that is mainly produced by the liver. The first step of RAS activation is the cleavage of angiotensinogen to angiotensin I (Ang I or Ang 1–10) by the protease renin, an enzyme produced by the kidney. In this context, "1–10" refers to the number of amino acid residues constituting the peptide. Ang I is subsequently cleaved to angiotensin II (Ang II or Ang 1–8) by angiotensin converting enzyme (ACE) (Skeggs et al., 1980; Corvol et al., 1995). Ang II-mediated effects are exerted through the Ang II type 1 (AT1) and 2 (AT2) receptors (Eckenstaler et al., 2021; Fatima et al., 2021). The production of Ang II by ACE is often called the "classical" activation of the RAS. Ang I and Ang II may also be enzymatically cleaved by other, "alternative" proteases including ACE2, resulting in a multitude of smaller peptides such as Ang 1–9 or Ang 1–7 (Imai et al., 2008; Santos et al., 2018). In addition to production of Ang 1–7 from Ang II by ACE2,

Ang 1–7 may be cleaved from Ang II by prolyl oligopeptidase (POP) in the circulation (Serfozo et al., 2020). Another enzyme, neprilysin, a neutral endopeptidase, cleaves Ang I to Ang 1–7 (Rice et al., 2004). The peptide Ang 1–7 in turn is a substrate for the N-domain of ACE and cleaved to the smaller peptide Ang 1–5 (Corradi et al., 2006). In a broader context, there is complex interaction between the RAS, the kinin–kallikrein system and the activity of chymase, which is expressed by mast cells and various tissues and may also cleave Ang I to Ang II (Abassi et al., 2021). Renin, ACE and AT1 receptors are important targets of anti-hypertensive and heart failure therapy (Aronson and Krum, 2012; Laurent et al., 2012). Recently, a neprilysin inhibitor/angiotensin receptor blocker combination was added to the spectrum of heart failure therapy (Gallo et al., 2021). ACE inhibitors most importantly block the production of Ang II from Ang I and therefore lead to an accumulation of Ang I and subsequently the peptide Ang 2–10 that is cleaved from Ang I by aminopeptidase A (Velez et al., 2009), and Ang 1–7 that is cleaved from Ang I by neprilysin. As ACE inhibitors also block the degradation of Ang 1–7 to Ang 1–5, this leads to a further increase in Ang 1–7 levels that is detectable in the plasma of patients treated with ACE inhibitors (Kovarik et al., 2015, 2017). Angiotensin receptor blockers lead to increased levels of Ang II (Vischer et al., 2021), whereas inhibition of renin downregulates the concentrations of all angiotensin peptides, since the production of Ang I is already inhibited. This review describes the involvement of the RAS in the biological reactions to mechanical ventilation and summarizes what is known about RAS regulation in critically ill and mechanically ventilated patients with ARDS and COVID-19 for the purpose of discovering new biomarkers and identifying new therapeutic targets.

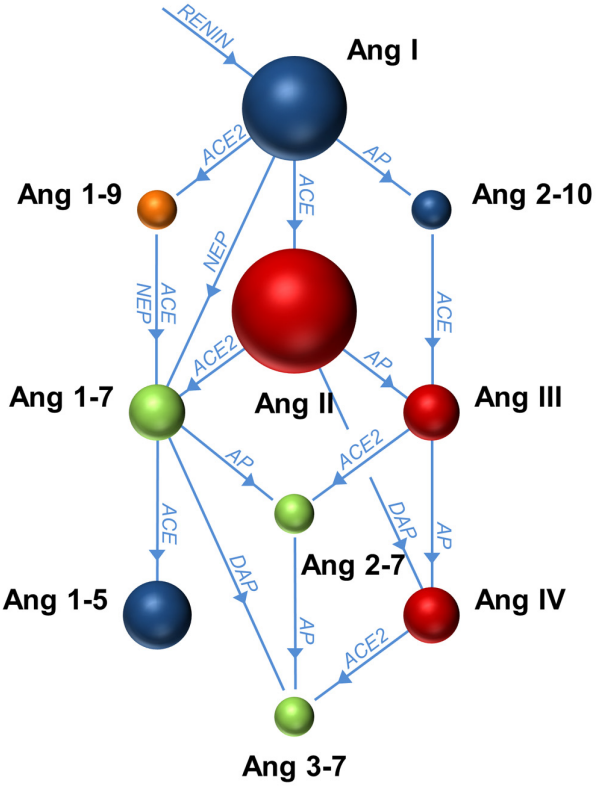

FIGURE 1 | The network of enzymes and peptides of the renin-angiotensin system in plasma. The enzymatic cascade is initiated by cleavage of angiotensinogen by renin to produce angiotensin (Ang) I. The network of enzymes that successively cleave the respective angiotensin metabolites is indicated in blue. ACE, angiotensin-converting enzyme; AP, aminopeptidase; NEP, neprilysin; DAP, diaminopeptidase.

CONCEPTS OF VENTILATOR-INDUCED LUNG INJURY

Mechanical ventilation is required in clinical conditions associated with acute pulmonary gas exchange deterioration, reduced vigilance, and loss of protective reflexes. The unphysiological state of positive pressure ventilation subsequently impacts the body further. The relevance of this impact increases with higher settings of mechanical power variables such as ventilation pressure, tidal volume and respiratory rate (Cressoni et al., 2016; Gattinoni et al., 2016). Components of ventilator-induced lung injury (VILI) include volutrauma and barotrauma, atelectrauma, and biotrauma (Gattinoni et al., 2010). Biotrauma involves upregulation of proinflammatory mediators and recruitment of neutrophils to the lungs which may augment pulmonary edema formation in VILI (Dreyfuss and Saumon, 1998). It is well established that experimental ventilation with high tidal volumes leads to increased levels of proinflammatory cytokines such as interleukin (IL)-6 and macrophage inhibitory protein (MIP)-2, a functional equivalent of IL-8 in rodents (Dreyfuss and Saumon, 1998; Halbertsma et al., 2005). This increase of proinflammatory mediators in the circulation caused by VILI may subsequently promote multiple system organ failure (Slutsky and Tremblay, 1998; Plotz et al., 2004). In a randomized controlled trial, Ranieri et al. (1999) showed that proinflammatory mediators in broncho-alveolar lavage fluid (BALF) and plasma were lower in a group of patients receiving lung-protective ventilation characterized by lower tidal volume and end-inspiratory plateau pressure as well as higher positive end-expiratory pressure (PEEP). The ARDS network study further underscored the clinical impact on ventilator-free days and survival of lung-protective ventilation with a reduced tidal volume of 6 ml/kg predicted body weight and an inspiratory plateau pressure of 30 cm of water at maximum (Acute Respiratory Distress Syndrome Network et al., 2000). At the interface of inflammatory activation and the fibroproliferative response that may occur following ARDS, scientists began to understand the relevance of Ang II as a mediator. Ang II is released from the lungs of patients suffering from ARDS (Wenz et al., 2000), and experimental data have shown its profibrotic effects in lung injury caused by bleomycin (Marshall et al., 2004). In the latter study, treatment with the ACE inhibitor ramipril or the AT1 receptor blocker losartan resulted in reduced pulmonary collagen deposition. Hypoxia as well as hyperoxia may occur in mechanically ventilated lungs – hypoxia for instance as a

consequence of hypoventilated areas attributable to the primary lung disease, hyperoxia as a consequence of the high inspiratory fraction of oxygen needed for treatment of hypoxemic patients. Hypoxia as well as hyperoxia induce collagen production in human pulmonary fibroblasts (Lang et al., 2010; Liu et al., 2013), and may be involved in the development of fibrotic changes following ARDS.

EXPERIMENTAL EVIDENCE ABOUT THE ROLE OF THE RENIN-ANGIOTENSIN SYSTEM IN LUNG INJURY

The RAS and potential sites of intervention have been studied in various experimental settings, sometimes yielding conflicting results that depend on the exact parameters of the experimental models. Ventilation with a very high tidal volume of 40 ml/kg in rats led to increased protein content in BALF, pulmonary expression and serum levels of MIP-2, and lung tissue levels of Ang II after 4 h, while mRNA expression levels of ACE2 were decreased (Jerng et al., 2007). The effects on BALF protein levels, MIP-2 and ACE2 expression were absent in a group of rats ventilated with a low tidal volume (7 ml/kg) and were reversible with the ACE inhibitor captopril. Beneficial effects on BALF protein levels in the high tidal volume group were also achieved by AT1 or AT2 receptor blockers in this study. Furthermore, studies have shown protective effects of ACE and AT1 receptor inhibition in rodent models of lung injury (Lukkarinen et al., 2005; He et al., 2007; Shen et al., 2009). In addition to increased levels of proinflammatory markers, Ang II may lead to an imbalance in the expression of epithelial sodium channel (ENaC) subunits that inhibits alveolar fluid clearance and thereby promotes pulmonary edema (Deng et al., 2012a,b). ACE2 is a natural counterregulator of classical RAS activation and the subsequent increase in Ang II levels. This homolog of ACE protects from ARDS, whereas increased ACE activity and signaling through AT1 receptors further aggravate lung injury (Imai et al., 2007). ACE2 inactivates Ang II and thus counteracts the deleterious effects of Ang II-signaling through AT1 receptors such as increased vascular tone and permeability (Imai et al., 2005, 2008). Mice with genetic inactivation of ACE were protected from acid-induced lung injury and revealed decreased Ang II levels in plasma and lung tissue, while mice with an ACE2 knockout genetic background developed more severe lung injury (Imai et al., 2005). In another study, mice infected with H7N9 influenza virus were found to have increased Ang II levels accompanied by decreased ACE2 protein expression in the lung tissue after 3 days (Yang et al., 2014). The enzymatic cleavage of Ang II by ACE2 produces Ang 1–7, which is in itself biologically active and initiates protective effects through the Mas receptor (Santos et al., 2018). Ang 1–7 has been recognized as the principal mediator of the beneficial effects of recombinant ACE2 in a murine model of Ang II-mediated myocardial fibrosis (Patel et al., 2015) and as a protective treatment against ARDS induced by bronchial acid instillation and high stretch ventilation in rats (Zambelli et al., 2015).

The role of ACE activity in VILI is somewhat controversial and may depend on the exact parameters of the model system, the biological compartments that were investigated, and the analytic methods. In mechanically ventilated rats with tidal volumes of 18 ml/kg aiming at moderate alveolar hyperdistension, lung ACE activity was significantly decreased (Behnia et al., 1996). In a more recent study, an imbalance between ACE and ACE2 activity was found in ventilated rats exposed to lipopolysaccharide (LPS), resulting in increased Ang II and reduced Ang 1–7 levels in BALF (Wosten-van Asperen et al., 2011). In this model, the proinflammatory effects were attenuated by treatment with losartan or cyclic Ang 1–7. In another study using a lung injury model induced by instillation of hydrochloric acid and increased tidal volumes of 18 ml/kg in rats, infusion of Ang 1–7 improved oxygenation and reduced inflammation in the acute setting and led to reduced pulmonary collagen deposition after 2 weeks if it was continued through osmotic minipumps (Zambelli et al., 2015). Plasminogen activator inhibitor (PAI)-1 is a biomarker of ARDS (Bhargava and Wendt, 2012) and was increased in conjunction with Ang II in a rat model of VILI (Chen et al., 2008). Systemic PAI-1 levels and VILI were attenuated by treatment with the ACE inhibitor captopril, and, interestingly, also hepatic ischemia/reperfusion-induced lung injury in rats was ameliorated by treatment with captopril (El-Sayed et al., 2020).

Age is an important factor for interpreting the results of animal lung injury models. In a systematic review, Schouten et al. (2015) noted that older animals developed more edema, a higher degree of histological pulmonary damage, and were found to have higher mortality than juvenile/adult animals in studies of VILI, pneumonia, and lung injury induced by hyperoxia or LPS. In a study in rats, the same group found higher wet/dry lung weight ratio, BALF protein content, and proinflammatory cytokine levels in older animals exposed to VILI (tidal volume 15 mL/kg) and LPS (Schouten et al., 2016). The same study also investigated ACE and ACE2 and found that treatment with LPS alone or in combination with injurious mechanical ventilation led to an age-dependent decrease in membrane-bound ACE activity in lung tissue and to an increase in soluble ACE activity in BALF. Levels of soluble ACE activity correlated well with indicators of lung injury severity. The study also discussed a link between increased levels of tumor necrosis factor-α in BALF and subsequent activation of ADAM9, one of the enzymes responsible for ACE shedding. In addition to lung injury, Ang II promotes monocyte/macrophage infiltration in other tissues, reactive oxygen species production, and ageing-related neurodegeneration (Benigni et al., 2010).

Expression levels of RAS enzymes seem to depend on partial pressure of oxygen. Experimental models in mice (FiO_2 = 12%) as well as primary murine alveolar epithelial type II cells and human small airway epithelial cells exposed to 1% oxygen have demonstrated a hypoxia-induced increase in ACE2 mRNA expression (Sturrock et al., 2021). Furthermore, a cell culture study with primary murine pulmonary endothelial cells showed even higher ACE2 mRNA expression after exposure to hyperoxia (95% oxygen) and oscillating oxygen conditions between 0 and 95% than with hypoxia (5% oxygen) (Wohlrab et al., 2021). Both

hypoxia and hyperoxia may occur in the lungs of patients with ARDS depending on regional ventilation.

CLINICAL EVIDENCE ABOUT THE ROLE OF THE RENIN-ANGIOTENSIN SYSTEM IN ACUTE RESPIRATORY DISTRESS SYNDROME

A beneficial effect of ACE inhibitor intake in patients was demonstrated for the first time by Mortensen et al. (2005) in hospitalized patients with community-acquired pneumonia. In this study, 30-day mortality was lower in patients with previous ACE inhibitor treatment. In a population-based study, the 90-day risk for hospitalization with pneumonia was also reduced in patients over 65 years of age with a new prescription of antihypertensive drugs if the prescribed drugs were ACE inhibitors of angiotensin receptor blockers (Shah et al., 2014). Another retrospective study addressed the impact of preexisting ACE inhibitor or angiotensin receptor blocker therapy during intensive care unit (ICU) admission on the course of ARDS (Kim et al., 2017). Although patients taking a RAS inhibitor required a longer duration of mechanical ventilation and a longer ICU stay, their survival was improved compared to patients who were not taking a RAS inhibitor. **Table 1** summarizes the literature on RAS-modifying drugs and risk and outcomes of pneumonia, ARDS and radiation pneumonitis, RAS activation in critical illness, ARDS and COVID-19, and randomized controlled clinical trials targeting the RAS in these conditions.

Actions of Ang II in ARDS are proinflammatory, profibrotic (Hrenak and Simko, 2020) and involved in regulation of alveolar fluid clearance in the lung (Deng et al., 2012a,b). It can therefore be speculated that RAS activation may be a relevant parameter in the pathogenesis and course of ARDS. Biomarker phenotyping of patients with ARDS has yielded at least two classes, including a "reactive" phenotype with higher proinflammatory activation associated with higher mortality (Calfee et al., 2014, 2015; Bos et al., 2017). Considering the association of increases in Ang II with proinflammatory activation in experimental lung injury (Jerng et al., 2007), it would be interesting to explore whether this phenotype is associated with higher RAS activation as well.

So far, few studies have systematically examined RAS activation in mechanically ventilated ICU patients. One study measured the plasma renin levels of 20 critically ill patients at multiple time points, and found an inverse correlation with urine output and mean arterial blood pressure (Gleeson et al., 2019). As a marker of tissue perfusion, renin outperformed lactate levels in prediction of prognosis in this study. Nearly two thirds of the included patients were in septic, hemorrhagic, or cardiogenic shock, but only two patients had a diagnosis of pneumonia, so that no conclusions about renin levels in ARDS can be drawn from this study. The angiotensin metabolite profile in mechanically ventilated patients with ARDS was investigated by Reddy et al. (2019) with mass spectrometry in protease-inhibited samples, and by Krenn et al. (2020) with RAS equilibrium analysis without protease inhibition. An analysis of plasma from blood samples that are immediately stabilized with a protease inhibitor cocktail yields the circulating concentrations of angiotensin metabolites. In contrast, RAS equilibrium analysis uses plasma or serum without protease inhibition. Before measurement with liquid chromatography tandem mass spectrometry (LC-MS/MS), the samples are incubated at 37°C to establish an equilibrium between production and cleavage of angiotensin metabolites. In serum or plasma, this is feasible due to the high molar surplus of angiotensinogen to renin that is physiologically present and the constant formation of the initial substrate Ang I of the RAS cascade during the 37°C incubation step. Nascent Ang I is immediately converted into downstream metabolites by soluble enzymes so that new equilibrium levels emerge. These levels arise from equal enzymatic formation and degradation rates of individual angiotensin metabolites in the sample and depend on all enzymes involved in the plasma angiotensin metabolism, so that ratios between product and precursor may be used as angiotensin-based markers of enzyme activities (Burrello et al., 2020; Zoufaly et al., 2020). Furthermore, it has been shown that the sum of Ang I and Ang II (PRA-S) is highly correlated with plasma renin activity (Goppner et al., 2019; Burrello et al., 2020; Krenn et al., 2020). A summary of angiotensin-based markers of RAS enzyme activities is presented in **Figure 2**. Overall, angiotensin metabolite concentrations in protease-inhibited samples show a strong correlation with their equilibrium levels (Basu et al., 2017). In the study by Reddy et al. (2019) Ang I (1–10) levels were higher in non-survivors than in survivors of ARDS, and the Ang II/Ang I ratio was lower in non-survivors than in survivors in both studies (Krenn et al., 2020). In comparison to postoperative patients, Ang I, Ang II, and Ang 1–7 plasma concentrations were increased in early ARDS, and the Ang II/Ang I ratio was inverted (Krenn et al., 2020). **Figure 3** illustrates the main changes in the plasmatic angiotensin metabolite profile associated with ARDS. These results pointed to endogenous ACE inhibition, as none of the patients with early ARDS were receiving RAS-blocking drugs, and active ACE concentrations measured in a mass spectrometry-based assay and ACE protein levels measured by ELISA were not changed. After 7 days of mechanical ventilation, RAS activation indicated by PRA-S as a marker of plasma renin activity correlated with driving pressure (Krenn et al., 2020), indicating that improvement of the respiratory situation with decreased driving pressure was associated with a larger decrease in RAS activation. The relatively low Ang II levels in contrast to Ang I concentrations may have been attributable to the following causes: Part of Ang II was metabolized to Ang 1–7 by ACE2, as our ACE2 inhibition experiments suggested (Krenn et al., 2020), but other proteases may have played a role as well. Annoni et al. (2019) actually found increased ACE protein levels in mechanically ventilated patients with early ARDS who did not survive, while ACE2 protein levels had not changed. This is at least further proof that the reduced Ang II/Ang I ratios in ARDS are more likely attributable to endogenous ACE inhibition, increased metabolization of Ang II by ACE2, or other, yet unknown, proteases than to reduced ACE expression. However, using another brand of ACE and ACE2 ELISAs, Gerard et al. (2021) found decreased ACE and increased ACE2 protein

TABLE 1 | Clinical data about effects of RAS-modifying drugs on outcome, characterization of RAS activation, and randomized clinical trials with RAS-modifying drugs in pneumonia, critical illness, ARDS, COVID-19 and radiation pneumonitis.

Authors and year	Type of study	Collective/cohort/eligibility for inclusion	No. of participants	Treatment groups/main outcomes
RAS modifying drugs and outcome				
Mortensen et al., 2005	Retrospective cohort study	Patients hospitalized with community acquired pneumonia	*n* = 787	ACEi therapy/30-day mortality ↓
Caldeira et al., 2012	Systematic review and meta-analysis	Studies including patients taking ACEi or ARB and analyzing risk and mortality of pneumonia	29 studies	ACEi or ARB therapy/Incidence of pneumonia ↓ with ACEi Pneumonia related mortality ↓ with ACEi and ARB
Shah et al., 2014	Population based study	New prescription of antihypertensive drugs in patients >65 years	*n* = 254.485	Drugs: ACEi, ARB, calcium channel blockers, beta blockers and thiazide diuretics/90-day risk of hospitalization with pneumonia ↓ with ACEi and ARB
Kim et al., 2017	Retrospective case control study	Patients admitted to the ICU with ARDS	*n* = 182	ACEi or ARB therapy/With RAS inhibitor: ICU mortality ↓ Duration of mechanical ventilation ↑ Length of ICU stay↑
Sun et al., 2018	Meta-analysis	Lung cancer patients with radiation therapy	*n* = 1.412	ACEi and ARB therapy/Incidence of symptomatic radiation pneumonitis ↓ with ACEi
RAS activation in critical illness and ARDS				
Gleeson et al., 2019	Prospective observational study	Patients admitted to the ICU	*n* = 20	Renin in plasma
Reddy et al., 2019	Pilot study, observational study	ICU patients with ARDS	*n* = 39	Angiotensin metabolite profile in plasma, protease inhibited
Annoni et al., 2019	Observational study	Mechanically ventilated ICU patients with ARDS	*n* = 96	ACE and ACE2 protein levels in plasma
Krenn et al., 2020	Observational study	Mechanically ventilated ICU patients with ARDS	*n* = 27	Angiotensin metabolite profile in RAS equilibrium analysis, active ACE levels, ACE and ACE2 protein levels in plasma
Gerard et al., 2021	Retrospective study	Patients with COVID-19 related/non-COVID ARDS	Tissue: COVID *n* = 15/non-COVID *n* = 13, Serum: COVID *n* = 35/non-COVID *n* = 24	ACE and ACE2 protein expression in lung tissue Ang II, Ang 1–7, ACE and ACE2 protein levels in serum
RAS activation in COVID-19				
Ozkan et al., 2021	Observational study	Hospitalized patients with COVID-19 and signs of pneumonia	*n* = 112	Ang II levels in serum
Kutz et al., 2021	Observational study with matched controls	Patients with COVID-19 admitted to the hospital and SARS-CoV-2 negative propensity-score matched controls	*n* = 43 in both groups	Angiotensin metabolite profile in RAS equilibrium analysis, angiotensin concentration-based markers of renin, ACE and ACE2 activities
Files et al., 2021	Observational study	Patients with moderate to severe acute respiratory failure due to COVID-19 and moderate acute respiratory failure negative for SARS-CoV-2	COVID-19: *n* = 22, SARS-COV-2 negative patients: *n* = 11	Ang II and Ang 1–7 plasma levels and ACE, ACE2 and POP activities in serum in fluorescent assays
Eleuteri et al., 2021	Observational study	Patients with COVID-19 related ARDS admitted to the ICU	*n* = 32	Renin, Ang I, Ang II, Ang 1–7 serum levels, Ang I/Ang II ratio as indicator of ACE activity
Reindl-Schwaighofer et al., 2021	Observational study	Patients hospitalized with COVID-19, and critically ill patients with influenza pneumonia	COVID-19: *n* = 126 Influenza: *n* = 27	Equilibrium plasma levels of Ang II, Ang 1–7 and active ACE2 levels
Wang et al., 2021	Observational study	Patients hospitalized with COVID-19	*n* = 242	Circulating Ang I, Ang II, Ang 1–7 and ACE2 levels
Osman et al., 2021	Observational study	Patients hospitalized with COVID-19 and healthy SARS-CoV-2 negative controls	COVID-19: *n* = 44 (16.6% male) Controls: *n* = 15 (46.7% male)	Ang I, Ang II, Ang 1–7 and ACE2 plasma levels, ACE2 mRNA expression and ACE2 and specific biomarker membrane protein expression in flow cytometry in peripheral blood mononuclear cells

(Continued)

TABLE 1 | (Continued)

Authors and year	Type of study	Collective/cohort/eligibility for inclusion	No. of participants	Treatment groups/main outcomes
RAS modifying drugs in randomized controlled clinical trials				
Boldt et al., 1995	RCT	Critically ill patients	*n* = 45	Enalapril/Hemodynamic and respiratory parameters
Khan et al., 2017	RCT	Patients with ARDS	*n* = 46	rhACE2/PaO_2/FiO_2 ratio, biomarkers, SOFA score
Small et al., 2018	RCT	Lung cancer patients with radiation therapy	*n* = 33	Captopril/Incidence of symptomatic radiation pneumonitis ↓
Sio et al., 2019	RCT	Lung cancer patients with chemo-radiation therapy	*n* = 23	Lisinopril/Incidence of chemo-radiation induced pulmonary distress ↓

ACE, angiotensin converting enzyme; ACEi, ACE inhibitor; Ang, angiotensin; ARB, angiotensin receptor blocker; ARDS, acute respiratory distress syndrome; ICU, intensive care unit; RAS, Renin-angiotensin system; RCT, randomized controlled trial; rhACE2, recombinant human ACE2; POP, prolyl oligopeptidase, SOFA score, sequential organ failure assessment score.

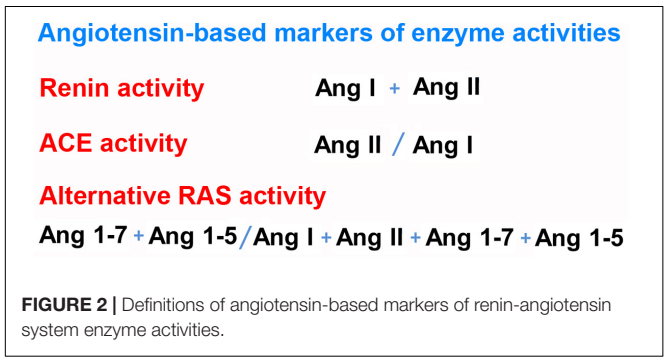

FIGURE 2 | Definitions of angiotensin-based markers of renin-angiotensin system enzyme activities.

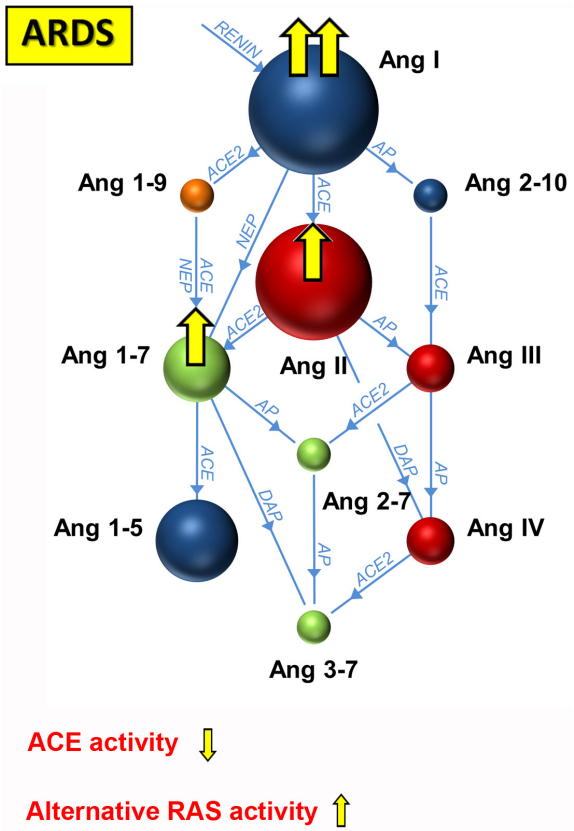

FIGURE 3 | Changes in the angiotensin metabolite profile in plasma of patients with ARDS. Studies in patients with ARDS revealed increased renin-angiotensin system (RAS) activation with higher Ang I than Ang II concentrations and increased Ang 1–7 levels. Decreased Ang II/Ang I ratios and increased alternative RAS activation may be caused be decreased ACE and increased ACE2 activities, or other, yet-to-be characterized, enzymes that process Ang II.

levels in the serum of patients with COVID-19-related and non-COVID ARDS. These seemingly conflicting results may be due to rather small sample sizes, a mix of underlying pathologies and biomarker phenotypes in patients with non-COVID ARDS and different specificities of ELISA kits.

Novel methods to measure active ACE and ACE2 concentrations offer new opportunities to study the role of these enzymes in a clinical context. These assays work by spiking the natural substrates to the samples to determine the specific product formation rate. For analysis of ACE activity, the samples are spiked with Ang I and incubated in the presence and absence of an ACE inhibitor, while the unspecific degradation of the substrate (Ang I) and product (Ang II) is prevented with protease inhibitors. Ang II is quantified by LC-MS/MS and the specific activity of ACE is calculated by determining the inhibitor-sensitive fraction of Ang II formation. The active ACE concentration is then calculated by relating the ACE activity in the sample to the activity of recombinant human ACE in plasma. The assay to determine active ACE2 follows an equivalent process, namely spiking Ang II, application of the ACE2-specific inhibitor MLN-4760, and quantification of Ang 1–7. The Ang 1–7 formation rate is calibrated to a standard curve of ACE2 from healthy human control plasma. With the help of this method, it was discovered that high concentrations of active ACE2 in the plasma of hospitalized patients with COVID-19 depend on disease severity (Reindl-Schwaighofer et al., 2021). Increased ACE2 protein expression in the lung tissue has recently been shown in patients with ARDS caused by COVID-19 as well as ARDS caused by other reasons and was primarily located in endothelial cells (Gerard et al., 2021). In this

study, the pulmonary ACE protein expression was diminished in COVID-19-related and non-COVID ARDS. The same tendency of changes with increased ACE2 and decreased ACE protein levels, paired with increased Ang 1–7 levels, were found in the serum of patients with COVID-19-related and non-COVID ARDS. This is actually the opposite to findings of decreased ACE2 expression in rodent models of lung injury (Jerng et al., 2007; Wosten-van Asperen et al., 2011), but regulation of ACE2 expression appears to differ among species (Sajuthi et al., 2020; Ziegler et al., 2020). Another difference may be the prolonged course of COVID-19 and ARDS in critically ill patients, which may be accompanied by a dysregulated interferon response as a stimulus for ACE2 expression, as opposed to the relatively short duration of experiments modeling ARDS in animals (Gerard et al., 2021).

THE RENIN-ANGIOTENSIN SYSTEM IN COVID-19

In light of the COVID-19 pandemic, interest in the function of the RAS in the context of ARDS has grown, as ACE2 serves as the primary cell entry receptor for SARS-CoV-2 (Walls et al., 2020; Yan et al., 2020; Zhang et al., 2020). Increased plasma levels of Ang II were reported in a small group of patients with COVID-19 in the early phase of scientific description of COVID-19 (Liu et al., 2020). In patients with H7N9 avian influenza, higher Ang II plasma levels predicted a fatal outcome (Huang et al., 2014), and in a mouse study on H7N9 infection, Ang II levels were increased and lung tissue protein expression of ACE2 was decreased after 3 days (Yang et al., 2014). Based on cell culture and animal data on SARS-CoV and SARS-CoV-2 infection, and human data of ACE2 levels in conditions with increased susceptibility to severe COVID-19, multiple reviews arrived at the conclusion that Ang II levels should be increased and ACE2 should by downregulated by SARS-CoV-2 infection (Edmonston et al., 2020; Verdecchia et al., 2020; Iwasaki et al., 2021; Triposkiadis et al., 2021). Risk factors for mortality in patients with COVID-19 include older age, male gender, hypertension, type 2 diabetes mellitus, cardiovascular disease, chronic obstructive pulmonary disease, chronic kidney disease and asthma (Aktar et al., 2021). Obesity and type 2 diabetes, especially with poor glycemic control, lead to immune cell dysfunction and foster chronic inflammatory states (Fishkin et al., 2021; Rea and Alexander, 2021). It has also been reported that ACE2 expression diminishes in advanced age and in individuals with hypertension, cardiac hypertrophy, and heart failure (Rea and Alexander, 2021). Lower ACE2 serum levels were described in patients with type 2 diabetes despite increased ACE2 expression in the pancreas and lungs as well as correlation of ACE2 serum levels with HbA1c (Elemam et al., 2021). Rising soluble ACE2 levels correlated with worsening symptoms, B-type natriuretic peptide levels and mortality in patients with heart failure (Garcia-Escobar et al., 2021), and the sum of Ang 1–7 and Ang 1–5 concentrations as marker of alternative RAS activation predicted adverse events in patients with heart failure and preserved ejection fraction (Binder et al., 2019). In a large cohort of 497 patients from the Atherosclerosis Risk in Communities Study elevated soluble ACE2 levels were associated with increased cardiac biomarkers, left ventricular hypertrophy, as well as risk for hospitalization because of heart failure, risk for cardiovascular disease events and death (Hussain et al., 2021). A similar link between increased ACE2 plasma levels and male sex as well as biomarkers of ageing, cardiovascular disease and diabetes was established in even larger cohorts of elderly patients with atrial fibrillation (Wallentin et al., 2020). Although many patients with cardiovascular diseases are treated with ACE inhibitors or angiotensin receptor blockers, no negative impact of these drugs on the risk for severe COVID-19 was found (Hippisley-Cox et al., 2020). Therefore, these drugs should only be discontinued in COVID-19 patients with hemodynamic compromise (Alexandre et al., 2020). Increased expression of ACE2 and transmembrane serine protease (TMPRSS) 2 in lung tissue together with decreased soluble ACE2 levels were also observed in patients with chronic obstructive pulmonary disease (Fliesser et al., 2021). While several patient characteristics and conditions are associated with reduced soluble ACE levels, worsening of comorbidities such as heart failure or diabetes leads to rising soluble ACE2 levels. However, there is still controversy whether increased or decreased soluble ACE2 levels are indeed a susceptibility factor for severe COVID-19 (Leow, 2020; Rahman et al., 2021).

At first glance, results from studies measuring circulating angiotensin peptides and soluble ACE2 in patients with COVID-19 do not completely fit this theoretical model of increased Ang II and downregulated ACE2 levels. A study of Ang II serum levels in a cohort of 112 patients with COVID-19 reported a decrease in Ang II levels that was more pronounced in patients with ARDS and in non-survivors (Ozkan et al., 2021). Another study reported lower Ang II equilibrium levels in hospitalized patients with COVID-19 than in propensity score matched controls negative for SARS-CoV-2 (Kutz et al., 2021). ACE and ACE2 activities were unchanged in patients with COVID-19 in this study. Other studies showed slightly decreased ACE activity in the blood of patients with moderate to severe acute respiratory failure due to COVID-19 (Files et al., 2021) and higher Ang II levels in survivors than in non-survivors of COVID-related ARDS (Eleuteri et al., 2021). This is in line with the results from a study on severe sepsis that indicated low levels of Ang II and ACE on day 1 as predictors of mortality (Zhang et al., 2014). As discussed above for non-COVID ARDS, low Ang II plasma levels, especially if they are lower than the Ang I levels, may be caused by reduced ACE activity or by increased processing of Ang II into Ang 1–7 by ACE2 (Krenn et al., 2020) or POP (Triposkiadis et al., 2021). Increased ACE2 levels in the blood of patients with COVID-19 have already been described by several studies using ELISA as well as mass spectrometry-based assays (Fagyas et al., 2021; Gerard et al., 2021; Lundstrom et al., 2021; Patel et al., 2021; Reindl-Schwaighofer et al., 2021; Wang et al., 2021)., and were higher in more severely ill patients (Fagyas et al., 2021; Patel et al., 2021; Reindl-Schwaighofer et al., 2021). An increasing trend in ACE2 plasma levels within 7 days from hospital admission indicated a higher 90-day mortality (Wang et al., 2021). Differences in soluble ACE2 levels between sexes may also play a role, as a study

involving only 16.6% men within the COVID-19 group found actually lower ACE2 and higher Ang I and Ang II plasma levels in patients with COVID-19 compared to SARS-CoV-2 negative controls (Osman et al., 2021). One explanation may be that ACE2 was shed from the lung tissue after infection with SARS-CoV-2 and therefore increasingly appeared in the circulation. However, the study by Gerard et al. (2021) showed that ACE2 protein expression in lung tissue was increased in patients who died from COVID-19-related ARDS, and that the pulmonary ACE2 expression was primarily localized to endothelial cells, while the number of alveolar type II cells was reduced. Whether the lung is indeed the source of circulating ACE2 is uncertain, but interestingly, the soluble ACE2 species found in the plasma of patients with COVID-19 had specific characteristics: There was less full-length ACE2, while the 70 kD species was increased (Garcia-Ayllon et al., 2021). It remains unknown whether this has functional implications for the pulmonary endothelium. Overall, the clinical findings at present contest the hypothesis of increased Ang II and decreased ACE2 in COVID-19 as a systemic phenomenon, while these changes might well play a role locally within the lung tissue (Gerard et al., 2021; Iwasaki et al., 2021). Furthermore, the above-mentioned clinical studies are difficult to compare to each other due to several limitations. The investigated patient cohorts differed in the severity of disease, grading of ventilatory support, and time points of sampling, so that differences may have been missed, for instance between patients requiring non-invasive versus invasive mechanical ventilation. The delay from the first positive test or onset of symptoms to sampling may also vary within and between studies. The studies offering information on circulating angiotensin metabolite concentrations in patients with COVID-19 are included in **Table 1**. In summary, RAS activation in severe COVID-19 may be the result of a variety of changes caused by COVID-19, ARDS, and critical illness with hemodynamic instability and acute kidney injury (Zarbock et al., 2021).

