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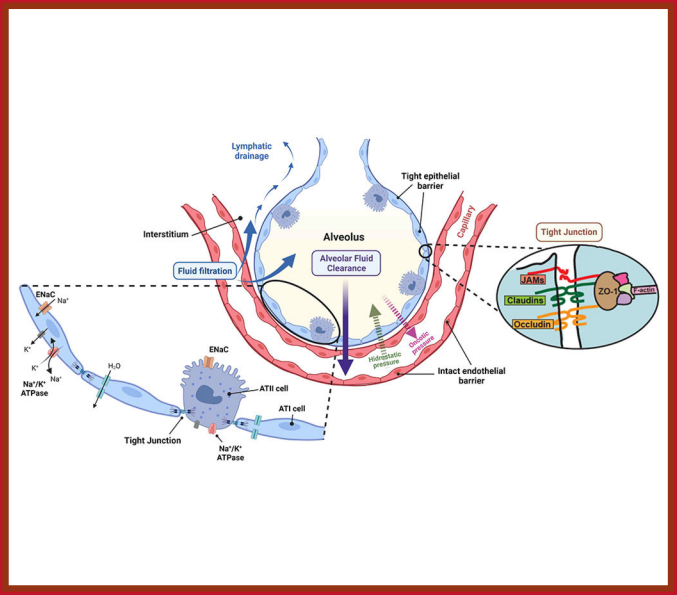
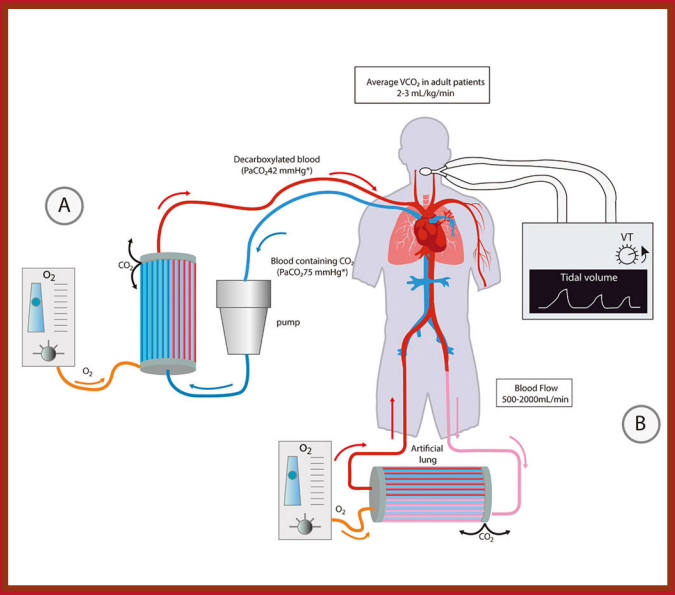
Special Issue

New Insights in

Acute Respiratory Distress Syndrome

Guest Editor: Prof. Dr. Antonio Artigas





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Prof. Antonio Artigas, MD, PhD
Guest editor

Antonio Artigas is a Professor of Applied Physiology and Chair of Sepsis and Acute Respiratory Failure Research at the Autonomous University of Barcelona. He was the Director of the Intensive Care Medicine Department at Corporacion Sanitaria Universitaria Parc Tauli 1988-2016 and now Emeritus Director and Director of the Pathophysiological Translational and Cell Therapy Laboratory. His research interests are primarily intensive care, ARDS and sepsis.

He completed his training in Barcelona University and became MD in 1973 and his PhD in 1992. He then was certified in internal medicine in 1976 and intensive care medicine in 1982.

Prof. Artigas is a member of several societies including ESA, ESCIM, ATS, SRLF (for which he was the vice president between the years 1991 – 1993), CIBER and chairman to the ERS critical care group and HERMES respiratory critical care program. He is an honorary member of the European Society of Intensive Care and was a member of the executive committee from the years 1982 – 1990. He is also heavily involved in sepsis research and is member of the Executive Committee of European Sepsis Alliance and member of the Advisory Committee of Sepsis of the Government in Catalonia. He was the chairman of the European-North American Consensus Conferences on ARDS and is member of the ATS Task Force on Experimental Models of ARDS.

In addition to this, Prof. Artigas is also on the editorial boards of many critical care and respiratory journals and reviews numerous papers. He has published over 200 articles in both national and international scientific journals and has published 20 books and wrote chapters in various others. He has an H-index of 49. He has presented at more than 200 conferences internationally. He has received 50 awards and more than 30 research grants funded by the Spanish Research Agency, the EU research programs and participate in many multicentre clinical trials of pharmaceutical companies. He is a member of the Educational Committee of the hospital and director of 20 doctoral thesis and tutor of residents and research fellowship program of intensive care medicine. He received the Educational Award of the European Respiratory Society in 2015. He is International Corresponding Member of Académie Nationale de Médicine since 2016.

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PREFACE

New Insights in Acute Respiratory Distress Syndrome

Antonio Artigas

Critical Care Center, Corporacion Sanitaria Universitaria Parc Tauli, Institut de Investigació I3PT, CIBER Enfermedades Respiratorias, Autonomous University of Barcelona, Sabadell, Spain.

Fifty Years Ago, Ashbaugh and colleagues described for the first time the term adult (later changed to acute) respiratory distress syndrome (ARDS). Since then substantial progress has been made in the care of affected patients and those at risk for the disorder, with reductions in both incidence and mortality. However, ARDS remains a relatively common and lethal or disabling syndrome.

Novel therapeutics have largely failed to translate from promising preclinical findings into improved patients outcomes in late phase clinical trials. Recent advances in personalized medicine, big data, causal inference using observational data, novel clinical trial designs, preclinical disease modeling, and understanding of recovery from acute illness promise to transform the methods of pulmonary and critical care clinical research. The recommendations for future research priorities and directions are: 1) focusing on understanding the clinical, physiological, and biological underpinnings of heterogeneity in ARDS with the goal of developing targeted, personalized interventions; 2) optimizing preclinical models by incorporating comorbidities, cointerventions, and organ support; 3) developing and applying novel clinical trial designs; 4) advancing mechanistic understanding of injury and recovery to develop and test interventions targeted at achieving long-term improvements in the lives of patients and families.

This special issue of Signa Vitae, we have drawn together international experts in different aspects of acute respiratory failure to examine and discuss some of the challenges in today's ARDS. Multiple experimental models have been developed in the last few decades, with major recent development in the fields of in vitro, ex vivo, and in vivo experimental ARDS: while some of these experiments failed, other succeeded in advancing our knowledge of the complex mechanisms of ARDS pathophysiology and the clinical translation of a few therapeutic interventions. Therefore, the judicious and imaginative use of broad range experimental and analytical approaches is a paramount importance in developing translational discovery research, with the goal of developing prediction medicine strategies to ultimately improve patients outcomes.

The recognition of ARDS heterogeneity has create an opportunity to identify various subphenotypes, associated with different clinical outcomes. Key challenges will be 1) the characterization of the lung compartment, and 2) integrating our subphenotypes related to clinical variables, lung morphology, gas-exchange abnormalities and biology in preclinical models and clinical trials. Deeper subphenotyping, with parallel use of prognostic and predictive enrichment strategies, will hopefully reveal mechanistic differences and treatable traits, marking the beginning of precise medicine in ARDS.

The ventilatory management of ARDS patients has changed over the years to mitigate the risk of ventilator induced lung injury (VILI) and improves outcomes. Several strategies have been proposed to individualize tidal volume. Driving pressure, transpulmonary pressure, and mechanical power have been proposed as markers to quantify risk of VILI and to optimize ventilator settings. Several rescue therapies, including neuromuscular blockade, prone position, recruitment maneuvers, vasodilators and extracorporeal oxygenation and carbon dioxide removal, may considered in severe ARDS. These new

ventilatory strategies and recommendations may guide physicians in an individually tailored rather a fixed approach based on physiological targets to achieve optimal ventilatory settings for each patient.

The development of new and effective therapies for ARDS is a relevant objective of biomedical research and cell therapies are among the novel approaches with the greatest potential. However, mechanistic studies will still be needed to fully understand the mechanisms of action that these therapies can be optimized.

In summary, there are many and varied challenges across the fields of ARDS. I hope our selection, will help trigger for the reflection of this important area, with the realization that as we move forward, further challenge will inevitably arise. Importantly, although often considered as a barrier of progress, challenges should rather be seen as providing an opportunity to encourage debate and discussion to resolve difficult issues and patient management.

I would like to thanks all the contributors for their excellent contributions and the editorial staff at Signa Vitae for their assistance and patience.

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REVIEW

Hypercapnia and extracorporeal carbon dioxide removal ($ECCO_2R$) in the acute respiratory distress syndrome

Luis Morales-Quinteros[1,2,3,*], Jordi Mancebo[1], Lorenzo Del Sorbo[4,5]

[1]Intensive Care Department, Sant Pau University Hospital, 08001 Barcelona, Spain
[2]Translational Research Laboratory, Institute for Research and Innovation Parc Tauli (I3PT) Universitat Autònoma de Barcelona Sabadell, 08208 Barcelona, Spain
[3]CIBER Enfermedades Respiratorias, Madrid, Spain
[4]Interdepartmental Division of Critical Care Medicine, University of Toronto, Toronto, Ontario M5R 2R3, Canada
[5]Department of Medicine, Division of Respirology, University Health Network, Toronto, Ontario M1E 4E6, Canada

***Correspondence**
lmoralesq@santpau.cat
(Luis Morales-Quinteros)

Abstract

As a result of technical improvements, extracorporeal techniques for carbon dioxide removal have become an attractive option in managing adults with acute respiratory failure. However, evidence to support its use is scarce, and several questions regarding the best way to implement this therapy remain unanswered, which can be associated with severe side effects. In this review, we will present the currently available knowledge on (1) $ECCO_2R$ as an adjuvant treatment to invasive mechanical ventilation, (2) the impact of hypercapnia in patients with acute respiratory distress syndrome (ARDS), (3) the pathophysiological rationale and evidence of $ECCO_2R$ in patients with ARDS.

Keywords

Acute respiratory distress syndrome; Extracorporeal carbon dioxide removal; Carbon dioxide; Lung-protective ventilation; Ventilator-associated lung injury

1. Background

Acute hypoxemic respiratory failure, and its most severe form, acute respiratory distress syndrome (ARDS), is a leading cause of admission to the intensive care unit (ICU). It is associated with significant mortality and long-term morbidity for survivors and considerable resource utilization for health care systems [1].

In critically ill patients with acute hypoxemic respiratory failure, mechanical ventilation is a life-saving treatment [2]. At the same time, this therapy can cause ventilator-induced lung injury (VILI), a lung injury condition inflicted or aggravated by mechanical ventilation during treatment. Multiple evidence demonstrated that excessive lung stress and strain, induced by excessive transpulmonary pressure, results in regional alveolar overdistension or cyclic opening and closing of distal airways, which cause lung injury [3]. In recent years, much effort has been invested in understanding the pathophysiology of VILI, which has led to notable changes in ventilation management and remarkable improvement in patient outcomes. For instance, while it was common practice to use "unphysiological large" tidal volumes to prevent atelectasis and target normal gas exchange, it is now widely accepted to use low pressures and low tidal volumes to protect the lungs against VILI [2, 4]. In a seminal study, the ARDSNet investigators showed significantly higher mortality with a high tidal volume (V_T) strategy of 12 mL/kg of predicted body weight (PBW), as compared to a low $V_T$strategy of 6 mL/kg PBW and limiting end-inspiratory plateau pressure (P_{PLAT}) to $\leq$ 30 cmH_2O [5]. However, the reduction in tidal volume and inspiratory pressures results in the development of respiratory acidosis, which is tolerated within certain safe limits, according to the notion of "permissive hypercapnia".

Nonetheless, in some patients, even lung-protective ventilation (LPV) settings may not be fully protective [6, 7]. Up to one-third of patients receiving lung-protective ventilation had evidence of tidal hyperinflation and, hence, risk of VILI [6]. Moreover, data from large observational studies suggest that there might not be a safe threshold for tidal volume or driving pressure due to the heterogeneity of lung injury [8, 9]. These data prompted the hypothesis that further reducing tidal volume and driving pressure could result in less VILI and patient-centered outcome improvement [10].

This strategy would potentially entail an unacceptably high risk of life-threatening respiratory acidosis [11] due to significantly reducing alveolar ventilation with tidal volumes equal to or inferior to physiologic dead space. To overcome this issue and facilitate "ultra" protective strategies of mechanical ventilation to minimize VILI, increasing interest has been focused on extracorporeal carbon dioxide removal ($ECCO_2R$) since the first reports in the 1980s [12–14].

2. Pathophysiologic rationale of $ECCO_2R$ in ARDS

One of the major clinical challenges in ARDS and hypoxemia is carbon dioxide (CO_2) clearance and the strategy to best achieve it. However, the optimal physiologic and metabolic

targets to provide adequate homeostasis without inducing VILI are not yet defined, as highlighted above, suggesting a potential role for $ECCO_2R$.

In patients with ARDS, hypercapnia develops due to decreased alveolar ventilation, determined by the variable combination of alveolar collapse/infiltrate and increased alveolar dead space. Alveolar infiltrates, and collapse is unevenly distributed throughout the lung, with smaller preserved aerated zones, defined as "baby lung" [15]. Physiological dead space (V_D/V_T) is the sum of the anatomical and alveolar dead spaces and is defined as all parts of the tidal volume that do not participate in gas exchange. V_D/V_T comes from respiratory units that receive disproportionately low perfusion compared with ventilation ($Q < V$), resulting in an increasing "West Zone 1" physiology [16]. High alveolar dead space (VD_{ALV}) may result from endothelial injury, microvascular thrombi, and overdistention of alveoli during mechanical ventilation [17, 18]. V_D/V_T during the first seven days after ARDS diagnosis is an independent lung-specific physiological variable associated with increased mortality [19, 20]. However, dead space measurements are not routinely performed in clinical practice to guide patient management due to the challenges of the various measurement strategies [21]. Other methods for estimating V_D/V_T, which do not require quantitative assessment of exhaled carbon dioxide, are easier to use at the bedside. Recently, the ventilatory ratio and end-tidal-to-arterial Partial pressure of carbon dioxide (PCO_2) ratio have been described as surrogates for V_D/V_T in ARDS patients [22–25].

2.1 Hypercapnia in ARDS

The effects of hypercapnia have been extensively studied in clinical and experimental investigations, but the results are conflicting. Thus, the definition of adequate CO_2 and pH clinical targets remains challenging.

Hickling *et al*. [26] were the first to propose protective ventilation strategies as the rescue therapy for patients with severe ARDS to limit VILI. These strategies include the following measures: (1) low peak inspiratory pressure and low V_T ventilation; (2) use of positive end-expiratory pressure (PEEP); and (3) acceptance of higher partial pressure of arterial carbon dioxide ($PaCO_2$) levels. Despite its limitations, this study showed significantly lowered hospital mortality by adapting the protective ventilation strategies. This finding led to a series of clinical investigations in patients with ARDS, including the potential protective role of permissive hypercapnia [5, 8, 27–29]. Regretfully, important limitations of these studies, such non-randomization of patients to receive normocapnia or hypercapnia, have precluded the conclusive demonstration of a direct protective effect of high CO_2 in these patients.

To advance the knowledge on this issue, several experimental studies have also investigated the potential protective effect of hypercapnia on mechanisms of acute lung injury [30]. In an experimental model of rabbit lungs ventilated *ex-vivo* with high pressures, hypercapnia decreased microvascular permeability, lung edema formation, and protein concentration in the bronchoalveolar lavage fluid [31]. The plausible mechanisms are (1) the CO_2 action, through nuclear factor-kappa ($NF\kappa B$) pathway activation, preventing p65 translocation and thereby reducing inflammation [32, 33]; (2) CO_2 inhibition of the ADAM-17 (a disintegrin and metalloprotease domain enzyme), which prevents the activation of the p44/p42 MAPK (mitogen-activated protein kinases pathway) [34].

Hypercapnia has also been shown to reduce apoptosis in rat lungs exposed to high-pressure ventilation by inhibiting the activation of the MAPkinase and stress-activated protein kinases (SAPK)/Jun amino-terminal kinases (JNK) pathways in alveolar epithelial cells [35].

In contrast to its beneficial effects, the potentially detrimental effects of hypercapnia on mechanisms of injury have also been studied. It has been observed that high levels of CO_2 impaired the phagocytic activity of neutrophils in rat models [36]. Furthermore, hypercapnia decreased alveolar cell proliferation and delayed wound repair in different types of human lung cells in pH-independent and dose-dependent ways [37]. Hypercapnic acidosis impairs membrane wound resealing [38, 39] in *ex-vivo* and *in-vitro* rat models of VILI. High CO_2 levels have been found to decrease the clearance of alveolar edema through inhibition of the Na^+-K^+-ATPase pump through an endocytosis process [40] that is pH independent [41]. Lastly, hypercapnia may modulate innate immunity and host defense via pH-independent or dependent mechanisms [42, 43]. High CO_2 levels suppress innate immunity by inhibiting mRNA and the expressions of inflammatory cytokines (IL-6 and TNF-α) and autophagy in alveolar macrophages in rats [43, 44]. The biological actions of CO_2 are depicted in Fig. 1.

Although progressively adopted or tolerated in patients with ARDS to facilitate protective mechanical ventilation settings, permissive hypercapnia has considerable pathophysiological effects, which need to be considered. Hypercapnic acidosis can increase pulmonary vascular resistance and worsen pulmonary hypertension, potentially increasing right ventricular afterload and triggering acute cor pulmonale. It also impairs diaphragmatic function through afferent transmission or integrity with short-term exposure to moderate hypercapnia in preclinical models [45, 46]. Hypercapnia causes precapillary cerebral arteriole dilation, increasing cerebral blood flow, a clear concern in the setting of reduced intracranial compliance, in which increased global cerebral blood flow may critically elevate intracranial pressure. Moreover, hypercapnic acidosis directly reduces the contractility of cardiac and vascular smooth muscle [47, 48]. However, this is counterbalanced by the hypercapnia-mediated sympathoadrenal effects, including increased preload and heart rate, increased myocardial contractility, and decreased afterload, leading to a net increase in cardiac output [48, 49].

A recent secondary analysis of three international studies on patients with ARDS showed that severe hypercapnia, defined as $PaCO_2$ 50 mmHg, was independently associated with higher ICU mortality and multiorgan failure [50]. Interestingly, the number of patients with severe hypercapnia progressively increased from 1998 to 2010, mirroring the progressively higher adoption of lung protective ventilation, which may reflect the belief of the beneficial effect of hypercapnia.

In another retrospective analysis of mechanically ventilated patients, it was observed that patients who developed respiratory acidosis (pH <7.35 and $PaCO_2$ >65 mmHg) during the first 24 hours of ventilation had a worse prognosis compared

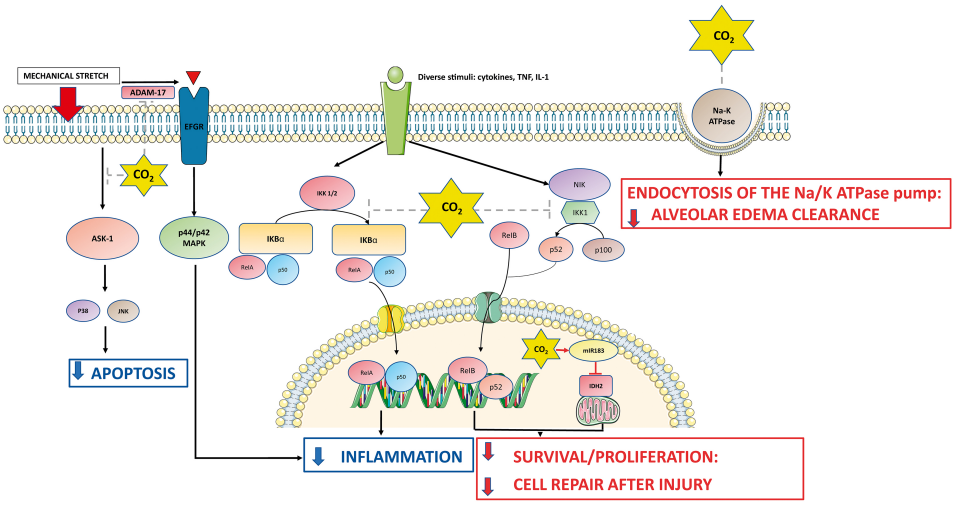

FIGURE 1. Schematic depiction of CO_2 actions at cellular level with its positive (BLUE) and negative effects (RED). Left: Mechanical stretch induced phosphorylation of p44/p42 is decreased by CO_2 inhibition of ADAM-17. Apoptosis is decreased by hypercapnia by impairment of ASK1-JNK/p38 MAPK pathway. Right: CO_2 acts upon the NF-κB pathway after inflammatory stimuli. Carbon dioxide inhibits IκB-α degradation, impairing ReIA/p50 translocation into the nucleus exerting its anti-inflammatory effects. On the other hand, CO_2 impairs alveolar cell proliferation by inhibiting IKK/NIK complex impairing ReIB/p52 formation via the NF-κB complex and also by inducing miR183 which down-regulates IDH2 producing mitochondrial dysfunction (independent of NF-κB pathway). Hypercapnia- induced endocytosis of the Na,K-ATPase transporter. ADAM-17: disintegrin and metalloproteinase 17; MAPK: mitogen-activated protein kinases; ASK: Apoptosis signal-regulating kinase 1; JNK: c-Jun N-terminal kinase; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; IL-1: interleukin1; TNF: Tumoral necrosis factor; IDH2: isocitrate dehydrogenase-2; NIK: NF-κB-inducing kinase; IKK: IκB kinase; EFGR: epidermal growth factor receptor; CO_2: carbon dioxide.

to those who had normocapnia or compensated hypercapnia [51].

The "Large observational study to UNderstand the Global impact of Severe Acute respiratory FailurE" (LUNG SAFE) study, a worldwide multicenter observational investigation in ventilation practice in patients with ARDS [52], reported the prevalence and impact of changes in CO_2 on ventilation management and outcomes in patients with early ARDS. This observational study showed that hypocapnia and hypercapnia are commonly present, and in approximately half of the patients, CO_2 derangements are sustained over the first two days of ventilation. Interestingly, there was no mortality difference between normocapnic and hypercapnic patients, concluding that there is no evidence for hypercapnia to be considered beneficial or harmful. Of note, the LUNG SAFE investigators also show ICU mortality to be higher in hypocapnic compared to normocapnic patients with mild-to-moderate ARDS, suggesting the need for caution with sustained hypocapnia.

The above-discussed evidence suggests that the application of $ECCO_2R$ could be beneficial to improving metabolic homeostasis and minimizing VILI, which is achieved by allowing the delivery of ultra-protective mechanical ventilation settings and avoiding the potentially detrimental hemodynamic and neurological consequences of hypercapnia. It is increasingly recognized that CO_2 is more than just a product of cellular metabolism and that hypercapnia can regulate several critical biological functions in the lung, which could be detrimentally altered by inadequate $ECCO_2R$ application.

3. Principles and technical aspects of $ECCO_2R$

3.1 Principles

The $ECCO_2R$ devices consist of a drainage cannula placed in a large central vein or artery (the latter if an arterio-venous configuration is used, which is not often), a pump, and a gas exchanger (artificial membrane lung), and a return cannula into the venous system. Gas exchange is achieved through an extracorporeal artificial lung unit containing a diffusion membrane. In this unit, blood is passed through hollow plastic fibers with a mesh-like pattern that increase the surface area for membrane-to-blood contact and gas exchange efficiency. Via the surface of the membrane fibers, the exchange of oxygen and CO_2 occurs by diffusion. The efficiency of each device (*i.e.*, the volume of CO_2 removed per minute, adjusted to blood flow) should be an important consideration for clinicians since it determines the blood flow rate and hence the catheter size needed for adequate CO_2 removal. To obtain an efficient membrane lung with the lowest necessary amount of membrane surface, a design incorporating short fibers that allows a maximal sweep gas ratio is required to keep the gradient over the entire length of the fiber at its highest possible level. This is in contrast to extracorporeal membrane oxygenation (ECMO), which requires high flow rates to increase arterial blood oxygenation. $ECCO_2R$ needs considerably lower blood flow rates as the gas dissociation curves in blood for oxygen and CO_2 are significantly different.

Theoretically, due to the higher diffusion coefficient of CO_2,

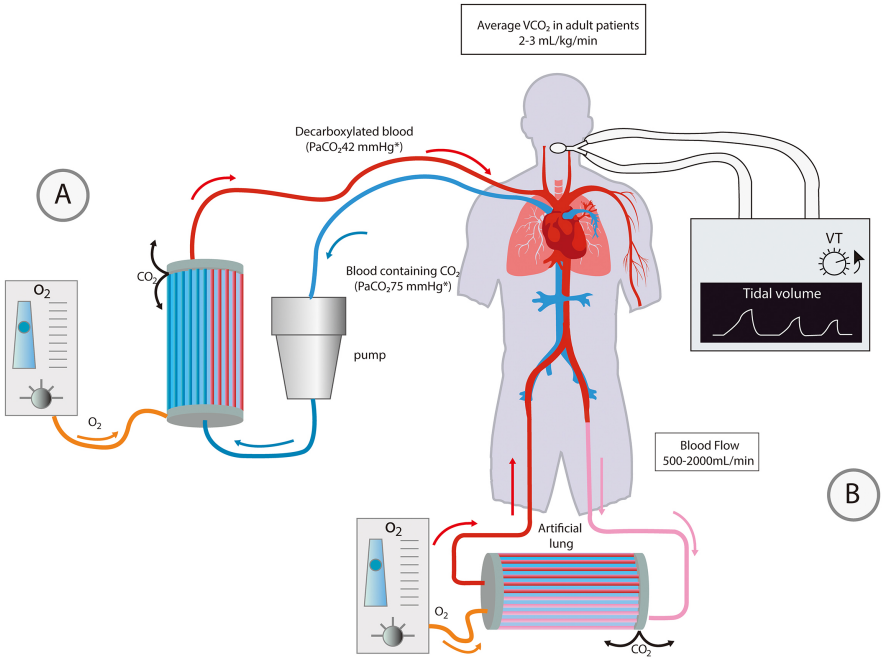

FIGURE 2. Use of $ECCO_2R$ to decrease the injury induced by mechanical ventilation. Figure depicts the common configurations used. A. Veno-venous $ECCO_2R$ configuration with a double-lumen catheter inserted into a central vein. B. Arterio-venous $ECCO_2R$ configuration with the positioning of the exchange membrane linking the femoral artery and vein. No pump is needed. $PaCO_2$: partial pressure of carbon dioxide in arterial blood; VCO_2: carbon dioxide production; CO_2: carbon dioxide; O_2: oxygen; V_T: tidal volume.

blood flow of ~1 L/min is sufficient to remove the entire CO_2 production of an average-sized patient effectively. In contrast, relevant oxygenation of the blood only occurs with blood flows of approximately 50–60% of the cardiac output. Therefore, an $ECCO_2R$ system requires smaller cannulas and lower blood flow. In $ECCO_2R$, the sweep gas flow is kept high to maximize the effectiveness of CO_2 elimination through the artificial membrane from the blood.