Angiotensin converting enzyme polymorphisms contribute to the relatively high standard deviation of Ang II plasma levels in patient cohorts and are associated with the severity of ARDS (Pabalan et al., 2021). From the beginning of the SARS-CoV-2 pandemic it has been obvious, that disease severity varies greatly between patients spanning a spectrum from no symptoms at all to critical illness with high mortality. A likely explanation for this phenomenon may be yet unknown genetic factors that predispose patients for one or the other outcome. Possible contributors may include the known polymorphisms in the genes of the RAS enzymes ACE and ACE2. The ACE gene is located on chromosome 17q23 and exhibits an insertion/deletion (I/D) polymorphism of a 287-base pair Alu repeat sequence in intron 16, giving rise to II and DD homozygotes, respectively, and ID heterozygotes. Different genotypes vary in the expression levels and plasma activities of ACE (Rigat et al., 1990; Gard, 2010), which can also influence the responsiveness to therapeutic ACE inhibitors (Haas et al., 1998). Several studies have shown considerable association between ACE genotype (as observed with variable prevalence in different ethnic populations) and various disease endpoints such as sepsis, ARDS (Pabalan et al., 2021), and risk of pneumonia (Nie et al., 2014). In the context of COVID-19, there may also be a connection between frequency of ACE genotypes in populations and severity and outcome of this disease (El-Arif et al., 2021). Furthermore, single-nucleotide polymorphisms in the ACE2 gene on chromosome Xp22.2 have been discussed as possible predetermining factors for COVID-19 severity. The best-characterized ACE2 polymorphism is the splice region variant (rs2285666, G > A, Intron 3/4), which has also been shown to be associated with hypertension, coronary heart disease and diabetes with cerebral stroke (Mohlendick et al., 2021). Other ACE2 polymorphisms may affect ACE2-spike protein binding affinity or binding of the co-factor TMPRSS2 that is needed for viral cell entry (El-Arif et al., 2021; Suryamohan et al., 2021). In a study including hospitalized patients with COVID-19 age, high soluble ACE2 levels, a low aldosterone to renin ratio and the TMPRSS2 rs2070788 non-AA genotype were factors that independently predicted disease severity (Akin et al., 2021). Allelic variants of ACE2 differ in serum levels of soluble ACE2, which also implies a possible altered susceptibility to SARS-CoV-2 infection (Mohlendick et al., 2021).

POTENTIAL FOR CLINICAL APPLICATION

An early randomized controlled trial on the hemodynamic and respiratory effects of enalapril in 45 critically ill patients was published in 1995 (Boldt et al., 1995). While enalapril dose-dependently decreased mean arterial pressure, the cardiac index and PaO_2/FiO_2 ratio were higher in patients treated with enalapril than in the control group.

After a successful phase I study of pharmacokinetics and pharmacodynamics of recombinant human (rh)ACE2 in healthy volunteers (Haschke et al., 2013), a clinical trial in patients with ARDS was terminated prematurely because the predefined effects on outcome were not achieved (Khan et al., 2017). In the phase I study, intravenous treatment with ACE2 decreased Ang II levels within 30 min of infusion. Ang 1–7 levels increased, decreased, or remained unchanged, and Ang 1–5 levels increased after all investigated doses of ACE2. Interestingly, the cardiovascular effects of ACE2 administration were absent in healthy individuals. In the phase II study, Ang II levels decreased within 12 h of rhACE2 (GSK2586881) infusion, whereas no change was observed in the placebo group. At the same time, Ang 1–7 and Ang 1–5 levels were increased in the treatment group. As in the phase I study, there were no episodes of hypotension associated with study treatment in the phase II trial. rhACE2 has also been applied in an international multicenter randomized controlled trial in hospitalized patients with COVID-19 (registered at clinicaltrials.gov as NCT04335136), but results are yet to be published. A case report on rhACE2 treatment in a patients with severe COVID-19 confirmed the intended effect of a reduction in Ang II levels during twice daily intravenous treatment with rhACE2 for 7 days (Zoufaly et al., 2020).

There is evidence that ACE inhibitors protect against radiation pneumonitis in lung cancer patients (**Table 1**). This includes

two small randomized controlled trials (Small et al., 2018; Sio et al., 2019) and several studies analyzing the incidence of radiation pneumonitis in patients with or without ACE inhibitor or angiotensin receptor blocker therapy (Sun et al., 2018).

CURRENT RESEARCH GAPS AND PERSPECTIVES

Experimental and clinical evidence shows that the RAS is involved in VILI and other types of pulmonary inflammation where it may offer novel therapeutic targets. Despite the increasing precision and detail of describing RAS activation with mass spectrometry-based assays, many aspects of RAS enzyme activity remain elusive. ACE inhibitors, angiotensin receptor blockers and Ang 1–7 supplementation seem to protect from VILI in experimental studies in rodents, not only by affecting levels of RAS components but also by acting on proinflammatory cytokines such as IL-6 and MIP-2, and there is evidence of decreased histological lung injury (Jerng et al., 2007; Wosten-van Asperen et al., 2011; Zambelli et al., 2015). However, clinical data suggest that decreased ACE activity indicated by a low Ang II/Ang I ratio in early ARDS in mechanically ventilated patients is a poor prognostic sign, and that this decreased Ang II/Ang I ratio likely does not come from reduced circulating ACE protein levels. One may speculate that this is an endogenous protective mechanism in patients with ARDS to limit generation of potentially harmful Ang II that is not sufficient for improvement in non-survivors. However, hypoxia only causes death in a minority of patients with ARDS (Esan et al., 2010). Thus, non-survivors of ICU-stays also include patients with multi-organ failure or acute complications such as intracranial bleeding or myocardial infarction. Reasons for the diminished Ang II/Ang I ratio in ARDS may include the presence of a yet-to-be characterized endogenous ACE inhibitor, increased ACE2 activity (Krenn et al., 2020; Reindl-Schwaighofer et al., 2021), or increased activity of other, yet-to-be characterized proteases that process Ang II. Future mechanistic investigations will have to study which molecular mechanisms are really involved. In addition, the activity levels and concentrations of ACE and ACE2 in plasma are regulated by shedding of parts of the molecules from their cells of origin in various organs. This may be the primary reason why ACE2 increases in the systemic circulation in certain disease states. The mechanisms that lead to ACE2-shedding involve proteases, e.g., tumor necrosis factor-α convertase (TACE, ADAM17) (Lambert et al., 2005). Interestingly, ADAM17 activation may be enhanced by Ang II acting on AT 1 receptors and by bradykinin (Dey et al., 2010; Rahman et al., 2021), while ACE is the most important enzyme for inactivation of bradykinin (Schmaier, 2002). Based on this mechanism, ACE inhibition may also be involved in increased shedding of ACE2. Novel assays for calculating active ACE and ACE2 concentrations will help to further study the impact of these enzymes in ARDS, but a future goal will also be to further clarify from where the enzymes are shed and by which mechanisms.

Another broad field for future study is applying the findings about involvement of the RAS in ARDS in randomized controlled clinical trials. Various trials currently listed in *clinicaltrials.gov* aim to include patients with COVID-19 and to test a treatment with angiotensin receptor blockers, ACE inhibitors, or Ang 1–7. Apart from selecting the most suitable substances, the challenge is to establish the most effective form of application. Since the lungs can be reached by circulation, systemic application of RAS-modifying drugs has a substantial impact on angiotensin metabolite concentrations in plasma (Kovarik et al., 2015; Khan et al., 2017). Inhalation therapy is another option for treating ARDS. Depending on the molecule, this method may offer the benefit of positive local effects on the bronchial/alveolar epithelium without systemic toxicity. As another advantage, inhalation of RAS-modifying drugs can be expected to have fewer effects on systemic hemodynamics. As an example, inhaled Ang 1–7 has already been studied as an anti-inflammatory therapeutic agent in a mouse model of ovalbumin-induced chronic asthma (Magalhaes et al., 2020).

CONCLUSION

In summary, knowledge about RAS activation in experimental lung injury and clinical ARDS is increasing in quantity and detail. The mechanisms of regulating RAS enzyme activities and their shedding remain elusive, especially in the clinical setting, and require further study with innovative measurement tools. However, even with the current conception of RAS activation in ARDS, clinical studies can be designed to counteract primary and ventilator-induced lung injury. Several clinical trials with RAS-modifying drugs are currently underway for COVID-19, with most results still pending.

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KK drafted the manuscript and prepared the figures. VT, FK, and RU assisted with literature search and revised the manuscript. FK drafted **Table 1**. All authors read and approved the submitted version of the manuscript.

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Lung-Protective Ventilation Attenuates Mechanical Injury While Hypercapnia Attenuates Biological Injury in a Rat Model of Ventilator-Associated Lung Injury

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Ismaiel N, Whynot S, Geldenhuys L, Xu Z, Slutsky AS, Chappe V and Henzler D (2022) Lung-Protective Ventilation Attenuates Mechanical Injury While Hypercapnia Attenuates Biological Injury in a Rat Model of Ventilator-Associated Lung Injury. Front. Physiol. 13:814968. doi: 10.3389/fphys.2022.814968

Background and Objective: Lung-protective mechanical ventilation is known to attenuate ventilator-associated lung injury (VALI), but often at the expense of hypoventilation and hypercapnia. It remains unclear whether the main mechanism by which VALI is attenuated is a product of limiting mechanical forces to the lung during ventilation, or a direct biological effect of hypercapnia.

Methods: Acute lung injury (ALI) was induced in 60 anesthetized rats by the instillation of 1.25 M HCl into the lungs via tracheostomy. Ten rats each were randomly assigned to one of six experimental groups and ventilated for 4 h with: 1) **Conventional HighV_E Normocapnia** (high V_T, high minute ventilation, normocapnia), 2) **Conventional Normocapnia** (high V_T, normocapnia), 3) **Protective Normocapnia** (V_T 8 ml/kg, high RR), 4) **Conventional iCO_2 Hypercapnia** (high V_T, low RR, inhaled CO_2), 5) **Protective iCO_2 Hypercapnia** (V_T 8 ml/kg, high RR, added CO_2), 6) **Protective endogenous Hypercapnia** (V_T 8 ml/kg, low RR). Blood gasses, broncho-alveolar lavage fluid (BALF), and tissue specimens were collected and analyzed for histologic and biologic lung injury assessment.

Results: Mild ALI was achieved in all groups characterized by a decreased mean PaO_2/FiO_2 ratio from 428 to 242 mmHg ($p < 0.05$), and an increased mean elastance from 2.46 to 4.32 cmH_2O/L ($p < 0.0001$). There were no differences in gas exchange among groups. Wet-to-dry ratios and formation of hyaline membranes were significantly lower in low V_T groups compared to conventional tidal volumes. Hypercapnia reduced diffuse alveolar damage and IL-6 levels in the BALF, which was also true when CO_2 was added to conventional V_T. In low V_T groups, hypercapnia did not induce any further protective effect except increasing pulmonary IL-10 in the BALF. No differences in lung injury were observed when hypercapnia was induced by adding CO_2 or decreasing minute

ventilation, although permissive hypercapnia decreased the pH significantly and decreased liver histologic injury.

Conclusion: Our findings suggest that low tidal volume ventilation likely attenuates VALI by limiting mechanical damage to the lung, while hypercapnia attenuates VALI by limiting pro-inflammatory and biochemical mechanisms of injury. When combined, both lung-protective ventilation and hypercapnia have the potential to exert an synergistic effect for the prevention of VALI.

Keywords: lung-protective mechanical ventilation, hypercapnia, ventilator associated lung injury, acute lung injury, mechanical ventilalion

INTRODUCTION

The role of lung-protective ventilation using low inspiratory pressures and low tidal volumes for attenuating ventilator-associated lung injury (VALI) is well established (Amato et al., 1998; Acute Respiratory Distress Syndrome et al., 2000; Slutsky and Ranieri, 2013). Protective ventilation is believed to attenuate VALI by limiting barotrauma and volutrauma, thereby reducing the stretch and strain to lung tissue during the inflation phase of the respiratory cycle. Barotrauma and volutrauma have been specifically attributed to mechanical power, which is directly proportional to the respiratory rate and transpulmonary driving pressures, and inversely proportional to lung elastance (Amato et al., 2015). Santos et al. (2018) recently demonstrated that increased ventilatory power, which is primarily a product of increased transpulmonary driving pressure, worsened VALI as expected. The transpulmonary pressure is calculated from the difference between airway and intrapleural pressure, measured clinically in the mid-esophagus. The transpulmonary driving pressure is derived from inspiratory and expiratory transpulmonary pressures which determine the achieved tidal volume. If no esophageal pressure is available, the airway driving pressure may be used as a surrogate and has been shown to correlate with outcome (Amato et al., 2015).

Two mechanisms of injury have been the focus of research in the field of VALI: 1) The direct structural damage caused by overdistension of lung units leading to volutrauma, barotrauma, and 2) biotrauma (i.e., the activation of biological pathways via mechanotransduction). Cyclic opening and closure of distal airways/alveoli during the ventilatory cycle, termed atelectrauma, may contribute to all of the above by increasing stress and strain. When ventilatory power and driving pressures are reduced using lung protective ventilation (Rocco et al., 2012; Santos et al., 2018) there may be a reduction in structural injury (pulmonary edema and histologic lung injury), a reduction in biotrauma (pro-inflammatory mediators) and improved clinical outcomes (Santos et al., 2018).

One of the consequences of limiting driving pressure, tidal volume ventilation and respiratory rate is the rise in arterial $PaCO_2$, a concept known as permissive hypercapnia (Hickling et al., 1994; Contreras et al., 2015; Costa et al., 2021). Although the differential mechanisms of biological vs mechanical effects of permissive hypercapnia have not been systematically investigated, several clinical studies have investigated lung protective ventilation with permissive hypercapnia as a tolerated side effect (Bidani et al., 1994; Hickling et al., 1994; Acute Respiratory Distress Syndrome et al., 2000). Hypercapnia has been associated with a reduction in the effects of excessive lung stretch by an intracellular mechanism that remains elusive (Ismaiel and Henzler, 2011). In view of the clinical benefits, lung protective ventilation allowing permissive hypercapnia has been adopted in current treatment guidelines for acute respiratory distress syndrome (ARDS), as well as neonatal respiratory failure, acute status asthmaticus (Darioli and Perret, 1984), and respiratory failure secondary to chronic obstructive pulmonary disease (Contreras et al., 2015).

The hypothesis that hypercapnia may no longer be viewed as a side effect but rather as a therapeutic concept (Kavanagh and Laffey, 2006) by adding a small fraction of inspired carbon dioxide (CO_2) to the gas mixture during ventilation was experimentally introduced in recent years (Ismaiel and Henzler, 2011). This therapeutic hypercapnia has been shown to attenuate pulmonary inflammation and free radical production, in addition to preserving pulmonary mechanics in an ischemia reperfusion-induced lung injury model in rabbits (Laffey et al., 2000). The resulting hypercapnic acidosis is characterized by a decrease in intracellular pH due to the accumulating CO_2 and has been associated with a reduction in pulmonary inflammation, oxidative stress (Broccard et al., 2001) and cell death (Laffey et al., 2000; Chonghaile et al., 2008). Specifically, there is evidence to suggest that hypercapnic acidosis reduces Xanthine Oxidase activity, thereby reducing the production of free radicals and reactive oxygen species (Shibata et al., 1998; Ismaiel and Henzler, 2011).

Further, antimicrobial and anti-inflammatory properties have been attributed to hypercapnia as evidenced by reduced inflammatory cell infiltrates and bacterial cell counts in the injured lungs (Chonghaile et al., 2008). The acidotic state resulting from hypercapnia is associated with a reduction in architectural damage and histologic injury (Chonghaile et al., 2008), decreased pulmonary edema (Sinclair et al., 2002) and reduced production of key pro-inflammatory mediators, including IL-1β, TNF-α, IL-6, MCP-1, MMP-9, and KC (Laffey et al., 2000; Peltekova et al., 2010). Potential harmful effects of hypercapnia and hypercapnic acidosis have been described to occur in impaired cell membrane and wound healing, depressed cardiac function and uncontrolled increase in intracerebral pressure in case of brain injury (Ismaiel and

Henzler, 2011). Despite the potentially therapeutic and protective effects of experimental hypercapnic acidosis noted previously, the true effect of acidosis has yet to be robustly studied in the clinical setting. It is well known that the acidotic state secondary to respiratory failure seen in ALI and ARDS is associated with deleterious effects and poor clinical outcomes (Tiruvoipati et al., 2017). Since the acidotic state in ALI and ARDS is often a mixture of respiratory and metabolic acidosis induced by multi-organ dysfunction, trying to compensate by hyperventilation increases VALI, while buffering the acidotic state with sodium bicarbonate remains controversial and potentially harmful (Chand et al., 2021). However, the true effect of hypercapnic acidosis either in the context of permissive hypercapnia or by adding a small fraction of inspired carbon dioxide (CO_2) to the gas mixture during ventilation remains elusive.

To date it's unclear whether the attenuation of VALI with lung protective ventilation and associated hypercapnia can be attributed mainly to reduction of the mechanical forces to the lung, to a direct effect of hypercapnia and acidosis, or a combination thereof. The aim of our study was thus to address five key questions:

1. Does lung-protective ventilation with low tidal volumes and limited driving pressures cause less VALI than conventional non-protective ventilation (i.e., higher tidal volumes) during normocapnia?
2. Does lung protective ventilation with permissive hypercapnia cause less VALI than normocapnic lung-protective ventilation?
3. Does exogenous CO_2 attenuate VALI during ventilation with high tidal volumes?
4. Does exogenous CO_2 attenuate VALI during lung protective ventilation with low tidal volumes?
5. Is there a different effect if hypercapnia is produced by exogenous delivery of carbon dioxide instead of permissive hypercapnia with endogenous rise in CO_2?

MATERIALS AND METHODS

Experimental Procedures

All experimental procedures were conducted with ethics approval from the Dalhousie University Committee on Laboratory Animals, and the care and handling of the animals was in accordance with the National Institutes of Health Guidelines for ethical animal treatment. Sixty male Sprague-Dawley rats (15–18 weeks old, weight 400–490 g) were used. Details of experimental procedures are outlined in the **Supplementary Material**.

In brief, anesthetized animals received a continuous IV infusion of 20 mcg/ml remifentanil and 25 mcg/ml pancuronium at 5 ml/h during controlled ventilation as described elsewhere (Ismaiel et al., 2012). Animals were tracheotomized with a 14G cannula and mechanically ventilated (EVITA4, Draeger Medical Canada Inc., Richmond, ON, Canada). The carotid artery and internal jugular vein were cannulated with 20G catheters for blood pressure monitoring and blood gas analysis (ABL510 and OSM3, Radiometer Copenhagen, Denmark). The femoral artery was cannulated with a thermocouple probe (ADInstruments Inc., Colorado Springs, CO, United States) for cardiac output measurements and cardiac index calculations. A pneumotachometer (Hans Rudolph Inc., Shawnee, KS, United States) was used to measure flow-related respiratory variables. A complete set of measurements was taken at baseline, and after 1 and 4 h of ventilation. The parameters measured at each time point included hemodynamics (MAP, CI, HR), respiratory variables (V_T, RR, V_E) and arterial blood gasses with 300 µl of blood per sample (PaO_2, $PaCO_2$, pH) with a FiO_2 of 1.0 (Ismaiel et al., 2012).

We used a model of mild ARDS that had been previously described in rats and rabbits (Imai et al., 2003; Henzler et al., 2011) (see **Supplementary Material**). After baseline measurements, acute lung injury (ALI) was induced by endotracheal instillation of 2.5 ml/kg of unbuffered hydrochloric acid (HCl, pH 1.25), and allowed to develop over 1 hour of controlled ventilation with tidal volume (V_T) 8 ml/kg, positive end expiratory pressure (PEEP) 5 cmH_2O and a partial pressure of carbon dioxide ($PaCO_2$) 40–55 mmHg, which was set by varying the respiratory rate. Establishment of ALI was defined by a PaO_2:FiO_2 (P/F) ratio ≤300 mmHg and a significant increase in respiratory system elastance.

Sixty rats were randomly assigned to receive one of six ventilation regimens (n = 10/group), each lasting 3 h. Three groups were ventilated to achieve relatively normocapnic conditions ($PaCO_2$ = 45–55 mmHg) and three groups to achieve hypercapnic conditions ($PaCO_2$ = 60–70 mmHg). All animals received a PEEP of 5 cmH_2O while being ventilated (**Table 1**, **3**).

Conventional HighV_E Normocapnia

Ventilation with high tidal volume (V_T 12 ml/kg) and increased minute ventilation (V_E). 1 ml of dead space was added to the respiratory circuit to prevent hypocapnia and respiratory rate (RR) adjusted to achieve normocapnia (RR was 72 ± 16 breaths per minute).

Conventional Normocapnia

Ventilation with high V_T (12 ml/kg) and RR adjusted to maintain normocapnia (RR was 42 ± 10 breaths per minute).

Protective Normocapnia

Ventilation with low V_T (8 ml/kg) and RR adjusted to maintain normocapnia (RR was 92 ± 14 breaths per minute).

Conventional iCO_2 Hypercapnia

Maintained V_E with high V_T (12 ml/kg) and inhaled CO_2 ($FiCO_2$ 1.6%) targeting hypercapnia (RR was 42 ± 11 breaths per minute).

Protective iCO_2 Hypercapnia

Maintained V_E with low V_T (8 ml/kg) and inhaled CO_2 ($FiCO_2$ 1.6%) targeting hypercapnia (RR was 69 ± 19 breaths per minute).

Protective Endogenous Hypercapnia

Reduced V_E with low V_T (8 ml/kg) and low RR, with endogenous rise in $PaCO_2$ by hypoventilation (RR was 52 ± 26 breaths per minute).

TABLE 1 | Summary of target ventilation settings, including tidal volume (V_T, ml/kg), minute ventilation (V_E), partial arterial pressure of carbon dioxide ($PaCO_2$, mmHg), and the addition of inspired CO_2 gas or dead space.

Group	V_T target (ml/kg)	V_E	$PaCO_2$ target (mmHg)	Additional CO_2 or dead space
Conventional HighV_E Normocapnia	High (12)	⇧	40–55	1 ml added dead space
Conventional Normocapnia	High (12)	⇔	40–55	
Protective Normocapnia	Low (8)	⇔	40–55	
Conventional iCO_2 Hypercapnia	High (12)	⇔	60–70	Inspired CO_2 (1.6%)
Protective iCO_2 Hypercapnia	Low (8)	⇔	60–70	Inspired CO_2 (1.6%)
Protective Endogenous Hypercapnia	Low (8)	⇩	60–70	

After final measurements and sample collection animals were killed with a 1 ml intravenous bolus of potassium chloride (150 mg/ml).

Tissue and Fluid Analyses

Lung tissue samples were evaluated using the Diffuse Alveolar Damage (DAD) scoring criteria (Castro, 2006; Henzler et al., 2011). The liver and kidney tissue samples were graded for tissue damage scores on a scale from 0–2 and 0–4, respectively (See **Supplementary Material**). The wet-to-dry lung ratio was calculated from the weight of the wet right middle lobe after excision, and the dry weight after a 48-h incubation at 40°C. Lung homogenates were prepared, and the Bradford assay was used to prepare the samples for western blot analysis to quantify Caspase-3 protein expression (Kruger and Walker, 2002). Caspase-3 protein expression in the lung homogenates was expressed as the ratio of active (17 kD band) to inactive (35 kD band) caspase-3 (See **Supplementary Material**).

The BALF and arterial plasma samples were analyzed using a multiplex immunoassay (BIO-RAD; Hercules, California, United States). Cytokines and chemokines (interleukin (IL)-1β, ICAM-1, IL-6, TNF-α, GM-CSF, IL-10, RANTES, KC, MCP-1, and MIP-1α) were analyzed using the Luminex Technology Analyzer 100 and BioPlex Manager software from BIO-RAD (Mississauga, ON, Canada).

Data are expressed as means ± SD or SEM. All statistical analyses were conducted using GraphPad Prism 5.0 software (La Jolla, CA, United States). Normality of data was tested with K-S test. Differences between groups and changes over time of physiologic variables were tested by two-way ANOVA with a mixed-effects model, biologic variables by one-way ANOVA or Kruskal–Wallis test, whichever was appropriate. Adjustments for multiple comparisons was done by the Bonferroni method for adjusted p values. The level of significance was set at $p < 0.05$.

RESULTS

Physiologic Measurements

Compared to baseline, 1 hour after induction of ALI the P/F ratio had decreased (428 ± 72 vs 287 ± 100 mmHg, $p < 0.0001$) and elastance (2.5 ± 0.6 vs 4.0 ± 0.9 cmH_2O/ml $p < 0.001$) and airway driving pressure (9.2 ± 2.1 vs 15.1 ± 3.2 cmH_2O, $p < 0.0001$) had increased significantly in all groups equally, confirming the development of mild ALI (**Figure 1**). The experimental setup in respect to V_T and V_E was achieved (**Table 3**). There was a significant drop in MAP after induction of ALI, but hemodynamic parameters (MAP, HR and CI) remained stable thereafter and were similar in all groups (**Table 2**). Respiratory variables (V_T, V_E, and RR) were similar in all groups at baseline and at the initiation of ALI.

Gas Exchange

Gas exchange parameters were similar at baseline and at establishment of ALI in all groups with P/F ratios that would be compatible with mild ARDS in patients. The $PaCO_2$ targets were achieved in all groups and by design, the hypercapnia groups had higher $PaCO_2$ than the normocapnia groups. The hypercapnia group had somewhat greater acidemia than the normocapnia groups) (**Figure 2A**).with the lowest pH in the Protective Endogenous Hypercapnia group ($p < 0.001$) (**Table 4**).

Comparison of Conventional and Protective Ventilation Targeting Normocapnia

There were no differences in oxygenation among groups (**Table 4**). The V_T and $delta_{TP}$ (transpulmonary driving pressure) were lower in protective vs conventional with normal or highV_E injurious ventilation ($p < 0.001$) (**Table 3**). Protective Normocapnia trended to a reduced W/D ratio compared to Conventional Normocapnia and Conventional HighV_E Normocapnia that failed significance in multiple comparisons (**Figure 2B**). Protective Normocapnia also exhibited a lower DAD score compared to Conventional HighV_E Normocapnia ($p = 0.0220$), with a notable decrease in the hyaline membrane subscore ($p = 0.0271$) (**Figures 2D,E**). The caspase-3 activation in the lung was higher in Protective Normocapnia compared to Conventional HighV_E Normocapnia in lung homogenates ($p = 0.0183$), however, liver or kidney histologic injury scores were not different (data not shown). In general, reduced V_T in protective ventilation was more likely to result in loss of airspace (**Figures 2F**, **5**).

Comparison of Protective Ventilation Targeting Normocapnia With Permissive Hypercapnia

The V_T and $delta_{TP}$ were similar, but V_E was significantly increased with protective ventilation to achieve normocapnia. In Protective Endogenous Hypercapnia, the DAD score and W/D ratio were similar to Protective Normocapnia (**Figures 2B,D**), and gas exchange was not improved, as shown by equal P/F ratios

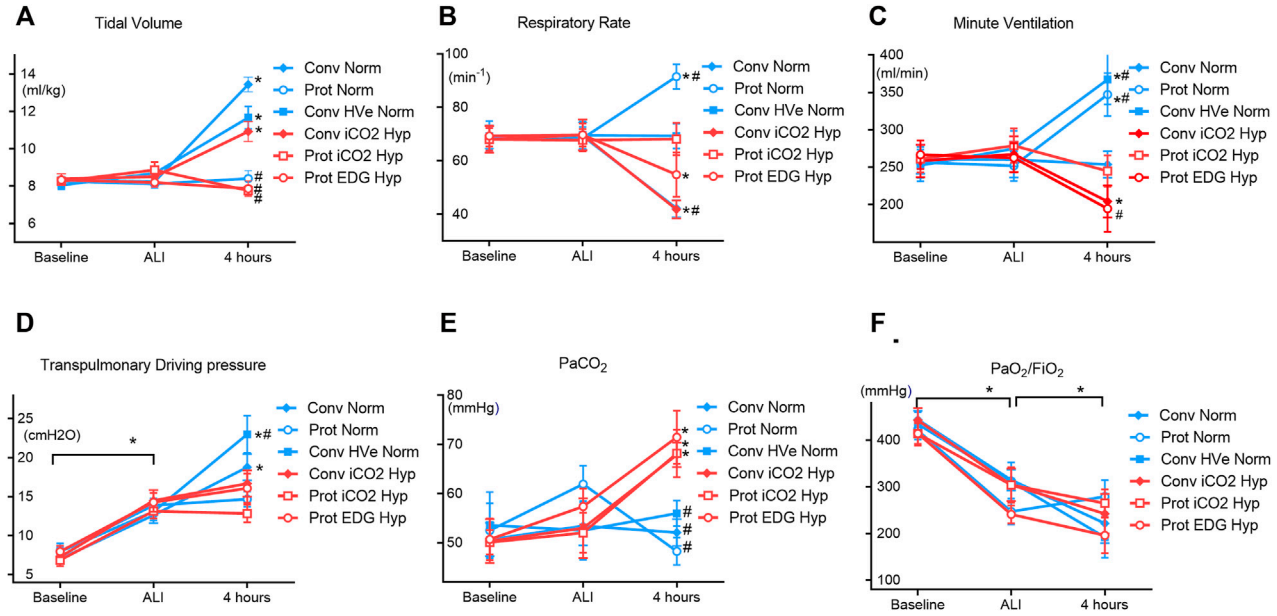

FIGURE 1 | Physiologic dataat Baseline, ALI, and 4 h of ventilation (Mean ± SEM). Closed symbols: high tidal volume; open symbols: low tidal volumes; blue color: normocapnia; red color: hypercapnia. * denotes significant differences between groups.

TABLE 2 | Hemodynamic measurements at baseline, ALI and after 4 h of ventilation in each group, including mean arterial pressure (MAP), heart rate (beats per minute, BPM), and cardiac index. Values are expressed as mean ± SD. Baseline data given for reference only, but not included into statistical analysis. There were no significant differences between groups. * denotes significance between ALI and 4 h measurements with no significant interaction between groups.

	Group	Baseline	ALI	4 hour	2-way ANOVA
MAP (mmHg)	Conventional HV_E Normocap	156 ± 13	117 ± 20	120 ± 28	Time p = 0.51 Group p = 0.38
	Conventional Normocapnia	158 ± 13	129 ± 19	116 ± 29	
	Protective Normocapnia	152 ± 13	123 ± 16	134 ± 40	
	Conventional iCO_2 Hypercapnia	150 ± 13	119 ± 20	121 ± 37	
	Protective iCO_2 Hypercapnia	146 ± 13	129 ± 14	140 ± 23	
	Protective EDG Hypercapnia	157 ± 11	119 ± 14	126 ± 28	
Heart Rate (BPM)	Conventional HV_E Normocap	401 ± 44	333 ± 39	406 ± 52	Time* p < 0.001 Group p = 0.212
	Conventional Normocapnia	422 ± 36	373 ± 56	428 ± 64	
	Protective Normocapnia	418 ± 44	376 ± 68	445 ± 63	
	Conventional iCO_2 Hypercapnia	414 ± 43	343 ± 50	411 ± 59	
	Protective iCO_2 Hypercapnia	404 ± 41	375 ± 55	420 ± 53	
	Protective EDG Hypercapnia	421 ± 30	365 ± 28	432 ± 55	
Cardiac Index ($Lmin^{-1}$ m^{-2})	Conventional HV_E Normocap	2.50 ± 0.55	2.40 ± 0.42	2.22 ± 0.40	Time* p = 0.046 Group p = 0.96
	Conventional Normocapnia	2.55 ± 0.89	2.36 ± 0.75	2.27 ± 0.71	
	Protective Normocapnia	2.59 ± 0.64	2.51 ± 0.56	2.19 ± 0.44	
	Conventional iCO_2 Hypercapnia	2.46 ± 0.48	2.44 ± 0.67	2.31 ± 0.70	
	Protective iCO_2 Hypercapnia	2.47 ± 0.67	2.45 ± 0.59	2.08 ± 0.38	
	Protective EDG Hypercapnia	2.33 ± 0.41	2.75 ± 1.10	2.24 ± 0.85	

after 4 h of ventilation (**Figure 1F**). Protective Endogenous Hypercapnia also did not alter caspase-3 expression in lung homogenates (**Figure 2C**) and did not improve liver or kidney histologic injury (data not shown). However, Protective Endogenous Hypercapnia produced profound hypercapnic acidosis compared to Protective Normocapnia (p = 0.0149) (**Figure 2A**), and also increased IL-10 concentrations in BALF (p = 0.0244) (**Figure 4D**).

Comparison of Conventional Ventilation Targeting Normocapnia With Hypercapnia

After 4 h of ventilation there were no significant differences in V_T or $delta_{TP}$, although $delta_{TP}$ increased further from the ALI time point in Conventional Normocapnia (p < 0.001) (**Figure 1A**). Adding inhaled CO_2 in Conventional iCO_2 Hypercapnia significantly reduced the DAD score (p = 0.0097) (**Figure 2D**), however it did not reduce the W/D ratio and did not improve gas exchange

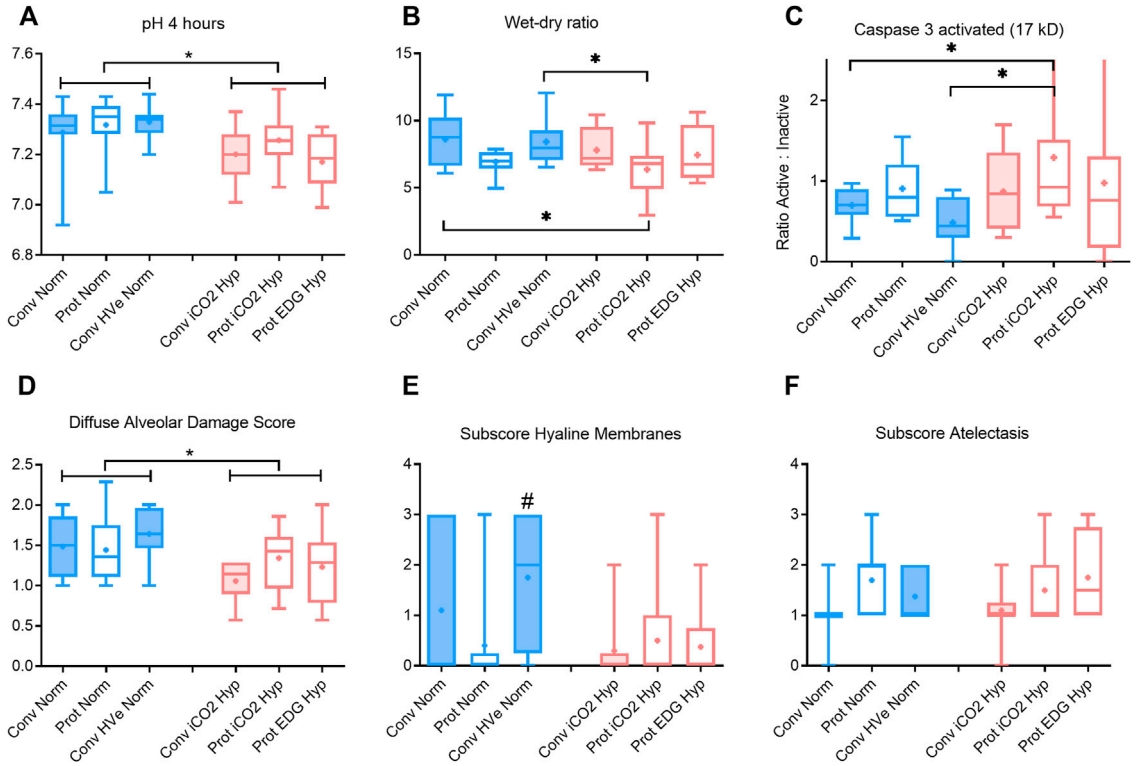

FIGURE 2 | pH, wet-dry ratio, caspase activation and alveolar damage scoring after 4 h of ventilation boxplot of IQR, mean, median, min-max (whiskers). Closed boxes: high tidal volumes; open boxes: low tidal volumes; blue color: normocapnia; red color: hypercapnia. * denotes significant differences between groups # denotes significant difference to all other groups.

(**Table 4**; **Figure 2B**). No differences were found in caspase-3 expression (**Figure 2C**) or liver and kidney histologic injury (data not shown). However, there was a notable decrease in the plasma IL-1β concentrations associated with hypercapnia compared to normocapnia (p = 0.0015) (**Figure 3A**) and pulmonary IL-6 and MCP-1 concentrations were significantly reduced in the BALF (p = 0.0202 and p = 0.0030, respectively) (**Figures 3E**, **4F**).