Before initiating the extracorporeal CO_2 elimination, it is necessary to estimate the patient's CO_2 production (on average, about 250 mL/min in the critically ill patient under resting conditions [53]) and, on the other hand, the therapeutic goal. With low flow rates in the 200–450 mL/min range, it is possible to eliminate an average of CO_2/min corresponding to about 20–30% of the average CO_2 production [54, 55] as demonstrated in recent clinical trials [56, 57].

Recent preclinical research has investigated ways to increase the efficiency in CO_2 removal by techniques that acidifies blood in the extracorporeal circuit and by using electrodialysis with encouraging results [58–60].

3.2 Technique

Due to the much higher diffusion capacity of CO_2 than O_2, different configurations of extracorporeal CO_2 elimination are possible. The system's configuration depends on the election of the vascular access (arterial or venous) and the type of cannulas that will be used. A distinction is made between pump-driven *vs.* arterio-venous pumpless systems (Fig. 2).

3.2.1 Arterio-venous $ECCO_2R$ (AV-$ECCO_2R$)

$ECCO_2R$ with arterio-venous configuration utilizes the patient's arterio-venous pressure gradient to pump blood through the artificial lung. Vascular access is most commonly obtained by cannulating the femoral artery and vein using the percutaneous technique. Mean arterial pressure greater than 60 mmHg and a cardiac index >3 L/min/m^2 provide flow rates ranging between 0.5 and 1.2 L/min. This configuration is unsuitable for hemodynamically unstable or heart failure patients [61, 62].

The major advantage of the system is the absence of blood trauma due to a pumpless system and thus pump-associated complications. However, this benefit is outbalanced by the risk of distal ischemia, which can occur on the side of the arterial cannulation. The pumpless arterio-venous system introduces a new vascular bed to the patient, which adds an additional burden to the heart that already has to pump blood through the brain, liver, kidneys, and other organs. Given the complications associated with cannulation, its use has fallen out of interest.

3.2.2 Veno-venous $ECCO_2R$ (VV-$ECCO_2R$)

Veno-venous $ECCO_2R$ systems utilize a pump to generate flow across a membrane. To date, pump-driven systems are by far the more used systems. They enable a jugular or femoral double lumen cannula of a size between 20 and 23–24 Fr, allowing blood flows around 500–1000 mL/min. Smaller cannulas can also be considered for lower blood flow, decreasing the cannulation risk. A hemodialysis catheter with 11.5 or 13.5 Fr can generate blood flows of up to 300 mL/min but has a relatively high recirculation rate [63], thus reducing the system's efficiency.

The pumps can be roller (peristaltic) or rotary (centrifugal). The latter has a rotating impeller which creates a suction vortex that draws blood into the center of the pump and propels it outwards from the outlet. The system, which evolved from dialysis, is driven by roller pumps and uses 200 to 450 mL/min of corresponding blood flows. In contrast, the systems developed from ECMO often have flow rates of 0.5 to a maximum of 2.0 L/min using a centrifugal pump [64].

Compared to the AV configuration, one of the gains of VV-$ECCO_2R$ is that it is less invasive as arterial cannulation is avoided and that patients can potentially be mobilized earlier. We recommend VV-$ECCO_2R$ over AV-$ECCO_2R$ in most circumstances unless the centers are already familiar with this technology.

4. Evidence of $ECCO_2R$ in ARDS

$ECCO_2R$ was first proposed in the 1980s when the detrimental effect of VILI was still vastly unrecognized and ignored. The evolving conceptual paradigm of $ECCO_2R$ clinical application was to use extracorporeal support to rest the lung and avoid VILI from high volume and pressure ventilation [14]. Interestingly, in small clinical series, the application of $ECCO_2R$ was reported to decrease barotrauma in patients with ARDS [13, 14] before large clinical trials could demonstrate the benefit of lung-protective ventilation. However, to date, no high-quality evidence has shown the efficacy of $ECCO_2R$ in improving patient outcomes.

A recent meta-analysis of 14 studies with pumpless and pump-driven $ECCO_2R$ [65] has shown that the technique can achieve a sustained reduced partial pressure of arterial CO_2 to 40–50 mmHg and increased blood pH to 7.30–7.45 and a significant increased PaO_2/FiO_2 ratios; these while decreasing V_T~3 mL/kg/IBW (ideal body weight), and P_{PLAT} by at least 5 cmH_2O, maintaining a PEEP level of around 15 cmH_2O. The device duration was between 7 to 14 days. However, there was no effect on mortality or clinically relevant outcome measures.

The SUPERNOVA study investigated the role of ultra-protective ventilation in patients with early moderate ARDS under invasive mechanical ventilation [66]. Ultra-protective ventilation consisted in targeting tidal volumes of 4 mL/kg and $P_{PLAT} \leq$ 25 cmH_2O. The main outcome was the proportion of patients achieving ultra-protective ventilation without developing respiratory acidosis (pH <7.30 while maintaining $PaCO_2$ around 20% of baseline values with Vt 6 mL/kg IBW). Devices with different CO_2 extraction rates were used. $ECCO_2R$ was kept for 3–8 days. $ECCO_2R$ was able to significantly reduce P_{PLAT} from 26 $\pm$ 5 cmH_2O to 23 $\pm$ 3 cmH_2O in 73% of patients, with a reduction of driving pressure from 13 $\pm$ 5 to 9 $\pm$ 4 cmH_2O. Few adverse effects were related to the use of $ECCO_2R$. These findings showed that in this study, $ECCO_2R$ was feasible and safe. A secondary analysis of the data from the SUPERNOVA study demonstrated that the magnitude of reduction in VT, driving pressure, and mechanical power permitted by $ECCO_2R$ is significantly higher in ARDS patients with higher dead space (determined by a ventilator ratio (VR) >2) or lower compliance of the respiratory system (Crs) or treated with a higher CO_2 extraction rate device [67].

Finally, although these data confirmed the technique's feasibility with consistent physiological effects, the lack of patient-centered outcomes warranted further investigation.

Several studies have shown the feasibility and efficiency of $ECCO_2R$ in removing significant amounts of CO_2 to facilitate very low tidal volume mechanical ventilation strategies [66, 68]. However, these studies were not designed to investigate the efficacy of this technique in improving patient-centered outcomes.

Recently a large, randomized, controlled, open, phase 3 pragmatic clinical and cost-effectiveness trial led by experienced clinical trials group [57] tried to respond to the clinical question of whether $ECCO_2R$ improves day 90 all-cause mortality in mechanically ventilated patients with acute hypoxemic respiratory failure. The original plan was for an interim analysis of 560 patients. However, this was moved forwards to 412 patients after the trial was paused to investigate an intracranial hemorrhage in the intervention arm. At this time point, the Data and Safety Monitoring Board (DSMB) performed a conditional power analysis and found that ongoing recruitment was unlikely to show benefit. 202 patients were randomized to the experimental arm and 210 to the control arm. Tidal volumes, inspiratory plateau pressure, and driving pressure were lower in patients randomized to the intervention arm than controls, as per the study design. However, although mean ventilator-free days were significantly lower in the $ECCO_2R$ group (mean difference, –2.1 (95% CI, –3.8 to –0.3); $p = 0.02$), no difference was found in the primary outcome of day 90 all-cause mortality, 41.5% in the lower tidal volume ventilation with $ECCO_2R$ group *vs.* 39.5% in the standard care group (Risk Ratio, 1.05 (95% CI, 0.83–1.33); difference, 2.0% (95% CI, –7.6% to 11.5%); $p = 0.68$). This was unchanged after adjusting for age, Sequential Organ Failure Assessment (SOFA) score, and baseline PaO_2/FiO_2. Higher rates of adverse events were observed in the intervention arm: 168 (52% of patients) *vs.* 61 (23% of patients), including higher rates of intracranial hemorrhage and infectious complications.

Moreover, several issues may have affected the outcome in the $ECCO_2R$ group. In fact, in the intervention arm of the trial, there were higher rates of mandatory modes of mechanical ventilation and neuromuscular blockade and less use of prone positioning than in the control arm. In addition, several participating centers had little experience with the clinical application of $ECCO_2R$. Furthermore, although driving pressure in the $ECCO_2R$ group was 2–3 cmH_2O lower than in controls, with the expected significant decrease of mechanical load, in both groups, driving pressure was maintained below 14 cmH_2O, which has been suggested as a protective threshold to minimize VILI [69]. Future studies will need to investigate whether targeting a lower respiratory rate by study design with $ECCO_2R$ results in improved outcomes, as demonstrated in an elegant experimental large animal model [70].

Overall, the data presented in this study confirmed that achieving lower tidal volumes using $ECCO_2R$ is possible and highlighted how translating this physiologic effect into clinical benefit is challenging due to the complex and not fully revealed pathophysiology of VILI.

Other relevant studies on $ECCO_2R$ in ARDS are summarized in Table 1.

TABLE 1. Relevant studies of $ECCO_2R$ in ARDS.

Study	No. of patients	$ECCO_2R$ Characteristics				Time on $ECCO_2R$	Major Results
		Configuration	Blood flow (mL/min)	Sweep flow (L/min)	Membrane (material); surface in m^2		
Terragni *et al.* [77]	32	RRT platform adapted to $ECCO_2R$ and a double lumen catheter (femoral)	191–422	8	PLP* (Decap®, Hemodec, Salerno, Italy); 0.33	6 (3.5–7) d	Prospective study. IMV + LPV to maintain P_{PLAT} 28–30 cmH_2O After 72 h of IMV, $ECCO_2R$ started with posterior decreasing of V_T. V_T successfully decreased to 4 mL/kg PBW and P_{PLAT} decreased to 25.0 cmH_2O ($p < 0.001$). $ECCO_2R$ prevented respiratory acidosis. Reduction of biomarkers of lung injury after 72 h of ultraprotective ventilation.
Bein *et al.* [68]	79	Femoral AV PECLA	1300	Not reported	PMP** (iLA AV, Novalung, Heil-bronn,Germany); 1.3	7.4 (3–11) d	Randomized controlled trial. AV-$ECCO_2R$ commencement after 24 h in moderate/severe ARDS. $ECCO_2R$ group aimed a V_T 3 mL/kg PBW. Control group aimed for a V_T 6 mL/kg PBW. No significant differences in VFDs at D-28 or D-60. $ECCO_2R$ + ARDS with P/F $\leq$150 had significantly shorter duration of ventilation at D-60. Significantly higher rate of bleeding in the $ECCO_2R$ group.
Fanelli *et al.* [56]	15	VV system and single double lumen catheter with femoral or jugular approach	435	10	PLP* based on siloxane layer (ALung Hemolung RAS); 0.59	2 h	Prospective study. Moderate/severe ARDS. V_T reduced to 4 mL/kg PBW. $ECCO_2R$ started after severe respiratory acidosis (pH $<$ 7.25 + $PaCO_2$ $>$ 60 mmHg). $ECCO_2R$ successfully reverted respiratory acidosis ECMO needed in 2 patients.
Augy *et al.* [78]	70	VV system and a double-lumen catheter	430	Not reported	PLP* based on siloxane layer (ALung Hemolung RAS) or PMP; 1.3 (Novalung iLA activve); 0.59	5 d	Multicenter, observational, prospective, cohort study. Ultraprotective ventilation for ARDS patients, rest of indications related to COPD patients. Significant reduction in V_T was observed in ARDS patients, up to 4 mL/kg PBW. Side effects related to the device: hemolysis, bleeding, and membrane clotting. 3 deaths related to $ECCO_2R$.
Schmidt *et al.* [79]	20	VV system managed with RRT platform via a 15.5-Fr single dual lumen catheter (femoral or jugular)	420	10	PMP** (Pris-maLung®; Gambro-Baxter); 0.32	31 h	Prospective multicenter study. Mild/moderate ARDS V_T progressively decreased to 4 mL/kg within 2 h + PEEP adjustment to aimed P_{PLAT} 23–25 cmH_2O using a RRT platform. No ECMO requirement. No worsening oxygenation. $ECCO_2R$ with RRT platform was feasible for ultraprotective ventilation.

TABLE 1. Continued.

Study	No. of patients	$ECCO_2R$ Characteristics				Time on $ECCO_2R$	Major Results
		Configuration	Blood flow (mL/min)	Sweep flow (L/min)	Membrane (material); surface in m^2		
Ding X *et al.* [80]	12	VV configuration with two 12-Fr two lumen hemodialysis into the right jugular vein and one of the femoral veins	342	10	PMP** (PrismaLung®; Gambro-Baxter); 0.32	Not reported	Single-center, prospective study. COVID-19 ARDS patients with refractory hypercapnia with compliance 13.29 $\pm$ 4.88 mL/cmH_2O. Low-flow $ECCO_2R$ system based on the RRT platform can reduce the $PaCO_2$ level <50 mmHg and significantly decrease the P_{PLAT}, driving pressure and mechanical power in moderate hypercapnic patients. Twenty-four hours later, the DP and P_{PLAT} slightly increased, but were still significantly reduced compared with the baseline.
Combes *et al.* [66]	95	VV configuration with a double-lumen catheter	300–500 *vs.* 800–1000	6–10	PLP* based on siloxane layer (ALung Hemolung RAS, iLA activve, Novalung, Cardiohelp® HLS 5.0, Getinge)	5 (3–8) d	Prospective multicenter international phase II study. Ultraprotective settings by 8 h and 24 h was achieved significantly in 78% at 8 h and 82% at 24 h of $ECCO_2R$ running. Two SAEs related to $ECCO_2R$ use (brain hemorrhage and pneumothorax). $ECCO_2R$- related AE were reported in 39% of the patients. Sixty-nine patients (73%) were alive at day 28. Fifty-nine patients (62%) were alive at hospital discharge.
McNamee JJ *et al.* [57]	405	VV configuration with a dual-lumen catheter inserted percutaneously into a central vein	350–450	10	PLP* based on siloxane layer (Alung Hemolung-RAS system); 0.59	4 d	Pragmatic, multi center, open label, randomized controlled and cost-effectiveness clinical trial. No difference in primary outcome of day 90 all-cause mortality 41.5% in the lower tidal volume ventilation with extracorporeal carbon dioxide removal group *vs.* 39.5% in the standard care group Risk Ratio, 1.05 (95% CI, 0.83–1.33); difference, 2.0% (95% CI, −7.6% to 11.5%); $p = 0.68$). Higher rates of adverse events: 168 (52% of patients) *vs.* 61 (23% of patients) 65 of these felt to be related to study intervention. Higher rates of intracranial hemorrhage: 10 *vs.* 25 were thought related to the intervention and 3 which resulted in death. Higher rates of infectious complications (7 *vs.* 1).

**PLP: polypropylene; **PMP: poly-4-methyl-1-pentene; AE: adverse effects; ARDS: acute respiratory distress syndrome; COPD: chronic obstructive pulmonary disease; LPV: lung protective ventilation; PECLA: pumpless extracorporeal lung assist; PEEP: positive end-expiratory pressure; P_{PLAT}: Plateau pressure; RRT: renal replacement therapy; V_T: tidal volume; SAE: serious adverse effects; IMV: invasive mechanical ventilation; PBW: predicted body weight; AV: arterio-venous; iLA: interventional lung assist; VFDs: ventilator free days; VV: veno-venous; RAS: Respiratory Assist System; HLS: Heart-Lung Support; ECMO: extracorporeal membrane oxygenation; DP: driving pressure.*

5. Complications

Although $ECCO_2R$ seems to improve or correct hypercapnic acidosis, its use is associated with a range of vascular, hematological, and other complications (Table 2). In a recent international feasibility trial, $ECCO_2R$-related adverse events such as catheter displacement or infectious complications were observed in 2% and membrane lung clotting or bleeding in 14% of patients, highlighting the coagulation/anticoagulation balance as a key issue [56].

TABLE 2. Complications associated with $ECCO_2R$.

Therapy-related
• Worsening of hypoxemia at the onset of low tidal ventilation
• Bleeding (pulmonary, gastrointestinal, cerebral)
• Hemolysis
• Consumption coagulopathy
• Thrombocytopenia/thrombopathy
• Air embolism
Catheter-related
• Vascular injury (bleeding)
• Catheter infection
• Thrombosis
• Hematoma, aneurism, pseudoaneurysm
• Distal limb ischemia (AV-$ECCO_2R$)
• Catheter malposition, dislodgement or kinking
• Compartment syndrome
• Accidental arterial insertion (AV-$ECCO_2R$ system)
• Recirculation
Device-related
• Pump malfunction
• Oxygenator malfunction
• Heat exchanger failure
• Clot plugging

AV-$ECCO_2R$: arterio-venous extracorporeal carbon dioxide removal.

$ECCO_2R$ can worsen hypoxemia and increase FiO_2 requirements due to derecruitment, which can be counteracted by applying higher levels of PEEP. Lower partial alveolar oxygen pressure can also result from a reduced lung respiratory quotient [71–73].

One of the most important differences between AV and VV configurations is the risk of complications related to the femoral artery catheterization with partial obstruction of blood flow and the potential occurrence of limb ischemia.

Hemorrhagic events related to vascular access and anticoagulation are the most frequent complications of $ECCO_2R$. The low flow makes systemic anticoagulation necessary, increasing significant bleeding risk, including cerebral, gastrointestinal, and nasopharyngeal bleeding. The contact between blood and the artificial surfaces of the circuit at very low flows can lead to a secondary consumption of clotting factors and associated bleeding complications. Clinically significant hemorrhagic complications are reported in the range between 2% and 50% [65, 74].

Although most systems are also coated with heparin to minimize thrombogenicity of the surface as little as possible, thrombus formation may build-up due to increased exposure time of the blood in contact with the artificial membrane lung and circuit due to lower flow rates. Clotting in the system may reduce or compromise the membrane efficiency or completely obstruct the circuit if anticoagulation is not achieved. This may reduce the membrane efficiency and consequently increase CO_2 levels rapidly. Membrane thrombosis must be considered a life-threatening event, requiring the immediate substitution of the circuit.

Heparin-induced thrombocytopenia is rarely observed. In this case, an albumin or phosphorylcholine/phosphatidylcholine coating can be requested [75].

The careful choice of adequate vascular access is critical in preventing thrombosis and detecting catheter kinking, precluding the achievement of target blood flow rates [56]. Catheter displacement or kinking may also result in pump malfunction and membrane thrombosis. Hence, subclavian or jugular vein cannulation is preferred over the femoral vein access when a high body mass index or intraabdominal hypertension is present. Intravascular hemolysis also has been reported.

6. Future perspectives

$ECCO_2R$ effectively allows the implementation of protective or ultra-protective ventilation in patients with ARDS. However, current data do not demonstrate efficacy in improving patient-centered outcomes. Further investigations, warranted to establish the overall clinical effect of $ECCO_2R$ in patients with ARDS, will need to address several important issues regarding, among others, the definition of optimal blood flow and hence circuit configuration, the definition of optimal target of pH, CO_2, tidal volumes and alveolar distending pressures, and the definition of optimal anticoagulation strategies. These advancements will also clarify whether $ECCO_2R$ should be applied in all patients with ARDS, only in specific subphenotypes, or whether a personalized mechanical ventilation strategy, including $ECCO_2R$, should be delivered to each patient based on specific disease characteristics and risk factors.

7. Summary and recommendations

$ECCO_2R$ may be a promising adjuvant therapeutic strategy to reduce the injury induced by mechanical ventilation.

In a recent European consensus on using $ECCO_2R$ for ultraprotective ventilation in ARDS patients, driving pressure with plateau pressure optimization was the main criteria for commencement of the technique. The clinical targets were pH >7.30, respiratory rate <20–25 breaths/min, P_{PLAT} <25 cmH_2O and driving pressure <14 cmH_2O [76]. At the moment, $ECCO_2R$ in patients with ARDS should not be used in patients outside clinical trials.

Future studies that harness the potential benefits of $ECCO_2R$

without increasing the risk of other complications are needed to progress this technology.

AUTHOR CONTRIBUTIONS

LMQ and LDS—designed the study, wrote original draft, reviewed and edited. LDS and JM—reviewed, edited and corrected English. JM—supervised and reviewed.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A , *et al*. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. The Journal of the American Medical Association. 2016; 315: 788–800.

[2] Fan E, Del Sorbo L, Goligher EC, Hodgson CL, Munshi L, Walkey AJ, *et al*. An official American thoracic society/European society of intensive care medicine/society of critical care medicine clinical practice guideline: mechanical ventilation in adult patients with acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. 2017; 195: 1253–1263.

[3] Slutsky AS, Ranieri VM. Ventilator-induced lung injury. New England Journal of Medicine. 2013; 369: 2126–2136.

[4] Rackley CR, MacIntyre NR. Low tidal volumes for everyone? Chest. 2019; 156: 783–791.

[5] The Acute Respiratory Distress Syndrome Network, Matthay MA, Morris A, Schoenfeld D, Thompson BT, Wheeler A. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. New England Journal of Medicine. 2000; 342: 1301–1308.

[6] Terragni PP, Rosboch G, Tealdi A, Corno E, Menaldo E, Davini O, *et al*. Tidal hyperinflation during low tidal volume ventilation in acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. 2007; 175: 160–166.

[7] Bellani G, Guerra L, Musch G, Zanella A, Patroniti N, Mauri T, *et al*. Lung regional metabolic activity and gas volume changes induced by tidal ventilation in patients with acute lung injury. American Journal of Respiratory and Critical Care Medicine. 2011; 183: 1193–1199.

[8] Amato MBP, Barbas CSV, Medeiros DM, Magaldi RB, Schettino GP, Lorenzi-Filho G, *et al*. Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. New England Journal of Medicine. 1998; 338: 347–354.

[9] Hager DN, Krishnan JA, Hayden DL, Brower RG. Tidal volume reduction in patients with acute lung injury when plateau pressures are not high. American Journal of Respiratory and Critical Care Medicine. 2005; 172: 1241–1245.

[10] Terragni PP, Del Sorbo L, Mascia L, Urbino R, Martin EL, Birocco A, *et al*. Tidal volume lower than 6 mL/kg enhances lung protection: role of extracorporeal carbon dioxide removal. The Journal of the American Society of Anesthesiologists. 2009; 111: 826–835.

[11] Fanelli V, Costamagna A, Ranieri VM. Extracorporeal support for severe acute respiratory failure. Seminars in Respiratory and Critical Care Medicine. 2014; 35: 519–527.

[12] Kolobow T, Gattinoni L, Tomlinson T, Pierce J. Control of breathing using an extracorporeal membrane lung. Anesthesiology. 1977; 46: 138–141.

[13] Gattinoni L, Pesenti A, Mascheroni D, Marcolin R, Fumagalli R, Rossi F, *et al*. Low-frequency positive-pressure ventilation with extracorporeal CO_2 removal in severe acute respiratory failure. The Journal of the American Medical Association. 1986; 256: 881–886.

[14] Gattinoni L, Pesenti A, Rossi GP, Vesconi S, Fox U, Kolobow T, *et al*. Treatment of acute respiratory failure with low-frequency positive-pressure ventilation and extracorporeal removal of CO_2. The Lancet. 1980; 316: 292–294.

[15] Gattinoni L, Mascheroni D, Torresin A, Marcolin R, Fumagalli R, Vesconi S, *et al*. Morphological response to positive end expiratory pressure in acute respiratory failure. Computerized tomography study. Intensive Care Medicine. 1986; 12: 137–142.

[16] West JB, Dollery CT, Naimark A. Distribution of blood flow in isolated lung; relation to vascular and alveolar pressures. Journal of Applied Physiology. 1964; 19: 713–724.

[17] Tomashefski JF Jr, Davies P, Boggis C, Greene R, Zapol WM, Reid LM. The pulmonary vascular lesions of the adult respiratory distress syndrome. The American Journal of Pathology. 1983; 112: 112–126.

[18] Greene R. Pulmonary vascular obstruction in the adult respiratory distress syndrome. Journal of Thoracic Imaging. 1986; 1: 31–38.

[19] Nuckton TJ, Alonso JA, Kallet RH, Daniel BM, Pittet J, Eisner MD, *et al*. Pulmonary dead-space fraction as a risk factor for death in the acute respiratory distress syndrome. New England Journal of Medicine. 2002; 346: 1281–1286.

[20] Kallet RH, Zhuo H, Liu KD, Calfee CS, Matthay MA. The association between physiologic dead-space fraction and mortality in subjects with ARDS enrolled in a prospective multi-center clinical trial. Respiratory Care. 2014; 59: 1611–1618.

[21] Doorduin J, Nollet JL, Vugts MPAJ, Roesthuis LH, Akankan F, van der Hoeven JG, *et al*. Assessment of dead-space ventilation in patients with acute respiratory distress syndrome: a prospective observational study. Critical Care. 2016; 20: 121.

[22] Sinha P, Calfee CS, Beitler JR, Soni N, Ho K, Matthay MA, *et al*. Physiologic analysis and clinical performance of the ventilatory ratio in acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. 2019; 199: 333–341.

[23] Gattinoni L, Chiumello D, Rossi S. COVID-19 pneumonia: ARDS or not? Critical Care. 2020; 24: 154.

[24] Kallet RH, L Lipnick MS. End-tidal-to-arterial P_{CO2} ratio as signifier for physiologic deadspace ratio and oxygenation dysfunction in acute respiratory distress syndrome. Respiratory Care. 2021; 66: 263–268.