Comparison of Protective Ventilation Targeting Normocapnia With Hypercapnia

Hypercapnia mediated by inhaled CO_2 did not reduce the DAD score or the W/D ratio, and did not improve gas exchange compared to normocapnia if lung-protective settings were applied (**Table 4**; **Figures 2B,D**). The Protective iCO_2 Hypercapnia group also did not differ in caspase-3 expression in lung homogenates (**Figure 2C**) or liver or kidney histologic injury (data not shown). However, plasma IL-1β concentrations were markedly reduced in Protective iCO_2 Hypercapnia compared to Protective Normocapnia (p = 0.0042) (**Figure 3A**).

Comparison of Protective Ventilation Targeting Hypercapnia Induced via Inhaled CO_2 With Permissive Endogenous Rise

The V_T and $delta_{TP}$ were similar between groups, but V_E and pH were reduced in Protective Endogenous Hypercapnia (**Table 4**; **Figures 1A**, **2A**). No differences were found in DAD score, W/D ratio, oxygenation or caspase-3 expression between protectively ventilated animals with endogenously or exogenously induced hypercapnia (**Table 4**; **Figure 2**). Protective iCO_2 Hypercapnia also did not alter the inflammatory cytokine profile in plasma and BALF (**Figures 3**, **4**). While Protective Endogenous Hypercapnia did not improve kidney histologic injury, it reduced liver histologic injury (0; 0–0.25) compared to Protective iCO_2 Hypercapnia (1.5; 0–2) (p = 0.035).

DISCUSSION

The purpose of this study was to differentiate whether the main mechanism by which VALI is attenuated in lung-protective ventilation is attributable to the limitation of mechanical driving forces to the lung itself, or the increase in $PaCO_2$ as a consequence of reduced minute ventilation. While gas exchange was impaired equally in all groups at 4 h of ventilation after establishing experimental ALI, we demonstrated that lung-protective ventilation targeting normocapnia limited histologic injury and the W/D lung ratio, and also increased caspase-3 activation compared to conventional ventilation, and especially injurious ventilation with high V_T and V_E. Again this proves important evidence that relevant injury to the lungs happens before it can be detected by clinical means, i.e., changes in gas exchange or respiratory mechanics. During ventilation with high V_T and high $delta_{TP}$, hypercapnia decreased histologic injury and pro-inflammatory cytokines in the plasma and BALF compared to

TABLE 3 | Respiratory measurements at baseline, ALI and 4 h of ventilation in each group, including tidal volume (V_T, ml/kg), respiratory rate (RR, breaths per minute, min^{-1}), minute ventilation (V_E, ml/min), respiratory system elastance (cmH_2O/L) and transpulmonary driving pressure (cmH_2O). Values are expressed as mean ± SD. Baseline data given for reference only, but not included into statistical analysis. * denotes significant difference vs ALI measurements; numbers denote significant differences between groups.

	Group	Baseline	ALI	4 hours	2-way ANOVA
V_T (ml/kg)	1 Conventional HV_E Normocap	7.9 ± 0.7	8.6 ± 1.0	12.3 ± 1.4*3,5,6	Time $p < 0.001$ Group $p < 0.001$
	2 Conventional Normocapnia	8.3 ± 0.7	8.2 ± 0.6	13.4 ± 1.2*3,5,6	
	3 Protective Normocapnia	8.3 ± 0.8	8.1 ± 0.7	8.4 ± 1.41,2	
	4 Conventional iCO_2 Hypercap	8.4 ± 0.9	8.5 ± 0.6	10.9 ± 1.7*5,6	
	5 Protective iCO_2 Hypercapnia	8.3 ± 0.5	8.9 ± 1.4	7.8 ± 0.71,2,4	
	6 Protective EDG Hypercapnia	8.3 ± 0.6	8.3 ± 0.7	8.1 ± 1.31,2,4	
Interaction Time × Group $p < 0.001$					
RR (min^{-1})	1 Conventional HV_E Normocap	72 ± 19	72 ± 18	72 ± 162,4	Time $p < 0.001$ Group $p = 0.004$
	2 Conventional Normocapnia	69 ± 14	69 ± 16	42 ± 10 *1,3,5	
	3 Protective Normocapnia	68 ± 13	68 ± 13	92 ± 14 *2,4,5,6	
	4 Conventional iCO_2 Hypercap	68 ± 16	69 ± 18	42 ± 11 *1,2,3,5	
	5 Protective iCO_2 Hypercapnia	68 ± 13	68 ± 13	68 ± 192,3,4	
	6 Protective EDG Hypercapnia	68 ± 11	68 ± 13	52 ± 263	
Interaction Time × Group $p < 0.001$					
V_E (ml/min)	1 Conventional HV_E Normocap	252 ± 67	275 ± 75	367 ± 104*2,4,5,6	Time $p = 0.350$ Group $p = 0.018$
	2 Conventional Normocapnia	261 ± 61	260 ± 76	254 ± 561	
	3 Protective Normocapnia	256 ± 65	252 ± 64	347 ± 91*4,6	
	4 Conventional iCO_2 Hypercap	258 ± 69	267 ± 76	204 ± 68*3,1	
	5 Protective iCO_2 Hypercapnia	261 ± 58	279 ± 73	245 ± 661	
	6 Protective EDG Hypercapnia	253 ± 36	256 ± 57	191 ± 1073,1	
Interaction Time × Group $p < 0.001$					
Respiratory System Elastance (cmH_2O/ml)	1 Conventional HV_E Normocap	2.6 ± 0.5	3.8 ± 0.8	4.9 ± 2.3	Time $p = 0.315$ Group $p = 0.471$
	2 Conventional Normocapnia	2.4 ± 0.4	3.8 ± 0.7	3.6 ± 1.0	
	3 Protective Normocapnia	2.5 ± 0.7	4.2 ± 0.7	4.5 ± 0.8	
	4 Conventional iCO_2 Hypercap	2.6 ± 0.7	4.3 ± 1.2	3.9 ± 1.4	
	5 Protective iCO_2 Hypercapnia	2.3 ± 0.5	3.7 ± 0.7	4.2 ± 0.8	
	6 Protective EDG Hypercapnia	2.4 ± 0.4	4.1 ± 1.1	4.8 ± 1.8	
Interaction Time × Group $p = 0.263$					
Trans-pulmonary Driving pressure (cmH_2O)	1 Conventional HV_E Normocap	7.9 ± 2.1	13.1 ± 3.6	23.0 ± 7.6*3,4,5,6	Time $p < 0.001$ Group $p = 0.078$
	2 Conventional Normocapnia	7.0 ± 1.6	12.6 ± 3.2	18.8 ± 5.1*	
	3 Protective Normocapnia	8.0 ± 3.1	13.8 ± 2.9	14.7 ± 2.91	
	4 Conventional iCO_2 Hypercap	7.3 ± 1.7	14.5 ± 4.0	16.7 ± 5.31	
	5 Protective iCO_2 Hypercapnia	6.9 ± 2.3	13.2 ± 2.8	12.8 ± 3.41	
	6 Protective EDG Hypercapnia	7.9 ± 2.3	14.3 ± 3.6	16.1 ± 6.01	
Interaction Time × Group $p = 0.001$					

normocapnic conditions. Hypercapnia during lung-protective ventilation had only small benefits in preventing cytokine activation as compared to normocapnia, regardless whether it was induced by inhaled CO_2 or endogenous rise during permissive hypoventilation. However, respiratory acidosis in Protective Endogenous Hypercapnia caused an increase in BALF IL-10 concentrations, possibly exerting protective effects in distant organs.

Does Lung-Protective Ventilation With Low Tidal Volumes Cause Less VALI Than Conventional ventilation in the State of Normocapnia?

This question may seem odd in the face of numerous experimental and clinical studies that have proven the superiority of a ventilatory concept in which stress and strain are reduced by limiting distending volumes and pressures to the lung. However, in most investigations, hypercapnia has been regarded as an undesirable side effect. Strategies have even been developed for extracorporeal CO_2 removal (ECCO2R), with quite dissimilar outcomes and no general recommendation for its use in current guidelines (Combes et al., 2017).

When compared to high V_T ventilation, lung-protective low V_T settings reduced histologic injury and the formation of hyaline membranes, which are fibrous eosinophilic structures made of fibrin, collagen, elastin and cellular debris from mechanical strain on the lungs (Castro, 2006). The formation of hyaline membranes along alveolar walls disrupts gas exchange by creating an additional diffusional barrier through which gas exchange must occur, thereby potentially worsening oxygenation (Henzler et al., 2011). Importantly, high V_T is associated with increased transpulmonary driving pressures and mechanical power delivered to the lung, which have been shown to

TABLE 4 | Gas exchange measurements at baseline, 1 h and 4 h of ventilation in each group, including pH, partial pressure of O_2 (PaO_2, mmHg), and partial pressure of carbon dioxide ($PaCO_2$, mmHg). Values are expressed as mean ± SD. Baseline data given for reference only, but not included into statistical analysis. * denotes significant difference vs ALI measurements; numbers denote significant differences between groups.

	Group	Baseline	ALI	4 hours	2-way ANOVA
pH	1 Conventional HVE Normocap	7.37 ± 0.12	7.37 ± 0.13	7.33 ± 0.07	Time* p = 0.001 Group p = 0.04
	2 Conventional Normocapnia	7.40 ± 0.06	7.35 ± 0.11	7.29 ± 0.14	
	3 Protective Normocapnia	7.38 ± 0.10	7.29 ± 0.05	7.32 ± 0.12	
	4 Conventional iCO2 Hypercap	7.38 ± 0.08	7.33 ± 0.10	7.20 ± 0.11*	
	5 Protective iCO2 Hypercapnia	7.38 ± 0.09	7.36 ± 0.11	7.26 ± 0.11*	
	6 Protective EDG Hypercapnia	7.37 ± 0.07	7.29 ± 0.08	7.17 ± 0.11	
					Interaction Time × Group p = 0.207
PaO_2 (mmHg)	1 Conventional HVE Normocap	431 ± 92	365 ± 72	242 ± 135	Time* p = 0.007 Group p = 0.527
	2 Conventional Normocapnia	444 ± 60	315 ± 71	222 ± 134	
	3 Protective Normocapnia	420 ± 61	247 ± 89	279 ± 111	
	4 Conventional iCO2 Hypercap	442 ± 84	304 ± 110	242 ± 135	
	5 Protective iCO2 Hypercapnia	414 ± 81	304 ± 120	265 ± 94	
	6 Protective EDG Hypercapnia	421 ± 78	239 ± 68	204 ± 127	
					Interaction Time × Group p = 0.248
$PaCO_2$ (mmHg)	1 Conventional HVE Normocap	52 ± 19	52 ± 16	55 ± 7 [6]	Time* p = 0.019 Group p = 0.088
	2 Conventional Normocapnia	51 ± 11	53 ± 12	52 ± 9 [6]	
	3 Protective Normocapnia	52 ± 17	61 ± 12	48 ± 9 [4,5,6]	
	4 Conventional iCO2 Hypercap	50 ± 14	53 ± 19	68 ± 8 [*3]	
	5 Protective iCO2 Hypercapnia	50 ± 8	52 ± 13	68 ± 15[*3]	
	6 Protective EDG Hypercapnia	50 ± 11	57 ± 11	71 ± 14[*3]	
					Interaction Time × Group p = 0.002

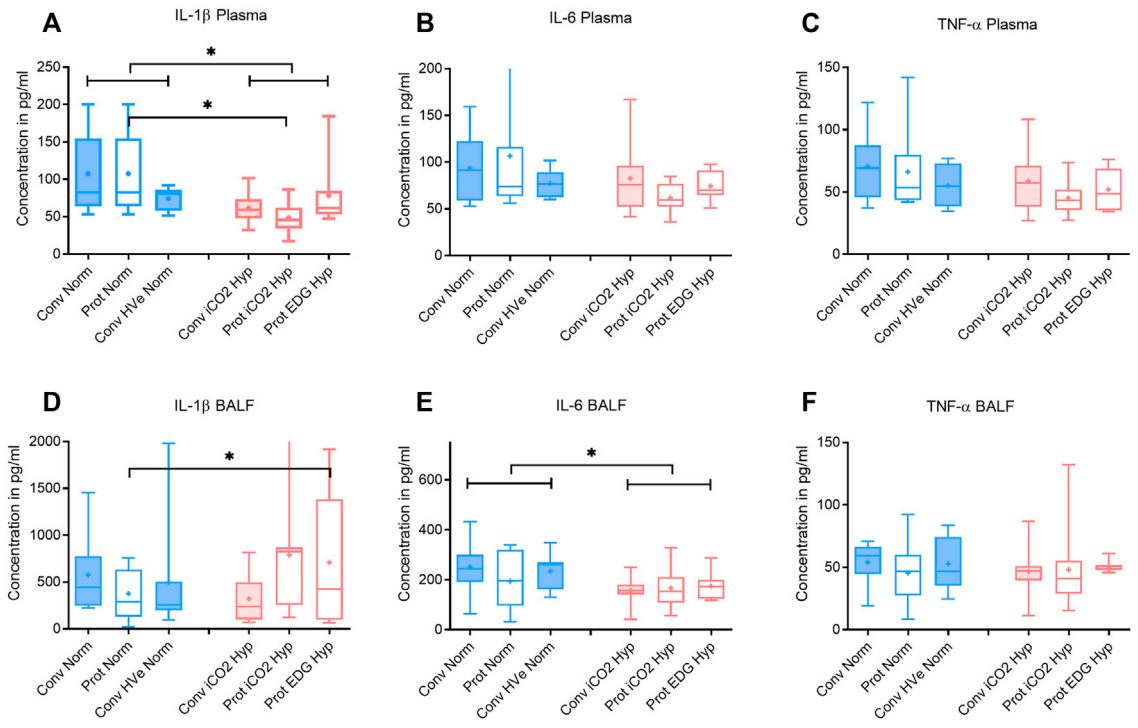

FIGURE 3 | Plasma and broncho-alveolar lavage fluid (BALF) cytokines after 4 h of ventilation. Boxplot of IQR, mean, median, min-max (whiskers). Closed boxes: high tidal volumes; open boxes: low tidal volumes; blue color: normocapnia; red color: hypercapnia. * denotes significant differences between groups.

worsen VALI in experimental (Santos et al., 2018) and clinical studies (Amato et al., 2015). Our results are consistent in that $delta_{TP}$ was higher in the high V_T groups exhibiting lung damage. We had previously demonstrated that transpulmonary pressure and not respiratory effort is the main determinant of VALI (Henzler et al., 2019); these findings were confirmed, since the

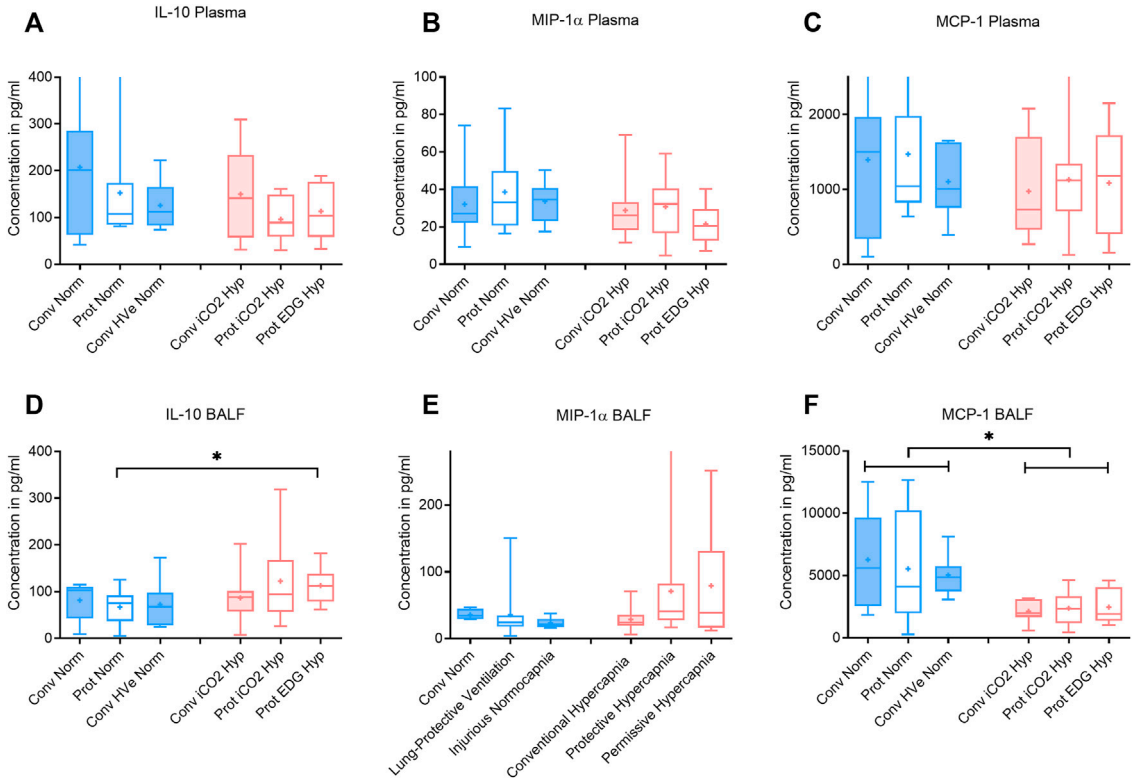

FIGURE 4 | Plasma and broncho-alveolar lavage fluid (BALF) cytokines after 4 h of ventilation. Boxplot of IQR, mean, median, min-max (whiskers). Closed boxes: high tidal volumes; open boxes: low tidal volumes; blue color: normocapnia; red color: hypercapnia. * denotes significant differences between groups.

higher RR and V_E in conventional ventilation (Conventional HighV_E Normocapnia group) did not further increase lung injury.

Protective Normocapnia also significantly increased caspase-3 activation compared to Conventional HighV_E Normocapnia (**Figure 1**). Caspase-3 is a pro-apoptotic protein representing the final step in the apoptosis common pathway onto which both the intrinsic and extrinsic apoptotic pathways converge. Lung-protective ventilation has previously been shown to reduce apoptosis in the lungs by TUNEL staining (Syrkina et al., 2008), which represents the detection of total caspase regardless of activity. In contrast, we determined the ratio of uncleaved (inactive) to cleaved (active) form by Western blotting. The reason for higher caspase-3 activation during Protective Normocapnia may be that apoptosis represents a protective rather than a harmful mechanism suggesting that it is more protective to induce programmed cell death by apoptosis than traumatic cell death by necrosis. It seems quite possible that the enhanced apoptotic activity is a product of limiting mechanical stretch without at the same time inhibiting a biochemical mechanism in the cells. This is supported by the observation that cytokine concentrations in the plasma and BALF of all animals were similar in the three normocapnia groups. Taken together, these findings indicate that VALI may be attenuated by limiting mechanisms of mechanical trauma in the lung that cause increased stretch and strain.

Does Lung-Protective Ventilation With Permissive Hypercapnia Cause Less VALI Than Lung-Protective Ventilation Targeting Normocapnia?

Protective ventilation with Low V_T and low $delta_{TP}$ allowing less V_E (Protective Endogenous Hypercapnia) did not further reduce VALI compared to higher RR and V_E (Protective Normocapnia). Permissive, low V_E associated increase in $PaCO_2$ did not provide any further decrease in lung weight, histologic lung injury or improvement in gas exchange, and did not alter caspase-3 activation (**Figure 2C**). This head-to-head comparison between inhaled CO_2 and permissive, endogenous hypercapnia has not been investigated before and confirms previous investigations that limiting $delta_{TP}$ is more important in protecting the lung than the injurious impact of a ventilatory pattern induced when there is a high work of breathing (Henzler et al., 2019). Vaporidi et al. (2008) had investigated the effect of V_T and RR in mice and found that reducing RR was more effective in prevention of VILI than reducing V_T in ventilated mice with equal $PaCO_2$ target. However, these were previously healthy animals without ALI and transpulmonary pressures were not monitored. Recently, a meta-analysis of patient-level data from observational and randomized studies investigated the influence of RR, driving pressure ($delta_P$) and mechanical power on mortality in >4.500 pts. with ARDS (Costa et al.,

2021). They found that there is a trade-off between reducing V_T and increasing RR to facilitate protective ventilation. RR was an independent predictor of death (odds ratio 1.15 (10.6, 1.25)); $delta_P$ (odds ratio 1.31 (1.14, 1.5)) and mechanical power (odds ratio 1.24 (1.15, 1.33)) were even greater predictors of mortality. Using isocapnic curves, reducing RR (and therefore increasing $delta_P$) reduced the odds ratio of death in patients with normal respiratory system compliance, but increased the odds ratio of death in patients with low compliance (Costa et al., 2021) if V_T < 7 ml/kg PBW were applied. In this respect, our results are not conflicting and may serve as the physiological basis to explain their results.

It is worth noting that the Protective Endogenous Hypercapnia group had the greatest degree of hypercapnic acidosis. This decrease in pH was associated with an increase in pulmonary IL-10 concentrations in BALF (**Figure 4D**). IL-10 is a potent anti-inflammatory mediator that is released in response to pro-inflammatory mediators that are produced secondary to tissue trauma (Goodman et al., 1996).

Does Exogenous CO_2 Attenuate VALI During Conventional Ventilation Using High Tidalvolumes?

Compared to Conventional Normocapnia, adding inhaled CO_2 significantly reduced histologic lung injury, but did not reduce pulmonary edema, improve gas exchange, or enhance caspase-3 activation (**Figure 2**). Since both the Conventional Normocapnia and Conventional iCO_2 Hypercapnia groups were ventilated with a high V_T and $delta_{TP}$, a reduction in pulmonary edema and altered caspase-3 activity were not expected, which was in keeping with our findings from the first research question and the model of lung injury used in this study.

However, exogenous CO_2 in the Conventional iCO_2 Hypercapnia group significantly reduced pro-inflammatory cytokines in the systemic circulation (IL-1β) and in the pulmonary lavage fluid (IL-6 and MCP-1) (**Figures 3**, **4**). MCP-1 is a potent chemoattractant substance that recruits pro-inflammatory cells to the site of injury and can be leased by epithelial cells, monocytes, and endothelial cells to signal tissue injury (Deshmane et al., 2009). The decreased MCP-1 suggests that hypercapnia has the potential to reduce recruitment of monocytes and other immune cells (T-cells, macrophages and dendritic cells) to the injured lung. The reduction in pulmonary MCP-1 may be related to the decreased pulmonary IL-6, given that many of the recruited immune cells release an abundance of IL-6 to potentiate the local inflammatory response. Peltekova et al. (2010) also demonstrated a similar decrease in MCP-1 and IL-6 in a mouse model of lung injury treated with hypercapnia (Peltekova et al., 2010). A recent investigation in previously healthy pigs with unilateral ligation of the pulmonary artery (Marongiu et al., 2021) found that inhaled CO_2 5% prevented an inflammatory reaction otherwise developing in both lungs. Of note, the driving pressures were significantly reduced in the iCO_2 5% group. The alveolar and arterial CO_2 was double in the intervention group and ventilation-perfusion relationships (Va/Q) were not measured; it remains unclear whether the observed effects were predominately caused by the exogenous CO_2 or by a more favorable Va/Q allowing protective ventilation settings (hen-or-egg theorem) (Marongiu et al., 2021).

While cytokine "spillover" theories have previously been proposed as a gateway from respiratory failure to systemic organ failure (Plötz et al., 2004), the decreased systemic IL-1β in the Conventional iCO_2 Hypercapnia group is unlikely related to pulmonary IL-1β since it was not different from the Conventional Normocapnia group. It suggests that the reduced IL-1β in the systemic circulation may be related to the global effects of hypercapnia on tissues beyond the lung. Together, these findings suggest that hypercapnia can be protective even in the presence of ventilation conditions with excessive stretch and strain such as those with high tidal volume. Importantly, these results also support the notion that hypercapnia is more protective at the cellular level with its anti-inflammatory effects than at the level of tissue mechanics.

Does Exogenous CO_2 Attenuate VALI During Lung Protective Ventilation With Low Tidal Volumes?

Adding CO_2 to lung-protective ventilation with a low V_T in the Protective iCO_2 Hypercapnia group did not exert an additional benefit compared to Protective Normocapnia. The hypercapnia did not further reduce histologic lung injury or pulmonary edema, and did not enhance caspase-3 activity or improve gas exchange (**Figure 2**). Given that both groups were ventilated with low V_T (8 ml/kg) and the forces on the lung were equally limited, the groups only differed in the $PaCO_2$ target. As such, significant differences in VALI could not be expected. However, Protective iCO_2 Hypercapnia reduced plasma IL-1β concentrations compared to Protective Normocapnia, once again confirming the anti-inflammatory benefits of hypercapnia. Coupled with our findings from research question 3, these results indicate that hypercapnia may protect the lungs from pro-inflammatory chemical mediators independent of ventilation settings. However, it remains unclear how hypercapnia can attenuate systemic cytokines but not pulmonary cytokines if the exogenous CO_2 is applied to the lungs through ventilation. Taken together, these findings support the notion that hypercapnia likely plays a minor role in limiting mechanical damage but may play a key role at the intracellular level by attenuating the biotrauma (pro-inflammatory) component of VALI.

Is There an Added Effect if Hypercapnia Is Caused by Exogenous CO_2 Instead of Permissive Hypercapnia With Endogenous Rise in CO_2?

Our findings indicate that there is no added effect of hypercapnia by exogenous CO_2 (Protective iCO_2 Hypercapnia) compared to the hypercapnia by endogenous CO_2 observed in the Protective Endogenous Hypercapnia group. Exogenous CO_2 did not improve gas exchange, histologic lung injury, pulmonary

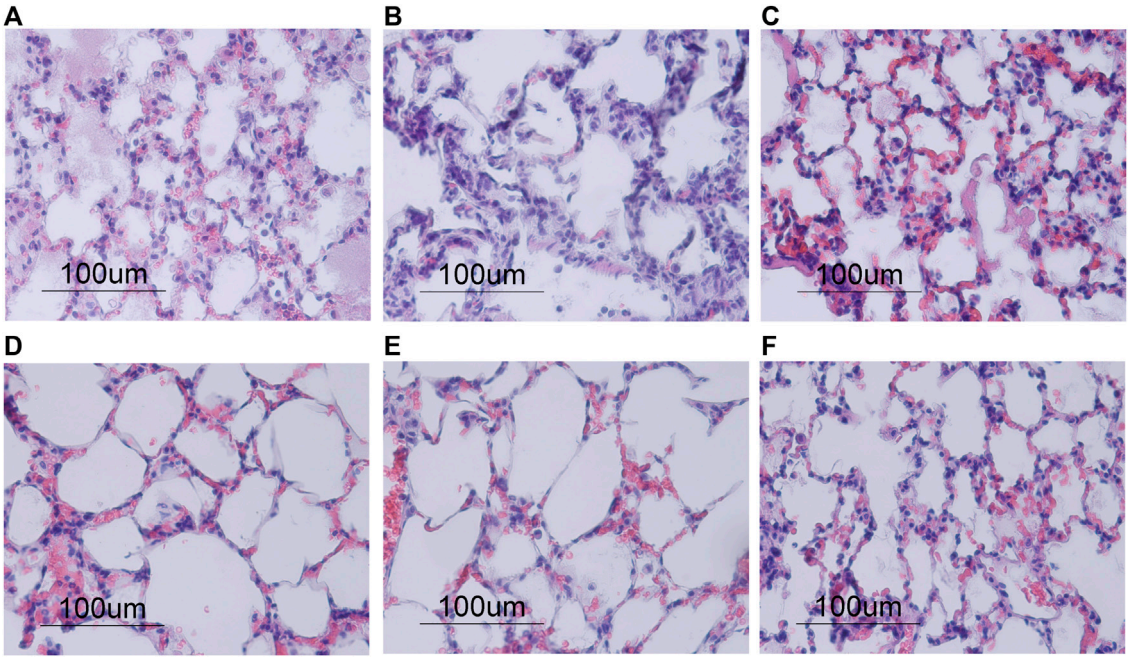

FIGURE 5 | Morphological changes in lung tissue stained with hematoxylin and eosin after 4 h of ventilation, shown in ×40 magnification. **(A–C)** Normocapnia groups showed an increase in polymorphonuclear (PMN) cell infiltration compared to the hypercapnia groups **(D–F)**. Conventional Normocapnia **(A)** and Conventional HV_E Normocapnia **(C)** showed significant pulmonary edema (e), PMN cell infiltrates (p), hemorrhages (h), with prominent hyaline membranes (hm) in Conventional HV_E Normocapnia **(C)**. Protective Normocapnia **(B)** showed a reduction in air space size (a). **(D–F)** Conventional iCO_2 Hypercapnia **(D)** and Protective iCO_2 Hypercapnia **(E)** showed larger and well-inflated air spaces (a) and less PMN infiltrates (p), however both produced hemorrhages (h). Protective EDG Hypercapnia **(F)** produced a loss in air spaces (a), some pulmonary edema (e) and PMN infiltrates (p).

edema or apoptotic activity (**Figures 2–5**). However, the significant reduction in liver histologic injury is a novel finding. Li et al. previously showed that exogenously induced hypercapnia improves liver histopathologic scores compared to normocapnia in a rat model of hepatic ischemia-reperfusion injury (Li et al., 2010). These findings further support the potentially important and perhaps poorly understood role of hypercapnia in attenuating histopathologic injury in the liver. Given that both groups were subject to equal $PaCO_2$ levels (achieved by different methods), the only difference was the somewhat lower pH in Permissive Hypercapnia. It is speculative to conclude and cannot directly be derived from our data that acidosis has an effect greater or independently from hypercapnia (Ismaiel and Henzler, 2011).

Limitations of the Study

While this study yielded important results that will contribute to better understanding the role of lung-protective ventilation and hypercapnia in attenuating VALI, it was not without its limitations. First, only male rats were used in this study, as this was traditionally believed to minimize the contribution of hormonal variability that can potentially result from including female rats in the study. However, it is possible that including only male rats may limit the generalizability of the results of this study. Second, the current experimental model of ALI in this study by endotracheal instillation of HCl (Imai et al., 2003; Henzler et al., 2011) simulates the aspiration of gastric contents that can often lead to ALI, though the authors acknowledge that ALI induced or aggravated by mechanical ventilation may have a higher incidence. In addition, it is possible that the short duration of ventilation (a total of 4 h, 1 h to establish acute lung injury *per definitionem* and 3 h in each respective group) may not have been long enough to identify additional benefits of the different ventilation modalities, especially in looking for an improvement in gas exchange. However, several previous investigations have shown improvements in gas exchange, inflammatory parameters and lung damage after 4 h of ventilation (Chiumello et al., 1999; Sinclair et al., 2002). In our study, a 4-h ventilation period was sufficient to detect early anti-inflammatory changes in cytokine profiles and lung damage, but not improvements in gas exchange. It's possible that a 6-h ventilation period could have unveiled more benefits of lung-protective ventilation and hypercapnia (Laffey et al., 2004).

Another limitation of our study may be related to the markers used to quantify the protective and beneficial effects of hypercapnia and hypercapnic acidosis. We have primarily investigated the changes in the cytokine profiles in the BALF and plasma, and caspase-3 activity in lung homogenates. It is possible that performing additional experiments to quantify free radical and reactive oxygen species production via changes in xanthine oxidase activity could have offered additional information about the protective and anti-inflammatory benefits of hypercapnia and hypercapnic acidosis, which had already been demonstrated before (Shibata et al., 1998).

Finally, the findings of our study may be limited to the ventilation settings, specifically those related to the $PaCO_2$ targets. It is possible that the $PaCO_2$ targets for normocapnia and hypercapnia were not distinct enough, and that the normocapnia target range (40–55 mmHg) may have been too high for normocapnia and likely approaching hypercapnia. Similar $PaCO_2$ targets have been used for normocapnia, although the $PaCO_2$ targets for hypercapnia were almost double those used in the present study (Peltekova et al., 2010). Similarly, V_T appeared to be higher in Conventional Normocapnia vs Hypercapnia, although these differences were not significant. We cannot rule out the possibility that the observed differences in biological injury were influenced by differences in V_T as well. We chose 8 ml/kg as the target volume for our low V_T groups for experimental reasons, although several data suggest 6–8 ml/kg predicted body weight as a safe range of tidal volumes for most protective settings (Vaneker et al., 2007; Güldner et al., 2015). However, there is no experimental data suggesting that 8 ml/kg is more protective than 6 ml/kg and the concept of using predicted body weight instead of actual body weight is cumbersome in rodent research. Choosing V_T was a compromise between prevention of volutrauma and mechanical power that would have increased with even higher RR to achieve the $PaCO_2$ targets. Although the differentiation between V_T and driving pressure as to the main protective mechanism remains controversial (Amato et al., 2015), it might have been possible to detect more significant differences between our low V_T and high V_T groups if we compared 6 ml/kg vs 10–12 ml/kg.

CONCLUSION

The aim of this study was to explore five key research questions about the role of hypercapnia in the prevention of VALI. Our findings indicate that there are two distinct mechanisms attached to protective ventilation in the attenuation of VALI: the limitation of mechanical damage and the limitation of biological damage. These mechanisms interact during mechanical ventilation to maximize protection against VALI. Consistent with previous investigations we found that mechanical damage is best attenuated by limiting tidal volume and transpulmonary driving pressures, to minimize excessive stretch and strain. The combined reduction of these mechanical mechanisms is believed to minimize traumatic cell death and promote apoptosis in the lung. The therapeutic effects of hypercapnia appear to extend beyond the lungs to distant organs, where the spontaneous rise in CO_2 attenuated histologic liver injury with permissive hypercapnia. These findings suggest that combining ventilation strategies that integrate lung-protective settings that limit tidal volumes, minute ventilation and driving pressures with permissive hypercapnia as an accepted side effect may offer the best protection against VALI. Lung-protective ventilation may limit mechanical damage to the lung, while hypercapnia attenuates VALI by limiting pro-inflammatory and biochemical mechanisms of injury. When combined, both have the potential to exert a synergistic effect for prevention of VALI and its systemic effects. Our results are encouraging and hold clinical implications for the future of research on VALI and clinical practice as the medical community makes the leap from the bench to the bedside.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available but physiological and biological data from animal study will be made available by reasonable request. Requests to access the datasets should be directed to DH, mail@d-henzler.de.

ETHICS STATEMENT

The animal study was reviewed and approved by Dalhousie University Committee on Laboratory Animals.

AUTHOR CONTRIBUTIONS

NI performed experimental work, participated in the analysis and prepared the manuscript. SW participated in experimental work and organization of animal studies. LG performed anatomical analysis. ZX performed anatomical analysis of lung tissue AS participated in cytokine analyses and reviewed the manuscript. VC participated in chemical and protein analyses. DH conceived the study, participated in analysis of data and writing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.814968/full#supplementary-material

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REVIEW
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The Physiological Basis of High-Frequency Oscillatory Ventilation and Current Evidence in Adults and Children: A Narrative Review

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High-frequency oscillatory ventilation (HFOV) is a type of invasive mechanical ventilation that employs supra-physiologic respiratory rates and low tidal volumes (V_T) that approximate the anatomic deadspace. During HFOV, mean airway pressure is set and gas is then displaced towards and away from the patient through a piston. Carbon dioxide (CO_2) is cleared based on the power (amplitude) setting and frequency, with lower frequencies resulting in higher V_T and CO_2 clearance. Airway pressure amplitude is significantly attenuated throughout the respiratory system and mechanical strain and stress on the alveoli are theoretically minimized. HFOV has been purported as a form of lung protective ventilation that minimizes volutrauma, atelectrauma, and biotrauma. Following two large randomized controlled trials showing no benefit and harm, respectively, HFOV has largely been abandoned in adults with ARDS. A multi-center clinical trial in children is ongoing. This article aims to review the physiologic rationale for the use of HFOV in patients with acute respiratory failure, summarize relevant bench and animal models, and discuss the potential use of HFOV as a primary and rescue mode in adults and children with severe respiratory failure.

Keywords: mechanical ventilation (lung protection) strategy, high-frequency ventilation with oscillations, high-frequency ventilation, children, ARDS, review (article), lung injury

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is a disease of acute onset characterized by significant hypoxemia and typical radiographic findings that affects both children and adults, and is an important cause of morbidity and mortality worldwide (Force et al., 2012; Pediatric Acute Lung Injury Consensus Conference, 2015; Thompson et al., 2017; Khemani et al., 2019). Whether pulmonary injury is the result of a direct (e.g., pneumonia, smoke inhalation, lung contusion) or indirect (e.g., sepsis, blood transfusion) insult, disease distribution in ARDS is heterogeneous, with more severe involvement of the dependent and relative sparing of the non-dependent lung regions (Ware and Matthay, 2000; Gattinoni et al., 2017b). This heterogeneous distribution of lung disease poses a challenge to the clinician instituting positive pressure ventilation, as different areas of the

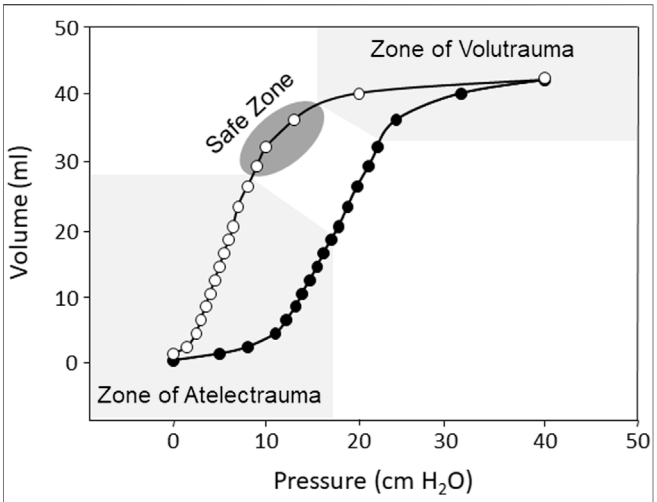

FIGURE 1 | Representation of the inspiratory (black circles) and expiratory (white circles) static pressure-volume curves from a rabbit saline lavage model of ARDS showing hysteresis between the inspiratory and expiratory curves, the zone of volutrauma and atelectrauma (light gray), and the theoretical safe zone of ventilation (dark gray).

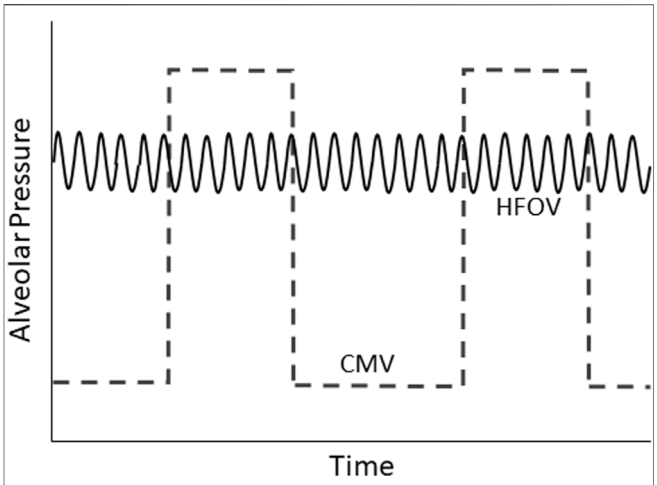

FIGURE 2 | Schematic representation of alveolar pressure over time during conventional mechanical ventilation (CMV) and high-frequency oscillatory ventilation (HFOV).

lung will have vastly different compliance and resistance. Non-dependent or uninjured alveoli (with better compliance) are at risk of overdistension, while dependent or injured alveoli (with worse compliance) are at risk of de-recruitment and repeated opening and closing with each respiratory cycle (Ware and Matthay, 2000; Gattinoni et al., 2017b).

When precisely employed, mechanical ventilation (MV) is a life-saving intervention, yet care must be taken to avoid ventilator-induced lung injury (VILI) (Tremblay and Slutsky, 2006; Beitler et al., 2016). Several factors have been identified as contributors to VILI. These include injury from the delivery of excessive pressure (barotrauma or stress) or tidal volume (V_T) (volutrauma or strain), injury from the cyclic opening and closing of alveoli (atelectrauma), toxicity caused by high inspired fraction of inspired oxygen (FiO_2), and injury resulting from cytokine release that can affect end-organ function (biotrauma) (Tremblay and Slutsky, 2006; Beitler et al., 2016). The landmark ARMA trial comparing mechanical ventilation with a tidal volume of 12 ml/kg to 6 ml/kg (both calculated using predicted body weight) found a significant mortality benefit with the application of a lower V_T strategy and confirmed the role of high V_T in VILI (Acute Respiratory Distress Syndrome et al., 2000). This study renewed interest in high-frequency oscillatory ventilation (HFOV) as an ultra-protective lung protective strategy capable of delivering very low V_T. MV strategies aimed at avoiding VILI are termed "lung protective," and generally operate in a theoretical "safe zone" on the deflating limb of the static pressure/volume curve (**Figure 1**).

A study by Amato et al., published in 2015, elegantly illustrated the direct association between driving pressure (plateau pressure minus measured PEEP) and mortality in ARDS (Amato et al., 2015). Subsequent studies have also implicated driving pressure as a key variable that is associated with mortality in ARDS (Guerin et al., 2016; Goligher et al., 2021). Recent studies have highlighted the role of total energy delivered during each tidal breath to be an important factor in VILI (Gattinoni et al., 2017a). This concept, referred to as mechanical power, incorporates all mechanical ventilator settings, including respiratory rate, driving pressure, PEEP, and inspiratory flow (Gattinoni et al., 2017a). Mechanical power is an appealing concept because it accounts for the energy required to distend the lung, move gas, and maintain lung volume (Gattinoni et al., 2017a). The lung injury resultant from energy transmission from the various elements that determine mechanical power is termed ergotrauma. Indeed, mechanical power and the resultant ergotrauma have been directly associated with unfavorable outcomes in both adults and children with ARDS (Costa et al., 2021; Bhalla et al., 2022). A recent study found only respiratory rate and driving pressure to be independent predictors of mortality among variables included in the calculation for mechanical power (Costa et al., 2021). The simplified equation using these variables for estimation of mechanical power [$(4 \times \Delta P) + RR$] had a similar predictive value for mortality as mechanical power calculated using the more complex original method (Costa et al., 2021). Thus, mechanical power is an intriguing concept, but its utility as a modifiable parameter needs confirmation in a clinical trial.

Although lung protective ventilation can certainly be achieved through carefully conducted conventional mechanical ventilation (CMV), the lower V_T, lower alveolar pressure swings, and higher mean airway pressure (mPaw) generally employed during various forms of high frequency ventilation make these modalities theoretically well suited for lung protection (**Figure 2**). There are four types of high frequency ventilation in clinical use: HFOV, high frequency jet ventilation, high-frequency percussive ventilation, and high frequency flow interruption (Keszler et al., 2015; Miller A. G. et al., 2021). This review will focus on the physiologic rationale for the use of HFOV in patients with

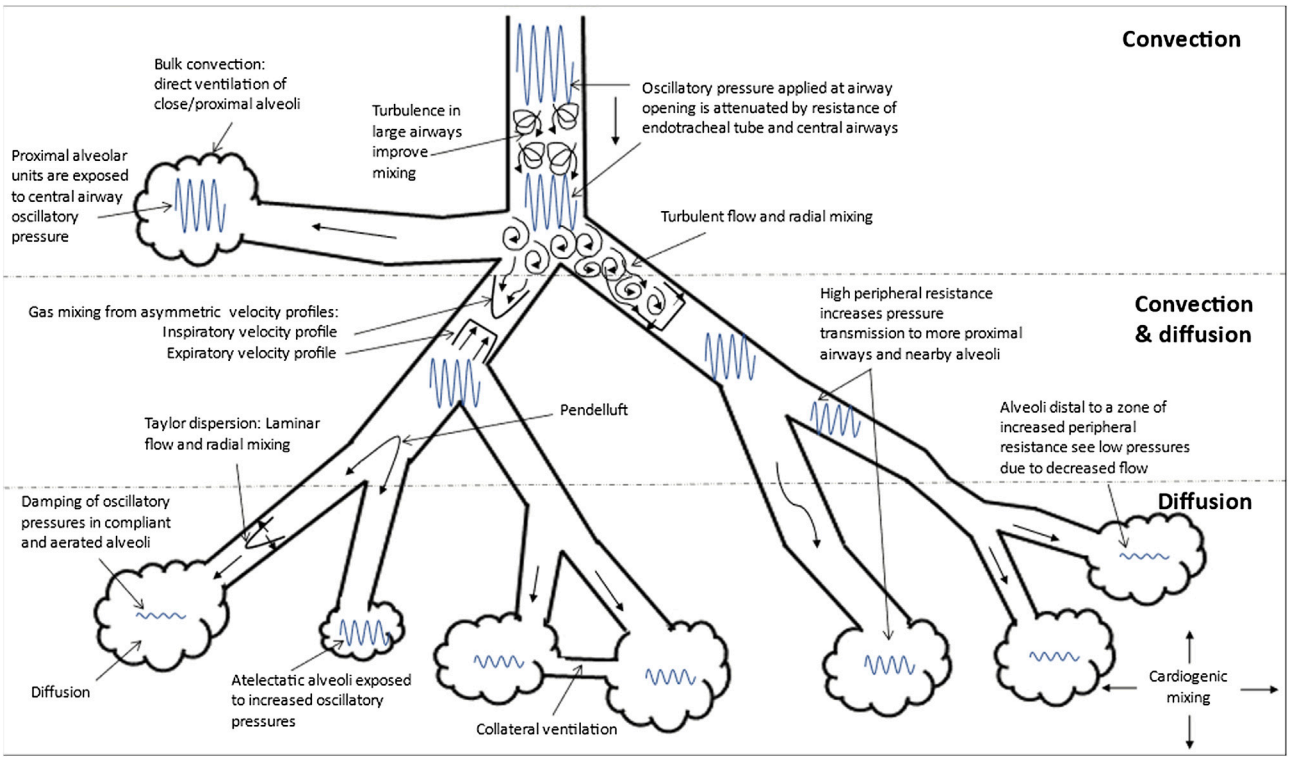

FIGURE 3 | Gas Transport Mechanisms During High Frequency Oscillatory Ventilation (HFOV). Adapted from references: (Slutsky and Drazen, 2002; Pillow, 2005). The gas exchange mechanisms that function in each region (convection, convection and diffusion and diffusion alone) are shown. The various mechanisms that contribute to gas transport during HFOV are: 1) turbulence in large airways producing improved mixing; 2) bulk convection (direct ventilation of close alveoli); 3) turbulent flow with lateral convective mixing; 4) pendelluft (asynchronous flow among alveoli due to asymmetries in airflow impedance); 5) asymmetric inspiratory and expiratory velocity profiles (gas mixing due to velocity profiles that are axially asymmetric resulting in streaming of fresh gas toward alveoli along the inner wall of the airway and the streaming of alveolar gas away from the alveoli along the outer wall); 6) Taylor dispersion (laminar flow with lateral transport by diffusion); 7) collateral ventilation through non-airway connections between neighboring alveoli; and 8) cardiogenic mixing (rhythmic, pulsatile nature of the heart conferring a mixing of gases). The extent to which the oscillatory waveform is attenuated is also shown in this figure. Atelectatic alveoli will experience higher oscillatory pressure and lesser damping compared to normally aerated alveoli. Increase in peripheral resistance, other the other hand increase pressure transmission to more proximal airways and nearby alveoli such that alveoli distal to this zone of increased peripheral resistance experience lower pressures due to decreased flow.

acute respiratory failure, summarize data from relevant bench and animal models, and discuss the potential use of HFOV as a primary and rescue mode in adults and children with severe respiratory failure.

Theory of HFOV Operation

HFOV is a form of MV that uses a constant distending pressure, usually reported as the mPaw, coupled with sinusoidal or square flow oscillations at supra-physiologic respiratory frequencies (Rettig et al., 2015; Miller A. G. et al., 2021). Respiratory frequencies used in clinical practice range from 5 to 15 Hz (i.e., 300 to 900 breaths per minute) with a small delivered V_T, generally around 1–3 ml/kg, or lower than the anatomic dead space (Rettig et al., 2015; Miller A. G. et al., 2021). The constant distending pressure allows for alveolar recruitment while avoiding repetitive opening and closing of alveoli (atelectrauma), and has been shown to improve oxygenation (Rettig et al., 2015; Meyers et al., 2019). HFOV may also decrease the occurrence of volutrauma and barotrauma (Rettig et al., 2015).

HFOV differs from CMV and high frequency jet ventilation in that both inspiration and expiration are active (Miller A. G. et al., 2021; Miller A. G. et al., 2021). Oxygenation and ventilation are fairly independent during HFOV with oxygenation being controlled by FiO_2 and mPaw while ventilation is controlled by V_T (amplitude) and frequency (f) (Kneyber et al., 2012; Miller A. G. et al., 2021). Various mechanisms contribute to gas exchange during HFOV; these include gas flow turbulence in large airways, bulk convection, turbulent flow with radial mixing, pendelluft, asymmetric inspiratory and expiratory velocity profiles, Taylor dispersion, collateral ventilation, and cardiogenic mixing (Slutsky and Drazen, 2002; Pillow, 2005) (**Figure 3**).

HFOV Mechanics

The mechanism of gas exchange during HFOV varies according to the method of oscillation generation, attenuation of the pressure waveform, and efficiency of volume delivery (Pillow et al., 2001; John et al., 2014). HFOV produces biphasic pressure waveforms and diverts fresh bias flow to the patient at frequencies

TABLE 1 | Characteristics of various high frequency oscillatory ventilators.

	Sensormedics 3100A	Sensormedics 3100B	Metran R100	Fabian HFO	Leoni plus	Stephan sophie	Sle 5000	Sle 6000	Drager babylog VN500
Principle of operation	Piston HFOV Oscillations generated by electro-magnetic diaphragm moving back and forth, similar to a permanent magnet loudspeaker		Oscillations provided from a diaphragm on the anterior side of the ventilator which is driven by a rotary valve mechanism	Oscillations generated by piston pushing a diaphragm back and forth (diaphragm principle)	Oscillations generated by piston pushing a diaphragm back and forth (diaphragm principle)	Valve oscillator with active exhalation	Non-piston HFOV Oscillations achieved by active exhalation, and rapid cycling of the forward and reverse jets		Oscillations generated by intermittent negative pressure generated by a venturi effect of the high-flow jet injector at the expiratory valve causing exhalation
Mode	HFOV only		CMV and HFOV	CMV and HFOV	CMV and HFOV	CMV and HFOV	CMV and HFOV		CMV and HFOV
Patient population	All, 3100A for patients <35 kg		CMV: VCV for neonates >8 kg HFOV: For infants, pediatrics and adults (upper limit of body weight not specified)	Up to 30 kg	Up to 30 kg	Up to 25 kg	SLE 5000: Up to 20 kg SLE 6000: Up to 30 kg		CMV: neonates, pediatrics and adults HFOV: up to 10 kg
Volume-targeted mode	No		No	Yes	Yes	Yes	Yes		Yes
V_T monitoring	No		V_T monitoring during CMV only	Hot-wire anemometer	Hot-wire anemometer	Hot-wire anemometer	Hot-wire anemometer		Hot-wire anemometer
Flow	0–40 L/min	0–60 L/min	10–40 L/min	5–20 L/min (neonatal, ≤10 kg) 5–30 L/min (pediatric, 10–30 kg)	7 L/min	0.2–10 L/min	8 L/min		2–30 L/min
Pressure amplitude setting range	1–90 cm H_2O		Pressure amplitude is a measured value. Stroke volume is set instead Stroke volume of 2–350 ml. (5 Hz: 14–350 ml. 10 Hz: 6–160 ml. 15 Hz: 2–100 ml) 5–100% (depth of oscillations expressed as percentage swing—peak to trough—around MAP)	4–80 cm H_2O	5–100 cm H_2O	5–100% (depth of oscillations expressed as percentage swing—peak to trough—around MAP)	4–160 cm H_2O	4–180 cm H_2O	5–90 cm H_2O
Mean airway pressure setting range	3–45 cm H_2O	3–55 cm H_2O	5–60 cm H_2O	5–50 cm H_2O	0–40 cm H_2O	0–30 cm H_2O	0–45 cm H_2O		5–50 cm H_2O
Frequency setting range	3–15 Hz		5–15 Hz	5–20 Hz	5–20 Hz	5–15 Hz	3–20 Hz		5–20 Hz
Inspiratory: Expiratory ratio	1:1 and 1:2		1:1	1:1 to 1:3	1:1 to 1:3	1:1 to 1:2	1:1 to 1:3		1:1 to 1:3

greater than 3 Hz (Keszler et al., 2015). The matching of positive and negative pressure deflections results in both inspiratory and expiration phases being active and can be achieved by a linear motor piston pump, an electromagnetically-driven vibrating diaphragm device, or an expiratory venturi jet (Keszler et al., 2015).

HFOV can be delivered via dedicated HFOV ventilator [e.g., Sensormedics 3100A and 3100B (Carefusion, Yorba Linda, California, United States)], or hybrid ventilators. Available HFOV ventilators and their mechanism of action are summarized in **Table 1**. Dedicated HFOV and hybrid ventilators differ in how they generate oscillations, ability to measure V_T, availability of volume-targeted mode, range of settings for flow, pressure amplitude, frequency, and I:E ratio (**Table 1**) (Grazioli et al., 2015; Keszler et al., 2015; Tingay et al., 2015).

Oxygenation during HFOV is directly correlated with alveolar recruitment (i.e. alveolar surface area available for gas exchange), which is controlled largely by mPaw (Meyers et al., 2019). Optimizing mPaw strikes a balance between avoidance of de-recruitment and overdistension (Meyers et al., 2019).

Ventilation efficiency during HFOV (Q) can be expressed as (Slutsky et al., 1980; Boynton et al., 1989; Pillow, 2005):

$$Q = f \times V_T^2$$

V_T is inversely proportional to frequency and directly proportional to amplitude (Sedeek et al., 2003; Miller A. G. et al., 2021). Therefore, the higher the frequency, the lower the V_T; the higher the amplitude, the higher the V_T (Sedeek et al., 2003; Miller A. G. et al., 2021). Similarly, a higher inspiratory time percentage results in increased ventilation due to higher V_T (Miller A. G. et al., 2021). However, with the commonly used inspiratory time percentage of 33% [inspiratory (I) to expiratory (E) ratio of 1:2], the effect of decreasing amplitude has a greater impact on decreasing V_T compared to increasing frequency (Sedeek et al., 2003). In contrast, with the inspiratory percentage set at 50% (I:E of 1:1), changes in frequency have a more pronounced effect on delivered V_T compared to changes in amplitude (Sedeek et al., 2003). Ventilation is also affected by endotracheal tube (ETT) length and diameter, presence of a leak around the ETT, airway resistance, and respiratory system compliance (Van de Kieft et al., 2005). V_T has been demonstrated in theoretical models, animals, and humans to have a greater effect on gas exchange than frequency during HFOV (Boynton et al., 1989; Pillow, 2005).

Diameter and length of the ETT affect V_T delivery during HFOV. An increase in resistance is observed as ETT diameter deceases and as the length of the ETT increases, resulting in smaller delivered V_T (Pillow et al., 2002; Van de Kieft et al., 2005; Custer et al., 2011). Creation of a cuff leak increases inhaled V_T but reduces exhaled V_T, and moves the source of fresh gas more distally towards the tip of the ETT (Van de Kieft et al., 2005; Van de Kieft et al., 2005; Bostick et al., 2012a). The Sensormedics 3100B has been shown to generate negative pressure during the exhalation and entrain CO_2 into the inspiratory limb of the circuit, which can be reduced with the creation of cuff leak (Bostick et al., 2012a). Lastly, in a bench and clinical study, V_T was found to be higher and CO_2 elimination greater when the piston position for the Sensormedics 3100A was displaced towards the left compared to when the piston was in the center or displaced to the right for any given amplitude, frequency and inspiratory time (Hamel et al., 2005).

Airway resistance and compliance affect CO_2 clearance during HFOV (Kneyber et al., 2012). The amplitude of the tracheal oscillatory pressure waveform decreases with increasing peripheral resistance, resulting in reduction of transmission of pressure over the airways to the alveoli (van Genderingen et al., 2001; Pillow, 2005; Kneyber et al., 2012). The opposite happens with reduced compliance, in which there is increase in pressure transmission to the alveoli and bronchi (van Genderingen et al., 2001; Pillow, 2005; Kneyber et al., 2012). Hence pressure transmission to the alveoli is the highest in patients with low compliance and low resistance.

Spontaneous breathing during HFOV may improve oxygenation and ventilation (van Heerde et al., 2009; van Heerde et al., 2010; Kneyber et al., 2012). However, due to limitations on maximal bias flow delivered during HFOV, spontaneous breathing may be challenging for older children who have higher inspiratory flow demands than the bias flow being delivered by the ventilator, resulting in an imposed increased work of breathing (van Heerde et al., 2006; Kneyber et al., 2012; Bordessoule et al., 2018). Hence, while neonates often tolerate spontaneous breathing during HFOV without the need for deep sedation or neuromuscular blockade, that is generally not the case for children and adults (Kneyber et al., 2012; Bordessoule et al., 2018).

While the physiology of gas exchange is similar between adults and children, some important differences exist between the two. Infants and children have shorter time constants, with resulting differing HFOV setting requirements. In general, children are managed with higher frequencies compared to adults, although significant variation in management exists (Arnold et al., 2000; de Jager et al., 2019). Thus, an infant on HFOV may be managed on a frequency of 10–12 Hz while an adult or larger child may require a frequency of 5–8 Hz. However, this will vary depending on the HFOV strategy used, severity of lung injury, and lung mechanics.

In clinical practice, oxygenation is managed by adjusting the mPaw or the fraction of inspired oxygen (FiO_2). If oxygenation is below goal, the mPaw generally is increased in 1–2 cmH_2O until oxygenation improves. Some centers may also employ a recruitment maneuver (e.g., rapid increase in mPaw by 10–15 cmH_2O for 30–60 s) to improve oxygenation; although this strategy can result in hemodynamic compromise. Other strategies include incremental recruitment/decruitment maneuvers to find the optimal mPaw (de Jager et al., 2019). Paradoxically, excessive mPaw may result in worsening of oxygenation due to overdistension and in some cases a trial of decreased mPaw may be warranted. If oxygenation is above goal and the FiO_2 is already within a non-toxic range (i.e., ≤0.50) mPaw generally is decreased in steps of 1–2 cmH_2O as part of a weaning strategy. The frequency of adjustments is dependent upon patient characteristics and local practice.

Ventilation is controlled by the pressure amplitude and frequency (respiratory rate). To increase ventilation and decrease $PaCO_2$, the amplitude can be increased or the frequency decreased. Different strategies are used depending on local practice, with some centers using a fixed frequency (higher frequencies being most protective) while adjusting amplitude to affect $PaCO_2$, some maximize amplitude and use frequency as the main variable affecting ventilation, and others use a combination of the two strategies. The ideal method is unknown as direct comparisons have not been performed. Similar to oxygenation, overdistension may impair ventilation while lung recruitment can result in increased CO_2 clearance without changes to frequency or amplitude.

Evidence for HFOV

Bench Models

Bench models of HFOV have demonstrated that adjustments to frequency have a larger effect on delivered V_T than changes to amplitude (Van de Kieft et al., 2005; Wong et al., 2017). For instance, a 2 Hz increase in frequency results in a 21% decrease in V_T, while a 10 cm H_2O increase in pressure amplitude is necessary for an equivalent decrease in V_T (Hager et al., 2007). When studied in patients, increasing frequency by 2 Hz decreased V_T by 23%, while increasing amplitude by 10 cmH_2O resulted in a 5.6% increase in V_T (Hager et al., 2007). V_T delivery also decreases as ETT size is reduced, due to the higher resistance across the smaller tube. Increasing bias flow from 20 to 30 L/min increases V_T by 11% but this relationship is not linear; further increasing the bias flow from 30 to 40 L/min only results in a 3% increase in V_T (Hager et al., 2007). Increasing bias flow has been shown to improve CO_2 clearance up to 30 L/min (Nagano et al., 2018). CO_2 clearance is most efficient with the R100 at 50% inspiratory time and least efficient with the 3100B at an inspiratory time 33% (Yumoto et al., 2019).

In the R100 ventilator with a 50% inspiratory time, V_T was lower with smaller ETTs and higher frequencies (Hirao et al., 2009). When comparing V_T delivery between the R100 and 3100B, V_T was higher at similar settings with the R100 but comparable at 9 Hz and when inspiratory time was set at 50% on the 3100B (Iguchi et al., 2010). Other studies have found similar differences in V_T delivery. s(Custer et al., 2011). Pressure delivery is attenuated when I:E is set at 1:2 using the 3100B (Hirayama et al., 2014).

Pendelluft has been observed between lung units, largely occurring during expiration (Lee et al., 2006). An adult bench model found negative pressure within the inspiratory limb of the circuit and CO_2 rebreathing that became detectable when amplitude was >70 cmH_2O and continued to increase as amplitude increased. CO_2 rebreathing was eliminated by instituting an ETT cuff leak and increasing bias flow (Bostick et al., 2012b). Pressure amplitude significantly decreases throughout the respiratory system and this pressure attenuation is directly proportional to resistance increase and inversely proportional to compliance (Rozanek et al., 2012).

A computational model found that the resonant frequency of the non-injured lung is 8 Hz while the injured lung has a resonant frequency of 17 Hz (Herrmann et al., 2016). Due to the heterogeneous nature of disease distribution in most patients, individual lung sections may have different optimal frequencies, making the frequency section challenging in clinical practice (Herrmann et al., 2016).

Animal Studies

Early animal models provided significant insights into the mechanisms of HFOV and were well summarized in a prior review (Kacmarek and Malhotra, 2005). These early models indicated that HFOV settings required to provide normocapnia were determined by $(V_T{*}f)\ n = 0.73{*}W{*}(V_T/V_L)^{-1.1}$ and that the $f{*}\ V_T$ during HFOV was higher than during CMV (Kacmarek and Malhotra, 2005). Early experiments also found that partial pressure of arterial CO_2 ($PaCO_2$) was held constant at different I:E ratio if mean lung volume and V_T were held constant up to a frequency of 9 Hz. This is due to gas velocity profiles being unaffected by bulk flow rate. Additional models found that as V_T increases, gas transport changes from dispersion (less efficient) to bulk gas flow (convection). Another study found that V_T *f remained constant with frequencies of 3, 6, and 9 Hz. Regional gas distribution was most homogenous at 9 Hz compared to CMV and to lower frequencies. The ability to adequately exchange gas with HFOV was demonstrated by Bohn et al., in 1980 (Bohn et al., 1980). Another seminal study in HFOV found that V_T *f was not the only determinant of CO_2 clearance and that Taylor laminar and turbulent dispersion, pendelluft and asymmetrical velocity profiles were also factors. Early animal studies also showed that V_T was directly related to amplitude and inversely proportional to frequency (Hz) (Kacmarek and Malhotra, 2005).

Studies using animal models have consistently found improved oxygenation with HFOV (Meyer et al., 2006; Ronchi et al., 2011; Li et al., 2015; Fioretto et al., 2019). However, HFOV did not offer an advantage over protective CMV for various markers of lung injury, both in a rabbit model of lung lavage (Rotta et al., 2001) or acid aspiration (Allardet-Servent et al., 2008). In an *ex-vivo* rabbit model of air leak, both stroke volume and mPaw influenced air leak flow, but mPaw appeared to be the main independent driver of air leak (Liu et al., 2007). Circuit disconnection was evaluated in a tween pig model of lung injury showing that disconnections resulted in worsening compliance and increase in FiO_2 requirement, with these effects persisting over time (Kubiak et al., 2010). A study evaluating stepwise decreases in mPaw in a tween pig model of lung injury found that the titrated HFOV group had more atelectasis, fibrin, congestion, PMN invasion, and regional overdistension (Maggio et al., 2010).

In a rat model of saline lavage or lung injury from lipopolysaccharide administration, HFOV use resulted in decreased lung inflammation compared to CMV with low PEEP, but was similar to the decrease in inflammation observed in CMV with optimized PEEP (Krebs et al., 2010). A saline lavage model of sheep found that transpulmonary pressure was lowest at 9 Hz, which coincided with the lowest degree of lung inflammation (Liu et al., 2013). A porcine model of oleic acid lung injury found greater lung strain at lower frequency, with the

lowest strain noted at a frequency of 20 Hz (Herrmann et al., 2020).

HFOV and protective MV have similar hemodynamic effects (Roosens et al., 2006). HFOV improves oxygenation without significant depression of cardiac function (Nakagawa et al., 2007). It also did not have deleterious effects on cerebral and systemic hemodynamics in a porcine model when mPaw was set 5 cmH_2O above the CMV mPaw (Heuer et al., 2012). A porcine model of saline lavage found that mean arterial pressure and cardiac output increased during a decremental mPaw maneuver while central venous and wedge pressure decreased (Liu et al., 2020).

A study in pigs using saline lavage to cause severe lung injury found that normocapnia could not be achieved by HFOV or conventional CMV without extracorporeal CO_2 removal (Brederlau et al., 2007). A similar study also found that high frequency improved lung recruitment (Muellenbach et al., 2008). HFOV with extracorporeal membrane oxygenation (ECMO) has been shown to attenuate lung inflammation in a saline lavage pig model compared to a pressure control strategy with a V_T of 6 ml/kg (Muellenbach et al., 2010).

Spontaneous breathing during HFOV was evaluated using a custom demand flow valve in a saline lavage pig model of lung injury and found improved gas exchange with spontaneous breathing (van Heerde et al., 2009), possibly by shifting ventilation to more dependent lung zones (van Heerde et al., 2010). Transpulmonary pressure monitoring may help identify the lowest mPaw required to improve oxygenation and may result in fewer hemodynamic adverse effects of HFOV that occur when higher than necessary mPaw is employed (Karmrodt et al., 2006; Klapsing et al., 2018).

Adult Evidence

Case Series and Observational Studies

Early case series of HFOV in adults reported improvements in oxygenation with variable effects on hemodynamics (Fort et al., 1997; Claridge et al., 1999; Mehta et al., 2001). Subsequent studies confirmed these results, but most enrolled less than 50 subjects (Fort et al., 1997; Claridge et al., 1999; Mehta et al., 2001; Andersen et al., 2002; David et al., 2003; Mehta et al., 2004; Ferguson et al., 2005; Finkielman et al., 2006; Pachl et al., 2006; Fessler et al., 2008; Kao et al., 2011; Niwa et al., 2011; Camporota et al., 2013; Jog et al., 2013; Naorungroj et al., 2015; Thind et al., 2021). HFOV has also been described in three small case series of burn patients involving 6 to 30 subjects with mortality between 32 and 83% (Cartotto et al., 2001; Cartotto et al., 2004; Cartotto et al., 2009). Two of these studies showed an improvement in arterial partial pressure of oxygen (PaO2)/FiO2 (P/F) during HFOV (Cartotto et al., 2001; Cartotto et al., 2004), but oxygenation index (OI) only improved in subjects without inhalation injury (Cartotto et al., 2009). HFOV has also been used successfully in patients with elevated intracranial pressure (David et al., 2005), chronic obstructive pulmonary disease exacerbation failing CMV (Frerichs et al., 2012), and in conjunction with a extracorporeal CO_2 removal (Lubnow et al., 2010).

The two largest case series included 156 (Mehta et al., 2004) and 102 (Camporota et al., 2013) subjects, with mortality rates of 63 and 48%, respectively. The first, published in 2004, found improvements in oxygenation during HFOV, and that mortality was associated with delayed HFOV initiation (Mehta et al., 2004). The second was published in 2013 and showed higher survival to be associated with younger age, greater initial improvement in P/F, and lower illness severity (Camporota et al., 2013).

While most studies have set the HFOV mPaw 3–5 cmH_2O above the CMV mPaw, others have evaluated strategies to optimize lung volumes and set optimal mPaw (Ferguson et al., 2005). The TOOLs study evaluated the combination of HFOV and recruitment maneuvers in 25 adults with early ARDS. A recruitment maneuver (40 cmH_2O for 40 s) was performed and mPaw was increased until F_IO_2 was <0.60, then targeted between 30 and 22 cmH_2O before decreasing F_IO_2 (Ferguson et al., 2005). This resulted in significant improvements in P/F and OI, and ICU mortality was 44%. The recruitment maneuvers were well-tolerated, with 3.3% were stopped due to hemodynamic instability (Ferguson et al., 2005). Casserly et al. evaluated a method to determine the optimal mPaw in seven subjects and assessed changes in end-expiratory lung volume by measuring chest wall dimensions (Casserly et al., 2013). After a recruitment maneuver (40 cmH_2O for 40 s), the mPaw was set at 35 cmH_2O for 15 min, then reduced by 2.5 cmH_2O every 15 min until the PaO_2 was <60 mmHg or mPaw was 15 cmH_2O. Lung volume was found to increase in a sigmoid shape, as did PaO_2, although $PaCO_2$ had a U-shaped curve as mPaw increased (Casserly et al., 2013).

In a study of 131 subjects with 60% mortality, HFOV was associated with significant increases in fentanyl, midazolam, and cisatracurium use, but no increase in propofol use over the first 4 days (Burry et al., 2013).

An early crossover study of HFOV in 16 adults with severe ARDS found that HFOV resulted in worsening right ventricular function and decreased cardiac index once mPaw was >5 cmH_2O above the mPaw on CMV (Guervilly et al., 2012). Another study in 12 adults with ARDS found improvement in P/F with HFOV and tracheal insufflation with no difference in cardiac index and higher central venous saturation (Vrettou et al., 2014). The relationship between mPaw and esophageal pressure has been shown to be linear and highly correlated with set mPaw (Guervilly et al., 2016).

A crossover trial evaluated short-term prone positioning during HFOV compared to supine/prone CMV. Patients undergoing CMV had the PEEP set 2 cmH_2O above the lower inflection point of the pressure-volume curve, while those undergoing HFOV had the mPaw was set 5 cmH_2O above the CMV mPaw. Prone positioning improved P/F in both groups, but P/F did not improve during HFOV when patients were supine. Inflammatory markers were lower in the prone HFOV group (Papazian et al., 2005). A different crossover trial evaluating HFOV plus prone positioning in 43 subjects with ARDS found that HFOV maintained lung recruitment from prone positioning, and that P/F was higher in the HFOV prone and CMV prone groups (Papazian et al., 2005).

Due to the significant improvement in oxygenation observed in case series and observational studies, there was great enthusiasm for the use of HFOV as a strategy to improve

TABLE 2 | Adult randomized controlled trials.

Trial	HFOV	HFOV mPaw Initial	Hz	Amplitude	Mortality (%)	Subjects	CMV PEEP	CMV V_T	Max plateau	Mortality (%)	Comment
MOAT Derdak et al. (2002)	75	CMV mPaw +5	5	For chest wiggle	37	73	≥10 cmH2O	10 ml/kg	None	52	No difference mortality, no lung protective ventilation in control
Bollen et al. (2005)	37	CMV mPaw +5	5	For chest wiggle	32	24	Up to 15 cmH_2O	8–9 ml/kg	None	38	HFOV mPaw increased for lung volume and PaO2
OSCAR Young et al. (2013)	397	CMV plateau +5	10, mean 7.8 on day 1	Max, then Hz adjusted	42	398	Table	6–8 ml/kg	N.R.	41	Each site had a single HFOV vent. CMV not controlled
OSCILLATE Ferguson et al. (2013)	275	Recruitment maneuver 30 cmH2O, mPaw: FIO2 table	Highest possible	90 mbar	47	273	—	6 ml/kg	≤35 cmH2O	35	High vasopressor use, high mPaw in HFOV

mortality. Two early RCTs evaluated the efficacy of HFOV in adult ARDS. The MOAT trial conducted between 1997 and 2000 (Derdak et al., 2002) enrolled 148 subjects with ARDS, with 75 in the HFOV group and 73 in CMV group. Groups were similar at baseline, although neither group was receiving lung protective ventilation (PIP 39 vs. 38 cmH_2O, V_T 10.5 vs. 10.1 ml/kg) at enrollment. Mortality was 37% in the HFOV group and 52% in the CMV group but did not reach statistical significance. Survivors had lower OI after 24 h, regardless of group assignment (Derdak et al., 2002). Another RCT enrolled 61 subjects with ARDS from 1997–2001 (Bollen et al., 2005); it was stopped due to slow enrollment and found no difference in mortality between groups. Of note, the control group in this trial did not receive lung-protective ventilation (Bollen et al., 2005).

In 2013, the OSCAR and OSCILLATE trials were published (Ferguson et al., 2013; Young et al., 2013). The OSCAR trial randomized 397 subjects with ARDS (P/F < 200 on a minimum of 5 cmH_2O PEEP, ventilated for <48 h) to HFOV and 398 to CMV in the United Kingdom. HFOV was set with a frequency of 10 Hz, mPaw 5 cmH_2O above plateau pressure on CMV, bias flow 20 L/min, and an inspiratory time of 50%. Ventilation was managed to keep pH > 7.25 by maximizing the amplitude prior to adjusting the Hz, with a minimum of 5 Hz. The oxygenation target was a PaO_2 between 60 and 75 mmHg. The CMV group was not controlled but centers were encouraged to target a V_T 6–8 ml/kg and use a PEEP:F_IO_2 table. The groups were well-matched prior to randomization. This study showed no differences in mortality or ventilator-free days between the HFOV and CMV groups (Young et al., 2013).