[25] Morales-Quinteros L, Neto AS, Artigas A, Blanch L, Botta M, Kaufman DA, *et al*. Dead space estimates may not be independently associated with 28-day mortality in COVID-19 ARDS. Critical Care. 2021; 25: 171.

[26] Hickling KG, Henderson SJ, Jackson R. Low mortality associated with low volume pressure limited ventilation with permissive hypercapnia in severe adult respiratory distress syndrome. Intensive Care Medicine. 1990; 16: 372–377.

[27] Brochard L, Roudot-Thoraval F, Roupie E, Delclaux C, Chastre J, Fernandez-Mondéjar E, *et al*. Tidal volume reduction for prevention of ventilator-induced lung injury in acute respiratory distress syndrome. The multicenter trail group on tidal volume reduction in ARDS. American Journal of Respiratory and Critical Care Medicine. 1998; 158: 1831–1838.

[28] Meade MO, Cook DJ, Guyatt GH, Slutsky AS, Arabi YM, Cooper DJ, *et al*. Ventilation strategy using low tidal volumes, recruitment maneuvers, and high positive end-expiratory pressure for acute lung injury and acute respiratory distress syndrome: a randomized controlled trial. Survey of Anesthesiology. 2008; 52: 271–272.

[29] Brower RG, Shanholtz CB, Fessler HE, Shade DM, White P, Wiener

CM, *et al*. Prospective, randomized, controlled clinical trial comparing traditional versus reduced tidal volume ventilation in acute respiratory distress syndrome patients. Critical Care Medicine. 1999; 27: 1492–1498.

[30] Laffey JG, Kavanagh BP. Carbon dioxide and the critically ill—too little of a good thing? The Lancet. 1999; 354: 1283–1286.

[31] Broccard A, Hotchkiss J, Vannay C, Markert M, Sauty A, Feihl F, *et al*. Protective effects of hypercapnic acidosis on ventilator-induced lung injury. American Journal of Respiratory and Critical Care Medicine. 2001; 164: 802-806.

[32] Contreras M, Ansari B, Curley G, Higgins BD, Hassett P, O'Toole D, *et al*. Hypercapnic acidosis attenuates ventilation-induced lung injury by a nuclear factor-κB-dependent mechanism. Critical Care Medicine. 2012; 40: 2622–2630.

[33] Cummins EP, Oliver KM, Lenihan CR, Fitzpatrick SF, Bruning U, Scholz CC, *et al*. NF-κB links CO_2 sensing to innate immunity and inflammation in mammalian cells. The Journal of Immunology. 2010; 185: 4439–4445.

[34] Otulakowski G, Engelberts D, Gusarova GA, Bhattacharya J, Post M, Kavanagh BP. Hypercapnia attenuates ventilator-induced lung injury via a disintegrin and metalloprotease-17. The Journal of Physiology. 2014; 592: 4507–4521.

[35] Yang W, Song C, Wang N, Zhang L, Yue Z, Cui X, *et al*. Hypercapnic acidosis confers antioxidant and anti-apoptosis effects against ventilator-induced lung injury. Laboratory Investigation. 2013; 93: 1339–1349.

[36] O'Croinin DF, Nichol AD, Hopkins N, Boylan J, O'Brien S, O'Connor C, *et al*. Sustained hypercapnic acidosis during pulmonary infection increases bacterial load and worsens lung injury. Critical Care Medicine. 2008; 36: 2128-2135.

[37] O'Toole D, Hassett P, Contreras M, Higgins BD, McKeown ST, McAuley DF, *et al*. Hypercapnic acidosis attenuates pulmonary epithelial wound repair by an NF-κB dependent mechanism. Thorax. 2009; 64: 976–982.

[38] Vohwinkel CU, Lecuona E, Sun H, Sommer N, Vadász I, Chandel NS, *et al*. Elevated CO_2 levels cause mitochondrial dysfunction and impair cell proliferation. Journal of Biological Chemistry. 2011; 286: 37067–37076.

[39] Bharat A, Angulo M, Sun H, Akbarpour M, Alberro A, Cheng Y, *et al*. High CO_2 levels impair lung wound healing. American Journal of Respiratory Cell and Molecular Biology. 2020; 63: 244–254.

[40] Lecuona E, Trejo HE, Sznajder JI. Regulation of Na,K-ATPase during acute lung injury. Journal of Bioenergetics and Biomembranes. 2007; 39: 391–395.

[41] Briva A, Vadász I, Lecuona E, Welch LC, Chen J, Dada LA, *et al*. High CO_2 levels impair alveolar epithelial function independently of pH. PLoS One. 2007; 2: e1238.

[42] Lardner A. The effects of extracellular pH on immune function. Journal of Leukocyte Biology. 2001; 69: 522–530.

[43] Lang CJ, Dong P, Hosszu EK, Doyle IR. Effect of CO_2 on LPS-induced cytokine responses in rat alveolar macrophages. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2005; 289: L96–L103.

[44] Oliver KM, Lenihan CR, Bruning U, Cheong A, Laffey JG, McLoughlin P, *et al*. Hypercapnia induces cleavage and nuclear localization of relb protein, giving insight into CO_2 sensing and signaling. Journal of Biological Chemistry. 2012; 287: 14004–14011.

[45] Nakahata K, Kinoshita H, Hirano Y, Kimoto Y, Iranami H, Hatano Y. Mild hypercapnia induces vasodilation via adenosine triphosphate-sensitive K+ channels in parenchymal microvessels of the rat cerebral cortex. Anesthesiology. 2003; 99: 1333–1339.

[46] Shiota S, Okada T, Naitoh H, Ochi R, Fukuchi Y. Hypoxia and hypercapnia affect contractile and histological properties of rat diaphragm and hind limb muscles. Pathophysiology. 2004; 11: 23–30.

[47] Tang W, Weil MH, Gazmuri RJ, Bisera J, Rackow EC. Reversible impairment of myocardial contractility due to hypercarbic acidosis in the isolated perfused rat heart. Critical Care Medicine. 1991; 19: 218–224.

[48] Kregenow DA, Swenson ER. The lung and carbon dioxide: implications for permissive and therapeutic hypercapnia. European Respiratory Journal. 2002; 20: 6–11.

[49] Cullen D, Eger E. Cardiovascular effects of carbon dioxide in man. Anesthesiology. 1974; 41: 345–349.

[50] Nin N, Muriel A, Peñuelas O, Brochard L, Lorente JA, Ferguson ND, *et al*. Severe hypercapnia and outcome of mechanically ventilated patients with moderate or severe acute respiratory distress syndrome. Intensive Care Medicine. 2017; 43: 200–208.

[51] Tiruvoipati R, Pilcher D, Buscher H, Botha J, Bailey M. Effects of hypercapnia and hypercapnic acidosis on hospital mortality in mechanically ventilated patients. Critical Care Medicine. 2017; 45: e649–e656.

[52] Madotto F, Rezoagli E, McNicholas BA, Pham T, Slutsky AS, Bellani G, *et al*. Patterns and impact of arterial CO_2 management in patients with acute respiratory distress syndrome: insights from the LUNG SAFE study. Chest. 2020; 158: 1967–1982.

[53] Kiiski R, Takala J. Hypermetabolism and efficiency of CO_2 removal in acute respiratory failure. Chest. 1994; 105: 1198–1203.

[54] Burki NK, Mani RK, Herth FJF, Schmidt W, Teschler H, Bonin F, *et al*. A novel extracorporeal CO_2 removal system: results of a pilot study of hypercapnic respiratory failure in patients with COPD. Chest. 2013; 143: 678–686.

[55] Wearden PD, Federspiel WJ, Morley SW, Rosenberg M, Bieniek PD, Lund LW, *et al*. Respiratory dialysis with an active-mixing extracorporeal carbon dioxide removal system in chronic sheep study. Intensive Care Medicine. 2012; 38: 1705–1711.

[56] Fanelli V, Ranieri MV, Mancebo J, Moerer O, Quintel M, Morley S, *et al*. Feasibility and safety of low-flow extracorporeal carbon dioxide removal to facilitate ultra-protective ventilation in patients with moderate acute respiratory distress syndrome. Critical Care. 2016; 20: 36.

[57] McNamee JJ, Gillies MA, Barrett NA, Perkins GD, Tunnicliffe W, Young D, *et al*. Effect of lower tidal volume ventilation facilitated by extracorporeal carbon dioxide removal *vs.* standard care ventilation on 90-day mortality in patients with acute hypoxemic respiratory failure: the REST randomized clinical trial. The Journal of the American Medical Association. 2021; 326: 1013–1023.

[58] Zanella A, Caironi P, Castagna L, Rezoagli E, Salerno D, Scotti E, *et al*. Extracorporeal chloride removal by electrodialysis. A novel approach to correct acidemia. American Journal of Respiratory and Critical Care Medicine. 2020; 201: 799–813.

[59] Scaravilli V, Kreyer S, Belenkiy S, Linden K, Zanella A, Li Y, *et al*. Extracorporeal carbon dioxide removal enhanced by lactic acid infusion in spontaneously breathing conscious sheep. Anesthesiology. 2016; 124: 674–682.

[60] Tapia P, Lillo F, Soto D, Escobar L, Simon F, Hernández K, *et al*. Liquid extracorporeal carbon dioxide removal: use of THAM (tris-hydroxymethyl aminomethane) coupled to hemofiltration to control hypercapnic acidosis in a porcine model of protective mechanical ventilation. American Journal of Translational Research. 2016; 8: 3493–3502.

[61] Cove ME, MacLaren G, Federspiel WJ, Kellum JA. Bench to bedside review: extracorporeal carbon dioxide removal, past present and future. Critical Care. 2012; 16: 232.

[62] Flörchinger B, Philipp A, Klose A, Hilker M, Kobuch R, Rupprecht L, *et al*. Pumpless extracorporeal lung assist: a 10-year institutional experience. The Annals of Thoracic Surgery. 2008; 86: 410–417.

[63] Karagiannidis C, Kampe K, Sipmann F, Larsson A, Hedenstierna G, Windisch W, *et al*. Veno-venous extracorporeal CO_2 removal for the treatment of severe respiratory acidosis: pathophysiological and technical considerations. Critical Care. 2014; 18: R124.

[64] Abrams D, Roncon-Albuquerque R, Brodie D. What's new in extracorporeal carbon dioxide removal for COPD? Intensive Care Medicine. 2015; 41: 906–908.

[65] Fitzgerald M, Millar J, Blackwood B, Davies A, Brett SJ, McAuley DF, *et al*. Extracorporeal carbon dioxide removal for patients with acute respiratory failure secondary to the acute respiratory distress syndrome: a systematic review. Critical Care. 2014; 18: 222.

[66] Combes A, Fanelli V, Pham T, Ranieri VM. Feasibility and safety of extracorporeal CO_2 removal to enhance protective ventilation in acute respiratory distress syndrome: the SUPERNOVA study. Intensive Care Medicine. 2019; 45: 592–600.

[67] Goligher EC, Combes A, Brodie D, Ferguson ND, Pesenti AM, Ranieri VM, *et al*. Determinants of the effect of extracorporeal carbon dioxide removal in the SUPERNOVA trial: implications for trial design. Intensive Care Medicine. 2019; 45: 1219–1230.

[68] Bein T, Weber-Carstens S, Goldmann A, Müller T, Staudinger T, Brederlau J, *et al*. Lower tidal volume strategy (≈3 mL/kg) combined with

extracorporeal CO_2 removal versus 'conventional' protective ventilation (6 mL/kg) in severe ARDS: the prospective randomized Xtravent-study. Intensive Care Medicine. 2013; 39: 847–856.

[69] Amato MBP, Meade MO, Slutsky AS, Brochard L, Costa ELV, Schoenfeld DA, *et al*. Driving pressure and survival in the acute respiratory distress syndrome. New England Journal of Medicine. 2015; 372: 747–755.

[70] Grasso S, Stripoli T, Mazzone P, Pezzuto M, Lacitignola L, Centonze P, *et al*. Low respiratory rate plus minimally invasive extracorporeal CO_2 removal decreases systemic and pulmonary inflammatory mediators in experimental Acute Respiratory Distress Syndrome. Critical Care Medicine. 2014; 42: e451–e460.

[71] Gattinoni L. Ultra-protective ventilation and hypoxemia. Critical Care. 2016; 20: 130.

[72] Gattinoni L, Kolobow T, Tomlinson T, Iapichino G, Samaja M, White D, *et al*. Low-frequency positive pressure ventilation with extracorporeal carbon dioxide removal (LFPPV-ECCO$_2$R): an experimental study. Anesthesia & Analgesia. 1978; 57: 470–477.

[73] Diehl J, Mercat A, Pesenti A. Understanding hypoxemia on ECCO$_2$R: back to the alveolar gas equation. Intensive Care Medicine. 2019; 45: 255–256.

[74] Sklar MC, Beloncle F, Katsios CM, Brochard L, Friedrich JO. Extracorporeal carbon dioxide removal in patients with chronic obstructive pulmonary disease: a systematic review. Intensive Care Medicine. 2015; 41: 1752–1762.

[75] Campbell EJ, O'Byrne V, Stratford PW, Quirk I, Vick TA, Wiles MC, *et al*. Biocompatible surfaces using methacryloylphosphorylcholine laurylmethacrylate copolymer. American Society for Artificial Internal Organs. 1994; 40: M853–M857.

[76] Combes A, Auzinger G, Capellier G, du Cheyron D, Clement I, Consales G, *et al*. ECCO$_2$R therapy in the ICU: consensus of a European round table meeting. Critical Care. 2020; 24: 490.

[77] Terragni P, Del Sorbo L, Mascia L, Urbino R, Martin E, Birocco A, *et al*. Tidal volume lower than 6 mL/kg enhances lung protection: role of extracorporeal carbon dioxide removal. Anesthesiology. 2009; 111: 826–835.

[78] Augy JL, Aissaoui N, Richard C, Maury E, Fartoukh M, Mekontso-Dessap A, *et al*. A 2-year multicenter, observational, prospective, cohort study on extracorporeal CO_2 removal in a large metropolis area. Journal of Intensive Care. 2019; 7: 45.

[79] Schmidt M, Jaber S, Zogheib E, Godet T, Capellier G, Combes A. Feasibility and safety of low-flow extracorporeal CO_2 removal managed with a renal replacement platform to enhance lung-protective ventilation of patients with mild-to-moderate ARDS. Critical Care. 2018; 22: 122.

[80] Ding X, Chen H, Zhao H, Zhang H, He H, Cheng W, *et al*. ECCO$_2$R in 12 COVID-19 ARDS patients with extremely low compliance and refractory hypercapnia. Frontiers in Medicine. 2021; 8: 654658.

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REVIEW

Therapeutic agents for ARDS

Elena Campaña-Duel[1,2], Marta Camprubí-Rimblas[1,3,*]

[1]Critical Care Research Center, Parc Taulí Hospital Universitari, 08208 Sabadell, Spain
[2]Facultat de Biologia, Universitat de Barcelona, 08028 Barcelona, Spain
[3]CIBER de Enfermedades Respiratorias (CIBERES), 28029 Madrid, Spain

***Correspondence**
mcamprubi@tauli.cat
(Marta Camprubí-Rimblas)

Abstract

In spite of supportive care of patients with acute respiratory distress syndrome (ARDS), morbidity and mortality of these patients are considerable and effective therapies centred in ARDS pathophysiology are needed. Substantial progress in pharmacological therapies has been noticed, however, several studies have not been successfully translated to the clinics. Nonetheless, many preclinical and clinical studies are ongoing. In this review, pharmacological therapies underlying ARDS pathophysiology are summarized: therapies targeting the alveolocapillary membrane, mucolytics, bronchodilators, immunomodulators, anticoagulants and fibrinolytics, aspirin, and other treatments are discussed, including both, studies with beneficial and controversial results, and ongoing trials. In addition, a section concerning preclinical studies is included. An enlarged understanding of ARDS pathophysiology and its fundamental pathways and mechanism, together with the identification of ARDS subsets of patients and phenotypes will maximise patient response to a specific treatment.

Keywords

Acute respiratory distress syndrome (ARDS); Acute lung injury; COVID-19; Sepsis

1. Introduction

Acute respiratory distress syndrome (ARDS) is an acute hypoxemic respiratory failure in critically ill patients of all ages [1]. This syndrome may originate from multiple insults that affect directly the lung (pneumonia or aspiration of gastric contents, among others), or systemic insults that will develop ARDS as a consequence of the primary disease (sepsis or trauma, among others) [1]. Recent clinical ARDS categories include patients with Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2]. ARDS is heterogenic based on its etiology, illness severity, duration, and individual patient characteristic, determining the course of the disease. Morbidity and mortality of ARDS remain high [3, 4], about 35–40%. Most surviving patients experience persistent and prolonged physical, mental and quality-of-life impairment, requiring specific medical attention after recovery of ARDS [5].

The pathophysiology of ARDS is characterized by the breakdown of the alveolar-capillary barrier, which leads to proteinaceous edema and neutrophils infiltration into the alveolar compartment, with pulmonary activated coagulation and inflammation, and decreased fibrinolysis [1, 6, 7]. Nowadays there is no single biomarker able to identify ARDS nor its underlying biology.

Currently, the management of ARDS patients is mainly supportive and preventive, and specific effective pharmacological therapy is not available yet. Despite years of research and knowledge, several preclinical and clinical studies have not been successfully translated. However, science is increasingly advancing day by day, and many treatments focused on ARDS pathophysiology are underway, and many others have emerged during the actual COVID-19 pandemic. Progressive understanding of the pathways and mechanisms involved in this disease, together with the identification of subsets of patients underlying ARDS might improve treatment response.

This narrative review is focused on the pharmacological therapies that have been proposed to treat adult ARDS, highlighting their beneficial and controversial effects, especially on those therapies that are ongoing but without excluding those that did not work. To better understand the mechanisms of the different therapies for adult ARDS, in some sections, studies on neonate/pediatric ARDS (soluble guanylate cyclase surfactant, budesonide) or studies for sepsis (Bevacizumab, Levosimendan Hydrocortisone, Vitamin C, Sivelestat Sodium, anti-TF antibody-836 (ALT-836), Antithrombin, thrombomodulin alfa-123 (ART-123), Drotrecogin alfa) have been introduced. Clinical studies have been found in Home-Clinical Trials. gov or PubMed (nih.gov).

The article is divided into therapies targeting the alveolocapillary membrane, mucolytics, bronchodilators, immunomodulators, anticoagulants and fibrinolytics, aspirin, and other treatments, including data of relevant preclinical and clinical studies and highlighting those that are ongoing (Fig. 1, Table 1). The different therapies are classified according to their main actions on target key processes and pathways of ARDS complex pathophysiology, but this does not exclude that one therapy do exert its effects through different systems. Also, there is a section for preclinical treatments which have not been

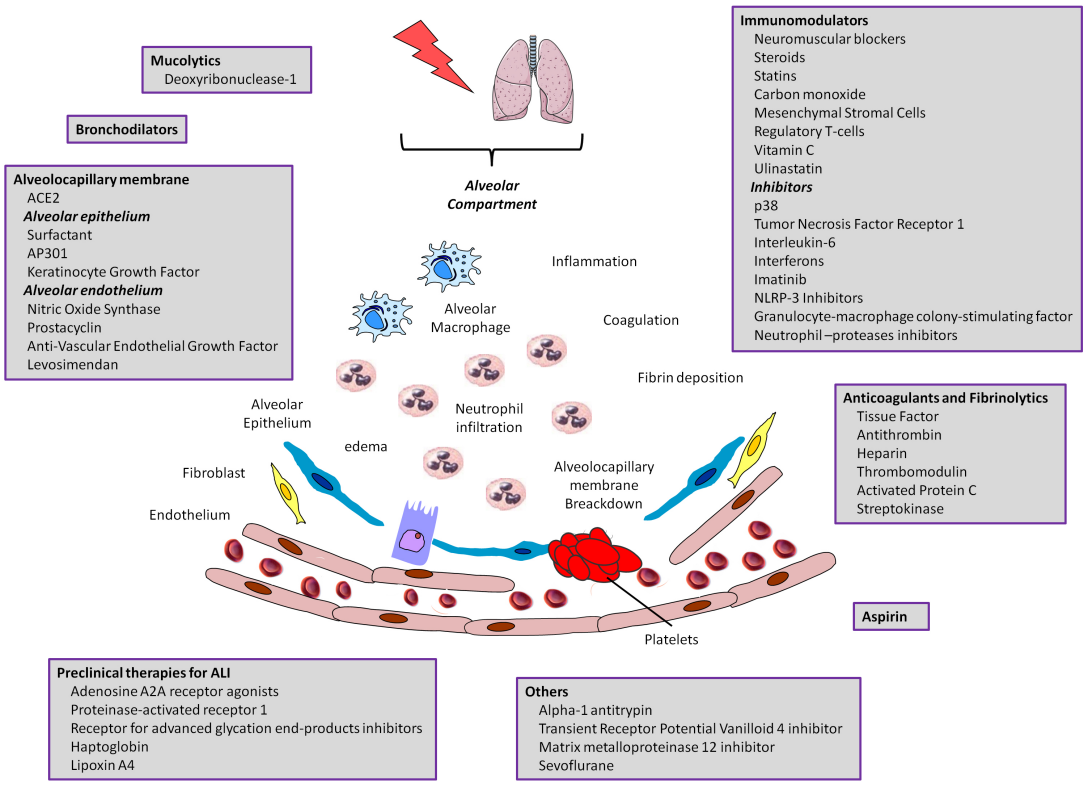

FIGURE 1. Acute respiratory distress syndrome and proposed preclinical and clinical specific pharmacological therapies.

tested yet in clinical trials.

2. Alveolocapillary membrane

Damage into the alveolocapillary membrane drives the loss of epithelial and endothelial barrier integrity, which leads to protein-rich edema extravasation and leukocytes infiltration into the alveolar compartment [8].

2.1 ACE2

The renin-angiotensin system (RAS) is involved in ARDS pathophysiology. Patients with ARDS present increased levels of Angiotensin II, a vasoconstrictor involved in inflammation and pulmonary edema that exerts its activities through angiotensin type I receptor [9]. Angiotensin Converting enzyme II (ACE2) hydrolyses Angiotensin II producing Angiotensin 1–7, which has been found to be protective in experimental models. In a randomized phase 2a clinical study, GSK2586881 (recombinant human ACE2) was administered as an exogenous ACE2, in order to hydrolize Angiotensin II, and proved safety but did not improve clinical outcomes in ARDS patients requiring mechanical ventilation [10].

In patients with COVID-19, SARS-CoV-2 is known to bind ACE2; both membrane-bound (mACE2) and soluble (sACE2) forms. However, only mACE2 mediates the virus entrance into the cell, but not sACE2. Angiotensin type I receptor blockers increase the levels of Angiotensin II, which stimulates ACE2 shedding; sACE2 catalyzes the conversion of Angiotensin II to Antiotensin 1–7 while also binds SARS-CoV-2 blocking its entrance to the host cells. Presently, there is an ongoing randomized phase 2 trial with oral 50 mg Losartan (an angiotensin type I receptor blocker) and 25 mg Spironolactone (a blocker of aldosterone secretion) in patients with COVID-19-ARDS (NCT04643619).

2.2 Alveolar epithelium

The alveolar epithelium has a key role in ARDS severity [11]. It is composed of alveolar type I cells (ATI cells), which cover the 95% of alveolar surface and are the major responsible of gas exchange, and alveolar type II cells (ATII cells), which are the progenitor cells of the alveolar epithelium and can proliferate and differentiate into ATII cells. ATII cells also produce surfactant. Both cell types are critical in ion transport and present immunologic functions [12, 13].

2.2.1 Surfactant

ATII cells produce and recycle pulmonary surfactant, which is composed of proteins and lipids. Surfactant maintains the alveolar surface tension and presents antimicrobial and host defense functions [14]. ATII cells injury together with the presence of proteins and enzymes in the edema induce surfactant dysfunction [15].

In pediatric patients, exogenous surfactant evidenced benefits. In the ULTRASURF randomised controlled trial, the lung ultrasound scores improve the time of surfactant administration and prove better oxygenation after early treatment with surfactant in premature newborns [16].

In a randomized controlled trial, continuously nebulized synthetic surfactant for five days in patients with sepsis-induced ARDS did not impact 30-day survival, duration

TABLE 1. Acute respiratory distress syndrome clinical studies of pharmacological therapies.

Therapeutic agent	Route of Administration	Mechanism of action	Severity of ARDS	Trial state	Stage of testing	Reference/ Identifier
ALVEOLOCAPILLARY MEMBRANE						
RAS related signalling						
Recombinant human ACE2 (GSK2586881)	Intravenous	Cleavage of Angiotensin II to Angiotensin 1–7	ARDS patients requiring mechanical ventilation for <72 h	Phase 2	Completed	[10]
Losartan and Spironolactone	Oral	Blocking angiotensin receptor and secretion of aldosterone.	COVID-19 ARDS	Phase 2	Recruiting	NCT04643691
Alveolar epithelium						
Synthetic surfactant	Aerosolized	Replace surfactant	Sepsis-induced ARDS	Not Applicable	Completed	[17]
Recombinant surfactant protein C	Intratracheal	Replace surfactant	Various etiologies	Phase 3	Completed	[18–20]
Poractant Alfa	Through bronchoscopy	Replace surfactant	COVID-19 ARDS	Not Applicable	Completed	[21]
Poractant Alfa	Endotracheal instillation or bronchial fibroscopy	Replace surfactant	COVID-19 ARDS	Phase 2	Recruiting	NCT04502433/ NCT04384731
AP301 (Solnatide)	Inhaled	Activation alveolar epithelium Na^+ channels	ARDS patients requiring mechanical ventilation	Phase 2a	Completed	[24, 25]
AP301 (Solnatide)	Inhaled	Activation alveolar epithelium Na^+ channels	Moderate-to-severe ARDS	Phase 2b	Recruiting	NCT03567577
Recombinant hKGF (palifermin)	Intravenous	ATII cell proliferation, migration, and regeneration	Not specified	Phase 2	Completed	[29]
Alveolar endothelium						
L-citrulline	Intravenous	Substate of NOS	Sepsis-induced ARDS	Phase 2	Completed, unpublished	NCT01474863
L-citrulline	Oral, dietary supplement	Substrate of NOS	COVID-19 ARDS	Not Applicable	Completed, unpublished	NCT04404426
sGC activator (BAY 1211163)	Inhaled	Conversion of GTP into cGMP	Moderate or severe ARDS	Phase 1	Recruiting	NCT04609943
Iloprost	Inhaled	Prostacyclin analogue, vasodilatation	Not specified	Phase 3	Completed, unpublished	NCT03111212
Epoprostenol	Inhaled	Prostacyclin analogue, vasodilatation	COVID-19 ARDS	Phase 2	Completed, unpublished	NCT04452669
Iloprost	Inhaled	Prostacyclin analogue, vasodilatation	COVID-19 ARDS	Phase 2	Recruiting	NCT04445246

TABLE 1. Continued.