The OSCILLATE trial randomized 548 subjects with ARDS and a P/F ≤ 200 with an $FiO_2 \geq 0.50$ to HFOV or CMV (Ferguson et al., 2013). HFOV was managed using a recruitment maneuver (40 cmH_2O for 40 s) at initiation, then mPaw was set at 30 cmH_2O and subsequently adjusted using a mPaw:F_IO_2 table, with the F_IO_2 needing to be ≤0.40 before the mPaw was decreased <30 cmH_2O. The highest frequency possible was used to maintain pH > 7.25. Groups were similar at baseline, although vasopressor use was high in both groups at enrollment. Vasopressor and neuromuscular blockage use increased over time in the HFOV group. HFOV subjects received higher mPaw throughout and had a more positive fluid balance, while CMV subjects received a V_T of 6.1 ml/kg and a PEEP of 18 cmH_2O (Ferguson et al., 2013). This trial was stopped early due to higher mortality in subjects randomized to HFOV. A post-hoc analysis of 4 RCTs of HFOV found it likely to be harmful in mild to moderate ARDS but possibly beneficial in those with severe disease (P/F < 64) (Meade et al., 2017). The adult RCTs are summarized in **Table 2**.

Post OSCAR and OSCILLATE

Few studies have been published after the publication of OSCAR and OSCILLATE, and there has been a significant decrease in HFOV use in clinical practice (Tatham et al., 2021). A large cohort study of rescue strategies in adult patients with severe acute hypoxemic respiratory failure found that HFOV was used in 6% of all subjects and was the second most common rescue strategy after inhaled pulmonary vasodilators (Moreno Franco et al., 2016). A survey of critical care specialists found that only 8% would consider HFOV as rescue while 26% would likely never utilize it (Alhurani et al., 2016). Additional studies have shown HFOV use to be rare in the current era, with one reporting 4.3% of rescue cases (Duan et al., 2017), another reporting 1.7% of ventilator epochs (Jabaley et al., 2018), and only 10% of ICUs in the UK reporting any HFOV use (Jha et al., 2021). In 2021, the use of HFOV was reported in a single center study of 48 subjects with a OI 36 and a mortality rate of 92% (Thind et al., 2021).

Systematic Reviews

Multiple meta-analyses following the publication of the OSCAR and OSCILLATE trials (Ferguson et al., 2013; Young et al., 2013) concluded that HFOV does not improve mortality compared to CMV; it also showed similar risk as CMV for barotrauma or hypotension but lower rate of treatment failure (Gu et al., 2014; Huang et al., 2014; Maitra et al., 2015; Sud et al., 2016; Goligher et al., 2017). Following those trials, multiple clinical practice

guidelines (CPGs) recommended against the routine use of HFOV in the adult population (Claesson et al., 2015; Cho et al., 2016; Fan et al., 2017). A CPG from the American Thoracic Society, European Society of Intensive Care Medicine, and the Society of Critical Care Medicine suggested that future research on HFOV should employ lower mPaw strategies, have HFOV settings titrated to lung mechanics, and focus on its role as a rescue therapy (Fan et al., 2017).

Non-systematic reviews have suggested HFOV has not been shown to improve outcomes due to inadequately low frequency, excessive mPaw, and the need for neuromuscular blockade (Nguyen et al., 2016). Others have suggested the failure of HFOV was related to overdistension of non-injured alveoli, transmission of high mechanical power, excessive mPaw in subjects with poor recruitability, and adverse hemodynamics, particularly right ventricular dysfunction/failure (Dreyfuss et al., 2015; Sklar et al., 2017). The resonant frequency of the lung may also factor into patient outcomes, although identifying the optimal frequency in clinical practice is still a major challenge as it will vary among patients or even within the same patient at different stages of lung disease (Sklar et al., 2017). Despite limited data, HFOV may benefit patients with P/F < 65 and should be used cautiously in subjects with significant (pH < 7.23) respiratory acidosis (Sklar et al., 2017; Fielding-Singh et al., 2018).

Despite the strong physiologic rationale for its use and reproducible improvements in gas exchange, available data has not demonstrated a major outcome benefit for the routine use of HFOV in adults. Enthusiasm for HFOV has significantly decreased following the publication of the OSCAR and OSCILLATE trials, and its use has been largely abandoned in adult patients. This is despite subgroup analyses suggesting HFOV may have of benefit in profoundly hypoxemic patients (i.e., P/F is <64) (Sklar et al., 2017; Fielding-Singh et al., 2018). These RCTs enrolled subjects with a P/F < 200, used aggressive recruitment maneuvers, and the OSCILLATE trial enrolled a large number of subjects with hemodynamic instability requiring vasopressor, along with an aggressive mPaw protocol (Ferguson et al., 2013; Young et al., 2013). It is possible that these trials enrolled subjects who were not ill enough to benefit from HFOV, or that subjects with poorly recruitable lung were harmed by the high mPaw strategy employed (both from a barotrauma and preload-dependency standpoint). No clinical trials performed to date have evaluated lung recruitability prior to enrollment. In addition, the OSCAR trial did not control CMV in the control group, only had a single ventilator available for each site, used an HFOV ventilator with the inspiratory time fixed at 50%, and staff received limited education to use the ventilator (Young et al., 2013). Importantly, the management of HFOV is complex and can be affected by the ventilator used, specific HFOV strategy, staff education, and operator familiarity with the device or strategy. Together, available data illustrate the inherent challenges of studying complex interventions such as HFOV, and the fact that optimization of HFOV settings may not have been achieved in the adult RCTs. In addition, existing studies are limited by the inherent heterogeneity of diseases and concurrent treatment strategies employed in HFOV studies.

For adult patients, it is uncertain whether additional large clinical trials will be performed. If that were the case, future trials should focus on patients with a greater disease severity (P/F < 75 or pH < 7.20) and enroll patients in units where ECMO is not readily available. Trials should also attempt to include transpulmonary pressure monitoring and electric impedance tomography, along with evaluation of lung recruitability prior to enrollment. Caution should be exercised if incorporating recruitment maneuvers into a trial protocol, as these have not been shown to improve outcomes (Pensier et al., 2019; Ibarra-Estrada et al., 2021).

Pediatric Evidence

Observational Studies

Early case series in children, published prior to 2000, found improved oxygenation, with variable effects on hemodynamics and mortality rates between 0 and 48% (Arnold et al., 1993; Goodman and Pollack, 1998; Duval et al., 1999; Fedora et al., 2000; Winters et al., 2000). These studies were small, single center studies that included patients receiving HFOV for a variety of indications and disease severity. These were followed by a large, multicenter study of 290 subjects that found a mortality rate of 32% in subjects with a P/F 75–90 and an OI 27–33 (Arnold et al., 2000). Oxygenation improved during HFOV and mortality was associated immunocompromised state, OI after 24 h of HFOV, sepsis, and chronic lung disease (Arnold et al., 2000). A subsequent study of 112 subjects in the era of lung-protective ventilation was unable to identify any risk factors for mortality but also found improved oxygenation during HFOV (Babbitt et al., 2012). A study of 34 subjects identified improvement in oxygenation and organ dysfunction score as independent predictors of mortality (Chattopadhyay et al., 2020).

Children often develop acute hypoxemic respiratory failure after hematopoietic stem cell transplant and 91% of centers performing stem cell transplants used HFOV as rescue (McArthur et al., 2011). A multi-center retrospective study on the use of HFOV in 85 children following hematopoietic stem cell transplant found that those treated with HFOV were 3 times more likely to die than those who did not receive HFOV (Rowan et al., 2016). They suggested that, when considering the use of HFOV in this population, it should be initiated within 5 days of respiratory failure. Another study of children with severe PARDS following hematopoietic stem cell transplantation found that HFOV use was associated with an odds ratio of 6.28 (95% confidence interval 1.16–34.12) for death (van Gestel et al., 2008).

A secondary analysis of the PARDIE study dataset evaluating rescue strategies for severe hypoxemia found that nearly all centers had HFOV available and used it in 9.3% of subjects (Khemani et al., 2019; Rowan et al., 2020). This rate was similar to the use of prone positioning (10%), slightly lower than inhaled nitric oxide (13%) but higher than ECMO (3%) (Rowan et al., 2020). HFOV was used more frequently in middle income countries, in patients with higher illness severity, and immunocompromised patients.

The feasibility of a physiologic, open-lung recruitment strategy of HFOV was described in 115 subjects treated in a single center in the Netherlands (de Jager et al., 2019). The HFOV strategy

consisted of a starting frequency of 12 Hz for all subjects and used incremental-decremental staircase adjustments to select the "optimal" mPaw on the deflation limb of pressure-volume curve (de Jager et al., 2019). Ventilation was controlled by adjusting the frequency, but only after maximizing the power (amplitude) setting (de Jager et al., 2019). The reported mortality for different PARDS severity was similar to the mortality reported in the PARDIE study (Khemani et al., 2019). A different report from the same group found minimal hemodynamic effects, with 88% sensitivity and 54% specificity for changes in lung volumes (de Jager et al., 2020). This is the HFOV strategy currently being investigated as part of the PROSPECT trial (https://prospect-network.org/).

The first pediatric RCT of HFOV, published in 1994, enrolled 70 children ≤35 kg with an OI > 13, acute diffuse lung injury, or barotrauma (Arnold et al., 1994); it showed significant improvements in oxygenation compared to CMV, but no difference in survival (59% for CMV vs. 66% for HFOV). The OI was the strongest predictor of mortality with an OR of 20.8 (95% confidence interval 3.4 to 128.4, $p < 0.001$)and HFOV subjects were less likely to require oxygen at discharge (Arnold et al., 1994).

More recently, three propensity score matched analyses of HFOV compared to CMV have been published. The first used the Virtual PICU Systems database and compared early vs. late HFOV to CMV (Gupta et al., 2014). Subjects were matched for age, weight, CPR, severity of illness, ECMO, dialysis, arterial catheter, central access, hemodynamics, diagnoses. This study found that HFOV was associated with an increased length of MV, ICU length of stay, and mortality (Gupta et al., 2014). Importantly, this database did not collect crucial variables such as ventilator settings, gas exchange, and measures of oxygenation. As such, the adequacy of propensity matching (and, thus, the study findings) should be taken with caution. A reanalysis of the RESTORE trial dataset used propensity score matching to compare early HFOV with CMV or late HFOV (Bateman et al., 2016) and found no significant association with mortality; however, secondary analyses revealed early HFOV was associated with a higher mortality after accounting for risk category, with the 2 highest risk groups having increased mortality. Subjects in the HFOV group spend more time on the ventilator (Bateman et al., 2016). HFOV use increased as OI increased, with subjects more likely to be placed on HFOV once OI was >8, and those with an OI ≥ 40 were 17 times more likely to be placed on HFOV (Bateman et al., 2016). The Pediatric Acute & Critical Care Medicine Asian Network (PACCMAN) group performed a propensity matched study using a large multicenter database of pediatric ARDS and found that, compared to CMV, HFOV was associated with increased mortality and fewer ICU free days, but no difference for ventilator free days (Wong et al., 2020).

These studies are significantly limited as most did not record granular respiratory details such as plateau pressure, V_T, driving pressure, and rationale for starting HFOV. Despite efforts to control for illness severity, it is likely that patients receiving HFOV were in fact sicker or failing CMV prior to placement on HFOV, thus resulting in mismatched acuity between the groups. Importantly, the presence of shock, vasopressor use, and renal failure were not included in the models.

Three additional RCTs have been published in recent years. A small trial, published in 2016, randomized 18 children with severe ARDS to HFOV ($n = 9$) or CMV ($n = 9$) with lung recruitment maneuvers. HFOV improved oxygenation and was well-tolerated hemodynamically. The overall survival was 89%, with one death in each group. Of note, 3 (33%) subjects randomized to the CMV group crossed over to HFOV (Samransamruajkit et al., 2016). The second trial, published in 2017, compared HFOV to protective CMV in 200 subjects with pediatric ARDS (El-Nawawy et al., 2017). HFOV resulted in improved oxygenation and more rapid increase in P/F but no differences in mortality (43% for CMV vs. 45% in HFOV), days of MV, OI difference after 24 h, and PICU length of stay (El-Nawawy et al., 2017).

A RCT of 61 infants with ARDS after high-risk atrial septal defect or ventricular septal defect repair compared HFOV to CMV; both groups also received surfactant replacement therapy (Zheng et al., 2021). The primary outcome was improvement in arterial blood gases. CMV strategy called for an inverse I:E ratio, with PIP 18–25 cmH_2O and PEEP 4–-6 cmH_2O but actual values were not reported. HFOV resulted in relatively small differences in PaO_2, P/F, $PaCO_2$, and OI. The HFOV group had shorter time on mechanical ventilation, ICU length of stay, and total hospital length of stay (Zheng et al., 2021). Pediatric RCTs are summarized in **Table 3**.

A recent systematic review of pediatric RCTs, propensity score matched studies, and observational studies failed to show an advantage of HFOV over CMV, with no demonstrable reduction in mortality, time on MV, or barotrauma (Junqueira et al., 2021). Of note, the GRADE certainty was low or very low for all studied outcomes (Junqueira et al., 2021). Recent non-systemic reviews suggest that, while HFOV strategies still require refinement, HFOV remains a viable rescue therapy for severe pediatric ARDS (Moerer et al., 2017; Nardi et al., 2017; Ng and Ferguson, 2017). Kneyber et al. advanced that HFOV was not optimized in prior RCTs (Kneyber et al., 2012) and suggested starting HFOV if SpO_2 <88%, PaO_2 < 50 mmHg with an F_IO_2 > 0.60 on sufficient support or in the presence of refractory respiratory acidosis. The suggested strategy involves increasing mPaw using incremental mPaw steps while following an expected rise in SpO2 until overdistension is observed. This is followed by a stepwise reduction in mPaw until the point of derecruitment, then the mPaw is set 2–4 cmH_2O above this decruitment point (Kneyber et al., 2012). This strategy also advocates the use of the highest tolerable frequency (Hz) to minimize V_T, and is currently being investigated in the PROSPECT trial (https://prospect-network.org/).

Similar to adults, available data do not support the use of HFOV in children with severe ARDS, despite consistently observed improvement in oxygenation. Unlike adults, however, these data are significantly limited by low quality RCTs and HFOV is still widely utilized in pediatric ICUs. Hopefully, the ongoing PROSPECT trial will provide more definitive answers on the role of HFOV in pediatric ARDS. Beyond the PROSPECT trial, the use of HFOV as a rescue strategy should be investigated, perhaps through a large registry or database that includes

TABLE 3 | Pediatric randomized controlled trials.

Trial	HFOV	HFOV mPaw Initial	Hz	Amplitude	Mortality	Subjects	CMV PEEP	CMV V_T	Max plateau	Mortality	Comment
Arnold et al. (1994)	29	CMV mPaw + 4–8	5–10	For chest wiggle	66%	29	Increased for oxygenation	10 ml/kg	None	59%	Control group did not receive LPV, PIP >40 at baseline in both groups
Samransamruajkit et al. (2016)	9	CMV mPaw + 5–8	5	3x CMV mPaw	NR	9	RM followed by decremental PEEP maneuver	6–8 ml/kg	None	NR	89% overall survival, between groups not reported
El-Nawawy et al. (2017)	100	CMV plateau + 3–5	5–12	1.-5-3 ml/kg	45%	100	NR	5–8 ml/kg	PIP ≤35	43%	No difference in PICU LOS, OI at 24 h 50% I:E
Zheng et al. (2021)	31	10–15, with slow recruitment maneuver	8–12	30–40	3.2%	30	4–6	NR	PIP ≤30	10%	Congenital heart disease, also received surfactant replacement, shorter time on MV in HFOV group CMV group received inverse I: E ventilation

granular variables to allow for improved patient-level matching of relevant characteristics. Physiologic studies should include electric impedance tomography to evaluate the continuous relationship between lung volume and gas exchange. We await the results of the PROSPECT trial, an international multicenter, two-by-two factorial, response-adaptive RCT evaluating CMV and HFOV, along with prone and supine positions in children with severe hypoxemic respiratory failure.

Effect of HFOV on the Right Ventricle and Hemodynamics

Positive pressure ventilation can have negative effects on right ventricular (RV) function and overall hemodynamics, with some investigators suggesting this as a possible reason why RCTs of HFOV have failed to show an outcome benefit (Dreyfuss et al., 2015; Sklar et al., 2017). When lung volume is excessively increased, there is a potential for an increase in West zone 1 (ventilation with no perfusion) lung units, which results in higher RV afterload from an increase in pulmonary vascular resistance (PVR). Conversely, the underinflated lung (i.e., below functional residual capacity), also lead to increased PVR and increased RV afterload (Simmons et al., 1961). Thus, the use of high mPaw during HFOV may be detrimental to RV function due to increased PVR. Available data, however, do not show significant hemodynamic effects during stepwise recruitment and de-recruitment maneuvers (de Jager et al., 2020). Additionally, the elevated mPaw used during HFOV may adversely affect preload and result in the need for intravascular fluid expansion that can lead to volume overload.

An observational study found that, when HFOV was initiated with a mPaw 5 cmH_2O above CMV mPaw, no differences in mean arterial pressure or heart rate were noted, but right atrial pressure increased, cardiac index slightly decreased, and left ventricular end-diastolic pressure decreased in a study of nine subjects with ARDS (David et al., 2004). Another study found HFOV did not appear to have a large effect on left and right ventricular function, but the cardiac index decreased by 13% when HFOV mPaw was set 5 cmH_2O above the CMV mPaw (Ursulet et al., 2015). Additional studies found no association between body mass index and mortality, and that the higher mortality observed in the OSCILLATE was not related to hemodynamic changes 2 h after HFOV initiation (Tlayjeh et al., 2019; Angriman et al., 2020). One study found that an acute cor pulmonale score ≥2 and a P/F ≥ 100 were directly associated with mortality during HFOV (Angriman et al., 2020).

Staff Education and Competency

Management of HFOV is complex and requires advanced skills, device specific training/competency, physiologic understanding, and critical thinking as the learning curve for HFOV is steep. HFOV management is challenging even for teams experienced in its use. Data evaluating HFOV education are sparse. Deficits in basic MV management and assessment for asynchrony by critical care physicians has been reported (Colombo et al., 2011). A narrative review concluded that there is a paucity of information describing MV education in graduate medical education (Keller et al., 2019). Likewise, there is a dearth of guidance to facilitate staff education and verify competency of the end user. Simulation based training has shown promise in improving the outcome of

learners as an addition to traditional didactic teaching (Cook et al., 2011). High-fidelity simulation has been shown to improve knowledge and skills related to MV in anesthesiology residents (Spadaro et al., 2017). There remains no standard approach to teach HFOV management nor to assess staff competency.

Staff education may be an underappreciated factor in prior clinical trials, particularly the OSCAR trial in which a new HFOV ventilator was used and some centers may have had limited experience with HFOV (Young et al., 2013). Education is even more critical when complex maneuvers, such as dynamic sustained inflation for lung recruitment or staircase titration of mPaw, are used to determine optimal mPaw (de Jager et al., 2019). Training and education in the use of HFOV settings, such as amplitude, require the user to assess subjective parameters such as "chest wiggle" as a surrogate for appropriate ventilation (Meyers et al., 2019). Inexperienced team members may have difficulty properly assessing the degree of chest wiggle or how to react appropriately, as frequency adjustments are counter-intuitive compared to CMV. Better feedback and assessment tools are needed to guide learning objectives and determine end-user competency. Future exploration into teaching and training methods utilizing HFOV are warranted; in the meantime, yearly education and competency assessment for centers that do not routinely utilize HFOV is suggested (Thind et al., 2021).

CONCLUSION

HFOV has largely been abandoned in adults following two large clinical trials despite a strong physiologic rationale and promising animal data. Available data do not support its routine use in adult or pediatric ARDS but it may have utility in more severe disease and pediatric data are of low quality. The mode is complex and patient outcomes may be affected by the ventilator used, HFOV strategy, and staff education.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Myths and Misconceptions of Airway Pressure Release Ventilation: Getting Past the Noise and on to the Signal

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In the pursuit of science, competitive ideas and debate are necessary means to attain knowledge and expose our ignorance. To quote Murray Gell-Mann (1969 Nobel Prize laureate in Physics): "Scientific orthodoxy kills truth". In mechanical ventilation, the goal is to provide the best approach to support patients with respiratory failure until the underlying disease resolves, while minimizing iatrogenic damage. This compromise characterizes the philosophy behind the concept of "lung protective" ventilation. Unfortunately, inadequacies of the current conceptual model–that focuses exclusively on a nominal value of low tidal volume and promotes shrinking of the "baby lung" - is reflected in the high mortality rate of patients with moderate and severe acute respiratory distress syndrome. These data call for exploration and investigation of competitive models evaluated thoroughly through a scientific process. Airway Pressure Release Ventilation (APRV) is one of the most studied yet controversial modes of mechanical ventilation that shows promise in experimental and clinical data. Over the last 3 decades APRV has evolved from a rescue strategy to a preemptive lung injury prevention approach with potential to stabilize the lung and restore alveolar homogeneity. However, several obstacles have so far impeded the evaluation of APRV's clinical efficacy in large, randomized trials. For instance, there is no universally accepted standardized method of setting APRV and thus, it is not established whether its effects on clinical outcomes are due to the ventilator mode *per se* or the method applied. In addition, one distinctive issue that hinders proper scientific evaluation of APRV is the ubiquitous presence of myths and misconceptions repeatedly presented in the literature. In this review we discuss some of these misleading notions and present data to advance scientific discourse around the uses and misuses of APRV in the current literature.

Keywords: APRV, myth, airway pressure release ventilation (APRV), TCAV, time controlled adaptive ventilation

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INTRODUCTION

"*Falsehood flies, and truth comes limping after it……*"—*Jonathan Swift*. Similarly, a myth about airway pressure release ventilation (APRV) can be published, perpetuated, and believed as fact before science has a chance to get out of the laboratory. Some APRV myths stem from what intuitively seems reasonable when making a mental comparison between APRV and the current conceptual model of delivering "lung protective ventilation." Unfortunately, this still revolves exclusively on the

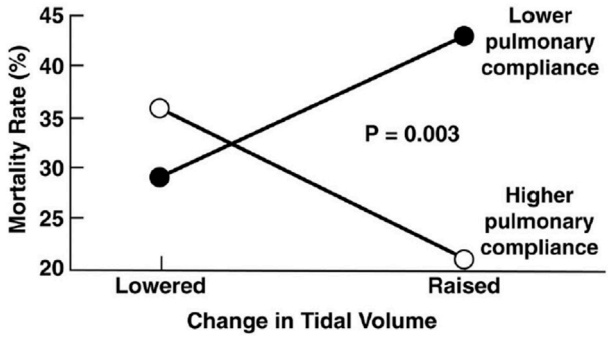

FIGURE 1 | Data from the original ARMA Trial shows correlation between tidal volume (V_T) and respiratory system compliance (C_{RS}) of individual patients on mortality. Using the volume assist control mode a lower V_T reduced mortality with low C_{RS} where a higher V_T increased mortality in patients with high C_{RS}. (Deans et al., 2005).

simplistic setting of a nominal and arbitrary value of "low" tidal volume (LV_T) and levels of pressures which promote further shrinking of the "baby lung" (Marini and Gattinoni, 2020). Data increasingly show this model is not only incorrect but may contribute to the unacceptably high mortality rate of patients with moderate and severe acute respiratory distress syndrome (ARDS) (Amato et al., 2015; Costa et al., 2021; Goligher et al., 2021; Raschke et al., 2021). Additional myths and misconceptions are generated from the confused lumping of different ventilator modes and methods under an umbrella term of APRV and the differing ventilator behavior from various implementations by ventilator manufacturers.

To scientifically study any ventilator mode, consistent methodology to set and adjust the mode is essential. This was clearly seen in the acute respiratory distress syndrome (ARDS) Network (ARDSNet) trial of low tidal volume ventilation study (ARMA) that used the volume assist–control (VAC) mode and compared lower with higher settings of tidal volumes (V_T) and plateau pressures (Pplat) (ARDSNet 2000). Changing just these two parameters resulted in a significant reduction in mortality even when using the same mode. Of equal interest, in a subsequent analysis of 2,587 patients from the ARMA study that met criteria but were not enrolled for technical reasons, it was shown that high or LV_T will either increase or decrease mortality, depending on respiratory system compliance (C_{RS}) of the individual patient as shown in **Figure 1** (Deans et al., 2005). These initial data are further supported by more recent studies (Amato et al., 2015; Costa et al., 2021; Goligher et al., 2021; Raschke et al., 2021) and make it clear that a protective ventilation strategy can only be interpreted in the context of respiratory mechanics. Undoubtedly, even small changes in mode settings can have a significant impact on outcome depending on the degree of lung pathophysiology and patient heterogeneity suggesting a need for personalization of lung protective strategies (Nieman et al., 2017a; Pelosi et al., 2021; Cheng et al., 2022).

Although APRV has been available on commercial ventilators since 1987, the method of applying the mode has varied widely in medical literature and clinical practice (**Figure 2**) (Jain et al., 2016; Habashi et al., 2021). Currently, APRV is an ill-defined initialism which identifies a mode without a consistent method of application. In fact, APRV is often used as a synonym for the biphasic positive airway pressure (BIPAP) mode so much so as to be found in the literature often indicated as a meaningless BIPAP/

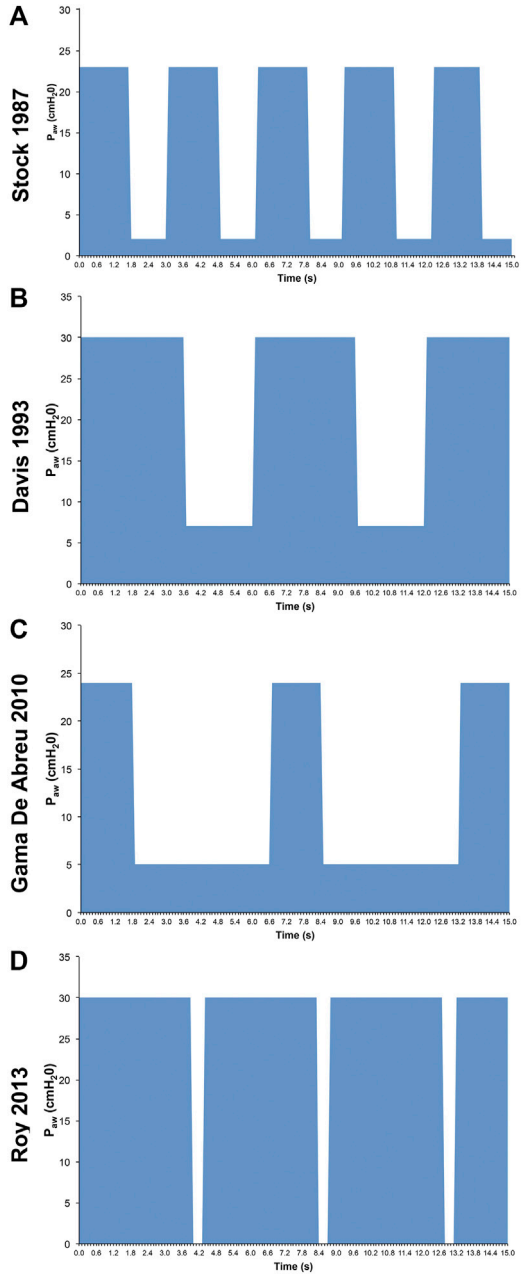

FIGURE 2 | Airway Pressure Release Ventilation (APRV) Pressure/Time waveforms from 4 studies: **(A)** Stock et al., 1987 set time at P_{Low} (T_{Low}) of 1.27s (Stock et al., 1987); **(B)** Davis et al., 1993 used an increased inspiratory to expiratory ratio (Davis et al., 1993); **(C)** Gama de Abreau 2010 simulated conventional ventilation (Gama de Abreau 2010); **(D)** Roy et al., 2013a used the Time Controlled Adaptive Ventilation method (Roy SK. et al., 2013). This illustrates the wide variability in methods used to set APRV, which may dramatically impact outcome (Jain et al., 2016).

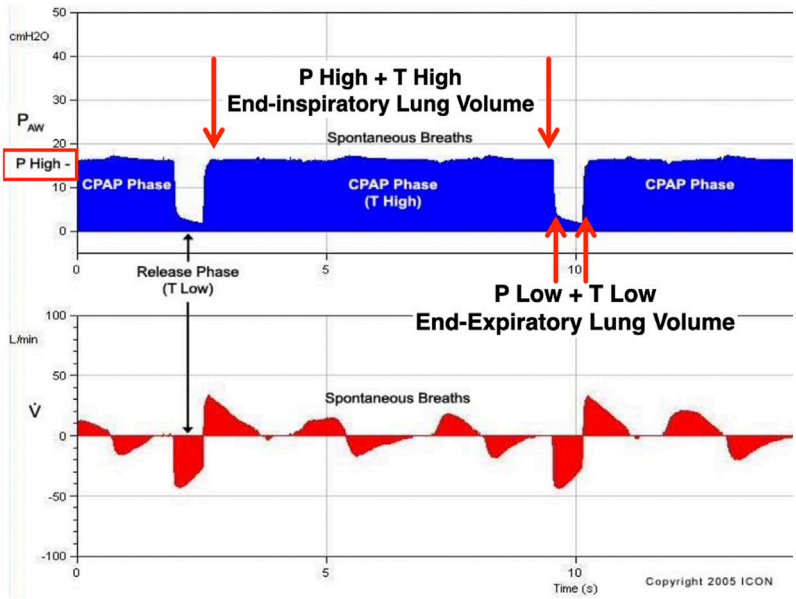

FIGURE 3 | Airway Pressure Release Ventilation (APRV) is a pressure-limited, time-cycled mode. The Time Controlled Adaptive Ventilation (TCAV™) method of setting the APRV mode includes the following settings: 1) upper airway pressure (P_{High}); 2) lower airway pressure (P_{Low}); 3) time spent at P_{High} (T_{High}); and 4) time spent at P_{Low} (T_{Low}). Combined, P_{High} and T_{High} form the continuous positive airway pressure (CPAP) Phase and impact end-inspiratory lung volume. The CPAP Phase releases to the combined P_{Low} and T_{Low}, which form the Release Phase and impact end-expiratory lung volume. During the TCAV™ method of APRV, the ventilator cycles between the CPAP and Release Phases. During the release phase, the T_{Low} set to terminate at 75% of the peak expiratory flow rate halts alveolar instability. Subsequently, the CPAP Phase maintains alveolar stability and recruits lung volume over time (hours to days).

APRV mode (Neumann et al., 2002; Dries and Marini, 2009; Kallet 2011; Daoud et al., 2012). Subsequently, the outcome in both basic science and clinical studies using APRV with different settings (Jain et al., 2016) has led to further confusion on the relative efficacy of individual components of the APRV settings–particularly the value of inspiratory (T_{High}) and expiratory (T_{Low}) time settings (Habashi et al., 2022). APRV was originally described as continuous positive airway pressure (CPAP) with a release phase. There are four basic settings to control in APRV other than FiO_2: 1) P_{High} (inspiratory pressure similar to Pplat; 2) T_{High} (duration of inspiratory time) - when combined with the P_{High} controls end-inspiratory lung volume and referred to as the CPAP Phase; 3) P_{Low} (expiratory pressure similar to PEEP); 4) T_{Low} (duration of expiratory time) - when combined with the P_{Low} controls end-expiratory lung volume (EELV) and referred to as the Release Phase (**Figure 3**). The method to set and adjust APRV that has been used most clinically, spanning over 30 years, and best studied consistently in translational animal models that exceed American Thoracic Society animal model guidelines (Matute-Bello et al., 2011) is the Time Controlled Adaptive Ventilation (TCAV™) method (Roy et al., 2012; Roy S. et al., 2013; Roy SK. et al., 2013; Andrews et al., 2013a; Andrews et al., 2013b; Emr et al., 2013; Kollisch-Singule et al., 2014a; Kollisch-Singule et al., 2014b; Kollisch-Singule et al., 2015; Kollisch-Singule et al., 2016a; Kollisch-Singule et al., 2019; Smith et al., 2015; Jain et al., 2017; Silva et al., 2018; Mahajan et al., 2019; Al-khalisy et al., 2020; Bates et al., 2020; de Magalhã¸es et al., 2021; Vasconcellos de Oliveira et al., 2022).

The TCAV™ method emphasizes *time control* of the upper and lower pressures and an adaptive methodology to personalize a lung protective strategy for each patient's respiratory mechanics throughout the evolution—or resolution—of their lung disease process (Habashi 2005; Habashi et al., 2011; Habashi and Andrews, 2013; Habashi et al., 2019). Unique to the TCAV™ method of setting APRV is using passive exhalation without a set PEEP and analyzing the slope of the expiratory flow-time curve ($SLOPE_{EF}$) (Dixon and Brodie, 1903; Rahn et al., 1946; Mead and Whittenberger, 1953; Brody, 1954; Comroe 1954; Brody and DuBois, 1956; McIlroy et al., 1963; Bergman 1966; Grimby et al., 1968; Ashutosh and Keighley, 1978; Behrakis et al., 1983; Richardson et al., 1989; Baydur and Carlson, 1994; Brunner et al., 1995; Guttmann et al., 1995; Nassar et al., 2012) to personalize V_T to C_{RS}, which has been validated experimentally and clinically (Roy et al., 2012; Roy S. et al., 2013; Roy SK. et al., 2013; Andrews et al., 2013b; Emr et al., 2013; Kollisch-Singule et al., 2014a; Kollisch-Singule et al., 2014b; Kollisch-Singule et al., 2015; Kollisch-Singule et al., 2016a; Kollisch-Singule et al., 2016b; Kollisch-Singule et al., 2019; Kollisch-Singule et al., 2020; Smith et al., 2015; Jain et al., 2017; Silva et al., 2018; Mahajan et al., 2019; Al-khalisy et al., 2020; Bates et al., 2020; de Magalhã¸es et al., 2021; Vasconcellos de Oliveira et al., 2022). The T_{Low} is tuned to the elastic recoil of the respiratory system (E_{RS}) to halt alveolar collapse aiding in distal airspace stability and when coupled with the P_{High} and T_{High}, the CPAP phase gradually normalizes lung volume over hours to days (Kollisch-Singule et al., 2014a; Boehme et al., 2015; Kollisch-

Singule et al., 2016a). This allows the lungs of each patient to determine the time-course to normalize lung volume rather that the clinician forcing it open such as with recruitment maneuvers (RMs).

We reviewed the current relevant literature identified using OvidSP and the National Library of Medicine's MEDLINE database via PubMed to locate published papers using APRV and identified myths and misconceptions consistently seen in the literature. This review discusses 10 myths and misconceptions about APRV, which are largely based on opinions or methodologic inconsistencies and lack evidence to support those inaccurate claims. Additionally, we found that many APRV myths originate in review articles, editorials, or the discussion section of papers. In other words, they reflect inferences, extrapolations, personal beliefs including hyperbole yet lack the furtherance of credible scientific evidence. These opinions then become an echo chamber that reverberates in the literature and become self-evident truths.

MYTH #1—AIRWAY PRESSURE RELEASE VENTILATION IS TOO DIFFICULT TO USE

Several papers include statements such as: "APRV evolved into a highly sophisticated, physiology-driven, dynamic mechanical breath profile with precise settings, which might cause a possibility of knowledge bias by the staff" (Zhong et al., 2020) and "APRV is more complex than it appears to be. It requires a lot more knowledge and skill than may be apparent from the descriptions in the literature (Chatburn et al., 2016)." These and other statements (MacIntryre, 2011) lead the reader to believe APRV is too difficult to use for the average practicing clinician. Further, it has been suggested that a simulator is the only practical way to gain understanding of APRV because equivalent experience with real patients could take years and put a lot of people at risk (*sic*) (Chatburn et al., 2016). This insinuates there is no risk in using any other ventilator mode nor is skill required and is dismissive of the mortality rate with current approaches to manage ARDS that continue to range from 35 to 49% (Villar et al., 2014; Bellani et al., 2016; Cavalcanti et al., 2017). Further, mechanical ventilation training in general suffers from a lack of structure, is non-standardized -leading to poor training and knowledge of mechanical ventilation- and often leaves the trainee dissatisfied (Goligher et al., 2012; Wilcox et al., 2016; Keller et al., 2019; Seam et al., 2021). Add to this the existence of a learning curve for any new medical device, procedure, technique, or ventilator mode including APRV (Govindarajulu et al., 2017). Indeed, like any other mode, using APRV for the first time without a general understanding of the rationale and settings on a critically ill and unstable patient with severe ARDS who is failing 'conventional therapies' may not be as successful as when applied by providers who have experience and use it daily as their primary mechanical ventilation strategy. In actuality, APRV has already been used successfully on tens of thousands of patients for over 30 years and continues to be a part of daily care in many hospitals amassing a large amount of empirical data (Sadowitz et al., 2011; Andrews et al., 2013b; Mallory and Cheifetz, 2020; Rola and Daxon, 2022). It is understandable that users who have never actually used APRV or are unfamiliar with this way of thinking about mechanical ventilation may consider it too difficult (Nieman et al., 2017a; Nieman et al., 2017b; Nieman et al., 2018b; Nieman et al., 2020a; Nieman et al., 2020b). However, there are many things in medicine and clinical practice that seem far more difficult but are used with proper education and training such as high frequency oscillatory ventilation (HFOV) and extracorporeal membrane oxygenation. In fact, after the ARMA trial, clinicians at the original 10 ARDSNet sites were surveyed on their use and experience of the ARDSNet protocol (Rubenfeld et al., 2004). The survey showed experienced bedside clinicians perceived important barriers to implementing lung protective ventilation. Obviously, such limitations can be overcome with education, training and experience and is not seen exclusively with APRV.