Bevacizumab	Intrvenous	Anti-VEGF	Sepsis-induced ARDS	Phase 2	Withdrawn	NCT01314066
Bevacizumab	Intrvenous	Anti-VEGF	COVID-19 ARDS	Phase 2	Completed, unpublished	NCT04275414
Levosimendan	Intravenous	K^+ channel activator	Sepsis	Phase 2	Recruiting	NCT04020003
			MUCOLYTICS			
N-acetylcysteine	Intravenous	Mucolytic	ARDS patients requiring mechanical ventilation	Not Applicable	Completed	[41]
N-acetylcysteine	Intravenous	Mucolytic	Moderate-to-severe COVID-19	Not Applicable	Completed	[42]
Dornase alfa	Inhaled	Cleaving extracellular DNA in NETs	COVID-19 ARDS	Phase 3	Terminated	NCT04355364
Dornase Alfa	Inhaled	Cleaving extracellular DNA in NETs	COVID-19 ARDS	Phase 2	Recruiting	NCT04402944
			BRONCHODILATORS			
Salbutamol	Intravenous	Beta-adrenergic agonist	ARDS patients requiring mechanical ventilation	Not Applicable	Completed	[45]
Salbutamol	Intravenous	Beta-adrenergic agonist	ARDS patients requiring mechanical ventilation, ICU	Phase 2	Completed	[46]
Salbutamol	Intravenous	Beta-adrenergic agonist	ARDS patients requiring mechanical ventilation	Phase 2	Completed	[47]
Albuterol	Inhaled	Beta-adrenergic agonist	ARDS patients requiring mechanical ventilation	Phase 3	Completed	[48]
Dornase Alfa and albuterol	Inhaled	Beta-adrenergic agonist	ARDS patients requiring mechanical ventilation, COVID-19 ARDS	Not Applicable	Completed, unpublished	NCT04387786
			NEUROMUSCULAR BLOCKERS			
Cisatracurium	Intravenous	Blocking cholinergic receptors	ICU patients	Not Applicable	Completed	[50]
Cisatracurium	Intravenous	Blocking cholinergic receptors	Moderate-to-severe ARDS	Not Applicable	Completed	[51]
			IMMUNOMODULATIONS			
			Steroids			
Dexamethasone	Intravenous	Anti-inflammatory and immunosupressor	Moderate-to-severe ARDS requiring mechanical ventilation	Not Applicable	Completed	[60]
Dexamethasone	Intravenous or oral	Anti-inflammatory and immunosupressor	COVID-19 ARDS	Not Applicable	Completed	[61]
Dexamethasone	Intravenous	Anti-inflammatory and immunosupressor	COVID-19 ARDS	Phase 4	Recruiting	NCT04663555
Dexamethasone and Methylprednisolone	Intravenous	Anti-inflammatory and immunosupressor	COVID-19 ARDS	Phase 3	Recruiting	NCT04499313

TABLE 1. Continued.

Hydrocortisone	Intravenous	Anti-inflammatory and immunosupressor	Sepsis-induced ARDS	Not Applicable	Completed	[62]
Methylprednisolone	Intravenous	Anti-inflammatory and immunosupressor	Severe	Not Applicable	Completed	[63]
Methylprednisolone	Intravenous	Anti-inflammatory and immunosupressor	Severe ARDS	Not specified	Completed	[64]
Methylprednisolone	Intrapleural	Anti-inflammatory and immunosupressor	ARDS and multi-organ dysfunction syndrome	Phase 2	Completed, unpublished	NCT01423864
Canrenone	Intravenous	Diuretic	COVID-19 moderate-to-severe ARDS	Phase 2	Not yet recruiting	NCT04977960
Budesonide	Inhaled	Anti-inflammatory and immunosupressor	Not specified	Phase 2	Completed	[65]
Budesonide	Inhaled	Anti-inflammatory and immunosupressor	ARDS patients requiring mechanical ventilation	Not Applicable	Completed	[66]
Budesonide	Inhaled	Anti-inflammatory and immunosupressor	Paediatric	Phase 2	Terminated	NCT04064684
Budesonide	Intratracheal	Anti-inflammatory and immunosupressor	Neonatal severe ARDS with mechanical ventilation	Not Applicable	Completed	[67]
Budesonide and surfactant	Intratracheal	Anti-inflammatory and immunosupressor	Preterm infants	Not Applicable	Completed	[68]
			Statins			
Simvastatin	Oral	HMG-CoA reductase inhibitor, immunomodulatory	ARDS diagnosed <48 h	Phase 2b	Completed	[70]
Rosuvastatin	Oral	HMG-CoA reductase inhibitor, immunomodulatory	ARDS and suspected infection	Not Applicable	Completed	[71]
			Carbon monoxide			
CO	inhaled	Down-regulation NLRP3, anti-apoptotic, anti-inflammatory	Intubated patients	Phase 2	Recruiting	NCT03799874
			Mesenchymal Stromal Cells			
MultiStem	Intravenous	Immunomodulatory and reparative effects	Moderate-to-severe ARDS patients requiring mechanical ventilation	Phase 1/2	Completed	[77]
hMSCs	Intravenous	Immunomodulatory and reparative effects	Moderate-to-severe ARDS patients requiring mechanical ventilation	Phase 2a	Completed	[78]

TABLE 1. Continued.

hMSCs	Intravenous	Immunomodulatory and reparative effects	Moderate-to-severe ARDS patients requiring mechanical ventilation	Phase 2b	Recruiting	NCT03818854
CD362 enriched MSCs	Intravenous	Immunomodulatory and reparative effects	ARDS patients requiring mechanical ventilation, COVID-19	Phase 1/2	Active	NCT03042143
ACE2^{-}MSCs	Intravenous	Immunomodulatory and reparative effects	COVID-19 ARDS	Not Applicable	Completed	[79]
			Regulatory T-cells			
T-regulatory cells	Intravenous	Promoting homeostasis	COVID-19 ARDS	Phase 1/2	Recruiting	NCT05027815/ NCT04468971
Treg/Th2 hybrid cells	Intravenous	Decreasing Th1 response	COVID-19 ARDS	Phase 1/2	Terminated	NCT04482699
			Vitamin C			
Vitamin C	Intravenous	Antioxidant	Sepsis-induced ARDS	Phase 2	Completed	[82]
Vitamin C	Oral	Antioxidant	COVID-19 ARDS	Not Applicable	Completed	NCT04570254
Vitamin C	Intravenous	Antioxidant	COVID-19 ARDS	Not Applicable	Completed	NCT04710329
Vitamin C	Intravenous	Antioxidant	Sepsis-induced ARDS	Phase 3	Not yet recruiting	NCT04404387
			Ulinastatin			
Ulinastatin	Intravenous	Urinary trypsin inhibitor	ARDS patients requiring mechanical ventilation	Not Applicable	Completed	[83]
			Inhibitors			
Dilmapimod	Intravenous	P39MAPK inhibitor	Patients at risk	Phase 2	Completed	[84]
Anti-TNRF1	Inhaled	Antagonizes TNF-α	LPS-induced experimentally	Phase 1	Completed	[88]
Tocilizumab	Intravenous	Blocking IL-6	COVID-19 ARDS	Phase 2/3	Completed	NCT04445272
Tocilizumab	Intravenous	Blocking IL-6	COVID-19 ARDS	Phase 3 /Not applicable	Recruiting	NCT04412772/ NCT05082714
Imatinib	Oral	Tyrosine kinase inhibitor	LPS-induced experimentally	Phase 1	Completed, unpublished	NCT03328117
Pirfenidone	Oral	Inhibition NLRP3	COVID-19 ARDS	Phase 3	Unknown	NCT04282902
Pirfenidone	Nasogastric	Inhibition NLRP3	COVID-19 ARDS, severe	Not Applicable	Recruiting	NCT04653831
Vadadustat	Oral	Increasing EPO production	COVID-19 ARDS	Phase 2	Recruiting	NCT04478071
Otilimab	Intravenous	Anti-GM-CSF	COVID-19 ARDS	Phase 2	Completed	NCT04376684
Lenzilumab	Intravenous	Anti-GM-CSF	COVID-19 ARDS	Phase 3	Completed, unpublished	NCT04351152
TJ0023234	Intravenous	Anti-GM-CSF	COVID-19 ARDS	Phase 2/3	Recruiting	NCT04341116
Sargramostim	Intravenous	Anti-GM-CSF	COVID-19 ARDS	Phase 2	Recruiting	NCT04400929
Mavrilimumab	Intravenous	Anti-GM-CSF	COVID-19 ARDS	Not Applicable	Completed	[107]
Sivelestat sodium	Intravenous	Neutrophil-proteases inhibitors	ARDS patients requiring mechanical ventilation, ICU	Not Applicable	Completed	[116]

TABLE 1. Continued.

Sivelestat sodium	Intravenous	Neutrophil-proteases inhibitors	not specified	Not Applicable	Completed	[117]
Sivelestat sodium	Intravenous	Neutrophil-proteases inhibitors	Sepsis-induced ARDS	Phase 3	Recruiting	NCT04973670
Sivelestat sodium	Intravenous	Neutrophil-proteases inhibitors	not specified	Phase 4	Not yet recruiting	NCT04909697
			Others			
SNG001 (Interferon β-1α)	Inhaled	Anti-inflammatory, anti-fibrotic, antiviral	COVID-19 ARDS	Phase 2	Completed	[92]
FP-1201 (Interferon β-1α)	Intravenous	Anti-inflammatory, anti-fibrotic, antiviral	Moderate-to-severe ARDS	Phase 3	Completed	[93]
GM-CSF	Intravenous	Immunomodulatory	not specified	Phase 2	Completed	[105]
rhGM-CSF	Inhaled	Immunomodulatory	Pneumonia	Phase 2	Active	NCT02595060
Molgramostim (rhGM-CSF)	Inhaled	Immunomodulatory	COVID-19 induced	Phase 2	Recruiting	NCT04569877
			ANTICOAGULANTS AND FIBRINOLYTICS			
ALT-836	Intravenous	Anti-TF	ARDS patients requiring mechanical ventilation	Phase 1	Completed	[123]
ALT-836	Intravenous	Anti-TF	Sepsis-induced ARDS	Phase 2	Completed	NCT00879606
Tifacogin	Intravenous	Recombinant TFPI, modulates extrinsic coagulation pathway	Pneumonia	Phase 3	Completed	[125]
Antithrombin	Intravenous	Inhibition procoagulant thrombin	Sepsis-induced ARDS	Phase 3	Completed	[128, 129]
Heparin	Inhaled	Anticoagulant	ARDS patients requiring mechanical ventilation	Phase 1	Completed	[133, 134]
Heparin	Inhaled	Anticoagulant	ARDS patients requiring mechanical ventilation	Phase 2	Completed	[135]
Heparin	Inhaled	Anticoagulant	ARDS patients requiring mechanical ventilation	Phase 3	Completed	[136]
Heparin	Inhaled	Anticoagulant	Inhalation trauma ARDS	Not Applicable	Completed	[137]
Heparin	Inhaled	Anticoagulant	COVID-19 ARDS patients requiring mechanical ventilation	Phase 2/3	Recruiting	NCT04545541
ART-123	Intravenous	Cleaving protein C	Sepsis-induced	Phase 2b	Completed	[138]
Rh thrombomodulin and sivelestat	Intravenous	Cleaving protein C	ARDS and disseminated intravascular coagulation	Not Applicable	Completed	[139]
ART-123	Intravenous	Cleaving protein C	Sepsis-induced ARDS, ICU	Phase 3	Completed	[140, 141]
Drotrecogin alfa	Inhaled	rh Protein C	not specified	Not Applicable	Completed	[142]

TABLE 1. Continued.

Drotrecogin alfa	Intravenous	rh Protein C	Sepsis-induced ARDS	Phase 3	Completed	[143]
Streptokinase	Nebulized	Conversion plasminogen to plasmin	Severe	Phase 3	Completed	[144]
			ASPIRIN			
Aspirin	Oral	Cyclooxygenase pathway inhibitor	not specified	Phase 2b	Completed	[145]
Aspirin	Oral	Cyclooxygenase pathway inhibitor	ARDS patients requiring mechanical ventilation	Phase 2	Terminated	NCT02326350
			OTHERS			
Alpha-1 antitrypsin	Intravenous	Serine protease inhibitor	COVID-19 ARDS	Not applicable	Completed	[147]
TRPV4 inhibitor	Inhaled	Decreasing inflammation	Healthy volunteers	Phase 1	Terminated	NCT03511105
FP-025		Inhibits MMP12	COVID-19 ARDS	Phase 2/3	Recruiting	NCT04750278
Sevoflurane	Inhaled	Anesthetic, anti-inflammatory	COVID-19 ARDS	Not applicable	Completed	[153]
Sevoflurane	Inhaled/ Intravenous	Anesthetic, anti-inflammatory	COVID-19 ARDS	Phase 3	Completed, unpublished	NCT04355962
Sevoflurane	Inhaled	Anesthetic, anti-inflammatory	Moderate-to-severe ARDS	Phase 3	Not yet recruiting	NCT04530188

ACE2: angiotensin converting enzyme 2; ALT-836: anti-TF antibody; ARDS: Acute Respiratory Distress Syndrome; ART-123: thrombomodulin alfa; ATII: alveolar type II epithelial cell; CD362: cluster of differentiation 362; cGMP: cyclic guanosine monophosphate; CO: carbon monoxide; COVID-19: Coronavirus Disease 2019; EPO: erythropoietin; FP-025: MMP12 inhibitor; FP-1201: recombinant human interferon-β; GM-CSF: granulocyte-macrophage colony-stimulating factor; GTP: guanosine-5'-triphosphate; hKGF: human keratinocyte growth factor; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A; hMSC: mesenchymal stem cell; ICU: intensive care unit; IL-6: interleukin-6; LPS: lipopolysaccharide; MMP12: matrix metallopeptidase 12; NETs: neutrophil extracellular traps; NLRP3: NOD-like receptor family pyrin domain containing 3; NOS: nitric oxide synthase; P39MAPK: P39 mitogen-activated protein kinase; RAS: rat sarcoma virus; rh: recombinant human; sGC: soluble guanylyl cyclase; SNG001: interferon-β drug; TFPI: tissue factor pathway inhibitor; Th2: T helper cell type 2; TNF: tumor necrosis factor; TNRF1: tumor necrosis factor receptor 1; Treg: T regulatory cell; TRPV4: transient receptor potential vanilloid 4; VEGF: vascular endothelial growth factor.

of mechanical ventilation nor physiologic function [17]. However, in a randomized controlled phase 3 trial intratracheal recombinant surfactant protein C in patients with ARDS from various etiologies did improve gas exchange but not survival [18, 19]. In a post hoc analysis, recombinant surfactant protein C proved to decrease mortality in patients with ARDS due to pneumonia or aspiration [20].

Surfactant replacement has also been proposed for COVID-19. In a retrospective analysis, poractant alfa (Curosurf), a surfactant replacement therapy, administered through a bronchoscopy, proved to be safe and produce a non-significant 28 days mortality reduction in adult COVID-19-ARDS patients [21]. Presently, phase 2 studies to evaluate efficacy and safety of three poractant alfa (Curosurf) administrations by endotracheal instillation every 24 h, or 3 mL/kg of poractant alpha (Curosurf) administered by bronchial fibroscopy in adult ARDS patients due to COVID-19 are being conducted (NCT04502433 and NCT04384731).

2.2.2 AP301

Transepithelial ion transport is affected by alveolar epithelium injury, which impairs excess liquid removal from the alveolar space [22]. AP301 (Solnatide) is a synthetic peptide that has been proved to activate alveolar epithelium sodium channels [23].

In a phase 2a randomized controlled trial inhaled AP301 every 12 h for 7 days in patients with ARDS requiring mechanical ventilation decreased extravascular lung water and ventilation pressures over 7 days in patients with Sepsis related Organ Failure Assessment (SOFA) scores $\geq$11 [24, 25]. Currently, there is a phase 2b randomized controlled dose-escalation study to determine the safety of multiple ascending doses (5 mg, 60 mg, 125 mg) inhaled every 12 h through 7 days in patients with moderate-to-severe ARDS (NCT03567577) [26].

2.2.3 Keratinocyte Growth Factor

Keratinocyte Growth Factor (KGF) is an epithelial growth factor that induces ATII cells proliferation and promotes migration

and regeneration of the alveolar epithelium. Because of its action on ATII cells, KFG also maintains ionic transport and surfactant functions of ATII cells [27]. In preclinical models of acute lung injury, KGF decreased infiltration of neutrophils in the alveoar space, edema, permeability and epithelial injury [28].

In the phase 2 of keratinocyte growth factor for the treatment of the ARDS (KARE) randomised clinical trial, intravenous palifermin, a recombinant human KGF, did not ameliorate physiological nor clinical outcomes in patients with ARDS. Although the study was not powered to assess ventilation and mortality, those were higher in patients that received palifermin [29]. Authors recommended not to use KGF to treat ARDS patients, however they also specified that the study was performed in a heterogeneous population regarding ARDS etiology, and that focus KGF therapy on an ARDS subphenotype might be a better option to determine KGF response.

2.3 Alveolar endothelium

The alveolar endothelium is exposed to higher oxygen tensions while maintaining low-pressure blood flow compared to the systemic vascular endothelium. When there is a damage, injured alveolar endothelium promotes the destruction of the vascular bed and the expression of proinflammatory, reactive oxygen species and recruitment molecules, together with enhanced procoagulant activity and clot formation [14].

2.3.1 Nitric Oxide Synthase

Citrulline is the substrate of nitric oxide synthase (NOS) and lower levels are linked to decreased functional gut mass [30]. A randomized phase 2 study of intravenous citrulline revealed no effectivity in ARDS patients with severe sepsis, although the completion of the study has not still been published (NCT01474863). Another randomized trial with dietary enterally L-citrulline administration in patients with COVID-19-ARDS has finished and the results have to be published (NCT04404426).

After the conversion of arginine into citrulline, the NOS produces the gas nitric oxide (NO). Inhaled NO has been demonstrated to improve oxygenation but does not reduce mortality and might be harmful in 14 randomized controlled trials in adults with ARDS [31].

NO activates soluble guanylate cyclase (sGC), which converts GTP into cGMP. Oxidative stress decreases the NO-sGC-cGMP pathway with sGC inactivation. The therapeutic use of sGC modulators is centered on ameliorations in alveolar and vascular development of premature neonatal lungs not properly developed [32]. In a chronic hypoxia-induced newborn rat model, the administration of BAY41-2272 (sGC-cGMP stimulator) or sildenafil (cGMP-specific phosphodiesterase 5 inhibitor) results in pulmonary vascular resistance, which is reduced when those treatments are combined [33]. Presently, in a phase 1 clinical study multiple doses (three times a day for a week) of BAY1211163 by inhalation are being administered in patients with ARDS, in order to determine the safest dose (NCT04609943).

2.3.2 Prostacyclin

Iloprost is a synthetic analogue of prostacyclin and its aerosolization results in selective pulmonary vasodilatation. A randomized phase 2 clinical trial with inhaled iloprost for 5 days in ARDS patients is being conducted (ThIlo) (NCT03111212) [34]. Concerning COVID-19, a phase 2 randomized controlled trials with inhaled epoprostenol in severe patients with COVID-19 (VPCOVID) (NCT04452669) was presently completed although results have not still been published, and a phase 2 randomized clinical trial with iloprost in COVID-19 patients (ILOCOVID) (NCT04445246) is being performed.

2.3.3 Anti-Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) increases lung vascular permeability [35]. In a preclinical model of increased permeability and pulmonary edema in mice, Bevacizumab (anti-VEGF) histological analysis revealed reduced edema fluid, decreased lung wet-to-dry ratio and bronchoalveolar lavage protein levels [36]. A phase 2 clinical trial with a single intravenous bevacizumab administration in patients with severe sepsis was withdrawn due to underfunding (NCT01314066). Nevertheless, in two cases of COVID-19 induced atypical pneumonia, Bevacizumab ameliorated patients outcome [37]. A phase 2 study with intravenous 500 mg Bevacizumab in patients with severe COVID-19 was just completed but results have not been published yet (NCT04275414).

2.3.4 Levosimendan

Levosimendan is a calcium sensitizer that opens adenosine triphosphate-dependent potassium channels with vasodilator effects [38]. A randomized phase 3 study with 0.5 mL/h of levosimendan in patients with ARDS is being conducted (NCT04020003). In a randomized controlled pilot study levosimendan ameliorates right ventricular performance and pulmonary vasodilator effect in septic patients with ARDS [39]. Secondary analysis of randomized controlled trials in septic patients reveal that the survival of the levosimendan group was lower [40].

3. Mucolytics

The respiratory tract contains secretions composed by mucin glycoproteins, but in patients with respiratory diseases the mucus presents a higher viscosity. N-acetylcysteine is an antioxidant derived from the amino acid cysteine and is the most widely recommended mucolytic. In a randomized clinical trial 150 mg/kg of N-acetylcysteine produced a significant difference in the consciousness of ARDS patients requiring mechanical ventilation [41]. A pilot study of intravenous N-Acetylcysteine in patients with mild-to-moderate COVID-19 did not prove benefit [42].

Neutrophil extracellular traps (NETs) and damage-associated molecular patterns (DAMPs) resulting from the inflammatory response contain extracellular DNA among other compounds [43]. Dornase alfa is a recombinant human Deoxyribonuclease (DNAse 1) commonly used in the treatment of cystic fibrosis. It acts as a mucolytic by cleaving

extracellular DNA, thereby facilitating airway clearance and reducing alveolar hyper-inflammation [44]. The terminated phase 3 COVIDornase study (NCT04355364) and another phase 2 study (NCT04402944) in recruitment stage propose inhaled Dornase alfa therapy for ventilated patients with COVID-19-related ARDS.

4. Bronchodilators

Beta-adrenergic agonists (β2 agonists) have a beneficial effect in alveolar fluid clearance and permeability. Salbutamol is a beta-adrenergic agonist. In a randomized controlled trial intravenous salbutamol for 7 days decreased extravascular lung water in patients with ARDS requiring mechanical ventilation [45]. However, in a randomized phase 2 trial intravenous salbutamol for up to 7 years was poorly tolerated and did not present benefit in patients with ARDS [46]. In another randomized phase 2 clinical trial, intravenous salbutamol early in the development of ARDS was not safe [47].

Nebulized bronchodilators have also been proposed. In randomized clinical trial nebulized albuterol did not improve clinical outcomes in patients with ARDS [48]. A clinical trial with nebulized Dornase Alfa co-administered with abuterol in patients with COVID-19 requiring mechanical ventilation has just been completed but results have not still been announced (NCT04387786). Also, there is an ongoing phase 1 study comparing nebulized lidocaine, salbutamol and beclomethasone plus salbutamol in patients with COVID-19-ARDS and non-invasive ventilation (NCT04979923).

5. Immunomodulation

5.1 Neuromuscular blockers

The neuromuscular transmission is blocked by neuromuscular blocking agents at the neuromuscular junction, in order to minimize volutrauma, ventilator-induced lung injury, and biotrauma [49].

In a multicenter randomized trial, the early administration of neuromuscular-blocking agent cisatracurium in patients with moderate to severe ARDS improved 90-day survival and the time off the ventilator [50]. However, in another clinical trial, and early and continuous infusion of cisatracurim did not decrease 90-day mortality in patients with moderate-to-severe ARDS [51]. Current evidence favors avoiding a continuous infusion of neuromuscular blockers in patients with mechanical ventilation but use a lighter sedation strategy, and for patients who need a deep sedation to facilitate lung protective ventilation or prone positioning, to infuse neuromuscular blockers for 48 h is a reasonable option [52].

5.2 Steroids

Steroids are powerful anti-inflammatory and anti-fibrotic drugs that may lead to high-risk infections due to the suppression they exert on the immune system.

Clinical trials suggest that steroid treatment in ARDS patients would be indicated at the onset of the pathology. Administered corticosteroids 72 h after ARDS diagnosis decreased lung damage and increased ventilator weaning [53]. A meta-analysis in ARDS patients concluded that low-dose corticosteroids in early ARDS significantly reduced mortality and the duration of mechanical ventilation, whereas high doses did not [54]. A different meta-analysis shows that steroid treatment improves mortality and promotes shorter ventilation periods [55]. In contrast, in patients with influenza pneumonia, the early use of steroid therapy is associated with increased mortality [56, 57]. Nonetheless, studies in patients with community-acquired pneumonia treated with corticosteroids showed a reduced risk of treatment failure [58], reduced mortality, hospital stay and need for mechanical ventilation [59].

Dexamethasone is one of the most clinically used steroids for treatment. In a phase 2/3 trial patients with moderate-to-severe ARDS requiring mechanical ventilation were intravenously administered with dexamethasone (20 mg for 5 days, then 10 mg for the next 5 days) and presented an increase in the number of ventilator-free days and reduced mortality [60].

In COVID-19 patients, treatment with dexamethasone (intravenous or oral, 6 mg/day for 10 days) resulted in a lower incidence of death in those patients requiring invasive mechanical ventilation compared to those not requiring ventilator support [61]. Some of the clinical trials now recruiting are the phase 4 REMED study (NCT04663555), which aims to test two different doses (6 mg *vs.* 20 mg) of intravenous dexamethasone in SARS-CoV-2-induced ARDS patients. Or a phase 3 study that aims to compare intravenous treatment with dexamethasone or methylprednisolone in COVID-19 patients with ARDS (NCT04499313).

Regarding hydrocortisone, in a trial, patients with ARDS-associated sepsis were treated with a dose of 50 mg every 6 h within 12 h of their ARDS diagnosis. The treated group showed improvements in pulmonary physiology, but not a decrease in mortality compared to the placebo group [62].

Another of the most investigated corticosteroids for future therapies is methylprednisolone. In 24 patients with severe ARDS methylprednisolone (2 mg/kg/day for 32 days) decreased in-hospital and ICU mortality [63]. In the first 72 h, patients with ARDS were treated with an infusion of methylprednisolone (1 mg/kg/day) for 28 days, and had decreased C-reactive protein, mechanical ventilation and mortality [64]. A phase 2 study proposed intrapleural administration of the steroid Solumedrol (methylprednisolone) versus conventional treatment with extracorporeal membrane oxygenation and intravenous steroid administration. Results are not yet available (NCT01423864).

The MINECRAFT phase 2 study, aims to study the efficacy of administering canrenone, a steroidal antimineralocorticoid, intravenously in moderate-to-severe ARDS patients due to SARS-CoV-2 infection (NCT04977960).

A recently explored field is the administration of inhaled steroids. Early treatment consisting of inhaled budesonide together with a beta-agonist in patients at risk of developing ARDS improved oxygenation [65]. Another study where nebulised budesonide was administered also improved oxygenation and reduced proinflammatory cytokines (Tumor necrosis factor-α (TNF-α), Interleukin (IL)-1β and IL-6) [66]. There is a phase 2 study in paediatric ARDS patients with inhaled budesonide (NCT04064684). In neonatal patients with severe ARDS requiring mechanical ventilation, intratracheal treat-

ment with budesonide and surfactant resulted in a decreased incidence of bronchial dysplasia or death and decreased inflammation [67]. In children on mechanical respiratory support, treatment with budesonide and surfactant did not improve survival or the development of bronchial dysplasia over the surfactant-treated group but decreased the need for mechanical ventilation [68].

5.3 Statins

Statins are β-Hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase inhibitors with immunomodulatory properties. A meta-analysis showed that treatment with statins prior to intensive care unit (ICU) admission or before a diagnosis of a specific pathology showed a decrease in 30-day mortality, but no association with in-hospital mortality [69].

The Hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in Acute lung injury to Reduce Pulmonary dysfunction (HARP-2) trial was a multicentre trial that sought to test simvastatin (80 mg/day) in ICU patients, 48 h after the onset of ARDS. Patients involved in the study could be divided into two different sub-phenotypes: hypo-inflammatory (65%) and hyper-inflammatory (35%), and only increased survival was found in patients who had a hyper-inflammatory sub-phenotype treated with simvastatin. This study highlighted the need to phenotype different types of ARDS patients [70]. In the Statins for Acutely Injured Lungs from Sepsis (SAILS) trial they were also able to identify different biological phenotypes but did not see phenotype-specific benefit from rosuvastatin treatment [71]. In recent years, clinical studies propose to investigate the role of statins in ARDS patients of different aetiologies, although those have been cancelled due to lack of enrolment or other causes.

5.4 Carbon monoxide

Carbon monoxide (CO) results from the catabolism of heme oxygenase within the body. Its anti-inflammatory and anti-apoptotic role has been described. CO down-regulates the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, thus preventing mitochondrial dysfunction, and protects against cellular oxidative stress in models of lung injury [72, 73]. In *in vivo* models that received lypopolysaccharide (LPS), 50 parts per million (ppm) inhaled CO restored arterial resistance and decreased NOS-2 expression, although no changes were seen in plasma levels of inflammatory cytokines [74]. In a nonhuman primate pneumonia model CO treatment (200 ppm of concentration for 60 minutes) reduced extravascular alveolar fluid [75].

In a phase 1 trial in patients with ARDS-induced sepsis, a low dose (100–200 ppm) of inhaled CO was found to be a well-tolerated and safe treatment during mechanical ventilation. A phase 2 trial is currently recruiting ARDS patients to be treated with inhaled carbon monoxide at 200 ppm (NCT03799874).

5.5 Mesenchymal Stromal Cells

Mesenchymal Stromal Cells (MSCs) have immunomodulatory properties and reparative effects on damaged tissue, presenting paracrine and cell-cell communication (see chapter Cell Therapies in ARDS). Their role as a treatment in ARDS depends on the microenvironment to which the cell therapy is exposed and what has caused the lung injury [76].

The phase 1/2 clinical trial MultiStem Therapy in ARDS (MUST-ARDS) evaluated the safety of intravenous 900 million bone marrow-derived multipotent adult progenitor cells administered within 96 h of the onset of moderate-to-severe ARDS patients requiring mechanical ventilation. Administration of the cells was well tolerated and tended to decrease the need for mechanical ventilation [77].

In the Human Mesenchymal Stromal Cells for ARDS (START) phase 2a trial, patients with moderate-to-severe ARDS requiring mechanical ventilation were given an intravenous dose of MSCs, which was safe but showed no improvement over the placebo-treated group. These findings have been attributed to the low viability of the administered cells [78]. The Mesenchymal Stromal Cells for ARDS (STAT) phase 2b trial, an extension currently recruiting, aims to test the safety and efficacy of 10 million MSCs/kg (NCT03818854).

The REALIST trial proposes to investigate whether a single infusion of MSCs (human umbilical cord-derived CD362 enriched MSCs) could help in the treatment of ARDS, a phase 1/2 study (NCT03042143).

Regarding COVID-19-induced ARDS, it has been shown that in 7 patients who received a transfusion of ACE2$^-$ MSCs, lung function and symptomatology improved two days after treatment, and inflammation was reduced by decreasing C reactive protein (CRP) and TNF-α [79].

5.6 Regulatory T-cells

Regulatory T-cells (Treg cells) act on the immune system by decreasing its activation and promoting homeostasis. Overexpression of Transforming Growth Factor (TGF) β1, the most secreted cytokine by Treg cells, in a murine model of acute lung injury (ALI) induces more Treg cells and decreases T helper 17 cells (Th17) cells, improving lung inflammation [80].

Several clinical trials are currently ongoing in COVID-19-ARDS patients proposing intravenous administration of Treg cells (NCT05027815 and NCT04468971), and a study in COVID-19-ARDS patients receiving intravenous Treg/Th2 hybrid cells has just been terminated, although results are not posted yet (NCT04482699).

5.7 Vitamin C

Vitamin C is an antioxidant molecule with protective effects. In one study, vitamin C levels were found to be undetectable in more than 90% of patients with SARS-CoV-2-associated ARDS [81]. In the Vitamin C in patients with Sepsis and Severe Acute Respiratory Failure (CITRIS-ALI) phase 2 trial, patients with sepsis and consequent ARDS were treated with a 96h-infusion of vitamin C. There was no difference between the vitamin C-treated group and the control group in terms of decreased inflammation, but secondary outcomes showed a decrease in 28-day mortality in the treated group [82].

Completed but unpublished clinical studies include COVID-19 patients with ARDS treated with vitamin C and other antiox-

idants (NCT04570254), and ascorbic acid (NCT04710329). Also, there is a phase 3 study in septic patients with ARDS that proposes to compare the effect of high-dose intravenous vitamin C, but is not yet enrolling patients (NCT04404387).

5.8 Ulinastatin

Ulinastatin, a glycoprotein known as urinary trypsin inhibitor, is an experimental drug with anti-inflammatory properties. A clinical study of 14 consecutive days of treatment with ulinastatin in ARDS patients requiring mechanical ventilation resulted in decreased TNF-α, IL-6 and CRP levels, increased antioxidant capacity, decreased ventilatory need and hospital-stay days [83].

5.9 Inhibitors

5.9.1 p38

The p38 mitogen-activated protein kinases (p38MAPK) are intracellular signals that play a crucial role in igniting inflammation through the release of proinflammatory cytokines such as IL-6, IL-1β and TNF-α [84].

In patients at risk of developing ARDS, a phase 2 study using dilmapimod, a specific inhibitor of p38MAPK, was shown to be well tolerated, with the highest dose (10 mg) administered as a continuous infusion over 24 h having the most favourable profiles and decreasing IL-6 and CRP [84].

The hyper-inflammatory response that occurs in SARS-CoV-2 infection may be caused by up-regulation of p38MAPK activity [85]. SARS-CoV-2 has previously been shown to act on the p38MAPK pathway, promoting inflammation, vasoconstriction and thrombosis and in turn favouring the continuation of the viral cycle. A preclinical study in which a p38 inhibitor was administered to SARS-CoV-infected mice showed an 80% survival rate in the treated group [86]. Among the proposed inhibitors, losmapimod is one of the most clinically studied inhibitors [85].

5.9.2 Tumor Necrosis Factor Receptor 1

Another pathway antagonised has been the TNF-α pathway, mainly by an anti-TNF-1 receptor (TNFR1) antibody that selectively binds to the TNFR1.

TNFR1 and TNFR2 levels are elevated in patients with critical COVID-19. In addition, markers of monocyte activation such as soluble cluster of differentiation 14 (sCD14) have been found to be directly correlated with TNFR1, suggesting an association with severe disease, and might be predictive for mortality in critically ill patients. The TNF/TNFR signalling pathway is an interesting target to improve survival in COVID-19 critical patients [87]. In healthy humans previously administered LPS, anti-TNFR1 treatment resulted in decreased inflammatory response, endothelial damage, and neutrophil infiltration into the lung [88].

5.9.3 Interleukin-6

IL-6 is secreted by T cells contributing to inflammation [89], which ends up in an increased ARDS pathophysiology. The administration of IL-6 blockers tocilizumab and sarilumab proved benefit in patients with ARDS [90]. Phase 2/3 clinical trials of intravenous tocilizumab in COVID-19-ARDS patients have been completed, but results have not still been published (NCT04445272), and other clinical trials are recruiting (NCT04412772, NCT05082714).

5.9.4 Interferons

Interferons comprise a set of molecules with different functions that may have opposing roles in ARDS. Interferon-γ (IFNγ) is notably involved in viral infections, being highly proinflammatory. In COVID-19 patients who develop ARDS, treatment with anti-IFNγ could be a potential treatment, since IFNγ has been observed to upregulate ACE2 expression in the lung epithelium, a receptor used by SARS-CoV-2 for cell entry [91].

On the contrary, interferon β-1α has anti-inflammatory, anti-fibrotic and antiviral properties. In a phase 2 trial Interferon β-1α (SNG001), nebulised inhaled interferon β-1α was administered to COVID-19 patients and proved a fast recovery from infection. It has also been recommended to test interferon β-1α in ventilated and critically ill patients [92]. In contrast, in a phase 3 study in patients diagnosed with moderate-to-severe ARDS, intravenous administration of FP-1201 (a recombinant human interferon β-1α), showed no improvement compared to placebo administration [93].

5.9.5 Imatinib

Imatinib, a tyrosine kinase inhibitor, attenuates oxidative damage by acting on lung endothelial catalase. Imatinib has been shown to attenuate ALI in preclinical double hit models (LPS- and ventilator-induced lung injury or VILI) [94] and to decrease mortality in models where intravenous LPS was administered [95].

In a phase 1 study, healthy individuals were treated orally with imatinib and then given inhaled LPS (NCT03328117). The results have not yet been published.

In silico studies have proposed imatinib as promising therapy for SARS-CoV-2 infection [96]. There is currently a phase 3 study enrolling hospitalised COVID-19 patients, which aims to evaluate the efficacy and safety of oral administration of imatinib [97].

5.9.6 NLRP-3 Inhibitors

The multiprotein cytosolic complex composed of NLRP3 oligomerization forms an inflammasome that causes the release of proinflammatory cytokines such as IL-1β and IL-18 through a dependent-caspase-1 mechanism.

In *in vitro* studies, the NLRP3 inflammasome inhibitor pirfenidone inhibited NLRP action by suppressing reactive oxygen species (ROS) generation. Furthermore, in a murine model instilled intratracheally with LPS, oral administration of pirfenidone mitigated lung inflammation and fibrosis [98].

Nowadays, there is a phase 3 study where pirfenidone is administered orally to COVID-19 patients (NCT04282902) and another in COVID-19 patients with severe ARDS, where it is administered through a nasogastric tube (NCT04653831).

Tetracycline, another NLRP3 inflammasome inhibitor, administered intraperitoneally in murine models has reduced mortality, lung injury and IL-1β concentration compared to those treated with phosphate-buffered saline (PBS) [99]. A

clinical trial is recruiting ARDS patients to evaluate the inhibition of human leukocyte immune response treated with tetracycline (NCT04079426).

A recent study in a murine ALI model has shown that intraperitoneal administration of erythropoietin (EPO) suppresses the NLRP3 inflammasome by inhibiting the Nuclear Factor kappa B (NF-κB) cellular pathway and consequently decreasing lung damage [100]. A phase 2 trial is studying a therapy with Vadadustat, a Hypoxia inducible factor prolyl-hydroxylase inhibitor drug that increases endogenous EPO production, in hospitalised COVID-19 patients with ARDS (NCT04478071).

5.9.7 Granulocyte-macrophage colony-stimulating factor

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is an immunomodulatory cytokine that has an important role in inflammation but has also been described to be crucial in antimicrobial defence in the lung and in surfactant homeostasis [101, 102]. More research is needed to define the role of cytokine in the course of ARDS.

In animal models, administration of GM-CSF proved benefit to the epithelium, restoring tissue homeostasis and barrier function, and limiting hyperoxic lung injury [103, 104]. In a randomised phase 2 trial of patients with ARDS GM-CSF infusion did not increase the number of ventilator-free days nor reduce mortality [105].

Inhaled GM-CSF administration might have improvements in the severity of ARDS [106]. A phase 2 study in patients with ARDS-associated pneumonia tested inhaled administration of a recombinant human GM-CSF, with no available results yet (NCT02595060). Now, the phase 2 GI-COVID study (NCT04569877) is recruiting COVID-19 patients to administer a nebulised solution of molgramostim (a human recombinant GM-CSF).

In contrast, GM-CSF polarises myeloid cells towards a proinflammatory phenotype and therefore it has been proposed to block its signalling. Due to the hyper-inflammatory situation in SARS-CoV-2 infection, clinical studies with anti-GM-CSF antibodies have been conducted in these patients. A phase 2 study with Otilimab (NCT04376684) and a phase 3 study with Lenzilumab (NCT04351152) have been carried out, although no results have been published yet. Others trials that propose anti-GM-CSF as a therapy for COVID-19 patients are recruiting right now (NCT04341116, NCT04400929). The Mavrilimumab in patients with severe COVID-19 pneumonia and systemic hyperinflammation (MASH-COVID) study showed that intravenous administration of mavrilimumab (anti-GM-CSF) showed no significant difference in survival compared to placebo [107]. The realisation of randomised trials will be essential to define the therapeutic effect of GM-CSF blockade in ARDS and COVID-19 [108].

5.9.8 Neutrophil-proteases inhibitors

In ARDS, neutrophils can promote cell damage through oxidative stress, the release of NETs and secretion of proteases [109].

Silvelestat is an inhibitor of neutrophil elastase, which has proteolytic activity and induces the production of inflammatory cytokines [110, 111]. The use of Silvelestat has been shown not to affect the bactericidal capabilities of neutrophils [112]. After several preclinical studies in which it has been shown to reduce mortality and parameters such as vascular permeability and inflammation [113–115], clinical studies aim to determine its protective role in ARDS. Favourable results have been observed in patients with mild ARDS [116], while another study showed no effect on 28–30 days mortality and ICU stays [117]. A phase 3 study in ARDS patients with sepsis (NCT04973670) and a phase 4 multicenter clinical trial in ARDS patients with systemic inflammatory response syndrome (NCT04909697) are ongoing.

Elafin is an endogenous protease inhibitor. The imbalance between neutrophil elastase and elafin is associated with mortality in ARDS [118]. In an LPS-induced mouse model of ALI, instillation of a cleavage-resistant variant of elafin (GG-elafin) was able to decrease neutrophil-induced inflammation as well as decrease protease activity compared to wild-type elafin [119].

6. Anticoagulants and Fibrinolytics

A major hallmark of ARDS is deregulated coagulation and fibrinolysis, leading to pulmonary coagulopathy in ARDS and systemically altered coagulation in septic patients [120, 121]. Coagulation and inflammation play an essential role in ARDS. Given the close interactions between these systems [122], anticoagulants might act on ARDS pathophysiology because of their anticoagulant and anti-inflammatory activities.

6.1 Tissue Factor

Tissue Factor (TF) is a transmembrane protein that is the major initiator of the extrinsic coagulation pathway when activated by the binding of factor VIIa. ALT-836 is an anti-TF antibody that blocks the binding of factor VIIa, and a single intravenous dose of ALT-836 (0.06, 0.08, 0.1 mg/kg) has proved to be safe in a randomized controlled phase 1 trial in patients with ARDS requiring mechanical ventilation [123]. A randomized phase 2 clinical trial in patients with sepsis and ARDS receiving a single (0.06 mg/kg) intravenous dose up to four doses has already been performed, although results have not been published yet (NCT00879606).

Tissue Factor Pathway Inhibitor (TFPI) modulates the initiation of the extrinsic coagulation pathway. A randomized controlled phase 3 clinical trial of intravenous tifacogin (recombinant TFPI) administration during 96 h in patients with severe sepsis did not reduce mortality and was associated with increased bleeding [124]. However, in a randomized controlled phase 3 clinical trial intravenous tifacogin administration during 96 h did not decrease mortality but reduced prothrombin fragment and thrombin antithrombin complexes levels in patients with severe community-acquired pneumonia [125].

6.2 Antithrombin

Antithrombin is a serine protease inhibitor synthesized in the liver [126, 127], and is known to inhibit procoagulant enzymes including thrombin, factor Xa, IXa, XIa and XIIa. When

heparin binds to antithrombin, its inhibitory activity is 1000-fold increased [127].

A randomized phase 3 clinical trial with intravenous 30,000 IU of antithrombin within 4 days had no effect on mortality in patients with severe sepsis (the KyberSept Trial), although an increased risk of hemorrage was detected when administering antithrombin and heparin together [128]. A post hoc analysis in patients with severe sepsis treated in a single center early after onset revealed increased bleeding due to antithrombin [129].

Nebulized antithrombin alone or combined with heparin attenuated lung injury in HCl/LPS-induced ALI in rats, reducing pulmonary coagulation and inflammation without altering systemic coagulation nor bleeding [130].

6.3 Heparin

Heparin is a natural anticoagulant produced by mast cells in the intestine or lungs, basophils in the blood and endothelial cells [131]. Heparin presents anticoagulant actions potentiating antithrombin inhibitory activity and enhancing TFPI, and anti-inflammatory actions both related or not to thrombin inhibition [132].

Controversial results have been determined in patients with ARDS while administering local heparin. In a phase 1 trial nebulized heparin (50,000 IU/day, 100,000 IU/day, 200,000 IU/day, 400,000 IU/day) did not produce adverse effects and attenuated pulmonary coagulopathy in patients with ARDS requiring mechanical ventilation [133, 134], and in a randomized phase 2 study nebulized heparin (25,000 IU) reduced days of mechanical ventilation in patients with ARDS [135]. Also, in a randomized phase 3 clinical trial (CHARLI) nebulized heparin (250,000 IU) every 6 h to day 10 was well tolerated with decreased lung injury progression and earlier return at home in patients with invasive ventilation [136]. In contrast, in a randomized controlled trial with nebulized heparin focused on the safety and efficacy of burn patients with inhalation trauma (HEPBURN), the trial was stopped because of increased systemic clotting times and adverse events [137].

Nebulized heparin has also been proposed for COVID-19 patients. A randomized phase 2/3 clinical trial with nebulized 25,000 IU of heparin every 6 h for up to 10 days in patients with COVID-19 requiring mechanical ventilation is being performed (NCT04545541).

6.4 Thrombomodulin

The Protein C Pathway also has a major role in coagulation and fibrinolysis regulation. Thrombomodulin is a thrombin receptor, and, when the complex is formed, protein C is cleaved and activated protein C is produced.

ART-123 is a recombinant human soluble thrombomodulin. In a randomized controlled phase 2b study intravenously administered ART-123 for 6 days proved to be safe and effective reducing prothrombin fragment and thrombin-antithrombin complex concentrations in patients with sepsis-associated disseminated intravascular coagulation [138]. In addition, in a retrospective study intravenously combined sivelestat and recombinant human soluble thrombomodulin improved 60-day survival and ventilator-free days in patients with ARDS and disseminated intravascular coagulation [139].

In the full analysis of the phase 3 multinational Scarlet study that evaluate the efficacy and safety of intravenous ART-123 during 6 days to treat sepsis-associated coagulopathy, no statistically differences were determined [140]. However, in post hoc analysis in patients with sepsis-associated coagulopathy that did not receive concomitant heparin, ART-123 proved more benefit, indicating that heparin administration could impact ART-123 efficacy, a fact that should be confirmed in further studies [141].

6.5 Activated Protein C

Alveolar epithelial cells release thrombomodulin from the cell surface, due to a metalloproteolytic process, and this decreases the ability of these cells to activate Protein C [120, 121].

Inhaled drotrecogin alfa (recombinant human activated Protein C) in patients with ARDS decreased coagulation, neutrophils recruitment and inflammation in the alveolar compartment and increased fibrinolysis without producing systemic effects [142]. However, in a randomized multicentre phase 3 Prowess-Shock trial intravenous drotrecogin alfa (recombinant human activated Protein C) in 1967 patients with septic shock did not reduce mortality [143], and no further studies have been performed becaause Activated Protein C was removed from the market.

6.6 Streptokinase

Plasminogen activator and inhibitor pathway regulate fibrin deposition. Streptokinase binds plasminogen and drives the conversion of plasminogen to plasmin, a fibrinolytic enzyme. In a randomized phase 3 trial nebulized streptokinase in patients with severe ARDS improved oxygenation and lung mechanics [144].

7. Aspirin

Coagulation cascade activation leads to increased platelet recruitment and thrombin formation in the lung. Aspirin is a non-selective inhibitor of the cyclooxygenase pathway, with reduced platelet recruitment, fibrinolytic and decreased inflammatory effect. In a randomized controlled phase 2 trial aspirin did not decrease the risk of ARDS [145]. Also, a randomized phase 2 clinical trial with enterally 75 mg aspirin administration in patients with ARDS requiring mechanical ventilation is terminated (STAR), although results have not been announced (NCT02326350).

8. Others

8.1 Alpha-1 antitrypsin

Alpha-1 antitrypsin is a serine protease inhibitor that has been found to ameliorate oxygenation [146]. A randomized phase 2 clinical trial with intravenous prolastin (plasma-purified alpha-1 antitrypsin) in patients with COVID-19 ARDS is presently being conducted [147].

8.2 Transient Receptor Potential Vanilloid 4 inhibitor

The mechanosensitive cation calcium channel Transient Receptor Potential Vanilloid 4 inhibitor (TRPV4) is an essential homeostasis regulator that is implicated in ARDS inflammation [148]. TRPV4 can induce alveolar endothelial and epithelial dysfunction, which results in increased permeability and edema [149]. Nevertheless, in a preclinical model with intratracheal *Pseudomonas aeruginosa* in mice, TRPV4 activity has demonstrated to enhance macrophages phagocytosis and decrease inflammation [150]. Various TRPV4 inhibitors have proved to decrease acute lung injury in preclinical models. In mice exposed to hydrochloric acid or chlorine gas, TRPV4 inhibitor reduced inflammation and vascular leakage [151]. In a first clinical study, TRPV4 inhibitor did not produce ameliorations in healthy patients receiving inhaled LPS (NCT03511105).

8.3 Matrix metalloproteinase 12 inhibitor

FP-025 inhibits matrix metalloproteinase-12 (MMP12), an enzyme that degrades and remodels the extracellular matrix but is also known to modulate the influx of monocytes and macrophages in the alveolar compartment [152]. There is an ongoing randomized phase 2/3 clinical study with FP-025 (100 or 300 mg) in patients with severe and critical COVID-19-ARDS (NCT04750278).

8.4 Sevoflurane

Sedation with the volatile anesthetic sevoflurane-induced anti-inflammatory processes in ventilated patients [153]. In a randomized controlled phase 3 clinical trial volatile or intravenous sedation with sevoflurane for 48 h has been administered in patients with COVID-19-ARDS, although results have not been published yet (NCT04355962). Another randomized phase 3 trial to determine the effects of inhaled sevoflurane sedation on extravascular lung water and pulmonary vascular permeability in ARDS patients is proposed, although the recruitment has not started (NCT04530188).

9. Preclinical therapies for ARDS

9.1 Adenosine A2A receptor agonists

The nucleoside adenosine has anti-inflammatory properties, and its deficiency has been shown to increase pulmonary oedema and inflammation in a murine model of VILI [154].

Pharmacological intervention with the adenosine A2A receptor agonist CGS-21680 in rat VILI models reduced pulmonary edema, respiratory elastance and neutrophil recruitment into the lung compared to vehicle-treated animals [155]. In different models of ALI induced by HCl, LPS or *Escherichia coli*, instillation of the agonist GW328267C led to alveolar fluid clearance [156].

Blockade of equilibrative nucleoside transporters (ENTs) with dipyridamole increases adenosine in the alveolus and decreases pulmonary edema and improves gas exchange during ALI [157, 158].

9.2 Protease-activated receptor 1

Coagulation activates inflammation through protease-activated receptors (PARs) [159]. PAR1 is expressed in epithelial lung cells and the endothelium and is associated with a prothrombotic state [160, 161]. Thrombin binds to PAR1 and stimulates neutrophil recruitment and the release of proinflammatory cytokines [162]. Nevertheless, in antigen-presenting cells, PAR1 activation decreases the production of proinflammatory cytokines [163].

In influenza virus infection in mice, after activating PAR1 receptors with an agonist, they found increased lung inflammation but did not affect survival. They also observed that activated PAR1 increased the conversion of plasminogen to plasmin [164]. PAR1 antagonists are only in clinical trials for other pathologies.

9.3 Receptor for advanced glycation end-products inhibitors

The soluble receptor for advanced glycation end-products (sRAGE) is a marker of epithelial damage, especially in ATI, and is a prognostic marker for ARDS [165, 166].