Although over emphasized, concern for APRV settings permeates the literature yet the more conventional approach to ventilator settings such as V_T, respiratory rate (RR), and PEEP remains controversial despite decades of research and debate (Deans et al., 2005; Amato et al., 2015; Sahetya et al., 2017; Algera et al., 2018; Costa et al., 2021; Goligher et al., 2021; Pelosi et al., 2021; Abrams et al., 2022; Dianti et al., 2022). In addition, important elements of mechanical ventilation such as RR, inspiratory time and flows, and expiratory time and flows are generally not reported or ignored–whereas they are essential components of the total energy delivered to the lung (Gattinoni et al., 2016; Bates et al., 2020) and the combination of these factors can promote lung healing or injury.

As for being a highly sophisticated ventilation mode or too difficult to learn, APRV does not require an in depth understanding of distinctive settings such as frequency (cycles per second) set in Hz and amplitude/power nor the use of a dedicated ventilator such as with HFOV. In fact, APRV is available on almost all intensive care unit (ICU) ventilators as either a standard mode or an option. Like any ventilator mode, APRV uses the same elements: 1) pressure, 2) flow and 3) volume. The key is the personalized configuration of the elements to create a stable airway pressure profile (CPAP Phase) that offers a rate (Release Phase). The airway profile of APRV highlights and leverages the use of time in a time-dependent viscoelastic organ such as the lung (Nieman et al., 2017a; Nieman et al., 2017b; Nieman et al., 2020a; Nieman et al., 2020b). Standard APRV settings include: 1) upper airway pressure (P_{High}), 2) time spent at P_{High} (T_{High}) [combined these define the CPAP Phase]; 3) lower airway pressure (P_{Low}), and 4) time spent at P_{Low} (T_{Low}) [combined these define the Release Phase] (**Figure 3**). With the TCAV™ method, the P_{High} is set to Pplat as you would in a pressure mode. The P_{Low} [typically referred to as PEEP in other ventilatory modes] is set to 0 cmH_2O because EELV is directly controlled with time instead of a set PEEP. This simplifies the quest for the optimal PEEP which has remained elusive despite over 50 years of study and debate and still lacks a refined approach to personalization (Sahetya et al., 2017). The adjustment for time is also simplified as the T_{Low} is used to balance the E_{RS} by retaining EELV and preventing expiratory

collapse (Kollisch-Singule et al., 2014a). Setting and personalizing the T_{Low} to achieve termination of the expiratory flow (E_{FT}) at 75% of the peak expiratory flow (E_{PF}) rate in normal to high E_{RS} -and 25% with low E_{RS} such as chronic obstructive pulmonary disease (COPD)—captures the majority of the closing time constants, thereby maintaining alveolar stability and ductal patency (Kollisch-Singule et al., 2014a, b; Vasconcellos de Oliveira et al., 2022). This personalization of the T_{Low} simplifies pairing V_T to C_{RS}, and provides a real-time, bedside, non-invasive assessment using the $SLOPE_{EF}$, which are all congruent with evolving or resolving changes in respiratory system mechanics. Since V_T does not correlate well with predicted body weight (PBW) in ARDS patients and appears that normalization of V_T to C_{RS} (i.e., driving pressure) relates to better outcome (Amato et al., 2015; Costa et al., 2021; Goligher et al., 2021; Pelosi et al., 2021), T_{Low} personalization of V_T to C_{RS} may be easier for real-time bedside monitoring and prove beneficial (Nieman et al., 2017a; Nieman et al., 2017b; Nieman et al., 2020a; Nieman et al., 2020b; Pelosi et al., 2021; Cheng et al., 2022; Habashi et al., 2022). Once the recoil forces of the lung are neutralized with the T_{Low}, the T_{High} is left to adjust for ventilation by controlling RR, which is common to all ventilator modes.

MYTH #2—AIRWAY PRESSURE RELEASE VENTILATION CAUSES BAROTRAUMA

One of the most common myths regarding APRV is that it causes barotrauma (Myers and Macintyre, 2007; Dries and Marini, 2009; Esan et al., 2010; Kallet 2011; Daoud et al., 2012; Mireles-Cabodevila and Kcmarek, 2016; Hirshberg et al., 2018; Kami et al., 2019), yet is not supported by scientific literature. We are not saying barotrauma does not occur with APRV, but we are saying it does not happen more frequently than in any other ventilatory condition–including in patients receiving non-invasive ventilation or high flow nasal cannula (Hamouri et al., 2021; Palumbo et al., 2021; Shrestha et al., 2022). In fact, there is no evidence demonstrating any component (alone or in combination) is the sole cause of barotrauma.

It would be difficult to establish causality solely from a specific ventilator setting or mode as barotrauma is multifactorial including the population heterogeneity, severity, and inhomogeneity of lung disease on which settings are applied. In fact, a study of 5,183 patients showed no correlation between barotrauma and the mode of ventilation or ventilator settings (Anzueto et al., 2004). Further, in 30 years of large randomized controlled trials (RCTs) comparing various ventilator modes, settings and parameters including 6 ml/kg vs 12 ml/kg V_T (ARDSNet, 2000), low vs high PEEP (Brower et al., 2004), and low vs high mean airway pressure (Paw) (Ferguson et al., 2013; Young et al., 2013) there has been no direct relationship linking barotrauma with a specific ventilator mode or settings. Additionally, a systematic review and meta-analysis of eight RCTs comparing higher versus lower PEEP strategies enrolling 2,728 patients with ARDS showed no difference in barotrauma rates (Fan et al., 2017). One exception is the 2017 Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial where a significant difference in barotrauma rates were seen between the group receiving lung RM with PEEP titration up to 45 cmH_2O (5.6%) compared to the low PEEP group (1.6%) (Cavalcanti et al., 2017).

The potential for barotrauma seems primarily associated with the severity of underlying (acute or chronic) lung disease, which may be aggravated by mechanical ventilation (Anzueto et al., 2004). More recently, barotrauma rates have been reported to occur with greater frequency in COVID related ARDS (CARDS) but not specific to any one ventilator mode (McGuinness et al., 2020; Gazivoda et al., 2021; Hamouri et al., 2021; Rajdev et al., 2021; Udi et al., 2021; Belletti et al., 2022; Shrestha et al., 2022). In a systematic review and meta-analysis, a linear association of increased barotrauma incidence with increasing disease severity was observed in COVID-19 patients requiring various forms of invasive and non-invasive respiratory support (Shrestha et al., 2022). Despite this increased risk of barotrauma with COVID-19, no difference in barotrauma was seen between APRV or ARDSNet low V_T (LV_T) in recent study of CARDS patients (Ibarra-Estrada et al., 2022).

To date, in RCTs comparing APRV with other ventilator modes where pneumothorax or pneumomediastinum was reported, there was no increased rate of barotrauma (Maxwell et al., 2010; Lim et al., 2016; Ganesan et al., 2018; Hirshberg et al., 2018; Lim and Litton, 2019; Zhong et al., 2020; Ibarra-Estrada et al., 2022). Conversely, Maxwell et al. (2010) showed the rate of pneumothorax was lower with APRV (0%) when compared with LV_T (3.1%) and a meta-analysis of seven RCTs with 405 eligible patients presented no statistical difference between LV_T and APRV in the incidence of pneumothorax (Zhong et al., 2020). In addition, a systematic review suggests mortality appears to be lower with APRV and no evidence of increased risk of barotrauma or other adverse consequences with APRV compared to LV_T in ARDS patients (Lim and Litton, 2019). Lastly, in three clinically applicable porcine models of sepsis-induced ARDS (Roy et al., 2012; Roy S. et al., 2013; Roy SK. et al., 2013; Kollisch-Singule et al., 2015) and a porcine neonatal infant respiratory distress syndrome model (Kollisch-Singule et al., 2016a) no barotrauma was noted, and lung injury prevented when using APRV.

Experimentally, micro-strain studies using APRV with the TCAV™ method vs LV_T suggest APRV has the lowest strain on distal air spaces (**Figure 4**) (alveoli and ducts), minimizes ductal dilatation (**Figure 5**) and restores alveolar homogeneity (**Figure 6**) after heterogenous lung injury when compared to LV_T with PEEP up to 24 cmH_2O (Kollische-Singule et al., 2014a; Kollische-Singule et al., 2014b; Kollische-Singule et al., 2015). These studies suggest lung tissue strain is lower with the TCAV™ method and could be favorable to lower barotrauma rates. In summary, the underlying lung disease is the key risk for barotrauma (Anzueto et al., 2004; McGuinness et al., 2020; Gazivoda et al., 2021; Hamouri et al., 2021; Rajdev et al., 2021; Udi et al., 2021; Shrestha et al., 2022) and is therefore difficult to implicate any one ventilator mode or setting.

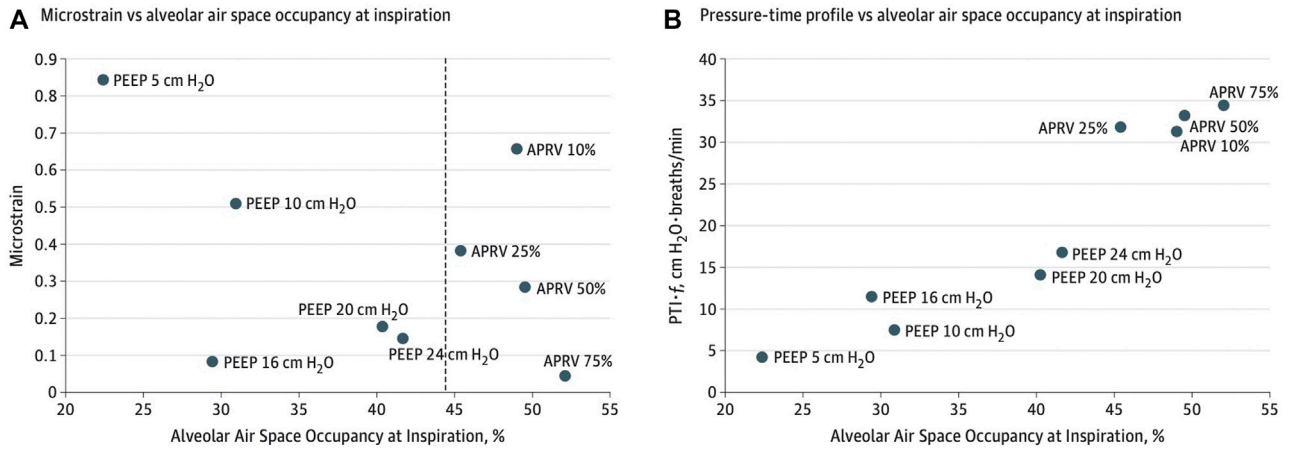

FIGURE 4 | (A) Microstrain vs alveolar air space occupancy (Aa) at inspiration. The dashed line shows the difference in Aa between airway pressure release ventilation (APRV) and controlled mandatory ventilation. **(B)** Normalized pressure-time profile over a minute vs Aa at inspiration. PEEP indicates positive end-expiratory pressure and % for APRV indicate ratio of termination of peak expiratory flow rate to peak expiratory flow rate.

MYTH #3—AIRWAY PRESSURE RELEASE VENTILATION GENERATES HIGH TIDAL VOLUMES LEADING TO VOLUTRAUMA

Several opinion papers (Kallet 2011; Modrykamien et al., 2011; Daoud et al., 2012) reference studies implying that APRV itself generates high V_T (Räsänen et al., 1991; Neumann et al., 2002; Varpula et al., 2004), which could potentially contribute to volutrauma. However, these studies demonstrate settings chosen by the operator (and not the mode) generated the high V_T and yet reported no evidence of volutrauma. For instance, in the Räsänen et al. (1991) study, it was not mentioned that although the V_T in the APRV group was 9 ml/kg, it was significantly lower than the conventional positive pressure ventilation group, which was 12 ml/kg. In the 2004 study by Varpula et al. (2004), APRV is singled out for high V_T, but a key point not mentioned is that both groups (synchronized intermittent mandatory ventilation-pressure control/pressure support (SIMV-PC/PS) and APRV) targeted V_T 8–10 ml/kg with no difference in V_T between modes. Interestingly, although these opinion papers reference Varpula et al. (2004) for high V_T in APRV, they neglect to cite a 2003 study [also by Varpula] comparing the same modes but the V_T in the APRV group was significantly lower than SIMV-PC/PS (Varpula et al., 2003). Some authors (Modrykamien et al., 2011) suggest an unvalidated claim of setting T_{Low} (*sic*) "40% of E_{FP} (around 0.6–0.8 s)." A T_{Low} of 40% of E_{FP} would not only assure a larger V_T than TCAV™ 75% but increases distal air space atelectrauma and induces lung injury (Kollische-Singule et al., 2014a; Kollische-Singule et al., 2014b; Kollische-Singule et al., 2016a; Jain et al., 2017). Lastly, a study by Neumann et al. (2002) is also frequently referenced regarding V_T greater than 1 L and large pleural pressure swings leading to large transpulmonary pressures that could contribute to volutrauma and ventilator induced lung injury (VILI) (Esan et al., 2010; Maxwell et al., 2010; Kallet 2011; Modrykamien et al., 2011; Daoud et al., 2012). However, what is not discussed is release times (T_{Low}) of up to 2.5 s were used, creating large V_T unlike when they decreased T_{Low} to 0.5 s (typically used with the TCAV™ method of APRV) and the subsequent decrease in V_T when T_{Low} was decreased from 2.5 to 0.5 s.

If the operator targets a V_T, then the mode cannot be blamed if this V_T is realized. Like any ventilator mode, high V_T may be generated with APRV as a result of variable methodologies as seen in several APRV studies (Jain et al., 2016). However, unlike the 2000 ARDSNet trial there have been no APRV studies linking an increase in mortality between groups even when V_T exceeds 6 ml/kg (Maxwell et al., 2010; Lim et al., 2016; Ganesan et al., 2018; Hirshberg et al., 2018; Lim and Litton, 2019; Zhong et al., 2020; Ibarra-Estrada et al., 2022).

With any pressure format mode of mechanical ventilation, the user selects the applied pressure and subsequent V_T is dependent on factors such as C_{RS}, gas volume, airway resistance (R_{AW}), and structural homogeneity of the lung. Therefore, the healthier the lung with a near normal C_{RS}, the more likely the V_T will increase beyond the "magic" number of 6 ml/kg. For instance, if V_T in VAC is set to 12 ml/kg, then high V_T will be generated and if set to 6 ml/kg, then LV_T will be generated. The fact that V_T and settings are determined more by mechanics than by guidelines is evident in the recent re-analysis of the LUNG-SAFE data where patients with a greater C_{RS} received higher V_T (averaging 8.5 ml/kg PBW) compared to patients with low C_{RS} who received lower V_T (averaging 7.5 ml/kg PBW) (Goligher et al., 2021). Which patients were ventilated more protectively? The value of driving pressures (ΔP) reveal that patients apparently ventilated more protectively (based on lower recorded values of V_T) were in fact exposed to significantly higher ΔP and therefore at higher risk given that ΔP—not V_T—is associated with greater risk of death (Amato et al., 2015; Bellani et al., 2016; Goligher et al., 2021). In addition, assigning very low V_T to patients with normal C_{RS} and R_{AW} leads to more asynchronies, breath stacking and ultimately

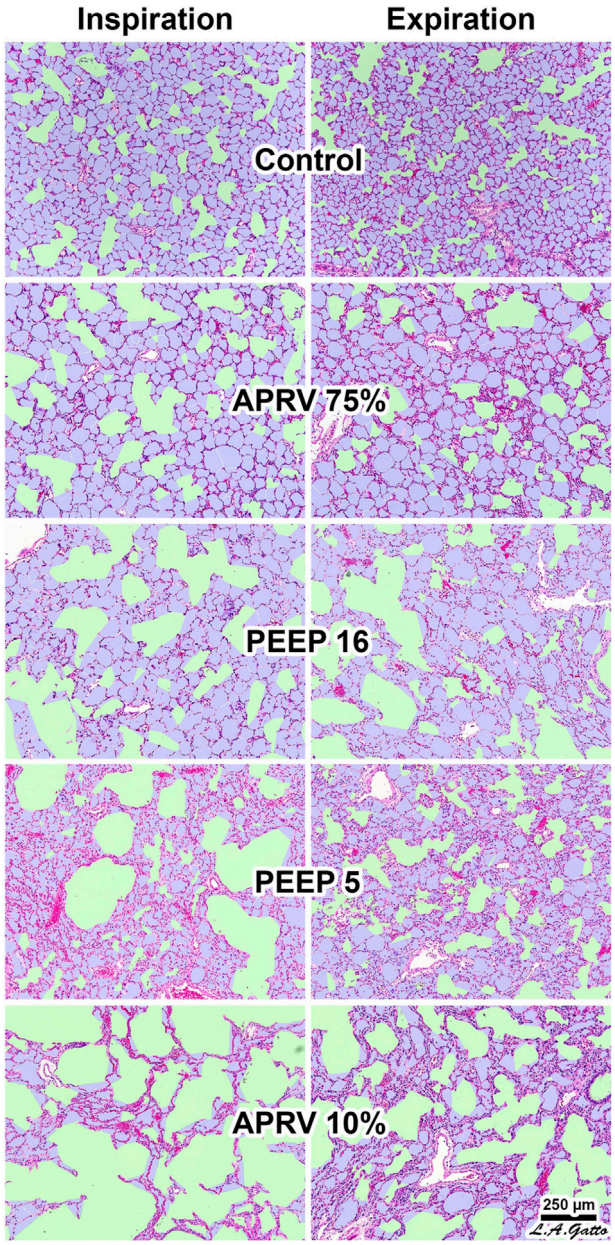

FIGURE 5 | The Airway Pressure Release Ventilation (APRV) 75% group produced the greatest alveolar air space occupancy (Aa) at both inspiration and expiration (I/E), with values similar to control ($p > 0.05$) and resulted in the least conducting airway micro-strain. The conducting airway air space occupancy (Ca) to alveolar air space occupancy Aa, Ca/Aa at I/E, closely matched uninjured normal lung terminal airway gas distribution. The APRV 10% (T_{Low} extended) group had the least Aa at both I/E and the greatest conducting airway micro-strain suggesting precise control of time is critical. In the conventional mechanical ventilation group increasing PEEP from 5 to 16 cmH_2O resulted in a greater degree of Ca rather than increasing Aa at I/E, suggesting increasing levels of PEEP primarily distend conducting airways rather than recruit alveolar gas and unable to restore the normal lung Ca/Aa.

higher risk of death (Deans et al., 2005; Bellani et al., 2016; Cavalcanti et al., 2017; Costa et al., 2021; Goligher et al., 2021; Raschke, et al., 2021).

In an uncontrolled sepsis-induced ARDS porcine model, preemptive application of APRV using the TCAV™ method was compared to ARDSNet LV_T (Roy S. et al., 2013). In this model of ARDS prevention, the lung was normal and uninjured at the onset of the experiment. In the APRV group, the lung C_{RS} remained normal throughout 48-h of uncontrolled sepsis, and V_T maintained at 12 ml/kg yet prevented the development of ARDS or volutrauma whereas the LV_T group with V_T of 6 ml/kg developed severe ARDS. This further supports that V_T should be normalized to C_{RS}, which was shown in the V_T data (Deans et al., 2005), ΔP data (Amato et al., 2015; Costa et al., 2021; Goligher et al., 2021; Raschke et al., 2021) and strenuous exercise data where V_T range from 36 to 40 ml/kg (Dominelli et al., 1985; Harms et al., 1998; Guenette et al., 2007; Guenette et al., 2009). With the TCAV™ method, when lung C_{RS} improves the V_T generally increases, which would then allow the P_{High} to be reduced and potentially the T_{High} to be extended. Additionally, in a mechanistic study with acute lung injury, APRV using TCAV™ had larger tracheal V_T displayed on the ventilator (macro-ventilation), yet the alveolar V_T (micro-ventilation) was lower than VAC with set and measured V_T of 6 ml/kg (Kollisch-Singule et al., 2014a). In this study, alveolar V_T was defined as the alveolar area change between inspiration and expiration (**Figure 7**). In the APRV group, area change was <5% with the T_{Low} set to 75% E_{FT}/E_{FP}; whereas the LV_T group demonstrated a 50% area change even with the most clinically used PEEP level (10 cmH_2O) (Bellani et al., 2016) (**Figure 8**) suggesting this commonly used PEEP level is associated with significant atelectrauma. Further, *in-vivo* microscopy of subpleural alveoli show the T_{Low} tuned to C_{RS} (i.e., 75% E_{FT}/E_{FP}) stabilizes alveoli within one breath cycle halting repetitive alveolar collapse and expansion (RACE)-induced atelectrauma (Kollisch-Singule et al., 2014a). With the TCAV™ method, the passive exhalation of the T_{Low} generates the $SLOPE_{EF}$ used to personalize V_T to C_{RS} (Dixon and Brodie, 1903; Rahn et al., 1946; Mead and Whittenberger, 1953; Brody 1954; Comroe 1954; Brody and Dubois, 1956; McIlroy et al., 1963; Bergman 1966; Grimby et al., 1968; Ashutosh and Keighley, 1978; Behrakis et al., 1983; Richardson et al., 1989; Baydur and Carlson, 1994; Brunner et al., 1995; Guttmann et al., 1995; Nassar et al., 2012). The $SLOPE_{EF}$ of T_{Low} characterizes elastic recoil (E_{RS}) including the chest wall and adapts to evolving lung mechanics, thereby optimizing alveolar stability and guides personalization of T_{Low}, normalizing EELV and V_T to C_{RS} which should not be set as a fixed duration or adjusted <75% E_{FT}/E_{FP} to achieve a desired V_T.

Finally, ventilators that can use pressure support with APRV incorporate a trigger window to attempt synchronization of inspiratory to expiratory (I:E) ratio creating an unstable T_{Low} that may randomly "kick out" beyond what is set (**Figure 9**). This has been shown to generate exceedingly high V_T where the actual T_{Low} displayed on the graphic waveform is a greater duration than the set T_{Low}. The example in **Figure 9** shows that despite a T_{Low} setting of 0.5 s (**Figure 9A**), the T_{Low} is extended to approximately 1.0 s (**Figure 9B**), subsequently creating a high V_T. The video shows the spontaneous changes in the duration of the T_{Low} without any changes to the T_{Low} setting.

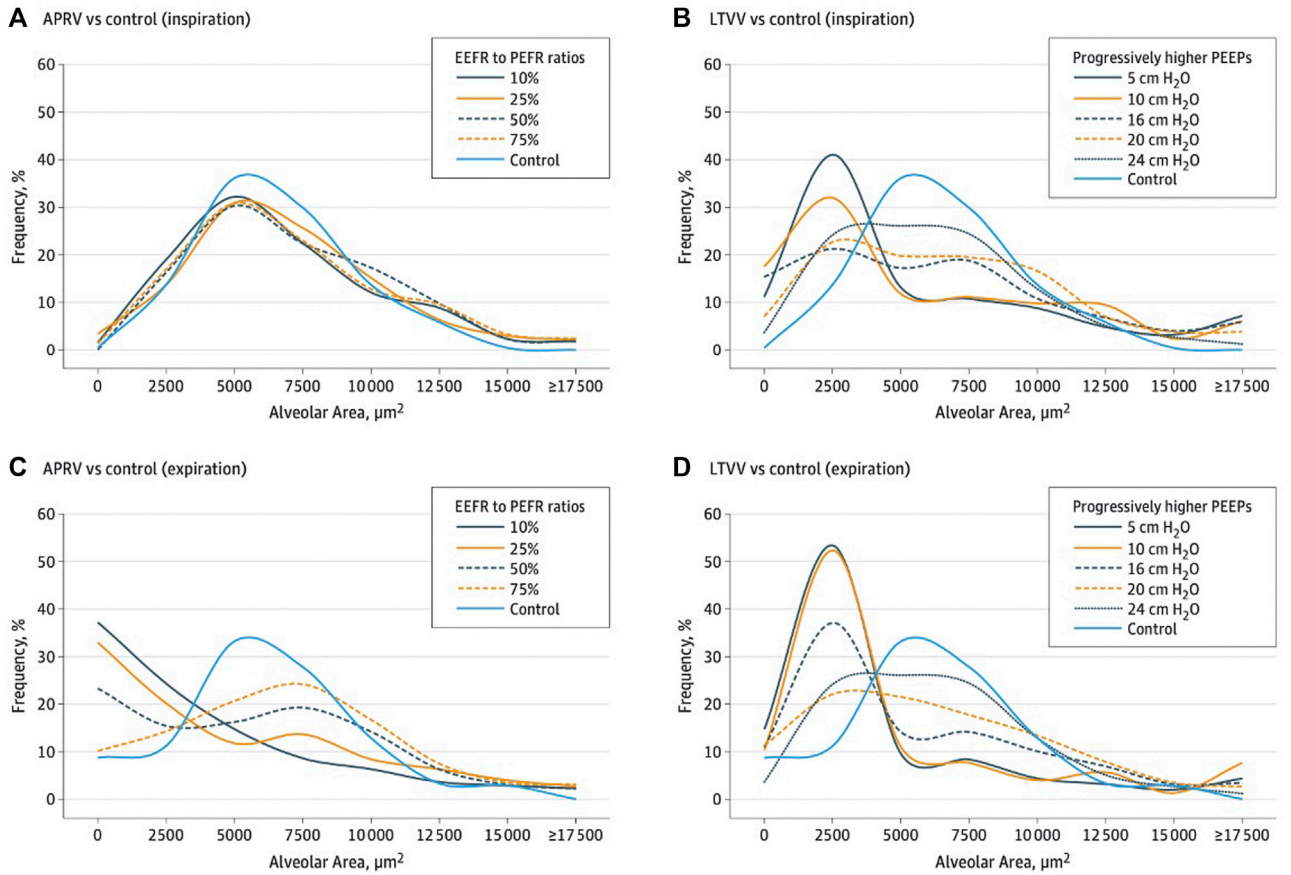

FIGURE 6 | Histogram overlying normal and injury alveolar area and frequency of distribution reflecting alveolar heterogeneity post lung injury. **(A,B)** show inspiration histogram with normal pre-injury (blue line) where remainder lines are post-injury demonstrating APRV normalizes post-injury heterogeneity. The LV_T group showing V_T with various positive end-expiratory pressure (PEEP) levels (5 to 24 cmH_2O) was not able to restore pre-injury homogeneity. **(C,D)** show expiration histogram with normal pre-injury (blue line) where remainder are post-injury demonstrating APRV normalizes post-injury heterogeneity. The LV_T group with various PEEP levels was not able to restore pre-injury.

(**Supplementary Video S1**). Additionally, in some variations of APRV (i.e., BiLevel on the Covidien ventilator) if the user sets T_{High} but not T_{Low}, subsequent RR changes unwittingly increase T_{Low} duration resulting in a larger V_T. This unintended consequence can be avoided by locking the T_{Low}, which eliminates linking the T_{Low} with the RR, keeps the T_{Low} fixed to the intended setting and avoids inadvertently generating a larger V_T.

MYTH #4—AIRWAY PRESSURE RELEASE VENTILATION INCREASES RIGHT VENTRICLE AFTERLOAD AND STRAIN

Several papers warn the use of APRV leads to an increase in right ventricular (RV) afterload, worsening of pulmonary hypertension and RV dysfunction, and reduction of venous return (VR) leading to systemic hypotension (Kallet, 2011; Modrykamien et al., 2011; Chatburn et al., 2016; Chen et al., 2017). There are even claims that APRV theoretically has an increased risk of cor pulmonale (Kallet, 2011; Chatburn et al., 2016; Chen et al., 2017). Indeed, applied airway pressure can result in a reduction of VR and cardiac output (CO). However, no scientific evidence exists this occurs more frequently with APRV than any other mode as these claims suggest. Although cor pulmonale is associated with increased mortality, no study has shown this increase in mortality is linked with APRV compared to LV_T. In fact, meta-analyses suggest the bias is towards greater survival in APRV (Lim and Litton, 2019; Zhong et al., 2020). This makes such claims implausible and uncredible leaving a basic review of physiology necessary to help navigate these misconceptions (Luecke and Pelosi, 2005). It must be realized that ventilator settings and lung-chest wall interactions have a key role in affecting the heart and these interactions may not be intuitive. Although some aspects of positive pressure may be beneficial, such as left ventricular afterload reduction with CPAP, most myths are related to RV function with inferences to systemic hypotension occurring more frequently with APRV than other ventilator modes.

Since the RV is incapable of generating significant pressure due to limitation of muscle mass, it relies on the large pressure drop

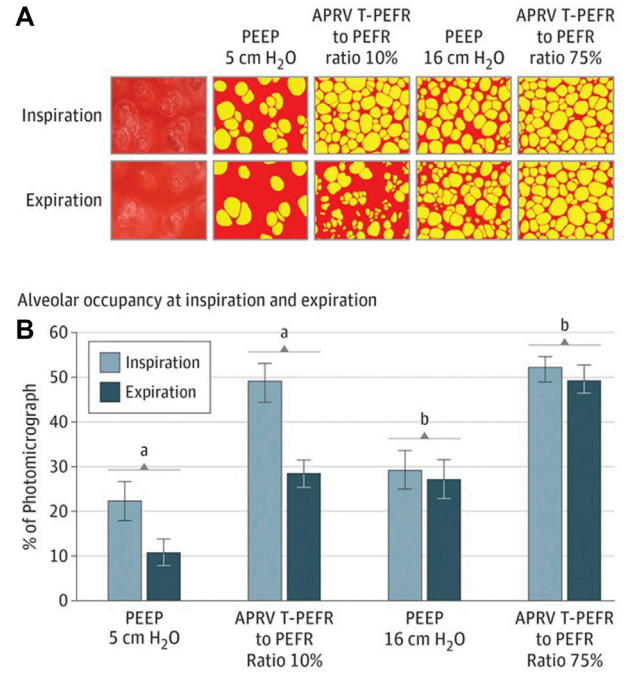

FIGURE 7 | (A) In vivo photomicrographs at inspiration and expiration (I/E) left to right: 1) positive end-expiratory pressure (PEEP) 5 cmH_2O; 2) airway pressure release ventilation (APRV) ratio of termination of peak expiratory flow rate (E_{FT}) to peak expiratory flow rate (E_{FP}) of 10%; 3) PEEP 16 cm H2O; and 4) APRV E_{FT}/E_{FP} 75% (original magnification ×10). Alveoli (yellow) and nonalveolar tissue (red). **(B)**, Alveolar air space occupancy is conveyed as a percentage of the photomicrograph containing inflated alveoli (yellow in A) at I/E. Data are shown as the mean; error bars indicate standard error of the mean. A) P<.0—PEEP 5 cmH_2O vs E_{FT}/E_{FP} 10%; B) P<.05—PEEP 16 cmH_2O vs E_{FT}/E_{FP} 75. Alveolar occupancy I/E shows that APRV 75% has the greatest number of open airspaces with inspiration, which is nearly double that of PEEP 16 cmH_2O and least loss of open airspace during exhalation resulting a less than 5% alveolar volume change between I/E. This results in the lowest micro-strain with APRV 75%.

across the vast highly distensible pulmonary vascular bed to limit flow resistance. The pressure drop occurs in small but numerous pulmonary vasculature, which are equally distributed between arterial and venous pulmonary circulation with the pulmonary artery having the highest resistance in the circuit (Gaar et al., 1967). Right heart loads are related to lung volume, pulmonary vascular resistance (PVR) and pleural pressure changes. Since the pulmonary circuit impedes RV output, anything affecting the lung can have an impact on right heart performance.

First, PVR and right heart load are increased at extremes of lung volume—1) residual volume (lung volume); and 2) total lung capacity (TLC) (Suresh and Shimoda, 2016) as seen in **Figure 10**. The lowest PVR and subsequent RV afterload is when the lung is at functional residual capacity (FRC) (Simmons et al., 1961). Many patients requiring mechanical ventilation have a loss of FRC (i.e., atelectasis) (Rahn et al., 1946; Puybasset et al., 1998; Rylander et al., 2004; Bikker et al., 2008; Bellani et al., 2011; Gonazalez-Lopez et al., 2012; Gommers, 2014; Hopkins and Sharma, 2022) and positive airway pressure to restore FRC generally results in decreased PVR and improved RV function by pulmonary artery wave-reflection (Sipmann et al., 2018) and echocardiogram (Duggan et al., 2003). Second, lung–chest wall interaction also influences hemodynamics and EELV. Similarly, this concept may also not be intuitive and goes beyond the oversimplified perception that RV load is solely a function of applied airway pressure or PEEP (Van Den Berg et al., 2001). For example, the chest wall springs out to a higher volume at the end of expiration while the lung simultaneously recoils to a lower volume with the abdominal cavity defining a boundary (diaphragm) of the chest wall and functioning as a fluid compartment rather than an elastic structure (Agostoni and Hyatt, 1973; Agostoni and Hyatt, 1986; West, 1989; Nunn, 1995; Lumb, 2010). Because of the spring out effect of the chest wall, a negative pleural pressure occurs at end-expiration even at high PEEP levels, which functionally results in the lung being suspended without any compressional forces from the chest wall at end-expiration (Stenqvist et al., 2012; Stenqvist et al., 2015; Persson et al., 2016; Persson et al., 2017). Increasing PEEP leads to lung inflation and displacement of the chest wall and diaphragm to a new pressure–volume equilibrium progressively lowering pleural pressure over subsequent breaths (Rahn et al., 1946; Katz et al., 1981; Stenqvist et al., 2012). Because right atrial pressure and VR are potentially influenced by pleural pressure, increased adaptation of the slow chest wall compartment allows EELV to increase without elevating pleural pressure (Stenqvist et al., 2012; Stenqvist et al., 2015; Persson et al., 2016; Persson et al., 2017). In fact, even RMs with high airway pressure are better tolerated hemodynamically if done incrementally rather than a sudden increase in pressure (Odenstedt et al., 2005; Santos et al., 2016). This may explain how patients with high potential for lung recruitment have less hemodynamic compromise in response to an increase in airway pressure compared to patients with non-recruitable lungs. However, data shows that ΔP (rather than PEEP per s) is associated with increased risk of cor pulmonale and the hemodynamic effect of PEEP is dependent on lung recruitability (i.e., the reduction in non-aerated lung in response to an increase in pressure) (McGuinness et al., 2020; Gazivoda et al., 2021; Hamouri et al., 2021; Rajdev et al., 2021; Udi et al., 2021).

The basic interaction between VR and positive pressure ventilation is also frequently misunderstood. Since VR is governed by the mean systemic pressure (MSP)-right atrial (RA) gradient, the application of PEEP and its impact on RA pressure would (in theory) reduce the MSP-RA gradient and decrease VR. However, many studies show the mechanism of PEEP on VR is not a reduction of the gradient as the applied pressure to the thorax is simultaneously transmitted to the abdominal compartment acting as a fluid filled compartment (Fessler et al., 1989; Nanas and Magder, 1992; Fessler et al., 1993). As a result, the pressure equally elevates the MSP, preserving the gradient for VR. Fessler et al. (1993) using MRI showed as PEEP and lung volume increases, an equal pressure point is reached compressing the vena cava as it enters the thorax from the abdomen, functioning as a starling resistor decreasing VR and impairing RV filling (Knowlton and Starling, 1912). Ultimately, lung volume is the main detriment of pleural pressure changes and can affect VR, right atrial pressure and RV afterload (O'Quinn et al., 1985).