Blockade of RAGE, using anti-RAGE antibody or sRAGE decoy receptor in acid-induced mice model of ALI reduced RAGE mRNA levels in the lung, restored alveolar-capillary barrier permeability after injury, decreased the total number of leukocytes in bronchoalveolar lavage (BAL) and restored membrane aquaporin-5 expression [167]. In piglets, blockade of RAGE decreased alveolar inflammation and induced alveolar fluid clearance [168]. A recent study in LPS-induced ALI murine model has shown that RAGE signalling mediates epithelial barrier dysfunction, enhancing lung inflammation and causing loss of adherent junctions [169].

9.4 Haptoglobin

Haptoglobin acts as a scavenger receptor for cell-free haemoglobin (CFH), which is elevated during sepsis and correlated with increased mortality. Haptoglobin decreases CFH levels and iron levels, leading to less oxidative damage to the lung in sepsis, but it has not been shown to reduce inflammation [170]. In a transgenic mouse overexpressing haptoglobin in alveolar macrophages, CFH clearance and decreased lung injury were observed, suggesting that haemoglobin catabolism is linked to iron mobilisation in macrophages [171].

9.5 Lipoxin A4

Lipoxin A4 (LXA4) is derived from arachidonic acid that promotes alveolar epithelial wound repair and the proliferation and differentiation of ATII cells into ATI cells [172]. *In vivo*, LPS-induced ALI model animals resulted in decreased levels of TNF-α and IL-1β, inhibition of neutrophil recruitment to the lung, inhibition of ATII cell apoptosis and epithelial-mesenchymal transition [173, 174].

Resolvin D1 is a specialised pro-resolving mediator that acts by stimulating the lipoxin A4 receptor on immune cells, reducing ROS generation, blocking nuclear factor kappa-light-chain-enhancer of activated B cells (NF-$\kappa\beta$) activation and

accelerating the production of antioxidant proteins. In a mouse model, resolvin D1 treatment reduces leukocyte infiltration and inflammatory cytokine release [175, 176]. In a rat ALI model, resolvin administration attenuated LPS-induced ALI and promoted alveolar fluid clearance by increasing the expression of sodium and Na, K-ATPase channels [177].

10. Conclusion

Management of patients with ARDS has substantially progressed, although this syndrome still remains relatively common, with high associated morbidity, mortality and persisting sequelae on survivors. All these expose the need for effective pharmacological therapies.

Although various treatments have failed when being translated to ARDS patients, other therapies are ongoing and proved efficacy in preclinical and clinical studies. However, there are different factors that should be taken into account for future research, in order to maximise patient treatment response.

Because of ARDS complex pathophysiology, to classify this heterogenic syndrome in identified patient subsets or phenotypes based on clinical, physiologic, radiologic and biologic criteria might result in a more feasible patient response, leading to personalized therapy [178]. In addition, time, dose and pathway administration of treatment are critical. Also, taking into account the complexity of the disease, a unique or combined therapy might encompass various pathways and mechanisms involved in the pathophysiology. Of no less importance, we should be aware that preclinical models reproduce human ARDS only in part, fact that could affect the relevance of the data [179].

Promising therapies for ARDS are underway. Increased knowledge on involved pathways and mechanism of ARDS pathophysiology and the identification of ARDS patient subsets will contribute on the development of effective therapies.

AUTHOR CONTRIBUTIONS

ECD and MCR contributed in the design of the review, searched the literature, studied and interpreted the data and wrote the manuscript. Both authors approved the last version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, *et al*. Acute respiratory distress syndrome. Nature Reviews Disease Primers. 2019; 5: 18.

[2] Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, *et al*. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Internal Medicine. 2020; 180: 934–943.

[3] Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, *et al*. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. JAMA. 2016; 315: 788.

[4] Villar J, Blanco J, Añón JM, Santos-Bouza A, Blanch L, Ambrós A, *et al*. The ALIEN study: incidence and outcome of acute respiratory distress syndrome in the era of lung protective ventilation. Intensive Care Medicine. 2011; 37: 1932–1941.

[5] Herridge MS, Tansey CM, Matté A, Tomlinson G, Diaz-Granados N, Cooper A, *et al*. Functional disability 5 years after acute respiratory distress syndrome. New England Journal of Medicine. 2011; 364: 1293–1304.

[6] Gonzales JN, Lucas R, Verin AD. The acute respiratory distress syndrome: mechanisms and perspective therapeutic approaches. Austin Journal of Vascular Medicine. 2015; 2: 1009.

[7] Ware LB, Matthay MA. The acute respiratory distress syndrome. New England Journal of Medicine. 2000; 342: 1334–1349.

[8] Millar FR, Summers C, Griffiths MJ, Toshner MR, Proudfoot AG. The pulmonary endothelium in acute respiratory distress syndrome: insights and therapeutic opportunities. Thorax. 2016; 71: 462–473.

[9] Hrenak J, Simko F. Renin-angiotensin system: an important player in the pathogenesis of acute respiratory distress syndrome. International Journal of Molecular Science. 2020; 21: 8038.

[10] Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, *et al*. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. Critical Care. 2017; 21: 234.

[11] Cross LJM, Matthay MA. Biomarkers in acute lung injury: insights into the pathogenesis of acute lung injury. Critical Care Clinics. 2011; 27: 355–377.

[12] Wong MH, Johnson MD. Differential response of primary alveolar type I and type II cells to LPS stimulation. PLoS One. 2013; 8: e55545.

[13] Lopez-Rodriguez E, Gay-Jordi G, Mucci A, Lachmann N, Serrano-Mollar A. Lung surfactant metabolism: early in life, early in disease and target in cell therapy. Cell and Tissue Research. 2017; 367: 721–735.

[14] Ware L. Pathophysiology of acute lung injury and the acute respiratory distress syndrome. Seminars in Respiratory and Critical Care Medicine. 2006; 27: 337–349.

[15] Lewis JF, Jobe AH. Surfactant and the adult respiratory distress syndrome. American Review of Respiratory Disease. 1993; 147: 218–233.

[16] Rodriguez-Fanjul J, Jordan I, Balaguer M, Batista-Muñoz A, Ramon M, Bobillo-Perez S. Early surfactant replacement guided by lung ultrasound in preterm newborns with RDS: the ULTRASURF randomised controlled trial. European Journal of Pediatrics. 2020; 179: 1913–1920.

[17] Anzueto A, Baughman RP, Guntupalli KK, Weg JG, Wiedemann HP, Raventós AA, *et al*. Aerosolized surfactant in adults with sepsis-induced acute respiratory distress syndrome. New England Journal of Medicine. 1996; 334: 1417–1422.

[18] Spragg RG, Lewis JF, Walmrath H, Johannigman J, Bellingan G, Laterre P, *et al*. Effect of recombinant surfactant protein c-based surfactant on the acute respiratory distress syndrome. New England Journal of Medicine. 2004; 351: 884–892.

[19] Meng H, Sun Y, Lu J, Fu S, Meng Z, Scott M, *et al*. Exogenous surfactant may improve oxygenation but not mortality in adult patients with acute lung injury/acute respiratory distress syndrome: a meta-analysis of 9 clinical trials. Journal of Cardiothoracic and Vascular Anesthesia. 2012;

26: 849–856.

[20] Taut FJH, Rippin G, Schenk P, Findlay G, Wurst W, Häfner D, *et al*. A search for subgroups of patients with ARDS who may benefit from surfactant replacement therapy. Chest. 2008; 134: 724–732.

[21] Piva S, DiBlasi RM, Slee AE, Jobe AH, Roccaro AM, Filippini M, *et al*. Surfactant therapy for COVID-19 related ARDS: a retrospective case–control pilot study. Respiratory Research. 2021; 22: 20.

[22] Ware L, Matthay M. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. 2001; 163: 1376–1383.

[23] Shabbir W, Scherbaum-Hazemi P, Tzotzos S, Fischer B, Fischer H, Pietschmann H, *et al*. Mechanism of action of novel lung edema therapeutic AP301 by activation of the epithelial sodium channel. Molecular Pharmacology. 2013; 84: 899–910.

[24] Krenn K, Croize A, Klein KU. Oral inhalation of AP301 peptide activates pulmonary oedema clearance: initial results from a phase IIa clinical trial in mechanically ventilated ICU patients. European Respiratory Journal. 2014; 44: 1386.

[25] Krenn K, Lucas R, Croizé A, Boehme S, Klein KU, Hermann R, *et al*. Inhaled AP301 for treatment of pulmonary edema in mechanically ventilated patients with acute respiratory distress syndrome: a phase IIa randomized placebo-controlled trial. Critical Care. 2017; 21: 194.

[26] Schmid B, Kredel M, Ullrich R, Krenn K, Lucas R, Markstaller K, *et al*. Safety and preliminary efficacy of sequential multiple ascending doses of solnatide to treat pulmonary permeability edema in patients with moderate-to-severe ARDS—a randomized, placebo-controlled, double-blind trial. Trials. 2021; 22: 643.

[27] Ware LB, Matthay MA. Keratinocyte and hepatocyte growth factors in the lung: roles in lung development, inflammation, and repair. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2002; 282: L924–L940.

[28] Franco-Montoya M, Bourbon JR, Durrmeyer X, Lorotte S, Jarreau P, Delacourt C. Pulmonary effects of keratinocyte growth factor in newborn rats exposed to hyperoxia. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2009; 297: L965–L976.

[29] McAuley DF, Cross LM, Hamid U, Gardner E, Elborn JS, Cullen KM, *et al*. Keratinocyte growth factor for the treatment of the acute respiratory distress syndrome (KARE): a randomised, double-blind, placebo-controlled phase 2 trial. The Lancet Respiratory Medicine. 2017; 5: 484–491.

[30] Cynober L. Citrulline: just a biomarker or a conditionally essential amino acid and a pharmaconutrient in critically ill patients? Critical Care. 2013; 17: 122.

[31] Afshari A, Brok J, Møller AM, Wetterslev J. Inhaled nitric oxide for acute respiratory distress syndrome and acute lung injury in adults and children. Anesthesia & Analgesia. 2011; 112: 1411–1421.

[32] Wagenaar GTM, Hiemstra PS, Gosens R. Therapeutic potential of soluble guanylate cyclase modulators in neonatal chronic lung disease. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2015; 309: L1037–L1040.

[33] Enomoto M, Jain A, Pan J, Shifrin Y, Van Vliet T, McNamara PJ, *et al*. Newborn rat response to single *vs*. combined cGMP-dependent pulmonary vasodilators. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2014; 306: L207–L215.

[34] Haeberle H, Prohaska S, Martus P, Straub A, Zarbock A, Marx G, *et al*. Therapeutic iloprost for the treatment of acute respiratory distress syndrome (ARDS) (the ThIlo trial): a prospective, randomized, multicenter phase II study. Trials. 2020; 21: 242.

[35] Ware LB. VEGF levels in the alveolar compartment do not distinguish between ARDS and hydrostatic pulmonary oedema. European Respiratory Journal. 2005; 26: 101–105.

[36] Watanabe M, Boyer JL, Crystal RG. Genetic delivery of bevacizumab to suppress vascular endothelial growth factor-induced high-permeability pulmonary edema. Human Gene Therapy. 2009; 20: 598–610.

[37] Patel G, Pielykh D, Patel SM, Patel MJ, Bhavsar K, Koritala T. COVID-19 coronavirus-induced atypical pneumonia: efficacy of the monoclonal antibody bevacizumab in moderate to severe cases. Cureus. 2021; 13: e18317.

[38] Häberle HA. Levosimendan—a 20-year experience. Anasthesiol Intensivmed Notfallmed Schmerzther. 2021; 56: 414–426. (In German)

[39] Morelli A, Teboul J, Maggiore SM, Vieillard-Baron A, Rocco M, Conti G, *et al*. Effects of levosimendan on right ventricular afterload in patients with acute respiratory distress syndrome: a pilot study. Critical Care Medicine. 2006; 34: 2287–2293.

[40] Shankar-Hari M, Santhakumaran S, Prevost AT, Ward JK, Marshall T, Bradley C, *et al*. Defining phenotypes and treatment effect heterogeneity to inform acute respiratory distress syndrome and sepsis trials: secondary analyses of three RCTs. NIHR Journals Library: Southampton. 2021.

[41] Ghorbi M, Rashidi M, Olapour A, Javaherforooshzadeh F, Akhondzadeh R. Effect of N-acetylcysteine on the treatment of acute respiratory distress syndrome in mechanically ventilated patients admitted to the intensive care unit. Medical Journal of the Islamic Republic of Iran. 2021; 35: 87.

[42] Taher A, Lashgari M, Sedighi L, Rahimi-bashar F, Poorolajal J, Mehrpooya M. A pilot study on intravenous N-acetylcysteine treatment in patients with mild-to-moderate COVID19-associated acute respiratory distress syndrome. Pharmacological Reports. 2021; 73: 1650–1659.

[43] Denning NL, Aziz M, Gurien SD, Wang P. DAMPs and NETs in sepsis. Frontiers in Immunology. 2019; 10: 2536.

[44] Paul K, Rietschel E, Ballmann M, Griese M, Worlitzsch D, Shute J, *et al*. Effect of treatment with dornase alpha on airway inflammation in patients with cystic fibrosis. American Journal of Respiratory and Critical Care Medicine. 2004; 169: 719–725.

[45] Perkins GD, McAuley DF, Thickett DR, Gao F. The beta-agonist lung injury trial (BALTI): a randomized placebo-controlled clinical trial: a randomized placebo-controlled clinical trial. American Journal of Respiratory and Critical Care Medicine. 2006; 173: 281–287.

[46] Gates S, Perkins GD, Lamb SE, Kelly C, Thickett DR, Young JD, *et al*. Beta-agonist lung injury TrIal-2 (BALTI-2): a multicentre, randomised, double-blind, placebo-controlled trial and economic evaluation of intravenous infusion of salbutamol versus placebo in patients with acute respiratory distress syndrome. Health Technology Assessment. 2013; 17: v–vi, 1–87.

[47] Smith FG, Perkins GD, Gates S, Young D, McAuley DF, Tunnicliffe W, *et al*. Effect of intravenous β-2 agonist treatment on clinical outcomes in acute respiratory distress syndrome (BALTI-2): a multicentre, randomised controlled trial. The Lancet. 2012; 379: 229–235.

[48] National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network, Matthay MA, Brower RG, Carson S, Douglas IS, Eisner M, *et al*. Randomized, placebo-controlled clinical trial of an aerosolized β_2-agonist for treatment of acute lung injury. American Journal of Respiratory and Critical Care Medicine. 2011; 184: 561–568.

[49] Hraiech S, Yoshida T, Annane D, Duggal A, Fanelli V, Gacouin A, *et al*. Myorelaxants in ARDS patients. Intensive Care Medicine. 2020; 46: 2357–2372.

[50] Papazian L, Forel J, Gacouin A, Penot-Ragon C, Perrin G, Loundou A, *et al*. Neuromuscular blockers in early acute respiratory distress syndrome. New England Journal of Medicine. 2010; 363: 1107–1116.

[51] Moss M, Huang DT, Brower RG, Ferguson ND, Ginde AA, Gong MN, *et al*. Early neuromuscular blockade in the acute respiratory distress syndrome. New England Journal of Medicine. 2019; 380: 1997–2008.

[52] Alhazzani W, Belley-Cote E, Møller MH, Angus DC, Papazian L, Arabi YM, *et al*. Neuromuscular blockade in patients with ARDS: a rapid practice guideline. Intensive Care Medicine. 2020; 46: 1977–1986.

[53] Choi H, Shin B, Yoo H, Suh GY, Cho JH, Kim HK, *et al*. Early corticosteroid treatment for postoperative acute lung injury after lung cancer surgery. Therapeutic Advances in Respiratory Disease. 2019; 13: 175346661984025.

[54] Sun S, Liu D, Zhang H, Zhang X, Wan B. Effect of different doses and time-courses of corticosteroid treatment in patients with acute respiratory distress syndrome: a meta-analysis. Experimental and Therapeutic Medicine. 2019; 18: 4637–4644.

[55] Mammen MJ, Aryal K, Alhazzani W, Alexander PE. Corticosteroids for patients with acute respiratory distress syndrome: a systematic review and meta-analysis of randomized trials. Polish Archives of Internal Medicine. 2020; 130: 276–286.

[56] Ni Y, Chen G, Sun J, Liang B, Liang Z. The effect of corticosteroids on mortality of patients with influenza pneumonia: a systematic review and meta-analysis. Critical Care. 2019; 23: 99.

[57] Tsai M, Yang K, Chan M, Kao K, Wang H, Perng W, *et al*. Impact of corticosteroid treatment on clinical outcomes of influenza-associated ARDS: a nationwide multicenter study. Annals of Intensive Care. 2020; 10: 26.

[58] Torres A, Sibila O, Ferrer M, Polverino E, Menendez R, Mensa J, *et al*. Effect of corticosteroids on treatment failure among hospitalized patients with severe community-acquired pneumonia and high inflammatory response. JAMA. 2015; 313: 677.

[59] Siemieniuk RAC, Meade MO, Alonso-Coello P, Briel M, Evaniew N, Prasad M, *et al*. Corticosteroid therapy for patients hospitalized with community-acquired pneumonia. Annals of Internal Medicine. 2015; 163: 519–528.

[60] Villar J, Ferrando C, Martínez D, Ambrós A, Muñoz T, Soler JA, *et al*. Dexamethasone treatment for the acute respiratory distress syndrome: a multicentre, randomised controlled trial. Lancet Respiratory Medicine. 2020; 8: 267–276.

[61] Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, *et al*. Dexamethasone in hospitalized patients with Covid-19. New England Journal of Medicine. 2021; 384: 693–704.

[62] Tongyoo S, Permpikul C, Mongkolpun W, Vattanavanit V, Udompanturak S, Kocak M, *et al*. Hydrocortisone treatment in early sepsis-associated acute respiratory distress syndrome: results of a randomized controlled trial. Critical Care. 2016; 20: 329.

[63] Meduri UG, Headley SA, Golden E, Carson SJ, Umberger RA, Kelso T, *et al*. Effect of prolonged methylprednisolone therapy in unresolving acute respiratory distress syndrome: a randomized controlled trial. Survey of Anesthesiology. 1999; 43: 135–136.

[64] Meduri GU, Golden E, Freire AX, Taylor E, Zaman M, Carson SJ, *et al*. Methylprednisolone infusion in early severe ARDS: results of a randomized controlled trial. Chest. 2007; 131: 954–963.

[65] Festic E, Carr GE, Cartin-Ceba R, Hinds RF, Banner-Goodspeed V, Bansal V, *et al*. Randomized clinical trial of a combination of an inhaled corticosteroid and beta agonist in patients at risk of developing the acute respiratory distress syndrome. Critical Care Medicine. 2017; 45: 798–805.

[66] Mohamed H, Meguid MA. Effect of nebulized budesonide on respiratory mechanics and oxygenation in acute lung injury/acute respiratory distress syndrome: Randomized controlled study. Saudi Journal of Anaesthesia. 2017; 11: 9.

[67] Yeh TF, Chen CM, Wu SY, Husan Z, Li TC, Hsieh WS, *et al*. Intratracheal administration of budesonide/surfactant to prevent bronchopulmonary dysplasia. American Journal of Respiratory and Critical Care Medicine. 2016; 193: 86–95.

[68] Kothe TB, Sadiq FH, Burleyson N, Williams HL, Anderson C, Hillman NH. Surfactant and budesonide for respiratory distress syndrome: an observational study. Pediatric Research. 2020; 87: 940–945.

[69] Oh TK, Song I, Choi S. Prior statin therapy and mortality among critically ill patients: a systemic review and meta-analysis of cohort studies. Annals of Translational Medicine. 2020; 8: 396–396.

[70] Calfee CS, Delucchi KL, Sinha P, Matthay MA, Hackett J, Shankar-Hari M, *et al*. Acute respiratory distress syndrome subphenotypes and differential response to simvastatin: secondary analysis of a randomised controlled trial. The Lancet Respiratory Medicine. 2018; 6: 691–698.

[71] Sinha P, Delucchi KL, Thompson BT, McAuley DF, Matthay MA, Calfee CS. Latent class analysis of ARDS subphenotypes: a secondary analysis of the statins for acutely injured lungs from sepsis (SAILS) study. Intensive Care Medicine. 2018; 44: 1859–1869.

[72] Ryter SW, Otterbein LE, Morse D, Choi AMK. Heme oxygenase/carbon monoxide signaling path-ways: regulation and functional significance. Molecular and Cellular Biochemistry. 2002; 234–235: 249–263.

[73] Jung S, Moon J, Xu J, Ifedigbo E, Ryter SW, Choi AMK, *et al*. Carbon monoxide negatively regulates NLRP3 inflammasome activation in macrophages. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2015; 308: L1058–L1067.

[74] Jahn N, Lamberts RR, Busch CJ, Voelker MT, Busch T, Koel-Simmelink MJA, *et al*. Inhaled carbon monoxide protects time-dependently from loss of hypoxic pulmonary vasoconstriction in endotoxemic mice. Respiratory Research. 2015; 16: 119.

[75] Fredenburgh LE, Kraft BD, Hess DR, Harris RS, Wolf MA, Suliman HB, *et al*. Effects of inhaled CO administration on acute lung injury in baboons with pneumococcal pneumonia. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2015; 309: L834–L846.

[76] Islam D, Huang Y, Fanelli V, Delsedime L, Wu S, Khang J, *et al*. Identification and modulation of microenvironment is crucial for effective mesenchymal stromal cell therapy in acute lung injury. American Journal of Respiratory and Critical Care Medicine. 2019; 199: 1214–1224.

[77] Bellingan G, Jacono F, Bannard-Smith J, Brealey D, Meyer N, Thickett D, *et al*. Primary analysis of a phase 1/2 study to assess multistem® cell therapy, a regenerative advanced therapy medicinal product (ATMP), in acute respiratory distress syndrome (MUST-ARDS). B14. Late Breaking Clinical Trials. 2019; 199: A7353.

[78] Matthay MA, Calfee CS, Zhuo H, Thompson BT, Wilson JG, Levitt JE, *et al*. Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial. The Lancet Respiratory Medicine. 2019; 7: 154–162.

[79] Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, *et al*. Transplantation of ACE2- mesenchymal stem cells improves the outcome of patients with COVID-19 Pneumonia. Aging and Disease. 2020; 11: 216.

[80] Chen J, Zhang X, Xie J, Xue M, Liu L, Yang Y, *et al*. Overexpression of TGFβ1 in murine mesenchymal stem cells improves lung inflammation by impacting the Th17/Treg balance in LPS-induced ARDS mice. Stem Cell Research & Therapy. 2020; 11: 311.

[81] Fowler AA 3rd, Truwit JD, Hite RD, Morris PE, DeWilde C, Priday A, *et al*. Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: the CITRIS-ALI randomized clinical trial: The CITRIS-ALI randomized clinical trial. JAMA 2019; 322: 1261–1270.

[82] Chiscano-Camón L, Ruiz-Rodriguez JC, Ruiz-Sanmartin A, Roca O, Ferrer R. Vitamin C levels in patients with SARS-CoV-2-associated acute respiratory distress syndrome. Critical Care. 2020; 24: 522.

[83] Ji M, Chen T, Wang B, Chen M, Ding Q, Chen L, *et al*. Effects of ulinastatin combined with mechanical ventilation on oxygen metabolism, inflammation and stress response and antioxidant capacity of ARDS. Experimental and Therapeutic Medicine. 2018; 15: 4665–4670.

[84] Christie JD, Vaslef S, Chang PK, May AK, Gunn SR, Yang S, *et al*. A randomized dose-escalation study of the safety and anti-inflammatory activity of the p38 mitogen-activated protein kinase inhibitor dilmapimod in severe trauma subjects at risk for acute respiratory distress syndrome. Critical Care Medicine. 2015; 43: 1859–1869.

[85] Grimes JM, Grimes KV. P38 MAPK inhibition: a promising therapeutic approach for COVID-19. Journal of Molecular and Cellular Cardiology. 2020; 144: 63–65.

[86] Jimenez-Guardeño JM, Nieto-Torres JL, DeDiego ML, Regla-Nava JA, Fernandez-Delgado R, Castaño-Rodriguez C, *et al*. The PDZ-binding motif of severe acute respiratory syndrome coronavirus envelope protein is a determinant of viral pathogenesis. PLoS Pathogens. 2014; 10: e1004320.

[87] Bowman ER, Cameron CMA, Avery A, Gabriel J, Kettelhut A, Hecker M, *et al*. Levels of soluble CD14 and tumor necrosis factor receptors 1 and 2 may be predictive of death in severe coronavirus disease 2019. The Journal of Infectious Diseases. 2021; 223: 805–810.

[88] Proudfoot A, Bayliffe A, O'Kane CM, Wright T, Serone A, Bareille PJ, *et al*. Novel anti-tumour necrosis factor receptor-1 (TNFR1) domain antibody prevents pulmonary inflammation in experimental acute lung injury. Thorax. 2018; 73: 723–730.

[89] McElvaney OJ, Curley GF, Rose-John S, McElvaney NG. Interleukin-6: obstacles to targeting a complex cytokine in critical illness. The Lancet Respiratory Medicine. 2021; 9: 643–654.

[90] Morrondo CD, Zarza LP, Gil JG, Pinto Tasende JA, Diez PD, López JMB. Benefit of tocilizumab therapy for adult-onset still disease complicated with acute respiratory distress syndrome. JCR: Journal of Clinical Rheumatology. 2016; 22: 291–293.

[91] Ziegler CGK, Allon SJ, Nyquist SK, Mbano IM, Miao VN, Tzouanas CN, *et al*. SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. Cell. 2020; 181: 1016–1035.e19.

[92] Monk PD, Marsden RJ, Tear VJ, Brookes J, Batten TN, Mankowski M, *et al*. Safety and efficacy of inhaled nebulised interferon beta-1a (SNG001) for treatment of SARS-CoV-2 infection: a randomised, double-blind,

placebo-controlled, phase 2 trial. The Lancet Respiratory Medicine. 2021; 9: 196–206.

[93] Ranieri VM, Pettilä V, Karvonen MK, Jalkanen J, Nightingale P, Brealey D, *et al*. Effect of intravenous interferon β-1a on death and days free from mechanical ventilation among patients with moderate to severe acute respiratory distress syndrome. JAMA. 2020; 323: 725.

[94] Rizzo AN, Sammani S, Esquinca AE, Jacobson JR, Garcia JGN, Letsiou E, *et al*. Imatinib attenuates inflammation and vascular leak in a clinically relevant two-hit model of acute lung injury. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2015; 309: L1294–L1304.

[95] Stephens RS, Johnston L, Servinsky L, Kim BS, Damarla M. The tyrosine kinase inhibitor imatinib prevents lung injury and death after intravenous LPS in mice. Physiological Reports. 2015; 3: e12589.

[96] Nejat R, Sadr AS. Are losartan and imatinib effective against SARS-CoV2 pathogenesis? a pathophysiologic-based in silico study. In Silico Pharmacology. 2021; 9: 1.

[97] Emadi A, Chua JV, Talwani R, Bentzen SM, Baddley J. Safety and efficacy of imatinib for hospitalized adults with COVID-19: a structured summary of a study protocol for a randomised controlled trial. Trials. 2020; 21: 897.

[98] Li Y, Li H, Liu S, Pan P, Su X, Tan H, *et al*. Pirfenidone ameliorates lipopolysaccharide-induced pulmonary inflammation and fibrosis by blocking NLRP3 inflammasome activation. Molecular Immunology. 2018; 99: 134–144.

[99] Bode C, Peukert K, Schewe J, Putensen C, Latz E, Steinhagen F. Tetracycline alleviates acute lung injury by inhibition of NLRP3 inflammasome. European Respiratory Journal. 2019; 54: PA2175.

[100] Cao F, Tian X, Li Z, Lv Y, Han J, Zhuang R, *et al*. Suppression of NLRP3 inflammasome by erythropoietin via the EPOR/JAK2/STAT3 pathway contributes to attenuation of acute lung injury in mice. Frontiers in Pharmacology. 2020; 11: 306.

[101] Hamilton JA. GM-CSF-dependent inflammatory pathways. Frontiers in Immunology. 2019; 10: 2055.

[102] Stanley E, Lieschke GJ, Grail D, Metcalf D, Hodgson G, Gall JA, *et al*. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. Proceedings of the National Academy of Sciences. 1994; 91: 5592–5596.

[103] Paine R, Wilcoxen SE, Morris SB, Sartori C, Baleeiro CEO, Matthay MA, *et al*. Transgenic overexpression of granulocyte macrophage-colony stimulating factor in the lung prevents hyperoxic lung injury. The American Journal of Pathology. 2003; 163: 2397–2406.

[104] Cakarova L, Marsh LM, Wilhelm J, Mayer K, Grimminger F, Seeger W, *et al*. Macrophage tumor necrosis factor-alpha induces epithelial expression of granulocyte-macrophage colony-stimulating factor: impact on alveolar epithelial repair: impact on alveolar epithelial repair. American Journal of Respiratory and Critical Care Medicine. 2009; 180: 521–532.

[105] Paine R, Standiford TJ, Dechert RE, Moss M, Martin GS, Rosenberg AL, *et al*. A randomized trial of recombinant human granulocyte-macrophage colony stimulating factor for patients with acute lung injury. Critical Care Medicine. 2012; 40: 90–97.

[106] Herold S, Hoegner K, Vadász I, Gessler T, Wilhelm J, Mayer K, *et al*. Inhaled granulocyte/macrophage colony-stimulating factor as treatment of pneumonia-associated acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. 2014; 189: 609–611.

[107] Cremer PC, Abbate A, Hudock K, McWilliams C, Mehta J, Chang SY, *et al*. Mavrilimumab in patients with severe COVID-19 pneumonia and systemic hyperinflammation (MASH-COVID): an investigator initiated, multicentre, double-blind, randomised, placebo-controlled trial. The Lancet Rheumatology. 2021; 3: e410–e418.

[108] Mehta P, Porter JC, Manson JJ, Isaacs JD, Openshaw PJM, McInnes IB, *et al*. Therapeutic blockade of granulocyte macrophage colony-stimulating factor in COVID-19-associated hyperinflammation: challenges and opportunities. The Lancet Respiratory Medicine. 2020; 8: 822–830.

[109] Zemans RL, Matthay MA. What drives neutrophils to the alveoli in ARDS? Thorax. 2017; 72: 1–3.

[110] Havemann K, Gramse M. Physiology and pathophysiology of neutral proteinases of human granulocytes. Advances in Experimental Medicine and Biology. 1984; XIII: 1–20.

[111] Bédard M, McClure CD, Schiller NL, Francoeur C, Cantin A, Denis M. Release of interleukin-8, interleukin-6, and colony-stimulating factors by upper airway epithelial cells: implications for cystic fibrosis. American Journal of Respiratory Cell and Molecular Biology. 1993; 9: 455–462.

[112] Zeiher BG, Matsuoka S, Kawabata K, Repine JE. Neutrophil elastase and acute lung injury: prospects for sivelestat and other neutrophil elastase inhibitors as therapeutics. Critical Care Medicine. 2002; 30: S281–S287.

[113] Hagio T, Nakao S, Matsuoka H, Matsumoto S, Kawabata K, Ohno H. Inhibition of neutrophil elastase activity attenuates complement-mediated lung injury in the hamster. European Journal of Pharmacology. 2001; 426: 131–138.

[114] Hagio T, Matsumoto S, Nakao S, Abiru T, Ohno H, Kawabata K. Elastase inhibition reduced death associated with acid aspiration-induced lung injury in hamsters. European Journal of Pharmacology. 2004; 488: 173–180.

[115] Xiao X-G, Zu H-G, Li Q-G, Huang P. Sivelestat sodium hydrate attenuates acute lung injury by decreasing systemic inflammation in a rat model of severe burns. European Review for Medical and Pharmacological Sciences. 2016; 20: 528–536.

[116] Maki C, Inoue Y, Ishihara T, Hirano Y, Kondo Y, Sueyoshi K, *et al*. Evaluation of appropriate indications for the use of sivelestat sodium in acute respiratory distress syndrome: a retrospective cohort study. Acute Medicine & Surgery. 2019; 7: e471.

[117] Pu S, Wang D, Liu D, Zhao Y, Qi D, He J, *et al*. Effect of sivelestat sodium in patients with acute lung injury or acute respiratory distress syndrome: a meta-analysis of randomized controlled trials. BMC Pulmonary Medicine. 2017; 17: 148.

[118] Wang T, Zhu Z, Liu Z, Yi L, Yang Z, Bian W, *et al*. Plasma neutrophil elastase and elafin as prognostic biomarker for acute respiratory distress syndrome. Shock. 2017; 48: 168–174.

[119] Small DM, Zani M, Quinn DJ, Dallet-Choisy S, Glasgow AM, O'Kane C, *et al*. A functional variant of elafin with improved anti-inflammatory activity for pulmonary inflammation. Molecular Therapy. 2015; 23: 24–31.

[120] Camprubí-Rimblas M, Tantinyà N, Bringué J, Guillamat-Prats R, Artigas A. Anticoagulant therapy in acute respiratory distress syndrome. Annals of Translational Medicine. 2018; 6: 36.

[121] Juschten J, Tuinman PR, Juffermans NP, Dixon B, Levi M, Schultz MJ. Nebulized anticoagulants in lung injury in critically ill patients—an updated systematic review of preclinical and clinical studies. Annals of Translational Medicine. 2017; 5: 444.

[122] Frantzeskaki F, Armaganidis A, Orfanos SE. Immunothrombosis in acute respiratory distress syndrome: cross talks between inflammation and coagulation. Respiration. 2017; 93: 212–225.

[123] Morris PE, Steingrub JS, Huang BY, Tang S, Liu PM, Rhode PR, *et al*. A phase I study evaluating the pharmacokinetics, safety and tolerability of an antibody-based tissue factor antagonist in subjects with acute lung injury or acute respiratory distress syndrome. BMC Pulmonary Medicine. 2012; 12: 5.

[124] Abraham E, Reinhart K, Opal S, Demeyer I, Doig C, Rodriguez AL, *et al*. Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis. JAMA. 2003; 290: 238.

[125] Wunderink RG, Laterre P, Francois B, Perrotin D, Artigas A, Vidal LO, *et al*. Recombinant tissue factor pathway inhibitor in severe community-acquired pneumonia. American Journal of Respiratory and Critical Care Medicine. 2011; 183: 1561–1568.

[126] Rezaie AR, Giri H. Antithrombin: an anticoagulant, anti-inflammatory and antibacterial serpin. Journal of Thrombosis and Haemostasis. 2020; 18: 528–533.

[127] Wiedermann CJ. Clinical review: molecular mechanisms underlying the role of antithrombin in sepsis. Critical Care. 2006; 10: 209.

[128] Warren BL, Eid A, Singer P, Pillay SS, Carl P, Novak I, *et al*. Caring for the critically ill patient. High-dose antithrombin III in severe sepsis: a randomized controlled trial. JAMA. 2001; 286: 1869–1878.

[129] Eid A, Wiedermann CJ, Kinasewitz GT. Early administration of high-dose antithrombin in severe sepsis: single center results from the kybersept-trial. Anesthesia & Analgesia. 2008; 107: 1633–1638.

[130] Camprubí-Rimblas M, Tantinyà N, Guillamat-Prats R, Bringué J, Puig

F, Gómez MN, *et al*. Effects of nebulized antithrombin and heparin on inflammatory and coagulation alterations in an acute lung injury model in rats. Journal of Thrombosis and Haemostasis. 2020; 18: 571–583.

[131] Cornet A, Smit E, Beishuizen A, Groeneveld ABJ. The role of heparin and allied compounds in the treatment of sepsis. Thrombosis and Haemostasis. 2007; 98: 579–586.

[132] Gray E, Hogwood J, Mulloy B. The anticoagulant and antithrombotic mechanisms of heparin. Heparin - a Century of Progress. 2012; 1: 43–61.

[133] Dixon B, Santamaria JD, Campbell DJ. A phase 1 trial of nebulised heparin in acute lung injury. Critical Care. 2008; 12: R64.

[134] Dixon B, Schultz MJ, Hofstra JJ, Campbell DJ, Santamaria JD. Nebulized heparin reduces levels of pulmonary coagulation activation in acute lung injury. Critical Care. 2010; 14: 445.

[135] Dixon B, Schultz MJ, Smith R, Fink JB, Santamaria JD, Campbell DJ. Nebulized heparin is associated with fewer days of mechanical ventilation in critically ill patients: a randomized controlled trial. Critical Care. 2010; 14: R180.

[136] Dixon B, Smith RJ, Campbell DJ, Moran JL, Doig GS, Rechnitzer T, *et al*. Nebulised heparin for patients with or at risk of acute respiratory distress syndrome: a multicentre, randomised, double-blind, placebo-controlled phase 3 trial. The Lancet Respiratory Medicine. 2021; 9: 360–372.

[137] Glas GJ, Muller J, Binnekade JM, Cleffken B, Colpaert K, Dixon B, *et al*. HEPBURN - investigating the efficacy and safety of nebulized heparin versus placebo in burn patients with inhalation trauma: study protocol for a multi-center randomized controlled trial. Trials. 2014; 15: 91.

[138] Vincent JL, Ramesh MK, Ernest D, LaRosa SP, Pachl J, Aikawa N, *et al*. A randomized, double-blind, placebo-controlled, Phase 2b study to evaluate the safety and efficacy of recombinant human soluble thrombomodulin, ART-123, in patients with sepsis and suspected disseminated intravascular coagulation. Critical Care Medicine. 2013; 41: 2069–2079.

[139] Miyoshi S, Ito R, Katayama H, Dote K, Aibiki M, Hamada H, *et al*. Combination therapy with sivelestat and recombinant human soluble thrombomodulin for ARDS and DIC patients. Drug Design, Development and Therapy. 2014; 8: 1211–1219.

[140] Vincent J, Francois B, Zabolotskikh I, Daga MK, Lascarrou J, Kirov MY, *et al*. Effect of a recombinant human soluble thrombomodulin on mortality in patients with sepsis-associated coagulopathy. JAMA. 2019; 321: 1993.

[141] François B, Fiancette M, Helms J, Mercier E, Lascarrou J, Kayanoki T, *et al*. Efficacy and safety of human soluble thrombomodulin (ART-123) for treatment of patients in France with sepsis-associated coagulopathy: post hoc analysis of SCARLET. Annals of Intensive Care. 2021; 11: 53.

[142] Heslet L, Andersen JS, Sengeløv H, Dahlbäck B, Dalsgaard-Nielsen J. Inhalation of activated protein C: a possible new adjunctive intervention in acute respiratory distress syndrome. Biologics. 2007; 1: 465–472.

[143] Ranieri VM, Thompson BT, Barie PS, Dhainaut J, Douglas IS, Finfer S, *et al*. Drotrecogin alfa (Activated) in adults with septic shock. New England Journal of Medicine. 2012; 366: 2055–2064.

[144] Abdelaal Ahmed Mahmoud A, Mahmoud HE, Mahran MA, Khaled M. Streptokinase versus unfractionated heparin nebulization in patients with severe acute respiratory distress syndrome (ARDS): a randomized controlled trial with observational controls. Journal of Cardiothoracic and Vascular Anesthesia. 2020; 34: 436–443.

[145] Kor DJ, Carter RE, Park PK, Festic E, Banner-Goodspeed VM, Hinds R, *et al*. Effect of aspirin on development of ards in at-risk patients presenting to the emergency department. JAMA. 2016; 315: 2406.

[146] Bai X, Hippensteel J, Leavitt A, Maloney JP, Beckham D, Garcia C, *et al*. Hypothesis: alpha-1-antitrypsin is a promising treatment option for COVID-19. Medical Hypotheses. 2021; 146: 110394.

[147] McEvoy NL, Clarke JL, Mc Elvaney OJ, Mc Elvaney OF, Boland F, Hyland D, *et al*. A randomised, double-blind, placebo-controlled, pilot trial of intravenous plasma purified alpha-1 antitrypsin for SARS-CoV-2-induced acute respiratory distress syndrome: a structured summary of a study protocol for a randomised, controlled trial. Trials. 2021; 22: 288.

[148] Scheraga RG, Southern BD, Grove LM, Olman MA. The role of transient receptor potential vanilloid 4 in pulmonary inflammatory diseases. Frontiers in Immunology. 2017; 8: 503.

[149] Alvarez DF, King JA, Weber D, Addison E, Liedtke W, Townsley MI. Transient receptor potential vanilloid 4-mediated disruption of the alveolar septal barrier. Circulation Research. 2006; 99: 988–995.

[150] Scheraga RG, Abraham S, Grove LM, Southern BD, Crish JF, Perelas A, *et al*. TRPV4 Protects the Lung from bacterial pneumonia via MAPK molecular pathway switching. The Journal of Immunology. 2020; 204: 1310–1321.

[151] Balakrishna S, Song W, Achanta S, Doran SF, Liu B, Kaelberer MM, *et al*. TRPV4 inhibition counteracts edema and inflammation and improves pulmonary function and oxygen saturation in chemically induced acute lung injury. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2014; 307: L158–L172.

[152] Abd-Elaziz K, Jesenak M, Vasakova M, Diamant Z. Revisiting matrix metalloproteinase 12: its role in pathophysiology of asthma and related pulmonary diseases. Current Opinion in Pulmonary Medicine. 2021; 27: 54–60.

[153] Jerath A, Ferguson ND, Cuthbertson B. Inhalational volatile-based sedation for COVID-19 pneumonia and ARDS. Intensive Care Medicine. 2020; 46: 1563–1566.

[154] Eckle T, Füllbier L, Wehrmann M, Khoury J, Mittelbronn M, Ibla J, *et al*. Identification of ectonucleotidases CD39 and CD73 in innate protection during acute lung injury. The Journal of Immunology. 2007; 178: 8127–8137.

[155] Chen C, Penuelas O, Quinn K, Cheng K, Li C, Zhang H, *et al*. Protective effects of adenosine A2A receptor agonist in ventilator-induced lung injury in rats. Critical Care Medicine. 2009; 37: 2235–2241.

[156] Folkesson HG, Kuzenko SR, Lipson DA, Matthay MA, Simmons MA. The adenosine 2a receptor agonist GW328267C improves lung function after acute lung injury in rats. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2012; 303: L259–L271.

[157] Eckle T, Hughes K, Ehrentraut H, Brodsky KS, Rosenberger P, Choi D, *et al*. Crosstalk between the equilibrative nucleoside transporter ENT2 and alveolar Adora2b adenosine receptors dampens acute lung injury. The FASEB Journal. 2013; 27: 3078–3089.

[158] Wang W, Chen N-Y, Ren D, Davies J, Philip K, Eltzschig HK, *et al*. Enhancing extracellular adenosine levels restores barrier function in acute lung injury through expression of focal adhesion proteins. Frontiers in Molecular Biosciences. 2021; 8: 636678.

[159] Coughlin SR. Thrombin signalling and protease-activated receptors. Nature. 2000; 407: 258–264.

[160] Li Z, Yin M, Zhang H, Ni W, Pierce RW, Zhou HJ, *et al*. BMX represses thrombin-PAR1–mediated endothelial permeability and vascular leakage during early sepsis. Circulation Research. 2020; 126: 471–485.

[161] Natarajan K, Gottipati KR, Berhane K, Samten B, Pendurthi U, Boggaram V. Proteases and oxidant stress control organic dust induction of inflammatory gene expression in lung epithelial cells. Respiratory Research. 2016; 17: 137.

[162] Shebuski RJ, Kilgore KS. Role of inflammatory mediators in thrombogenesis. Journal of Pharmacology and Experimental Therapeutics. 2002; 300: 729–735.

[163] Okajima K. Regulation of inflammatory responses by activated protein C: the molecular mechanism(s) and therapeutic implications. Clinical Chemistry and Laboratory Medicine (CCLM). 2004; 42: 132–141.

[164] Khoufache K, Berri F, Nacken W, Vogel AB, Delenne M, Camerer E, *et al*. PAR1 contributes to influenza a virus pathogenicity in mice. Journal of Clinical Investigation. 2013; 123: 206–214.

[165] Jabaudon M, Berthelin P, Pranal T, Roszyk L, Godet T, Faure J, *et al*. Receptor for advanced glycation end-products and ARDS prediction: a multicentre observational study. Scientific Reports. 2018; 8: 2603.

[166] Uchida T, Shirasawa M, Ware LB, Kojima K, Hata Y, Makita K, *et al*. Receptor for advanced glycation end-products is a marker of type I cell injury in acute lung injury. American Journal of Respiratory and Critical Care Medicine. 2006; 173: 1008–1015.

[167] Blondonnet R, Audard J, Belville C, Clairefond G, Lutz J, Bouvier D, *et al*. RAGE inhibition reduces acute lung injury in mice. Scientific Reports. 2017; 7: 7208.

[168] Audard J, Godet T, Blondonnet R, Joffredo J, Paquette B, Belville C, *et al*. Inhibition of the receptor for advanced glycation end-products in acute respiratory distress syndrome: a randomised laboratory trial in piglets. Scientific Reports. 2019; 9: 9227.

[169] Li J, Wang K, Huang B, Li R, Wang X, Zhang H, *et al*. The receptor

for advanced glycation end products mediates dysfunction of airway epithelial barrier in a lipopolysaccharides-induced murine acute lung injury model. International Immunopharmacology. 2021; 93: 107419.

[170] Remy KE, Cortés-Puch I, Solomon SB, Sun J, Pockros BM, Feng J, *et al*. Haptoglobin improves shock, lung injury, and survival in canine pneumonia. JCI Insight. 2018; 3: e123013.

[171] Yang F, Haile DJ, Berger FG, Herbert DC, Van Beveren E, Ghio AJ. Haptoglobin reduces lung injury associated with exposure to blood. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2003; 284: L402–L409.

[172] Zheng S, D'Souza VK, Bartis D, Dancer RCA, Parekh D, Naidu B, *et al*. Lipoxin A4 promotes lung epithelial repair whilst inhibiting fibroblast proliferation. ERJ Open Research. 2016; 2: 00079–02015.

[173] Mei HX, Ye Y, Xu HR, Xiang SY, Yang Q, Ma HY, *et al*. LXA4 inhibits lipopolysaccharide-induced inflammatory cell accumulation by resident macrophages in mice. Journal of Inflammation Research. 2021; 14: 1375–1385.

[174] Yang J, Li M, Chen X, Lian Q, Wang Q, Gao F, *et al*. Lipoxin a4 ameliorates lipopolysaccharide-induced lung injury through stimulating epithelial proliferation, reducing epithelial cell apoptosis and inhibits epithelial-mesenchymal transition. Respiratory Research. 2019; 20: 192.

[175] Zhang HW, Wang Q, Mei HX, Zheng SX, Ali AM, Wu QX, *et al*. RvD1 ameliorates LPS-induced acute lung injury via the suppression of neutrophil infiltration by reducing CXCL2 expression and release from resident alveolar macrophages. International Immunopharmacology. 2019; 76: 105877.

[176] Liao Z, Dong J, Wu W, Yang T, Wang T, Guo L, *et al*. Resolvin D1 attenuates inflammation in lipopolysaccharide-induced acute lung injury through a process involving the PPARγ/NF-κB pathway. Respiratory Research. 2012; 13: 110.

[177] Yang Q, Xu HR, Xiang SY, Zhang C, Ye Y, Shen CX, *et al*. Resolvin conjugates in tissue regeneration 1 promote alveolar fluid clearance by activating alveolar epithelial sodium channels and Na, K-ATPase in lipopolysaccharide-induced acute lung injury. The Journal of Pharmacology and Experimental Therapeutics. 2021; 379: 156–165.

[178] Heijnen NFL, Bergmans DCJJ, Schultz MJ, Bos LDJ. Precision medicine in Acute Respiratory Distress Syndrome. Signa Vitae. 2021. (in press)

[179] Zhai R, Bonda WLM, Matute-Bello G, Jabaudon M. From preclinical to clinical models of acute respiratory distress syndrome. Signa Vitae. 2022; 18; 3–14.

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REVIEW

Respiratory infections and acute respiratory distress syndrome

Adrian Ceccato[1,2,*], Elena Campaña-Duel[1], Aina Areny-Balagueró[1], Saad Nseir[3], Antoni Torres[2,4]

[1]Critical Care Center, Parc Taulí University Hospital, Parc Taulí Research and Innovation Institute I3PT, 08208 Sabadell, Spain
[2]CIBER of Respiratory Diseases (CIBERES), Institute of Health Carlos III, 28029 Madrid, Spain
[3]Critical Care Center, Lille University Hospital, F-59000 Lille, France
[4]Department of Pneumology, Hospital Clinic of Barcelona, August Pi i Sunyer Biomedical Research Institute—IDIBAPS, University of Barcelona, 08036 Barcelona, Spain

***Correspondence**
aaceccato@tauli.cat
(Adrian Ceccato)

Abstract

Respiratory infections and acute respiratory distress syndrome (ARDS) are closely related. Pneumonia is the most common cause of ARDS, and patients with ARDS usually develop infectious respiratory complications. Sixty percent of the cases of ARDS are due to pneumonia, but only some patients with pneumonia will develop ARDS. Viral pneumonia is a common cause of ARDS, especially in seasonal outbreaks or pandemics. Patients admitted with ARDS could present a secondary infection, and ventilator-associated pneumonia may impair prognosis. Several conditions that predispose patients with ARDS to respiratory infections are present. Decisions regarding antimicrobial treatment should be based on epidemiology, risk factors and current recommendations. Corticosteroids may be used as adjunctive therapy in both pathologies in selective patients.

Keywords

Pneumonia; VAP; ARDS

1. Introduction

Respiratory infections and acute respiratory distress syndrome (ARDS) are closely related. Pneumonia is the most common cause of ARDS, and patients with ARDS will often develop complications, such as respiratory infections. In the LUNG-SAFE study [1], 60% of patients presented pneumonia as the most common risk factor for ARDS. However, ARDS is usually under-recognized by clinicians, which suggests that its incidence may be higher [2]. The inflammatory environment present in ARDS leads to an impaired host defense response [3, 4], resulting in a more elevated risk of infectious complications. The recent coronavirus disease 2019 (COVID-19) pandemic saw an increased prevalence of respiratory infections in patients receiving invasive mechanical ventilation [5].

In this narrative review, we describe the closely relation between ARDS and respiratory infections, analyze common points and management of both conditions.

2. ARDS due to respiratory infections

While pneumonia is the most frequent cause of ARDS [1, 6, 7], only a few patients with community-acquired pneumonia (CAP) will develop ARDS. In a study by Cillóniz *et al.* [8] including 5334 hospitalized patients with CAP, only 125 patients met Berlin criteria for ARDS. Among those patients hospitalized for pneumonia, 930 (17%) required admission to the intensive care unit (ICU) and only 137 received invasive mechanical ventilation. Patients who developed ARDS had higher severity scores (Sepsis-related Organ Failure Assessment (SOFA) and Pneumonia Severity Index (PSI)) and lower ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO_2/FiO_2). Interestingly, those patients with severe CAP who did not develop ARDS more frequently received inhaled corticosteroids. Outcomes were similar between patients with severe CAP and those who developed ARDS; there was no reported differences in etiology. In a study evaluating risk factors for acute lung injury (ALI) [9], 102 of 1234 patients with pneumonia presented ALI. The Lung injury prediction scores (LIPS) score [10] was validated in this study and included pneumonia as a risk factor as well.

Ichikado *et al.* [11] described two clinical phenotypes for those patients with fatal outcomes due to pneumonia-causing ARDS. Those patients who died early (<5 days) presented a higher Acute Physiology and Chronic Health Evaluation II (APACHE II) score, and more commonly, disseminated intravascular coagulation. Those who died later (>5 days) had an early fibroproliferation pattern in the computerized tomography (CT) scan and disseminated intravascular coagulation as well.

Several pathogens may cause pneumonia and ARDS (Table 1). The most common pathogen isolated in patients with CAP is Streptococcus pneumoniae [12]. ARDS could be present in 3% of patients with pneumococcal pneumonia [13] and in 45% of patients with severe CAP due to pneumococcus [14]. The incidence of ARDS does not seem to be higher among patients with pneumococcal pneumonia or other types of bacterial pneumonia [15].

Viruses are an increasing cause of pneumonia that could

TABLE 1. Pathogens that may cause ARDS and pneumonia.