These physical concepts of lung-heart interactions apply to all modes of ventilation. In particular, extremes of lung volume should

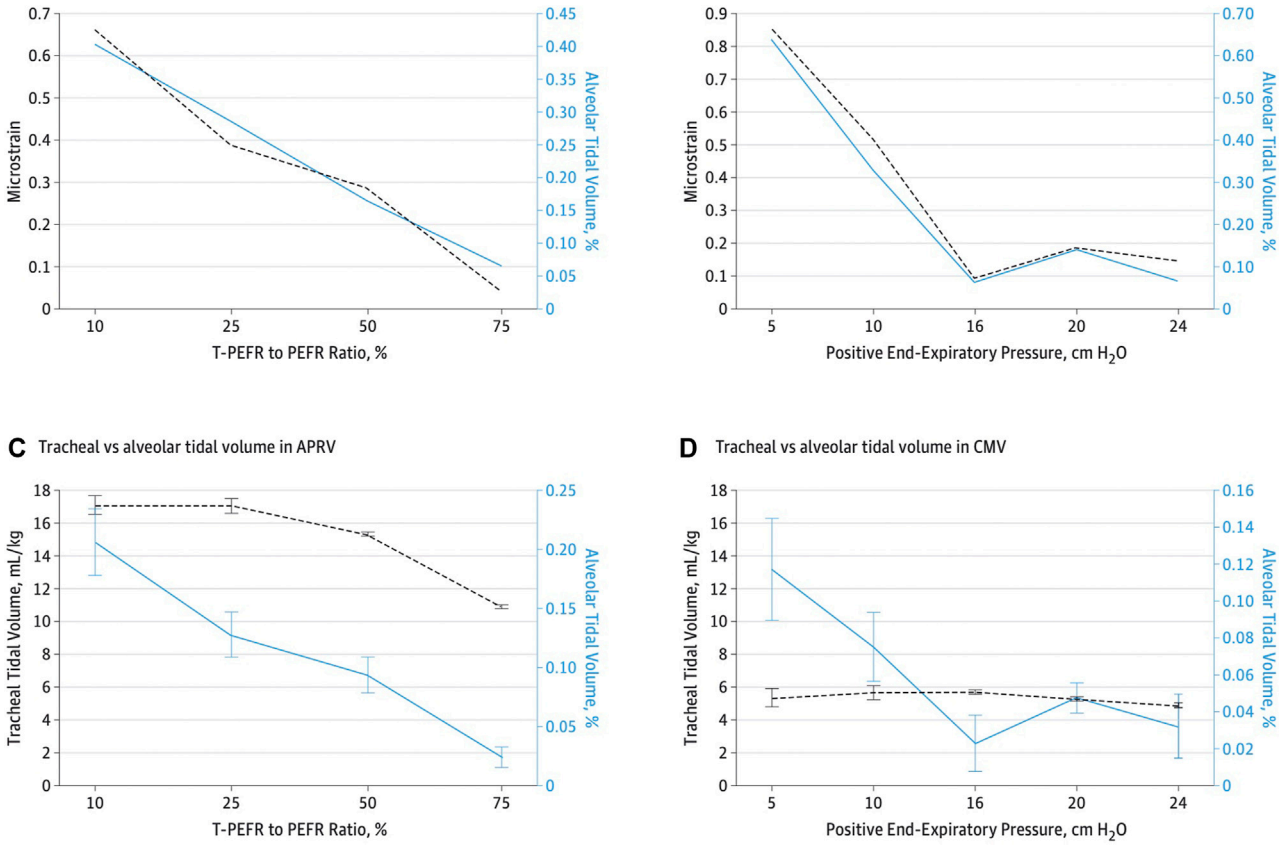

FIGURE 8 | (A,C)—As T_{Low} is adjusted towards 75% termination of peak expiratory flow rate (E_{FT}) to peak expiratory flow rate (E_{FP}), alveolar tidal volume (V_T) decreases despite tracheal volume 11 mL/kg. **(B,D)** with low V_T strategy, the opposite is true despite 6 ml/kg tracheal V_T with higher alveolar V_T. At peep of 10 cmH2O, the alveolar V_T and a tracheal V_T of 6 ml/kg is more that 3 times higher than alveolar V_T with APRV 75 % despite a tracheal V_T of 11 ml/kg.

be avoided and maintaining lung volume at FRC has the best effect on cardiopulmonary status (**Figure 10**). If lung volume is significantly below FRC (ie. residual volume), the airway pressure required to increase EELV will not increase heart strain; conversely if lung volume is above FRC (i.e., TLC), increased airway pressure will increase heart strain. In fact, as lung volume improves with recruitment, the size of the right heart is reduced (Duggan et al., 2003). Duggan, et al. (2003) showed that 150 min of derecruitment in rats resulted in marked dilation of the RV, paradoxical position of the interventricular septum and an underfilled left ventricle. Once the lung was recruited with an increase in applied airway pressure, there was a reduction in RV overload and improved left ventricular filling and lactate clearance. Many studies show an increase in lung volume with RMs or an appropriate increase in PEEP level improves RV function and pulmonary artery pressure (Reis Miranda et al., 2004; Reis Miranda et al., 2006; Longo et al., 2017). In general, the prevalence of cor pumonale during LV_T seems to increase in patients ventilated with lower PEEP levels (Boissier et al., 2013). A prospective sample of 200 patients receiving various ventilator modes, showed APRV was associated with the lowest ΔP when compared to VAC or pressure control ventilation (PCV) (Andrews et al., 2019).

To date, there have been no studies demonstrating increased hypotension or increased vasoactive use with APRV compared to any other ventilator mode whereas several studies show no difference or improved hemodynamics in APRV compared to other modes. For instance, in ARDS patients with cardiac dysfunction, APRV was shown to reduce vasoactive requirements while improving cardiac index, urine output, and lactate clearance (Kaplan et al., 2001). Additionally, a meta-analysis of seven RCTs with 405 eligible patients, showed APRV had a significantly higher mean arterial pressure on day 3 (Zhong et al., 2020) and a RCT comparing APRV to PCV in post cardiac bypass patients showed there was a significantly higher stroke volume, CO, and PaO_2/FiO_2 (P/F) ratio with APRV (Ge et al., 2021). More recent data showed a reduction of vasoactive support in CARDS patients managed with APRV (Joseph et al., 2020). Additionally, pediatric data includes a pediatric case series that showed APRV could safely be used in pediatric ARDS patients without significant hemodynamic deterioration (Kawaguchi et al., 2015), no difference in hemodynamic instability in pediatric patients when comparing APRV with LV_T (Ganesan et al., 2018) and Walsh et al. (2011) showed pulmonary blood flow, oxygen delivery and CO [in Tetralogy of Fallot group] were all significantly improved with APRV compared to PCV in children undergoing cardiac surgery.

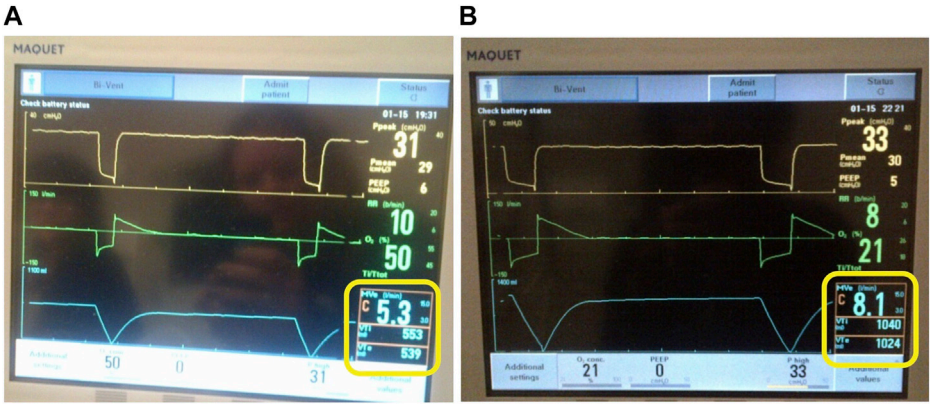

FIGURE 9 | Ventilator set in the Bi-Vent (APRV) Mode. **(A)** T_{Low} set to 0.5 s and release time is 0.5 s with V_Te 539 ml. **(B)** T_{Low} (release time) is kicking out to 1.0 s despite being set at 0.5 s with dramatically increased to V_Te 1024 ml. This occurs in ventilators that allow pressure support (inherent trigger and trigger windows) to be added on top of the P_{High}.

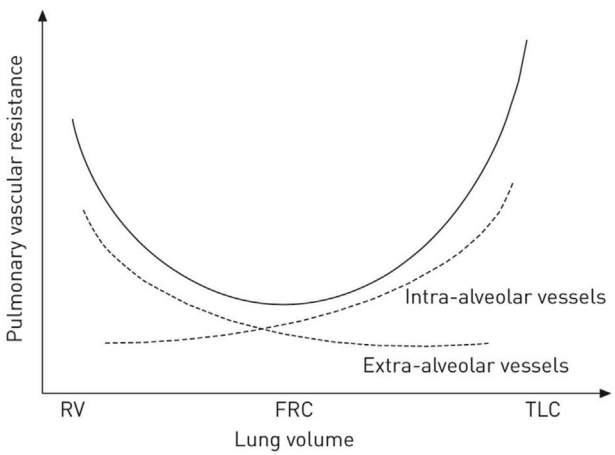

FIGURE 10 | Pulmonary vascular resistance (PVR) is at its lowest at functional residual capacity (FRC). At extremes of lung volume from residual volume (RV) to total lung capacity (TLC), PVR is increased, thereby increasing RV afterload.

Lastly, experimental studies have shown no difference or an improvement in hemodynamics with less vasoactives and a higher MAP in APRV using the TCAV™ method compared to conventional modes including LV_T (Roy et al., 2012; Roy S. et al., 2013; Roy SK. et al., 2013; Emr et al., 2013; Kollisch-Singule et al., 2015; Kollisch-Singule et al., 2016b; Jain et al., 2017; Vasconcellos de Oliveira et al., 2022).

MYTH #5—IT IS DIFFICULT TO CONTROL $PaCO_2$ WITH AIRWAY PRESSURE RELEASE VENTILATION

The misconception regarding inability to control partial pressure of arterial CO_2 ($PaCO_2$) leads clinicians to believe it is the ventilator mode that controls the settings and not the operator. For instance, it has been said "In APRV, some degree of CO_2 retention is not unusual" (Modrykamien et al., 2011), "mandatory breaths in APRV are intentionally set at a lower frequency (i.e., 10 breaths/min) than for conventional modes" (Mireles Cabodevila and Kacmarek, 2016), and the RR with APRV is usually 8–12 breaths/minute (b/min) (*sic*) (Daoud et al., 2012). These claims are simply not true as there is just as much ability to control $PaCO_2$ and set a higher RR in APRV as any other ventilator mode. In fact, APRV has been shown to be more efficient with $PaCO_2$ removal. A review of literature specific to $PaCO_2$ clearance with APRV spanning 25 years demonstrates APRV is associated with lower $PaCO_2$ when minute ventilation (MVe) is matched or a similar $PaCO_2$ with less MVe (Stock et al., 1987; Valentine et al., 1991; Smith and Smith, 1995; Maung et al., 2011). In other words, the volume of CO_2 (VCO_2) per liter of exhaled V_T is greater in APRV as compared to conventional ventilation (Bratzke et al., 1998). In addition, $PaCO_2$ depends on two phenomena: 1) physiological dead-space *per se;* and 2) the increased $PaCO_2$ seen in the case of high shunt fraction particularly when there is an increased gradient between the mixed-venous blood and $PaCO_2$. Increasing the inspiratory time allows more time for diffusive exchange of $PaCO_2$ where expiration begins when alveolar CO_2 ($PACO_2$) is close to equilibrium with mixed venous blood. Conversely, with a brief inspiratory time, expiration begins when $PACO_2$ is at its nadir. Physiologic data demonstrate optimizing diffusive and convective gas exchange [bulk flow of exhaled gas into the environment] increases ventilation efficiency, thus lowering MVe requirements for equivalent $PaCO_2$ clearance (Haycroft and Edie, 1891; Knelson et al., 1970; Engel et al., 1973; Fukuchi et al., 1976; Fuleihan et al., 1976; Fredberg, 1980; Valentine et al., 1991; Falkenhain et al., 1992; Smith and Smith, 1995; Mercat et al., 2001; Tsuda et al., 2011; Aboab et al., 2012). The concept that alveolar recruitment and derecruitment is time-dependent is often overlooked by clinicians. Although there is variability in alveolar recruitability among ARDS patients, time remains a critical element of distal airspace reopening and closure (Allen

et al., 2002; Allen and Bates, 2004; Allen et al., 2005; Albert et al., 2009).

In addition to controlling the RR, the T_{High} promotes gradual time-dependent alveolar recruitment throughout the lung, thereby reducing shunt fraction and increasing lung surface area for exchange of $PaCO_2$ based on Fick's Laws of Diffusion (Fick 1855; Wagner 1977). However, this does not imply that all patients, particularly those with significant lung dysfunction (i.e., ARDS) should have APRV initiated at a rate of 8–12 b/min (ie., T_{High} 4–6 s). Rather, the T_{High} should be adjusted to provide adequate ventilation and $PaCO_2$ for a given degree of pulmonary dysfunction. As surface area increases and alveolar stability improves, diffusive gas exchange increases and need for convective gas exchange (i.e., RR) decreases. Progressively, ventilation becomes more efficient over time (12–36 h) enabling an appropriate T_{High} increase. Correcting hypercarbia with T_{Low} manipulations to generate a larger V_T may briefly improve $PaCO_2$, but reduction in diffusive surface area from lung volume loss occurs, ultimately sacrificing alveolar stability and subsequently the mode is blamed for the high V_T and hypercarbia simultaneously.

In studies criticizing the inability of APRV to manage $PaCO_2$ (Batchinsky et al., 2011; Ibarra-Estrada et al., 2022), the T_{Low} was increased [E_{FT}/E_{FP} <75%] to adjust for hypercarbia, which resulted in a V_T increase that has been shown to subsequently increase alveolar collapse, worsen alveolar instability and heterogeneity, micro-strain and stress risers throughout the lung (Kollische-Singule et al., 2014a; Kollische-Singule et al., 2014b; Kollische-Singule et al., 2016a; Jain et al., 2017). Consequently, when the T_{Low} is adjusted to E_{FT}/E_{FP} <75%, alveolar collapse and instability ensues, ultimately resulting in further hypercarbia. In addition, rather than adjusting the T_{High} to increase the RR, these studies used a much lower RR in the APRV group compared to conventional modes (Batchinsky et al., 2011; Ibarra-Estrada et al., 2022). Conversely, in a large study of 411 patients in a burn unit using APRV, pH and $PaCO_2$ were maintained in the normal range with improved P/F ratios (Foster et al., 2021) and Maxwell, et al. (2010) reports the most interesting finding in their study was the LV_T group had a higher $PaCO_2$ than the APRV group despite a significantly higher MVe.

If patients with pulmonary dysfunction receiving APRV are treated the same as stable mechanically ventilated patients in terms of convective ventilation (i.e., RR 8–12 with T_{High} 4–6 s) hypercarbia would be expected. This was shown in a recent study of CARDS (Ibarra-Estrada et al., 2022) where more patients in the APRV group had transient (≤24 h) episodes of severe hypercapnia (42% vs 15%; $p = 0.009$) but were not associated with hemodynamic changes. However, the APRV group was managed with a T_{High} 4–6 s, which translated into ~10–12 b/min resulting in a significantly lower RR as compared with LV_T group ($p < 0.001$). It is important to note these patients had moderate to severe ARDS P/F ratios (per Berlin criteria) (ARDS Definition Task Force, 2012) from COVID, a pulmonary pathology with high dead-space fraction (Morales-Quinteroset al., 2021).

MYTH #6—AIRWAY PRESSURE RELEASE VENTILATION IS THE SAME AS INVERSE RATIO PRESSURE CONTROL

Several papers remark that APRV is functionally the same and indistinguishable from inverse ratio PCV (IR-PCV) in the absence of spontaneous breathing (Dries and Marini, 2009; Esan et al., 2010; Kallet 2011; Mireles Cabodevila and Kacmarek, 2016). Although it is true that both modes share similarities with settings that control pressure and time, there are key differences that are often overlooked. The first key difference is the inspiratory and expiratory times in APRV are controlled directly, independently and precisely, whereas I:E ratios of time are utilized in IR-PVC with the expiratory phase a "by-product" resulting indirectly from a set inspiratory time and RR. Comparable to the RR setting in IR-PCV, APRV uses the T_{High} to control RR where counterintuitively a decrease in T_{High} increases RR and an increase in T_{High} decreases RR. In addition, like the inspiratory time in IR-PCV, the T_{High} regulates the duration of the P_{High} creating a CPAP Phase to promote gradual expansion of collapsed alveoli (Syring et al., 2007; Boehme et al., 2015). However, because of the brief T_{Low} duration, APRV with an equal RR typically has a much higher I:E than is possible with IR-PCV on most ICU ventilators (**Figure 11**), which becomes progressively more limited with IR-PCV as the set RR increases. **Figure 11** shows conventional VAC (11A) with a set RR of 16 and I:E ratio of 1:3.2 transitioned to APRV (BiLevel on the Covidien) (11B) with same RR and the T_{Low} set to 0.32 s to achieve 75% E_{FT}/E_{FP} yielding an I:E ratio of 11:1. Subsequently, the V_T decreased from 408 to 308 ml (Nieman et al., 2020a; Nieman et al., 2020b).

The second key difference is that unlike IR-PCV, PEEP is not typically set with APRV because EELV is controlled with *time* (T_{Low}) rather than *pressure* (P_{Low}). Although studies show a P_{Low} in APRV may be set at any level, it is generally set at 0 cmH_2O when the T_{Low} is used as the controller of EELV (Habashi, 2005; Habashi et al., 2022). In fact, we have shown that personalizing the T_{Low} to E_{FT}/E_{FP} 75% in acute restrictive lung disease (i.e., increased E_{RS}) allows for quick stabilization of alveoli by halting alveolar collapse, loss of EELV and RACE-induced atelectrauma (Roy et al., 2012; Roy S. et al., 2013; Roy SK. et al., 2013; Andrews et al., 2013b; Emr et al., 2013; Kollisch-Singule et al., 2014a; Kollisch-Singule et al., 2014b; Kollisch-Singule et al., 2015; Kollisch-Singule et al., 2016a; Kollisch-Singule et al., 2016b; Kollisch-Singule et al., 2019; Smith et al., 2015; Jain et al., 2017; Silva et al., 2018; Bates et al., 2020; de Magalhães et al., 2021; Vasconcellos de Oliveira et al., 2022). Additionally, when the P_{Low} is set to 0 cmH_2O, the $SLOPE_{EF}$ is used to analyze the expiratory recoil forces, which allows personalization with fine-tuning of the T_{Low} to a patient's lung mechanics. This allows the clinician to adjust T_{Low} for changes in EELV and C_{RS}, based on the $SLOPE_{EF}$. This real-time breath to breath bedside monitoring of respiratory mechanics is not possible with IR-PCV as the PEEP valve attenuates the recoil force distorting the $SLOPE_{EF}$, which no longer reflects E_{RS} of a passive

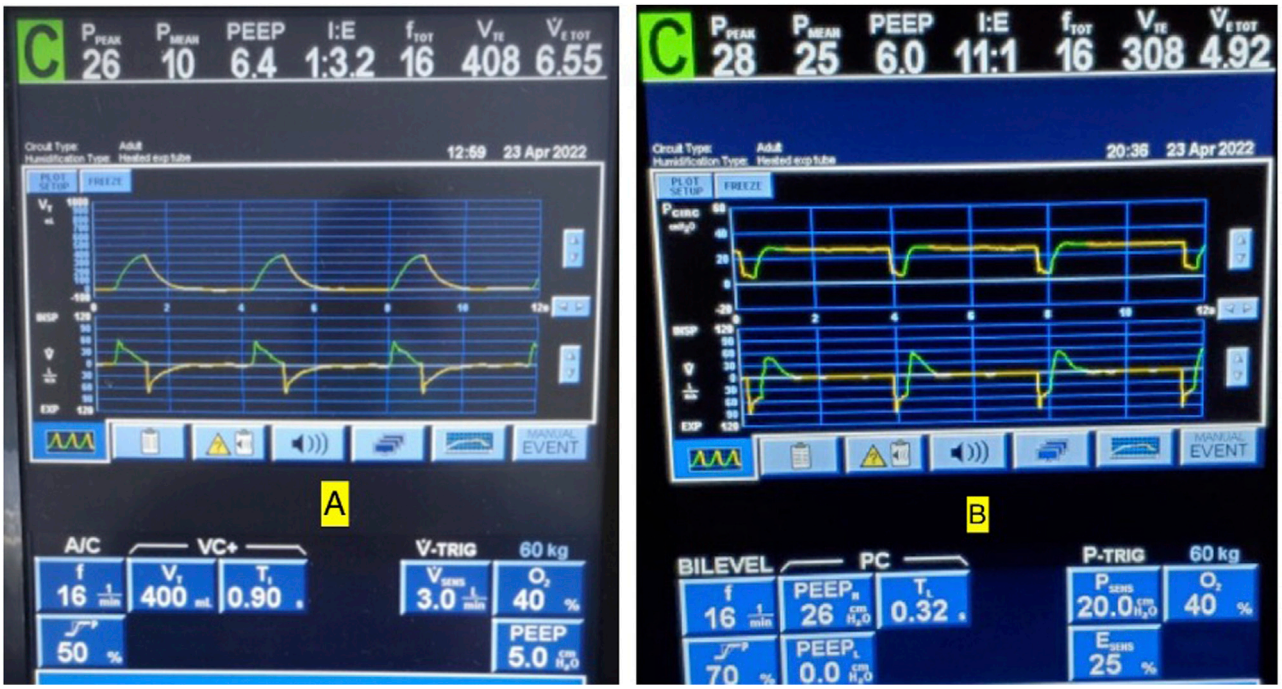

FIGURE 11 | (A) Conventional volume assist control (VAC) mode with a set respiratory rate (RR) of 16 and inspiratory to expiratory (I:E) ratio of 1:3.2. **(B)** Same patient transitioned to BiLevel (APRV) with same rate and T_{Low} set to 0.32 s to terminate at 75% of peak expiratory flow rate (E_{FT}/E_{FP}) yields an I:E ratio of 11:1. Note also that at E_{FT}/E_{FP} 75%, the tidal volumes decreased from 408 to 308 ml to match current C_{RS}.

exhalation (Dixon and Brodie, 1903; Rahn et al., 1946; Mead and Whittenberger, 1953; Brody 1954; Comroe 1954; Brody and Dubois, 1956; McIlroy et al., 1963; Bergman 1966; Grimby et al., 1968; Ashutosh and Keighley, 1978; Behrakis et al., 1983; Richardson et al., 1989; Baydur and Carlson, 1994; Brunner et al., 1995; Guttmann et al., 1995; Nassar et al., 2012).

Lastly, the name of the ventilator mode and what is configurable by the user varies among ventilator brands. For instance, APRV is often confused with variants of APRV (i.e. BiPAP, Bilevel) as manufacturers have their own branding of the mode APRV such as: Bi-Vent/APRV (Servo/Maquet), BiLevel/PC (Puritan Bennett/Covidien), APRV/BiPhasic (Avea/CareFusion), and APRV/PC-APRV (Dräger) to name a few. The crucial element when selecting the APRV mode, is the ability to set and adjust T_{High} and T_{Low} independently and precisely.

MYTH #7—AIRWAY PRESSURE RELEASE VENTILATION CREATES UNSAFE AUTO-PEEP

It has been the view of some that APRV leads to uncontrollable and even unsafe auto-PEEP (Dries and Marini, 2009; Modrikyniem et al., 2011; Daoud et al., 2012). Although a common statement, no data exists to support uncontrolled auto-PEEP and dynamic hyperinflation (DHI) occurs solely or with greater frequency in APRV (i.e., CPAP with release) than any other ventilator mode. Both terms and perception about auto-PEEP as it applies to APRV are assumed to equal DHI, which can cause barotrauma and hemodynamic instability and—by definition - increases over time. However, retaining static EELV should not be conflated to be equivalent to DHI and in fact, static lung volume with CPAP (i.e., without release) can decrease DHI in COPD (Petrof et al., 1990; Fessler et al., 1995; O'Donahuhe et al., 2002; Lopes et al., 2011). This opinion about auto-PEEP arises because APRV does not conform to the canonical practice of a set PEEP. Rather, in the TCAV™ method the T_{Low} prevents airway closure and retains EELV with brief, precise time control personalized to an individual's respiratory system mechanics [recoil force]. As EELV is a function of E_{RS} and the PEEP-volume, which is proportional to FRC and determined by T_{Low} duration and given that T_{Low} is adjusted based on a fixed percentage of the expiratory flow, which is an integral of volume, rather than a fixed or arbitrary time, volume displacement and EELV are therefore controlled directly.

Normally, lung volume at end-expiration approximates relaxation volume of the respiratory system. This defines FRC where the recoil forces of the lung towards the hilum are neutralized by outward forces of the chest wall and functions to maintain stable gas exchange, minimize elastic work of breathing (WOB) and optimize cardiopulmonary function (Rahn et al., 1946). Loss of FRC is common in hospitalized patients receiving mechanical ventilation (termed EELV) (Puybasset et al., 1998; Rylander et al., 2004; Bikker et al., 2008; Bellani et al., 2011; Albert, 2012; Gonazalez-Lopez et al.,

2012; Gommers, 2014; Albert, 2022; Hopkins and Sharma, 2022) and is magnified in ARDS where the role of static EELV is not only essential for cardiopulmonary benefits but may improve effectiveness of lung protective strategies as it minimizes lung strain, which can be high despite LV_T strategy (Chiumello et al., 2008; Gonzalez-Lopez et al., 2012; Xie et al., 2017).

Although set PEEP is intended to maintain or increase EELV by producing an expiratory retard, this view of creating auto-PEEP portends that only in APRV is the increase in EELV uncontrollable. Since adequate EELV during mechanical ventilation is necessary in protective ventilation, a reasonable question remains whether to use a set pressure (PEEP) to *indirectly* maintain EELV or guiding flow-time (T_{Low}) to *directly* retain and control EELV, as volume is an integral of flow. Since both PEEP and T_{Low} can maintain EELV, the key distinction is between static inflation (wanted) vs DHI (unwanted). The general concern for auto-PEEP, "air trapping" and DHI with APRV seems to be a reaction to the brief expiratory release time (T_{Low}). However, the role of expiratory time during mechanical ventilation has little impact on relieving DHI even in COPD (Leatherman et al., 2004; Ku, 2016; Natalini et al., 2016). Leatherman et al. (2004) noted extending expiratory time to >7 s did not significantly change DHI even in status asthmatics patients. Similarly in 186 patients with air flow limitations/obstructive lung disease, Natalini et al. (2016) states "Surprisingly, we observed that in our sample of mechanically ventilated subjects, the variables that characterized the breathing pattern (f, TE, VT, and minute ventilation) appeared to have a marginal role in auto-PEEP" and "It appears that even in patients with airflow limitations, Auto-PEEP can be more effectively reduced by acting primarily on modifiable characteristics of the patient, whereas manipulation of the breathing pattern might only have a negligible effect on the overall auto-PEEP value."

The equal pressure point contributes to increased airway resistance in addition to elastic recoil producing airflow limitations and delaying lung emptying allowing the next inspiratory effort/breath to occur before static equilibrium volume is reached resulting in DHI (Voets and Van Helvoort, 2013). Additionally, in patients with airflow limitations EELV may exceed predicted FRC (Kimball et al., 1982; Pepe and Marini, 1982). Despite being well described in the literature, the incidence of auto-PEEP remains unknown; however, most cases of DHI occur in patients with airflow limitations even without mechanical ventilation or typically receiving conventional ventilation (Wright and Gong, 1990; O'Donnell and Laveneziana, 2006). Whereas as "low level" auto-PEEP has been described with LV_T (Marini et al., 1985; de Durante et al., 2002; Patroniti and Pesenti, 2003). Bergman (1972) first described progressive air trapping using the term DHI that was induced by increasing RR up to 66 b/min coupled with increase in V_T up to 1 L in seven anesthetized patients. Subsequently, Pepe and Marini (1982) described the "auto-PEEP effect" in a case series describing DHI in three patients, two with known COPD and one with active bronchospasms using 11–12 ml/kg V_T with VAC. Because patients with COPD exhibit a decrease rate of lung emptying toward the end of expiration due to an increase in R_{AW} and are at greatest risk for DHI, a set PEEP is used to decrease R_{AW} and as a result DHI. This set PEEP results in a faster and more uniform rate of lung emptying (Kondili et al., 2004), which seems to be beneficial in decreasing DHI and may improve ventilator triggering (Chao et al., 1997). Likewise, in patients with ARDS the respiratory system deflation rate progressively decreases due to a considerable increase in expiratory resistance at low lung volume (Koutsoukou et al., 2000; Kondili et al., 2002) as airway caliber decreases during lung volume loss (Wilson, et al., 1993). Thus, application of PEEP in ARDS decreases the expiratory resistance similar to that seen in COPD patients and results in a relatively constant and fast rate of lung emptying (Koutsoukou et al., 2000; Kondili et al., 2002). Additionally, lung ultra-structure data shows PEEP dilates ducts as a possible mechanism of decreasing R_{AW} (Kollisch-Singule et al., 2014b).

Since PEEP decreases R_{AW} in COPD and ARDS, increasing lung emptying may be beneficial to reduce DHI in COPD where E_{RS} is low (i.e., low recoil force); however, when E_{RS} is high (i.e., ARDS) the lung may degas rapidly promoting atelectrauma. Thus, patients with high E_{RS} accommodate less inspired lung volume and maintain high recoil forces and in the absence of significant airflow limitations make DHI less likely (Gottfried, et al., 1985; Gottfried, 1991; Marini 2011). For instance, in a saline-lavage rabbit model cyclical lung recruitment was assessed with a fast PaO_2 probe comparing brief exhalation time (TExp) (0.83 s) and low PEEP (3 cmH_2O) to a prolonged TExp (2.9 s) and high PEEP (14cmH_2O) (Syring et al., 2007). Results showed compared to the low PEEP/brief TExp group, the high PEEP/prolonged TExp group experienced more cyclical recruitment (P 0.001). Furthermore, the low PEEP/brief TExp did not generate intrinsic PEEP (PEEPi). The authors summarize "Prevention of end-expiratory derecruitment without PEEPi suggests another mechanism, distinct from PEEPi, plays a role in the dynamic behavior of atelectasis." In addition, CO was increased on average 13% in the brief TExp compared with the high PEEP group (P 0.001), as was mixed venous saturation (P 0.001). In a lavage model of ARDS in juvenile pigs Boehme et al. (2015) found a prolonged inspiratory phase leads to higher average PaO_2 while the shortened Texp reduces tidal oscillations in PaO_2 suggesting a reduction in cyclic recruitment - derecruitment (c-R/D) with brief Texp. Shortening the Texp with inverse ratio ventilation (IRV) reduced the time available to derecruit, resulting in more average recruitment. Using electrical impedance tomography, as the I:E increased from 1:4 to 4:1 changes in regional ventilation occurred producing a redistribution from nondependent toward dependent lung regions. Boehme et al. (2015) also found negligible intrinsic PEEP as the Texp decreased in all settings. The authors conclude "Time constants for recruitment and derecruitment, and regional ventilation distribution, reflect these findings and highlight the time dependency of cyclic recruitment and derecruitment" (Boehme et al., 2015).

Although EELV is traditionally managed with PEEP, it remains unclear what PEEP level prevents airway closure in a given patient at a given time (Kalenka et al., 2016). Although increasing PEEP shows a linear correlation with oxygenation and is commonly used as a surrogate of recruitment, it remains a poor marker of alveolar stability as seen with *in-vivo*

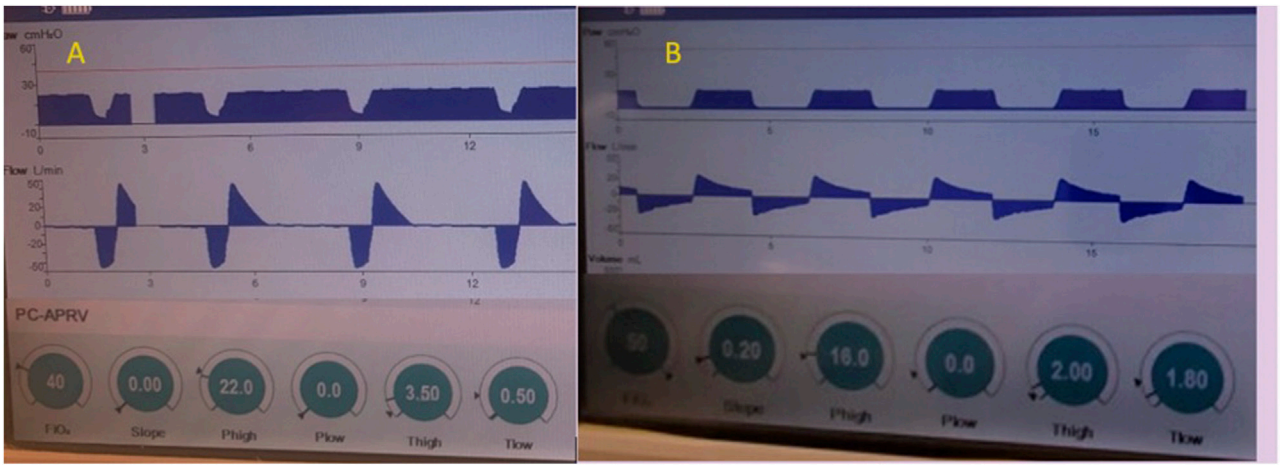

FIGURE 12 | Passive exhalation to determine lung mechanics in APRV - The Time Controlled Adaptive Ventilation (TCAV™) method of Airway Pressure Release Ventilation (APRV) uses the slope of the expiratory flow curve of passive exhalation to determine respiratory mechanics. Example **(A)** (left) is a patient with high elastance of the respiratory system (E_{RS}) denoted by the expiratory flow rate >50 liters/minute and the acute slope deceleration angle. The slope deceleration is affected by inspiratory lung volume and downstream resistance (native and artificial airways and P_{Low} >0 cmH_2O). Changes in E_{RS} (i.e., recoil force per unit of volume) or increase in airway resistance (airflow limitations) alters peak expiratory flow (E_{FP}) and slope angle. The T_{Low} with high E_{RS} is adjusted to terminate the expiratory flow (E_{FT}) at 75% of the peak expiratory flow (E_{FP}). End-expiratory lung volume (EELV) is controlled through precise and personalized adjustment of flow-time as an integral of volume. Because personalization of the T_{Low} is adjusted based on elastic recoil of the lung and E_{RS}, it should not be adjusted to achieve tidal volume (V_T) or control $PaCO_2$. The 75 E_{FT}/E_{FP} has be calibrated experimentally, validated clinically, and shown to optimize EELV, prevent airway closure and lower lung strain in lungs with normal to increased E_{RS}. Example **(B)** (right) is a patient with low E_{RS}, low recoil forces and high resistance denoted by the expiratory flow rate <20 liters/minute and the less acute slope deceleration angle where the T_{Low} is adjusted to achieve 25% of E_{FT}/E_{FP}, which has been calibrated to decrease alveolar heterogeneity, lung inflammation, edema, and gene expression of biological markers related to ventilator induced lung injury and improve right ventricular performance by personalizing a COPD model.

microscopy (Andrews et al., 2015). Decremental PEEP studies show the loss of EELV at each level of PEEP reduction making one PEEP level difficult to control time dependent lung behavior (Maggiore et al., 2001; Sahetya et al., 2017; Bates and Smith, 2018; Baumgardner 2019; Broche et al., 2019; Scaramuzzo et al., 2019). Data on the effect of PEEP on lung micro-architecture suggest PEEP primarily causes ductal dilatation rather than preventing alveolar collapse and increases alveolar heterogeneity (Kollisch-Singule et al., 2014b; Kollisch-Singule et al., 2016a) (**Figure 5**). In fact, the PEEP-FiO_2 scale has recently been challenged as dangerous for CARDS patients (Gattinoni et al., 2020a, 2020b; Tsolaki et al., 2020; Barthélémy et al., 2021; Ceruti et al., 2021).

Alternatively, the T_{Low} set to the prevailing time constants (Bates et al., 2020) demonstrated that APRV with the TCAV™ method increases alveolar stability, decreases micro-strain and alveolar heterogeneity and normalizes the airspace with less ductal dilation than PEEP (Kollisch-Singule et al., 2014a; Kollisch-Singule et al., 2014b; Kollisch-Singule et al., 2016a) (**Figures 4–7**). With the TCAV™ method, the T_{Low} is adjusted to target E_{FT}/E_{FP} 75% in normal to high E_{RS} (i.e., ARDS) and <50%–25% for patients with low E_{RS} (i.e., COPD, asthma) (**Figure 12**). Lastly, analyzing the $SLOPE_{EF}$ with the TCAV™ method provides real-time assessment of respiratory mechanics as a patient's disease process evolves rather than the arbitrary PEEP selection or attempts to use oxygenation as a marker of alveolar stability and a surrogate for low lung strain (Andrews et al., 2015). In APRV with a P_{Low} set to 0 cmH_2O, air flow limitations and changes in EELV are seen in real-time with changes in E_{RS} or resistance including experimental models of COPD (Vasconcellos de Oliveira et al., 2022). **Figure 13** shows the evolution of the T_{Low} in a patient with acute bronchospasm (status asthmaticus), which was captured with real-time bedside monitoring of airflow limitations and corresponding T_{Low} adjustments (**Figure 13**). When acceptable levels of spontaneous breathing occur and because the release phase in APRV-TCAV™ is so brief, there are three major implications: 1) spontaneous breaths occur primarily during the CPAP Phase preserving neural inspiratory time; 2) CPAP in patients with airflow limitations is associated with a decrease in DHI allowing patients to defend their lung volume making uncontrolled DHI unlikely (Petrof et al., 1990; Fessler et al., 1995; O'Donahuhe et al., 2002; Lopes et al., 2011); and 3) the active exhalation valve compared to a closed expiratory valve during the inspiratory phase allows patients to exhale beyond the set release frequency and gain an inspiratory assistance by using abdominal expiratory muscles (Torres et al., 1993).

MYTH #8—A P_{LOW} OF 0 CMH_2O LEADS TO INJURY AND ALVEOLAR COLLAPSE

Although which PEEP level is protective remains undefined, it has been suggested the abrupt transition from P_{High} to a P_{Low} of 0 cmH_2O is uncontrolled in APRV creating potential for mechanical injury, which is otherwise protected by PEEP

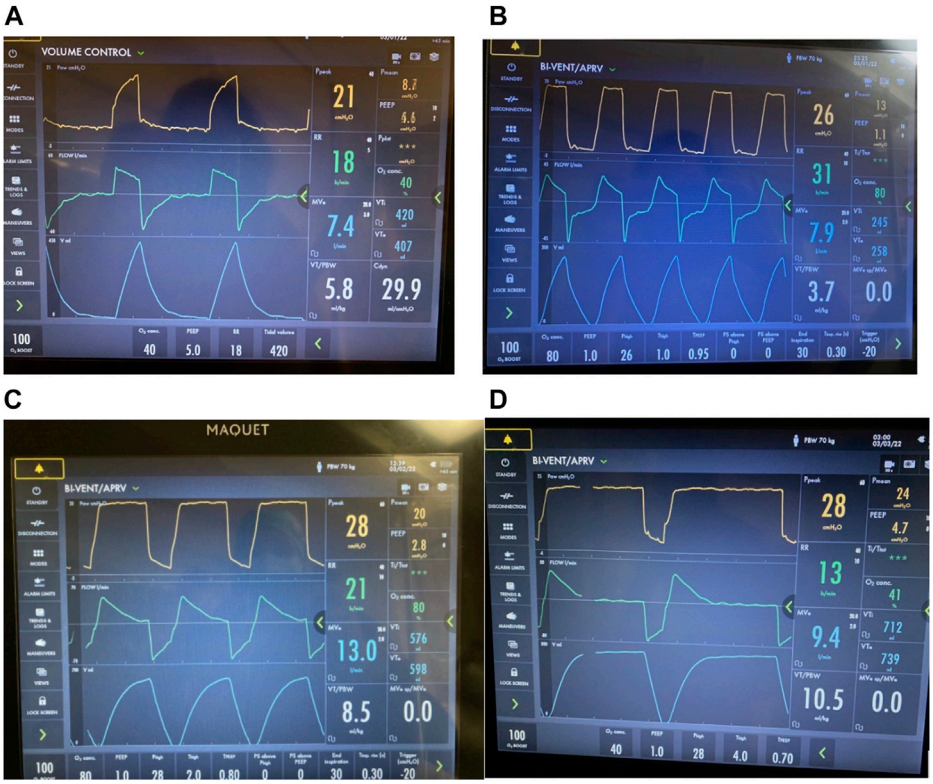

FIGURE 13 | T_{Low} setting in patient with acute bronchospasm (status asthmaticus). Bedside monitoring of airflow limitations with real-time T_{Low} adjustments with airway pressure release ventilation (APRV) BI-VENT in a patient with active bronchospasm **(A)** Volume Control mode where intrinsic dynamic (Dyn) positive end expiratory pressure (PEEP) is not seen in the expiratory flow waveform **(B)** Mode changed to BI-VENT/APRV with peak expiratory flow rate (E_{FP}) measured -20 L/min, which is consistent with severe airflow limitation. Note, E_{FP} is measured at onset of deceleration and not artifact from immediate loss circuit gas compression. T_{Low} is adjusted to 0.95 s targeting termination of flow rate (E_{FT}) >25% to <50% for patients with airflow limitations. **(C)** Resolving acute bronchospasm, E_{FP} is nearly 70 l/min allowing T_{Low} to be decreased to 0.8 seconds while continuing to target E_{FT}/E_{FP} >25% to <50%. **(D)** Continued improvement of bronchospasm where E_{FP} is nearly 80 l/min allowing T_{Low} to be decreased to 0.7 s while continuing to target E_{FT}/E_{FP} >25% to <50%. Progressive increase in tidal volume and minute ventilation allows gradual reduction of P_{High} (not shown). Note, this ventilator does not allow a P_{Low} of 0 cmH_2O with 1 cmH_2O the lowest setting possible.

(Neumann et al., 2002; Dries and Marini, 2009) and a P_{Low} of 0 cmH_2O allows for alveolar collapse even with a brief T_{Low} (Myers and Macintyre, 2007; Modrikyniem et al., 2011; Daoud et al., 2012). Fundamentally, when using the TCAV™ method of APRV the P_{Low} is set to 0 cmH_2O because time [rather than pressure] is used to control EELV. Additionally, a P_{Low} >0 cmH_2O alters the flow-time course of passive exhalation, thus dampening the recoil force where the $SLOPE_{EF}$ no longer represents mechanics of the respiratory system.

Like PEEP, there is no consensus of the P_{Low} setting in APRV. However, studies have shown that APRV a P_{Low} 0 cmH_2O and T_{Low} set to E_{FT}/E_{FP} 75% maintains EELV, prevents end expiratory airspace collapse, produces lowest micro-strain on distal air spaces (alveoli and ducts) (**Figure 4**), minimizes ductal dilatation (**Figure 5**) and restores alveolar homogeneity after heterogenous lung injury compared to LV_T with PEEP up to 24 cmH_2O (**Figure 6**) (Kollisch-Singule et al., 2014a, Kollisch-Singule et al., 2014b, Kollisch-Singule et al., 2016a, Kollisch-Singule et al., 2016b showed in a model of acute lung injury alveolar area change between inspiration and expiration was <5% in the APRV group with a P_{Low} of 0 cmH_2O and T_{Low} set to E_{FT}/E_{FP} 75%, mimicking the area change of uninjured lung; whereas the LV_T group with the most commonly clinically used PEEP of 10 cmH_2O (Bellani et al., 2016) demonstrated a 50% area change between inspiration and expiration suggesting a 10-fold greater RACE-induced atelectrauma (**Figure 8**). In addition, to determine APRV efficacy, a translational model comparing APRV with LV_T showed the APRV group with T_{Low} set to 75% E_{FT}/E_{FP} and P_{Low} 0 cmH_2O did not produce lung injury by P/F ratio, histology, or inflammatory markers, whereas the LV_T group developed ARDS in all animals by P/F ratio, histology, and inflammatory markers (Roy et al., 2012; Roy S. et al., 2013; Roy SK. et al., 2013; Silva et al., 2018; de Magalhã;es et al., 2021). Further, an observational study of ARDS prevention looked at 231 patients set with an APRV protocol using T_{Low} 75% E_{FT}/E_{FP} and P_{Low} 0 cmH_2O (Andrews et al., 2013b) and did not show a higher ARDS rate or mortality as would be assumed if using T_{Low} 75% E_{FT}/E_{FP} and P_{Low} 0 cmH_2O could not limit collapse, subsequently worsening lung injury.

It has also been claimed that a P_{Low} of 0 cmH_2O does not increase the E_{FP} (Zhou and Chatburn, 2012). However, this is based on data generated from a simulator model, which is unable to quantify the viscoelastic tissue behavior of the lung and chest wall, where it was speculated E_{FP} would remain unchanged with no delay when comparing P_{Low} settings. To achieve their goal, the P_{High} was increased with each increase in P_{Low} to maintain a Δ 25 cmH_2O. This of course does not represent the clinical application of APRV and would be analogous to increasing the inspiratory pressure in PCV each time a PEEP increase is made. It is not surprising their results showed an increase in E_{FP} with each increase in P_{High} and simultaneous increase in P_{Low} as the increased recoil in a single compartment model would be expected. However, this same concept was tested in 20 patients where only the P_{Low} was increased and not the P_{High} (Madden et al., 2016), reflecting standard clinical practice when using the T_{Low} to control EELV and showed a progressive decrease in the E_{PF} as the P_{Low} was sequentially increased >0 cmH_2O.

Because expiratory flow rates are critical for secretion removal, Mahajan et al. (2019) further validated setting a P_{Low} of 0 cmH_2O in a model of preserved pig lungs fitted with an endotracheal tube. Multiple combinations of peak inspiratory and E_{FP} rates were used to compare APRV (TCAV™ method) with LV_T (ARDSnet protocol). The P_{High}/Pplat was set equally in both groups. In the APRV group, only the P_{Low} was adjusted from 0 to 5 to 10 cmH_2O incrementally and in the LV_T group, the PEEP was adjusted from 5 to 10 to 20 cmH_2O incrementally. As the P_{Low} was increased, both E_{PF} and mucus movement decreased, which is important as studies suggest clearance of mucus is facilitated with increased E_{PF} (Kim et al., 1987; Dennesen, et al., 2003; de Prost et al., 2007; Powell et al., 2018). The APRV-TCAV™ group resulted in the greatest proximal mucus movement compared to no mucus movement in the LV_T group at any PEEP level as seen in **Figure 14**. Further, in a study comparing APRV-TCAV™ with VAC in experimental pneumonia (de Magalhã;es et al., 2021), APRV-TCAV™ was associated with less lung damage, less bacteremia and reduced gene expression of mediators associated with inflammation.

To summarize, when using the TCAV™ method of APRV setting a P_{Low} is not necessary and varied P_{Low} levels would be just as arbitrary as varied PEEP levels, which continues to be unsettled and may remain so for the foreseeable future. However, all experimental models show less injury or complete prevention of lung injury when using a P_{Low} 0 cmH_2O with a T_{Low} 75% E_{FT}/E_{FP} (Roy et al., 2012; Roy S. et al., 2013; Roy SK. et al., 2013; Kollisch-Singule et al., 2014a; Kollisch-Singule et al., 2014b; Kollisch-Singule et al., 2016a; Kollisch-Singule et al., 2016b; Silva et al., 2018; de Magalhã;es et al., 2021). Clinically, trials show APRV with P_{Low} 0 cmH_2O with a T_{Low} 75% E_{FT}/E_{FP} is not inferior to LV_T, which would be unlikely if APRV induced lung injury (Andrews et al., 2013a; Andrews et al., 2013b). There is a common mistake to assume linearity between macro-ventilatory parameters displayed on the ventilator and what's happening in the micro-environment of the lung) where APRV actually produces minimal dynamic strain (Kollisch-Singule et al., 2018).

MYTH #9 –IT IS NOT POSSIBLE TO MEASURE DRIVING PRESSURE IN APRV

In a study by Zhou, et al. (2017), the authors stated, "In order to make the ΔP of APRV and LTV comparable, the ΔP of APRV was measured under the same conditions as with LTV" (*sic*). The authors temporarily changed modes in the APRV group to VAC to measure ΔP. The belief it is not possible to measure ΔP in APRV may be in part a result of a study by Kacmarek et al. (1995) using a single compartment test lung model comparing set vs auto-PEEP is often referenced and data extrapolated to imply APRV would produce heterogenous distribution of end expiratory pressure and EELV thereby rendering ΔP inaccurate. Unfortunately, this study does not provide details as to the duration used for expiratory pressure equilibration and more importantly a test lung does not capture the behavior of tissue stress recovery within the lung and chest wall tissues due to their viscoelastic behavior (Bates et al., 1988; Kochi et al., 1988). Subsequently, authors have extrapolated the test lung model data further by theorizing APRV would produce such heterogenous ventilation to increase risk of volutrauma and atelectrauma (Chatburn et al., 2016). However, biologic data exist that make these opinions inaccurate speculations.

In an animal model, IRV producing V_T 8–12 ml/kg was used to produce intrinsic PEEP (PEEPi) and compared static (PEEPi stat) vs dynamic (PEEPi Dyn) measurement (Hernandez et al., 1994). The PEEPi stat was measured using an expiratory hold for 5 s and PEEPi Dyn was measured using expiration to no flow state. Results show that PEEPi Dyn underestimates PEEPi stat only in acute non-homogeneous airway obstruction induced with methacholine (MCh) whereas PEEPi stat approximates PEEPi Dyn without MCh induced bronchospasm. This suggests airflow limitations, such as with bronchoconstriction, have the greatest effect on PEEPi Dyn [not PEEPi stat] measurements and the ability to estimate alveolar pressure and measure ΔP. Kollisch-Singule et al. (2016a), showed in a lung injury model with APRV that alveolar heterogeneity after lung injury was normalized to match normal uninjured alveolar size distribution thereby restoring alveolar homogeneity whereas LV_T was unable to normalize alveolar heterogeneity using external PEEP ranging from 5 to 24 cmH_2O. In summary, APRV was superior to LV_T with external PEEP in producing uniform alveolar size and distribution after lung injury. In another study investigating alveolar to duct gas distribution ratio of conducting airway air space occupancy to alveolar air space occupancy in the distal airspace, APRV with T_{Low} set to E_{FT}/E_{FP} 75% restored alveolar to duct gas ratios closely resembling pre-injury ratios. In contrast, LV_T and PEEP demonstrated inability to restore alveolar gas volume but progressively overdistended alveolar duct volume (Kollisch-Singule et al., 2014b). Lastly, in a sophisticated porcine lung injury model producing a "baby lung" with injured dependent and normal non-dependent lung regions, using APRV overdistension was simulated with P_{High} of 40 cmH_2O. With APRV E_{FT}/E_{FP} 75% neither atelectrauma nor overdistension resulted and produced the least injury to both normal and injured lung regions (Jain et al., 2017).

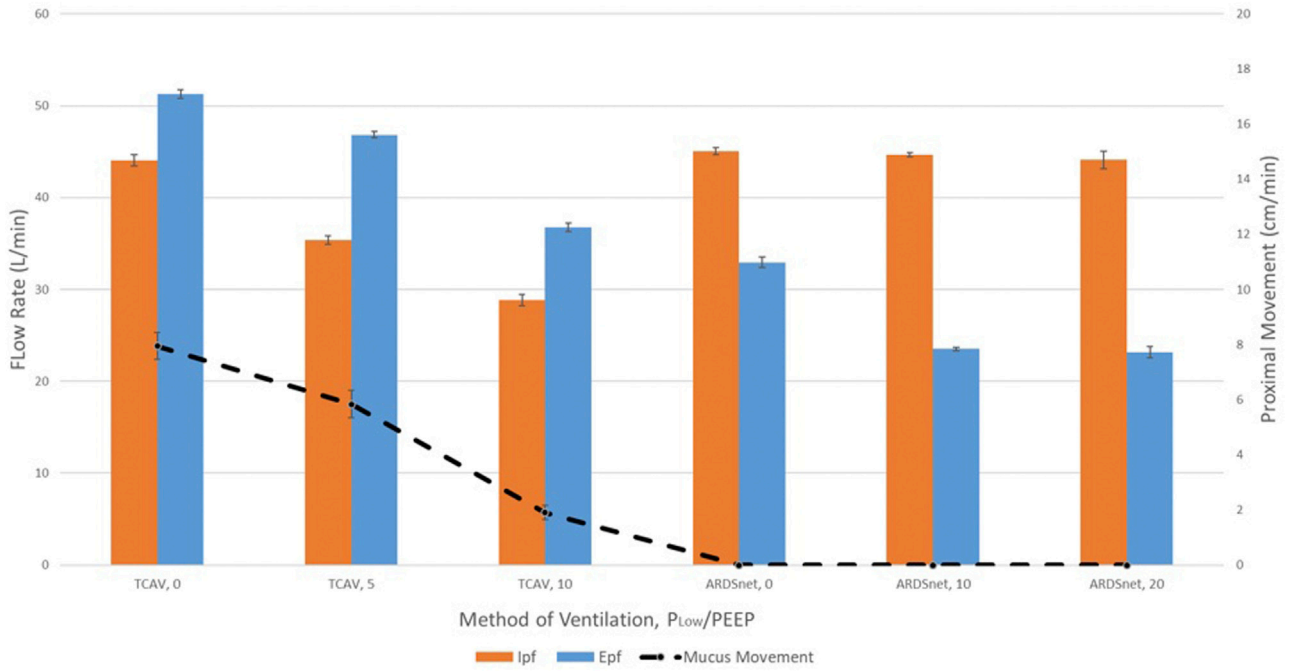

FIGURE 14 | Peak inspiratory flow (I_{PF}), peak expiratory flow (E_{PF}), and proximal mucus movement for experimental groups comparing Airway Pressure Release Ventilation (APRV) and Low Tidal Volume (LV_T). Orange and blue colored bars demonstrate the I_{PF} and E_{PF} respectively (left vertical axis). Proximal mucus movement is denoted by the dotted line connecting data points (vertical axis). TCAV protocol groups 1, 2, and 3 used APRV with varying P_{Low} (standard APRV-TCAV™ with 0 cmH_2O) and 5 and 10 cmH_2O, respectively. The ARDSNet LV_T groups 4, 5, and 6 varied positive end expiratory pressure (PEEP) settings of 0, 10 and 20 cmH_2O, respectively.

It has also been implied that APRV caused RV failure likely due to high ΔP from APRV with no supporting data (Chen et al., 2017). Conversely, ΔP was measured in 200 patients with a passive respiratory system such as post-operative patients (i.e., anesthesia or neuromuscular blocking agents) using a 4 s expiratory hold for equilibration (Andrews et al., 2019). Data was obtained and divided between four ventilator modes: 1) n = 86 VAC); 2) *n* = 28 PCV; 3) *n* = 74 APRV-TCAV™ as standard of care (S-APRV); and 4) *n* = 12 APRV-TCAV™ as a rescue mode (R-APRV) used when patients failed conventional ventilator modes. The ΔP was lowest in the S-APRV group compared to both the VAC group (p- value = 0.0010) and PCV group (*p*-value = 0.0002) but not statistically different than R-APRV group (*p*-value = 0.3379). Lastly, although Neumann et al., is often referenced regarding large pleural pressure swings with APRV increasing the risk of volutrauma and atelectrauma, they showed ΔP of mechanical breaths without spontaneous breathing decreased when T_{Low} was reduced from 2.5 to 0.5 s, such as that with the TCAV method where the E_{FT}/E_{FP} is set to 75% (Neumann et al., 2002).

The power of mechanical ventilation, defined as the amount of energy transmitted from the ventilator to the lungs, is comprised of the driving pressure (delta P), V_T (delta V), RR, PEEP, and inspiratory gas flow such that a fast inspiratory delivery with a slow expiratory flow adds to the power (Gattinoni et al., 2016). The two variables found to be most associated with increased mortality are the ΔP and RR (Costa et al., 2021). Given the time settings used in APRV (T_{High} and T_{Low}) ventilation is usually delivered with lower RR and ΔP. The T_{Low} and P_{High} depend on C_{RS}, recruitability and because T_{Low} is brief, T_{High} is generally 1–1.5 x greater duration than conventional ventilation for a give RR. The extended T_{High} gradually restores lung volume improving gas exchange efficacy leading to progressive lower RR and when coupled with pairing V_T to C_{RS} (ie delta P), these time settings reduce key mechanical power variables. Mechanical power was found to be significantly reduced in APRV (11.9 J/min) compared to LV_T (20.7 J/min) in an experimental model of blast lung injury (Scott et al., 2020).

MYTH #10—PATIENTS MUST BE SPONTANEOUSLY BREATHING FOR APRV TO BE EFFECTIVE/SPONTANEOUS BREATHING DURUNG APRV IS DANGEROUS

This myth comes full circle with both pro and con statements about spontaneous breathing with APRV. It has been claimed

that without spontaneous breathing, APRV is not effective, and the key physiologic advantages are lost (Dries and Marini, 2009; Modrykamien et al., 2011), yet others claim APRV can have a significant effect on work of breathing (WOB) and potential for harm with the cost of spontaneous breathing during APRV being markedly elevated. (Kallet, 2011; Daoud et al., 2012; Mireles Cabodevila and Kacmarek, 2016).

First, we address the claim regarding the benefits of APRV being lost without spontaneous breathing. Although spontaneous breathing may be facilitated with APRV, it is certainly not a prerequisite. Experimental and clinical data show benefits of APRV without spontaneous breathing (Roy S. et al., 2013; Roy SK. et al., 2013; Emr et al., 2013; Kollisch-Singule et al., 2014a; Kollisch-Singule et al., 2014b; Kollisch-Singule et al., 2016a; Kollisch-Singule et al., 2016b; Jain et al., 2017). Because APRV with the TCAV™ method can prevent or halt the progression of VILI and subsequently stabilize the lung, it can be used successfully even in brain dead donors who are obviously not spontaneously breathing (Roy et al., 2017; Koch et al., 2019). In fact, in a study of donors managed with APRV, lung utilization occurred more in the APRV group (84%) than the VAC group (18%) ($p < 0.001$) (Hanna et al., 2011). Additionally, organ recovery data demonstrate an increase in overall organs recovered in donors where APRV has been used during donor management (Roy et al., 2017; One Legacy. 2022).

Second, claims that spontaneous breathing during APRV is harmful and associated with an increased WOB have no supporting data nor validation of this occurring with greater frequency in APRV than with any other ventilator mode (Kallet 2011; Daoud et al., 2012; Mireles Cabodevila and Kacmarek, 2016; Yoshida et al., 2017). The 2002 Neumann, et al. paper (Neumann et al., 2002) is cyclically referenced to indict spontaneous breathing with APRV as always being harmful. Misquoted as a clinical trial of 35 patients (Myers and Macintyre, 2007), this is in fact is an observational study with a mixed population of 28 patients: COPD (25%), Acute Lung Injury (32%) (moderate ARDS by Berlin criteria), and Non-specific Pathology (43%). In the Neumann protocol, the Dräger Evita 1 ventilator was used in the BIPAP mode, which has a trigger and flow termination fixed to 25% and could increase WOB for the COPD patients. The P_{High} was stepwise increased until the patient stopped breathing spontaneously and then reduced by 25% of an unknown P_{High} to induce spontaneous breathing, potentially causing derecruitment and distress. In fact, the authors express concern in the manuscript that releasing the pressure from P_{High} to P_{Low} [even for a brief duration] could provoke lung collapse. However, they proceed to use a protocol that precipitously reduced P_{High} and extended the duration at P_{Low} at the onset of data collection, potentially creating a greater magnitude of collapse and respiratory distress. The authors also point out that: "Thus, if such very short release times are used in critically ill patients, adequate ventilatory support has to be assured". Additionally, they state: "This can either be obtained by an increase of P_{High} to increase the driving pressure of the mechanical breaths or an increase of the release cycles" (i. e., forcing patients to breathe spontaneously at the onset of distress would most likely uncover excessive WOB). Lastly, because each ventilatory setting was used for only ~30 min, the authors make the acknowledgement: "Thus, no conclusion can be drawn about potential long-term effects (e.g., development of respiratory muscle fatigue or improvement of oxygenation) of the respiratory settings used in the present study."

This paper is also hindered by numerous methodologic and statistical issues such as a small sample size with lack of power and high standard deviations, particularly with the transpulmonary data [used to assess WOB] and Simpson's paradox conflating a spectrum of lung pathologies. For instance, it is unlikely that optimal ventilator parameters for patients with ALI would be identical to that of patients with COPD. No major adverse effects were observed with brief T_{Low} settings on hemodynamics, the ability to spontaneously breath or gas exchange in any of the three patient groups. Most importantly the authors never conclude that APRV increased WOB or is harmful and the actual conclusion of the study was: "Airway pressure release ventilation is an open system which allows patients to maintain the "time control" over the respiratory cycle independent of the chosen duration for P_{High} and P_{Low}."

Excessive WOB is important, yet often underappreciated, in patients during mechanical ventilation (Yoshida et al., 2017). Unfortunately, dyspnea and distress are common and occur even in conventional modes of mechanical ventilation (Schmidt et al., 2011, 2014). Dyspnea during mechanical ventilation is often associated with anxiety, pain as well as inappropriate ventilator settings (Schmidt et al., 2011, 2014; Worsham et al., 2021) and has been linked with higher mortality during a patient's hospital admission in addition to the 2 years after discharge (Stevens et al., 2021). Further, dyspnea after extubation has been associated with an increased risk of recurrent respiratory failure (Dres et al., 2021). Patients can also experience mental discomfort and a form of post traumatic distress syndrome (PTSD) during mechanical ventilation (Schmidt et al., 2014; Worsham et al., 2021; Schwartzstein and Campbell, 2022). However, despite the risk of spontaneous breathing causing dyspnea and excessive breathing efforts, absence of spontaneous breathing during mechanical ventilation is equally detrimental. In fact, both excessive breathing or elimination of spontaneous breathing prolong duration of mechanical ventilation and impact patient outcome (Goligher et al., 2018). When considering spontaneous breathing it is important to understand it should never be viewed as a binary event–i.e., only described as present or absent - or depend on the solely on the acronym of the ventilator mode - but more importantly depend on the ventilator interactions with the patient's respiratory system.

Autonomic spontaneous breathing originates and is controlled in the brainstem. The brainstem controls the drive to breath and the respiratory muscles' output, which are regulated by the abundant feedback of chemical and mechanoreceptors that must be satiated in order to attain the many benefits and efficiency of spontaneous breathing. Hierarchical control of factors such as gas exchange, lung volume, C_{RS}, degree of respiratory muscle dysfunction, and diaphragm position all affect the patient's ability to perform acceptable spontaneous breathing without excessive WOB. No

one mode of mechanical ventilation including APRV, or ventilatory condition for that matter, can guarantee the promise of not inducing excessive WOB. Rather it is the clinician's role to support, select and prepare the patient who is capable of undergoing unharmful spontaneous breathing and eliminate complications such as VILI and ventilator induced diaphragm dysfunction (Goligher et al., 2015; Goligher et al., 2018; Goligher et al., 2020). Since both excessive and absence of spontaneous breathing are now recognized as a detriment to patient outcome, more than ever this demands that optimal approaches and understanding of the respiratory system is required. We can no longer be complacent allowing patients to stagnate on the ventilator while admiring ventilatory parameters, pulse oximetry and arterial blood gas.

Increased inspiratory effort may have deleterious effects on the lung—this is the concept of patient-self-inflicted lung injury (P-SILI) (Brochard et al., 2017). Since P-SILI is determined by the changes in transpulmonary pressure which are not dependent on a specific ventilatory mode and can occur even in the absence of a ventilator. Therefore, appropriately set APRV and patient selection should not expose patients to a higher risk of P-SILI. Because high flow demands, and air hunger are the worst form of dyspnea it is important to understand which ventilator settings may relieve or worsen distress. Air hunger is reduced with increased levels of PEEP by increasing EELV (Vovk and Binks, 2007) For example, spontaneous breathing at low levels of PEEP is associated with greater lung and diaphragm injury whereas spontaneous breathing at higher levels of PEEP is protective (Yoshida et al., 2016; Morias et al., 2018). Physiologically, as lung volume increases mechanical receptors provide feedback to the brainstem, which depresses inspiratory effort and signals expiratory muscles for active exhalation if needed (Road and Leevers, 1988; Road and Leevers, 1990; Torres et al., 1993). Conversely, at low lung volume expiration is suppressed with maximum inspiratory drive activated (Dempsey 1994). Lung volume and diaphragm contraction are optimally positioned for breath initiation at FRC. Lung volume changes from FRC alter the curve of the diaphragm and change the force generating capabilities (Road and Leevers, 1988; Road and Leevers, 1990). As lung volume increases, the diaphragm curvature diminishes (flattens) such that the force generating capacity decreases (ie force-length relationship) (Braun et al., 1982; Road and Leevers, 1988; Road and Leevers, 1990). The opposite is true at low lung volume where both the force generation of the diaphragm is maximal and is synergized with the high inspiratory drive at the brainstem (Yoshida et al., 2017). In fact, a case report by Kallet et al. (1999) illustrates this in a patient transitioning from PCV to VAC LV_T for the ARMA trial who developed rapid onset of negative pressure pulmonary edema and decrease in C_{RS} and subsequent rapid resolution of the pulmonary edema with the removal of LV_T. This led the authors to say: "……exacerbation of acute pulmonary edema coincided with the institution of a lung-protective strategy. The fact that pulmonary edema quickly appeared and resolved with the institution and removal of low V_T ventilation strategy led us to suspect that vigorous inspiratory efforts were responsible for the sudden deterioration in the patient's cardiorespiratory status." What these authors document is well understood in the physiology of dyspnea and the brainstem's control over lung volume. Many respiratory pathologies such as impaired gas exchange, lung injury and activation of chest wall and other receptors can increase respiratory drive and the sensation of dyspnea, reflexively stimulating further attempts to increase V_T. Patients with ARDS receiving LV_T strategy are more likely to experience air hunger, dyspnea and remain on the ventilator for a prolonged period of time potentially increasing the risk for PTSD symptoms (Schmidt et al., 2011, 2014; Worsham et al., 2021; Schwartzstein and Campbell, 2022). However, the LV_T strategy opposes the inherent physiologic mechanism to resolve dyspnea–increased lung volume (Worsham et al., 2021). Additionally, lower lung volumes position the diaphragm to generate high force and pressure to satisfy the high inspiratory drive. Alternatively, spontaneous breathing in APRV with the TCAV™ method targets breath initiation at or slightly above FRC. Once FRC is reestablished, the CPAP phase of APRV decreases inspiratory effort and air hunger provided proper lung volume and flow demands are met (Gregory et al., 1971; Gherini et al., 1979). Inspiratory efforts are usually minimal as the typical breathing pattern is to defend lung volume where expiratory muscles provide inspiratory assistance (Torres et al., 1993). The CPAP Phase (or P_{High}) permits lung volume titration that allows the patient to traverse between active exhalation and minimal inspiratory effort. Coupled with the open breathing system, the ability of the patient to have unrestricted spontaneous breathing preserves the neural inspiratory time making the patient less distressed. These features improve patient-ventilator interaction during properly set APRV and properly selected patients to satiate respiratory demand, allow control over ventilation and increase comfort suggesting that APRV may facilitate spontaneous breathing with an appropriate level of work.

MISCONCEPTION OF APRV TRIALS

We believe we have provided sufficient evidence to demystify 10 myths that are perpetuated in the literature through unsubstantiated statements, but other myths remain that were not addressed in this review. We do, however, wish to discuss the frequent objection with the use of APRV, which is the recall bias of the negative studies. Although there is no multi-center RCT to date showing APRV is superior to LV_T, there is equally no multi-center RCT to date showing LV_T to be superior to APRV. In fact, recent meta-analyses suggest a point estimate in favor of APRV–although the heterogeneity is high (Lim and Litton, 2019; Zhong et al., 2020).

Three trials are often highlighted as APRV failures. It is important to note that prior to the 2000 ARDSNet ARMA trial (ARDSNet 2000), three LV_T studies using various methods failed to show any benefit (Brochard et al., 1998; Stewart et al., 1998; Brower et al., 1999). Subsequently, it took the 41 million-dollar ARMA trial comparing two methods of setting V_T in VAC with the LV_T method (6 ml/kg) vs (HV_T) method (12 ml/kg) (ARDSNet 2000) to show a reduction in mortality. When reading beyond the

'headlines' in the alleged negative three trials with APRV, a critical review reveals the following:

1) The Hirshberg et al. (2018) trial was stopped for futility but not because the mode APRV was futile or for patient harm. The goal of the study was to conform APRV V_T to ≤6 ml/kg similar to that of LV_T and was stopped because this goal could not be met. There were three groups: 1) LV_T with VAC targeting V_T 6 ml/kg (n = 17); 2) APRV targeting V_T 6 ml/kg (n = 18); 3) APRV with no target V_T (n = 17). Allowing the T_{Low} to be adjusted to 50–75% EF_T/EF_P could explain the V_T exceeding 12 ml/kg. Interestingly, even with V_T exceeding 12 ml/kg in both APRV groups, there was no increase in barotrauma or mortality. Besides the V_T goal not being met, there was no significant difference in hemodynamics or vasoactive requirements, barotrauma rates, sedation or NMBA use, reintubation, ventilator-free days, hospital mortality or ICU length of stay.

2) The Ibarra-Estrada, et al. (2022) trial has been referred to as the APRV study of CARDS patients that was stopped for mortality. Here are the facts. After four episodes of barotrauma, a review by the data safety monitoring board recommended stopping recruitment for patients with COVID-19, although the decision was not unanimous. Prior to the study being stopped, there was no difference in mortality or difference in barotrauma rates between the groups, which was the impetus to recommend stopping the trial. Shrestha et al. (2022) showed in a systematic review and meta-analysis that increased barotrauma incidence was associated with increasing disease severity in COVID-19 and not linked with a particular mode. We previously reviewed the Ibarra-Estrada, et al. (2022) study and the incidence of hypercapnia in the APRV group in detail where the RR was lower than in the LV_T group. The authors admit clinicians were reluctant to use a T_{High} lower than the typical 4–6 s that is used in less ill patients, thereby decreasing the set RR in the APRV group.

3) The Ganesan et al., trial reports APRV was associated with a trend toward higher mortality compared to LV_T when used as a primary ventilation strategy in children with ARDS (Ganesan et al., 2018). However, patients in the APRV group with a primary cause of lung injury/ARDS had a longer duration of respiratory complaints and more cases of severe ARDS at enrollment indicating a sicker group of patients. Further there was an increased number of contaminated cases in the APRV group, which was defined as those who required an alternative mode of ventilation but is not fully explained. Conversely, the trend of barotrauma rates, use of sedation and analgesia and hemodynamic instability was lower in the APRV group. The authors point out that spontaneous breathing is a prerequisite for APRV, which we previously reviewed is not necessary. However, similar to the Ibarra-Estrada et al. (2022) study the T_{High} was set at 4.0 s, which would significantly decrease the set RR and force patients to assume the majority of the total MVe. A T_{High} of 4.0 s is exceedingly high in pediatric ARDS patients when this would translate to a RR of [the highest] 14 b/min as the T_{Low} on the ventilator used (Hamilton Galileo) cannot be set <0.2 s. Not only will a T_{High} of 4.0 s create a prolonged CPAP Phase where the patient assumes a greater portion of the total MVe and metabolic load, but without setting the T_{Low} to achieve E_{FT}/E_{FP} of 75%, alveolar instability and RACE is never stopped so that recruitment can begin. Lastly, if a reliable Pplat was not attained in this study, the P_{High} was initially set at 15cmH_2O and adjusted incrementally up to a P_{High} of 28 cmH_2O to achieve correlate P/F ratios. However, if the V_T exceeded 6–7 ml/kg ideal body weight (IBW), the P_{High} was decreased, which may have led to further derecruitment, loss of surface area and subsequently worsening hypercarbia and excessive WOB. In summary, alveolar instability may never have been halted leading to the worse P:F ratios and subsequent trend to increased mortality in the APRV group could have resulted from settings where the T_{High} was most likely much too high for a pediatric ARDS patient, the T_{Low} was possibly not set to achieve 75% if <0.2 s was required and the P_{High} reduced if the V_T exceeded 6–7 ml/kg IBW. These and additional statistical analysis issues have been addressed by other authors (Daxon 2018).

SUMMARY

Science should be based on evidence. Negative and sometimes Pavlovian responses regarding APRV are published without supporting data. Some authors even declare "APRV is a dangerous mode" (Kallet et al., Respir. Care, 2011, 56(2), 190–203), "there is no reason to consider this approach to ventilator support" and "APRV is the devil's spawn" (Mireles Cabodevila and Kacmarek, 2016) without any science to validate these claims. In fact, some of the most enthusiastic objections towards APRV were followed by the admission of little to no clinical experience using the mode (Mireles Cabodevila and Kacmarek, 2016). It would be "anti-science" to ignore or condemn new data because they do not fit one's prior ideas. The appropriate approach would be to review and consider all scientific and clinical information carefully to understand it wholly and become a useful critic.

The goal of this review was to highlight the most published myths and misconceptions and evaluate if any of these claims are supported scientifically. What we found were recurring statements that lack support and that many were recycled logical fallacies. Although APRV is far from being adequately studied scientifically, the TCAV™ method highlights non-traditional concepts of lung management that warrant further exploration to expand our knowledge of the lung in general and lung—ventilator interactions. We believe we have shown APRV is not an overly complex mode that is too difficult to understand, is distinguishable from IR-PCV, does not itself create barotrauma or volutrauma and itself generate high V_T, does not cause increased RV strain or unsafe auto-PEEP, does not cause alveolar collapse if P_{Low} is set to 0 cmH_2O, can control $PaCO_2$ and set a sufficient RR, can obtain a ΔP and can be used whether the patient is or is not spontaneously breathing. Science has always benefited from competitive ideas, debate, and the constant refinement of our concept all of which are advanced by knowledgeable critics. Unfortunately, misinformation is the nemesis of science.

As Albert Einstein said, "The important thing is to never stop questioning".

AUTHOR CONTRIBUTIONS

PA- manuscript drafting. JS, MM, GN, LC, NH: critical revisions. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.928562/full#supplementary-material

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Conflict of Interest: NH has patents in the field of mechanical ventilation including APRV.

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

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