Streptococcus pneumoniae
Mycoplasma pneumoniae
Pseudomonas aeruginosa
Staphylococcus aureus
Rhinovirus
Parainfluenza virus
Metapneumovirus
Influenza
Respiratory syncytial virus
Coronavirus
Adenovirus
Varicella-Zoster Virus
Hanta virus

ARDS: Acute respiratory distress syndrome.

induce ARDS [16]. Influenza virus may lead to seasonal disease—mainly fall or winter—or, in some cases, pandemic disease. In the world occurred 4–8.8 deaths per 100,000 individuals by Influenza [17]. The last influenza pandemic disease was in 2009 due to influenza A (H1N1) virus that emerged in Mexico and originated in swine [18]. ARDS is commonly diagnosed in patients with severe pneumonia due to influenza virus [19].

Adenovirus, metapneumovirus and the syncytial respiratory virus may also cause ARDS [20–22]. Hantavirus is a less common cause of pneumonia but may induce severe disease, characterized by severe respiratory and cardiovascular failure [23].

In the last two decades, three coronaviruses (CoV) have emerged as causing severe acute respiratory syndrome (SARS). In 2003, SARS-CoV was found to cause severe disease with ARDS, mainly in southeastern Asia [24], while Middle East respiratory syndrome (MERS) was described in Saudi Arabia in 2012 [25]. At the end of 2019, SARS-CoV-2 appeared in China, spreading quickly and triggering a pandemic. Almost all patients admitted to ICU due to COVID-19—the disease caused by SARS-CoV-2—presented ARDS [26–28]. Mortality associated with COVID-19 reached rates higher than 45% in patients receiving invasive mechanical ventilation [29]. Older age, high fever, comorbidities, neutrophilia, lymphocytopenia, elevated end organ-related indices (*e.g.*, aspartate aminotransferase, urea, lactate dehydrogenase) and inflammatory biomarkers, and coagulation disorders were significantly associated with a higher risk of ARDS onset [30]. COVID-19-related may cause lung sequelae [31, 32] in the long term; however, mortality rates have not been reported to significantly increase after discharge [33]. Alveolar-capillary microthrombi, microangiopathy, angiogenesis and classical diffuse alveolar damage were found in lung samples obtained during autopsies of patients with COVID-19 [34]. A possible cause for such observations is the release of procoagulant factors from injured endothelial cells [35, 36]. Patients with COVID-19 may also have a co-infection when admitted to the ICU. In a study comparing the prevalence of co-infections among patients with either COVID-19 or influenza, the rate was significantly lower in those with COVID-19 [37]. It is worth noting, though, that this prevalence may be higher if more sensitive tests are performed. In a study where bronchoalveolar lavage was obtained and analyzed using a multiplex polymerase chain reaction (PCR) panel, 21% of patients presented co-infections within 48 hours of undergoing invasive mechanical ventilation [38].

Co-infections are common in patients with pneumonia-related ARDS. In a study by Kao *et al.* [39] including 902 patients with ARDS due to any cause, microbiological isolation of the causative agent occurred in 142 patients with pneumonia. Twenty-nine percent (n = 41) presented a co-infection with a virus, fungus or bacteria (having been isolated in bronchoalveolar lavage samples). No differences in ARDS severity were observed among patients with either a co-infection or only viral infection; however, mortality was higher in those individuals with co-infections.

Respiratory infections that cause ARDS may be nosocomial or community-acquired. In a study analyzing community-, hospital- or ICU-acquired ARDS, Kao *et al.* [40] observed that ICU-acquired ARDS was the most common and patients with community-acquired ARDS had the lowest mortality.

Barbeta *et al.* [41] analyzed the characteristics and outcomes of patients with ventilator-associated pneumonia (VAP) who did and did not develop ARDS. Of the 302 patients with VAP, 41 (14%) presented ARDS. These patients were younger, with lower severity scores at admission and increased severity at VAP diagnosis. The most frequently isolated pathogen in both groups was Pseudomona aeruginosa. Interestingly, no differences in 28- and 90-day mortality were found between groups.

3. Secondary respiratory infections in patients with ARDS

Ventilator-associated lower respiratory tract infections (VA-LRTI) are common complications in patients with ARDS [42]. VAP and ventilator-associated tracheobronchitis (VAT) fall within the definition of VA-LRTI.

Several conditions that facilitate the onset of respiratory infections are present in patients with ARDS. Also, diagnosing VA-LRTI in patients with ARDS is difficult, given the presence of bilateral infiltrates in chest x-rays and, in many cases, colonization of the airway by pathogens.

VAP must be suspected when there is an impairment in the clinical condition, the onset of fever, changes in respiratory secretions, or higher requirements for oxygen or positive end-expiratory pressure levels [43, 44]. Increased levels of biomarkers such as C-reactive protein may help in diagnosing VAP [45, 46].

In a study published by Chastre *et al.* [47], VAP was the most frequent condition occurring in patients with ARDS compared to ventilated patients without ARDS. Patients with ARDS had a more elevated incidence of VAP due to methicillin-resistant Staphylococcus aureus. Mortality was

also higher in patients with VAP and ARDS.

Incidence of VAP was analyzed in a post-hoc study including patients from a randomized controlled trial (RCT) of cisatracurium besilate in patients with severe ARDS [48]. VAP occurred in 98 (29%) patients, and mortality was higher in those individuals who developed the condition (41% *vs.* 31%). However, when analyses were adjusted for severity and plateau pressure, VAP was not associated with ICU mortality. In a separate post-hoc analysis of a clinical trial evaluating prone position in ARDS [49], incidence of VAP was 1.18 (0.86–1.60) per 100 days of invasive mechanical ventilation; it was similar to that reported in patients included in the prone position arm. VAP was, furthermore, associated with higher mortality, with a hazard ratio of 2.2 95% CI 1.39–3.52 $p < 0.001$ after adjusting for position group, age, SOFA score, McCabe score and immunodeficiency.

A model of VAP prediction in patients with ARDS was developed using data from the EDEN trial [50]. Use of neuromuscular blocking agents, severe ARDS, admission for an unscheduled surgery, and trauma as primary causes of ARDS constituted independent risk factors for VAP.

An inflammatory environment present in the lungs of patients with ARDS may predispose them to developing VA-LRTI [4, 51–53]. An impaired host defense—including innate and acquired immune response—or the use of immunomodulatory drugs such as glucocorticoids may provide an explanation for this observation. Also, inflammatory patterns could be related to changes in microbiota as well [54]: dysbiosis observed in patients with ARDS may facilitate the development of VAP [55–57]. Finally, a low level of positive end-expiratory pressure and hyperoxia may increase the risk of VAP [58, 59], warranting consideration during the management of patients with ARDS.

Recently, critically ill patients with COVID-19 have been observed to present a higher incidence/prevalence of VA-LRTI [60, 61]. In a multicenter study evaluating both patients with COVID-19 or influenza and mechanically ventilated patients without non-viral pneumonia, Rouze *et al*. [5] observed an incidence of 50% of VA-LRTI in patients with COVID-19. This incidence was significantly higher even after being adjusted for confounders. VAP was associated with poor outcomes [62, 63].

Less common secondary pulmonary infections could be found in patients with severe viral pneumonia. Pulmonary aspergillosis may complicate ARDS caused by influenza or COVID-19 [64–67].

Combining novel microbiological tests and routine Gram staining and cultures may help diagnose VAP early and accurately and promote prompt and adequate treatment. Gram staining has good accuracy in diagnosing S. aureus [68], and multiplex PCR may reduce exposure to broad-spectrum antibiotics, especially if testing for resistance mechanism genes occurs [69, 70]. Bronchoalveolar lavage may promote better diagnoses and should be performed when possible [43, 71]. Diagnosing infections due to aspergillus is a challenge. However, detection of galactomannan in serum and bronchoalveolar lavage may help [72].

4. Managing ARDS due to or complicated by respiratory infections

ARDS must be managed according to current recommendations [73]. Protective mechanical ventilation and the use of prone positions have shown benefits in this population. Extracorporeal membrane oxygenation (ECMO) should be used in selective populations [7], and clinicians should consider the risks and benefits of neuromuscular blocking agents [74–78]. For respiratory infections, current guideline recommendations may help choose early and adequate antimicrobial treatments (Table 2) [43, 44, 79]. Local data about multidrug-resistant (MDR) pathogens and individual risk factors must be considered. ARDS was included as a risk factor for MDR pathogens in the last American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) guidelines [44]. However, in a study validating this particular guideline, ARDS had poor accuracy in relation to MDR pathogen prediction (high specificity (81%) and low sensitivity (24%)) [80].

Corticosteroids have been tested in several trials for both ARDS and pneumonia. Potent anti-inflammatory medications [81], corticosteroids may play a role as an immunomodulatory drug in the exacerbated inflammatory response observed in patients with either ARDS or pneumonia. In 1986, methylprednisolone was tested at high doses in patients with ARDS, conferring no benefits [82]. The same was found at intermediate doses in patients with prolonged ARDS (more than seven days since onset) [83]. In an analysis including data from four RCT, prolonged treatment (>25 days) with corticosteroids improved outcomes with fewer ventilator- and ICU-free days, and lower ICU mortality. Villar *et al*. [84] performed an RCT evaluating dexamethasone 10 mg per day for 10 days in patients with moderate or severe ARDS according to the Berlin definition [85]. Two hundred and seventy-seven patients were randomized to dexamethasone or placebo. Ventilator-free days were higher in the dexamethasone arm, and mortality decreased by 15.3% in those patients who received corticosteroids.

Corticosteroids have been extensively tested in patients with pneumonia [86, 87]. Several trials [88–91] and a meta-analysis [92–101] reported heterogeneous results about the efficacy of corticosteroids in reducing mortality and improving outcomes in patients with CAP. Benefits regarding mortality were observed mainly in patients with severe CAP [95, 96, 99–101]. Nonetheless, concerns about the reproducibility of these results have limited applicability thereof [79]. Recently, in a propensity score matching study using real-life data [102], our group observed significantly lower mortality in those patients with severe CAP criteria per ATS/IDSA guidelines [79]. Corticosteroids also reduced the risk of disease progression to ARDS [97]. Special considerations should be made for some cases of viral pneumonia. Corticosteroids showed impaired outcomes in patients with severe CAP due to influenza or MERS [103–105]. Conversely, though, corticosteroids have served as the main treatment drug for patients with severe or critical COVID-19. Corticosteroids have shown efficacy in reducing mortality in patients with COVID-19 when treatment was started after seven days since symptom onset [106, 107]. Most critically ill patients experienced higher benefits. Corticosteroids were described as a risk factor for ICU-acquired

TABLE 2. Recommended antimicrobial treatments for pneumonia.

Community-acquired pneumonia	Nosocomial pneumonia (HAP and VAP)
β-lactam plus macrolides or fluoroquinolone.	Higher prevalence of MDR pathogens (>25% of GNEB or 10% of MRSA), septic shock, high mortality risk or risk factors for MDR pathogens: double coverage with antipseudomonal agents and MRSA coverage: β-lactams/aminoglycosides +/− linezolid or vancomycin.
If MDR pathogens are suspected based on risk factors and local epidemiology: antipseudomonal β-lactams +/− linezolid or vancomycin.	If patient does not have septic shock and one agent is active against 90% of isolated pathogens in the ICU, a single agent could be administered.
Oseltamivir must be added if influenza is suspected or confirmed.	

Based on recommendations from ATS/IDSA [44, 79] and European respiratory society/European Society of Intensive Care Medicine/European Society of Clinical Microbiology and Infectious Diseases/Latin American Thoracic Society (ERS/ESICM/ESCMID/ALAT) [43] guidelines. Abbreviations: HAP: Hospital-acquired pneumonia; MDR: multidrug-resistant; VAP: ventilator-associated pneumonia; GNEB: Gram negative entero bacteriace; MRSA: Methicillin-resistant staphylococcus aureus; ICU: Intensive care unit. Note that ARDS may be considered as a risk factor for MDR pathogens according to ATS/IDSA guidelines.

pneumonia [108].

Corticosteroids should be used with caution in patients with VAP, given that the drug was associated with lower survival in an observational study by Ranzani *et al.* [109].

5. Future perspectives

ARDS is a heterogeneous condition [110], which means that not all measures could be of benefit. ARDS due to respiratory infections may differ in terms of the causative agent, previous condition or host-pathogen interaction (*e.g.*, inflammatory response) [111]. Thus, several aspects should be taken into account before recommending a treatment or measure. Knowing different phenotypes may allow clinicians to adjust treatment according to risks and benefits [112]. Platform or adaptive trials taking place during the last pandemic have demonstrated the possibility of evaluating several treatments, with quick results changing clinical practice (*e.g.*, the use of corticosteroid for COVID-19). Predictive and prognostic enrichment might improve clinical trials, increasing effect sizes; however, results may not be generalizable [113].

Given that many clinical trials have failed to improve clinical practice, future studies should be designed in a way that acknowledge the varying components and implement enrichment. Adaptive trials should be considered, mainly in those conditions with high prevalence.

6. Conclusions

Pneumonia is the most common cause of ARDS. Community- or hospital-acquired pneumonia can trigger ARDS. Patients admitted with ARDS could present a secondary infection. Antimicrobial treatment must be based on epidemiology, risk factors and current recommendations. Corticosteroids can be used as adjunctive therapy in both pathologies.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, *et al.* Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. The Journal of the American Medical Association. 2016; 315: 788–800.

[2] Ferguson ND, Frutos-Vivar F, Esteban A, Fernández-Segoviano P, Aramburu JA, Nájera L, *et al.* Acute respiratory distress syndrome: underrecognition by clinicians and diagnostic accuracy of three clinical definitions. Critical Care Medicine. 2005; 33: 2228–2234.

[3] Meduri GU, Kanangat S, Stefan J, Tolley E, Schaberg D. Cytokines IL-1beta, IL-6, and TNF-alpha enhance in vitro growth of bacteria. American Journal of Respiratory and Critical Care Medicine. 1999; 160: 961–967.

[4] Meduri GU. A paradigm shift: the bidirectional effect of inflammation on bacterial growth. Sepsis and Organ Dysfunction. 2000; 19: 145–154.

[5] Rouzé A, Martin-Loeches I, Povoa P, Makris D, Artigas A, Bouchereau M, *et al*. Relationship between SARS-CoV-2 infection and the incidence of ventilator-associated lower respiratory tract infections: a European multicenter cohort study. Intensive Care Medicine. 2021; 47: 188–198.
[6] Guérin C, Reignier J, Richard J, Beuret P, Gacouin A, Boulain T, *et al*. Prone positioning in severe acute respiratory distress syndrome. New England Journal of Medicine. 2013; 368: 2159–2168.
[7] Combes A, Hajage D, Capellier G, Demoule A, Lavoué S, Guervilly C, *et al*. Extracorporeal membrane oxygenation for severe acute respiratory distress syndrome. New England Journal of Medicine. 2018; 378: 1965–1975.
[8] Ferrer Monreal M, Cilloniz C, Liapikou A, Garcia-Vidal C, Gabarrus A, Ceccato A, *et al*. Acute respiratory distress syndrome in mechanically ventilated patients with community-acquired pneumonia. European Respiratory Journal. 2018; 51: 1702215.
[9] Gajic O, Dabbagh O, Park PK, Adesanya A, Chang SY, Hou P, *et al*. Early identification of patients at risk of acute lung injury: evaluation of lung injury prediction score in a multicenter cohort study. American Journal of Respiratory and Critical Care Medicine. 2011; 183: 462–470.
[10] Trillo-Alvarez C, Cartin-Ceba R, Kor DJ, Kojicic M, Kashyap R, Thakur S, *et al*. Acute lung injury prediction score: derivation and validation in a population-based sample. European Respiratory Journal. 2011; 37: 604–609.
[11] Ichikado K, Kawamura K, Johkoh T, Fujimoto K, Shintani A, Hashimoto S, *et al*. Clinical phenotypes from fatal cases of acute respiratory distress syndrome caused by pneumonia. Scientific Reports. 2021; 11: 20051.
[12] Cillóniz C, Ewig S, Polverino E, Marcos MA, Esquinas C, Gabarrus A, *et al*. Microbial aetiology of community-acquired pneumonia and its relation to severity. Thorax. 2011; 66: 340–346.
[13] Ceccato A, Torres A, Cilloniz C, Amaro R, Gabarrus A, Polverino E, *et al*. Invasive disease versus urinary antigen confirmed pneumococcal community-acquired pneumonia. Chest. 2017; 151: 1311–1319.
[14] Mongardon N, Max A, Bouglé A, Pène F, Lemiale V, Charpentier J, *et al*. Epidemiology and outcome of severe pneumococcal pneumonia admitted to intensive care unit: a multicenter study. Critical Care. 2012; 16: R155.
[15] Georges H, Leroy O, Vandenbussche C, Guery B, Alfandari S, Tronchon L, *et al*. Epidemiological features and prognosis of severe community-acquired pneumococcal pneumonia. Intensive Care Medicine. 1999; 25: 198–206.
[16] Luyt C, Combes A, Trouillet J, Nieszkowska A, Chastre J. Virus-induced acute respiratory distress syndrome: epidemiology, management and outcome. La Presse MéDicale. 2011; 40: e561–e568.
[17] Iuliano AD, Roguski KM, Chang HH, Muscatello DJ, Palekar R, Tempia S, *et al*. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. The Lancet. 2018; 391: 1285–1300.
[18] Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, Hernandez M, Quiñones-Falconi F, Bautista E, *et al*. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. New England Journal of Medicine. 2009; 361: 680–689.
[19] Domínguez-Cherit G, Lapinsky SE, Macias AE, Pinto R, Espinosa-Perez L, de la Torre A, *et al*. Critically ill patients with 2009 influenza A (H1N1) in Mexico. The Journal of the American Medical Association. 2009; 302: 1880–1887.
[20] Lee N, Qureshi ST. Other Viral Pneumonias: coronavirus, respiratory syncytial virus, adenovirus, hantavirus. Critical Care Clinics. 2013; 29: 1045–1068.
[21] Hasvold J, Sjoding M, Pohl K, Cooke CR, Hyzy RC. The role of human metapneumovirus in the critically ill adult patient. Journal of Critical Care. 2016; 31: 233–237.
[22] Wu Z, Zhang R, Liu D, Liu X, Zhang J, Zhang Z, *et al*. Acute respiratory distress syndrome caused by human adenovirus in adults: a prospective observational study in Guangdong, China. Frontiers in Medicine. 2021; 8: 791163.
[23] Figueiredo LTM, Souza WMD, Ferrés M, Enria DA. Hantaviruses and cardiopulmonary syndrome in South America. Virus Research. 2014; 187: 43–54.
[24] Peiris JSM, Yuen KY, Osterhaus ADME, Stöhr K. The severe acute respiratory syndrome. The New England Journal of Medicine. 2003; 349: 2431–2441.
[25] Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. New England Journal of Medicine. 2012; 367: 1814–1820.
[26] Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, *et al*. Baseline characteristics and outcomes of 1591 patients infected with sars-cov-2 admitted to ICUs of the Lombardy Region, Italy. The Journal of the American Medical Association. 2020; 323: 1574–1581.
[27] Botta M, Tsonas AM, Pillay J, Boers LS, Algera AG, Bos LDJ, *et al*. Ventilation management and clinical outcomes in invasively ventilated patients with COVID-19 (PRoVENT-COVID): a national, multicentre, observational cohort study. The Lancet Respiratory Medicine. 2021; 9: 139–148.
[28] Torres A, Motos A, Riera J, Fernández-Barat L, Ceccato A, Pérez-Arnal R, *et al*. The evolution of the ventilatory ratio is a prognostic factor in mechanically ventilated COVID-19 ARDS patients. Critical Care. 2021; 25: 331.
[29] Lim ZJ, Subramaniam A, Ponnapa Reddy M, Blecher G, Kadam U, Afroz A, *et al*. Case fatality rates for patients with covid-19 requiring invasive mechanical ventilation. A meta-analysis. American Journal of Respiratory and Critical Care Medicine. 2021; 203: 54–66.
[30] Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, *et al*. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. The Journal of the American Medical Association. 2020; 180: 934–943.
[31] González J, Benítez ID, Carmona P, Santisteve S, Monge A, Moncusí-Moix A, *et al*. Pulmonary function and radiologic features in survivors of critical COVID-19: a 3-month prospective cohort. Chest. 2021; 160: 187–198.
[32] Barbeta Viñas ER, Benegas M, Sánchez M, Motos A, Ferrer M, Ceccato A, *et al*. Risk factors and clinical impact of fibrotic-like changes and the organizing pneumonia pattern in patients with COVID-19- and non-COVID-19-induced acute respiratory distress syndrome. Archivos de Bronconeumologia. 2022; 58: 183–187.
[33] Ceccato A, Pérez-Arnal R, Motos A, Barbé F, Torres A, Saera MB, *et al*. One-year mortality after ICU admission due to COVID-19 infection. Intensive Care Medicine. 2022; 48: 366–368.
[34] Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, *et al*. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. New England Journal of Medicine. 2020; 383: 120–128.
[35] José RJ, Williams A, Manuel A, Brown JS, Chambers RC. Targeting coagulation activation in severe COVID-19 pneumonia: lessons from bacterial pneumonia and sepsis. European Respiratory Review. 2020; 29: 200240.
[36] Iba T, Warkentin TE, Thachil J, Levi M, Levy JH. Proposal of the definition for COVID-19-associated coagulopathy. Journal of Clinical Medicine. 2021; 10: 191.
[37] Rouze A, Martin-Loeches I, Povoa P, Metzelard M, Du Cheyron D, Lambiotte F, *et al*. Early bacterial identification among intubated patients with COVID-19 or influenza pneumonia: a European multicenter comparative cohort study. American Journal of Respiratory and Critical Care Medicine. 2021; 204: 546–556.
[38] Pickens CO, Gao CA, Cuttica MJ, Smith SB, Pesce LL, Grant RA, *et al*. Bacterial superinfection pneumonia in patients mechanically ventilated for COVID-19 pneumonia. American Journal of Respiratory and Critical Care Medicine. 2021; 204: 921–932.
[39] Kao K, Chiu L, Hung C, Chang C, Yang C, Huang C, *et al*. Coinfection and mortality in pneumonia-related acute respiratory distress syndrome patients with bronchoalveolar lavage: a prospective observational study. Shock. 2017; 47: 615–620.
[40] Kao K, Hu H, Hsieh M, Tsai Y, Huang C. Comparison of community-acquired, hospital-acquired, and intensive care unit-acquired acute respiratory distress syndrome: a prospective observational cohort study. Critical Care. 2015; 19: 384.
[41] Barbeta E, Ceccato A, Artigas A, Ferrer M, Fernández L, López R, *et al*. Characteristics and outcomes in patients with ventilator-associated pneumonia who do or do not develop acute respiratory distress syndrome. An observational study. Journal of Clinical Medicine. 2020; 9: 3508.
[42] Iregui MG, Kollef MH. Ventilator-associated pneumonia complicating the acute respiratory distress syndrome. Seminars in Respiratory and Critical Care Medicine. 2001; 22: 317–326.
[43] Torres A, Niederman MS, Chastre J, Ewig S, Fernandez-Vandellos P,

Hanberger H, *et al*. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia: guidelines for the management of hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) of the European Respiratory Society (ERS), European Society of Intensive Care Medicine (ESICM), European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Asociación Latinoamericana del Tórax (ALAT). European Respiratory Journal. 2017; 50: 1700582.

[44] Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, *et al*. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clinical Infectious Diseases. 2016; 63: e61–e111.

[45] Salluh JIF, Souza-Dantas VC, Póvoa P. The current status of biomarkers for the diagnosis of nosocomial pneumonias. Current Opinion in Critical Care. 2017; 23: 391–397.

[46] Póvoa P, Martin-Loeches I, Ramirez P, Bos LD, Esperatti M, Silvestre J, *et al*. Biomarker kinetics in the prediction of VAP diagnosis: results from the BioVAP study. Annals of Intensive Care. 2016; 6: 32.

[47] Chastre J, Trouillet J, Vuagnat A, Joly-Guillou M, Clavier H, Dombret M, *et al*. Nosocomial pneumonia in patients with acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. 1998; 157: 1165–1172.

[48] Forel J, Voillet F, Pulina D, Gacouin A, Perrin G, Barrau K, *et al*. Ventilator-associated pneumonia and ICU mortality in severe ARDS patients ventilated according to a lung-protective strategy. Critical Care. 2012; 16: R65.

[49] Ayzac L, Girard R, Baboi L, Beuret P, Rabilloud M, Richard JC, *et al*. Ventilator-associated pneumonia in ARDS patients: the impact of prone positioning. A secondary analysis of the PROSEVA trial. Intensive Care Medicine. 2016; 42: 871–878.

[50] Wu Z, Liu Y, Xu J, Xie J, Zhang S, Huang L, *et al*. A ventilator-associated pneumonia prediction model in patients with acute respiratory distress syndrome. Clinical Infectious Diseases. 2020; 71: S400–S408.

[51] Meduri GU, Kohler G, Headley S, Tolley E, Stentz F, Postlethwaite A. Inflammatory cytokines in the BAL of patients with ARDS: persistent elevation over time predicts poor outcome. Chest. 1995; 108: 1303–1314.

[52] Headley AS, Meduri GU, Tolley E. Infections and the inflammatory response in acute respiratory distress syndrome. Chest. 1997; 111: 1306–1321.

[53] Song M, Liu Y, Lu Z, Luo H, Peng H, Chen P. Prognostic factors for ARDS: clinical, physiological and atypical immunodeficiency. BMC Pulmonary Medicine. 2020; 20: 102.

[54] Kyo M, Nishioka K, Nakaya T, Kida Y, Tanabe Y, Ohshimo S, *et al*. Unique patterns of lower respiratory tract microbiota are associated with inflammation and hospital mortality in acute respiratory distress syndrome. Respiratory Research. 2019; 20: 246.

[55] Zakharkina T, Martin-Loeches I, Matamoros S, Povoa P, Torres A, Kastelijn JB, *et al*. The dynamics of the pulmonary microbiome during mechanical ventilation in the intensive care unit and the association with occurrence of pneumonia. Thorax. 2017; 72: 803–810.

[56] Fromentin M, Ricard J, Roux D. Respiratory microbiome in mechanically ventilated patients: a narrative review. Intensive Care Medicine. 2021; 47: 292–306.

[57] Luyt C, Bouadma L, Morris AC, Dhanani JA, Kollef M, Lipman J, *et al*. Pulmonary infections complicating ARDS. Intensive Care Medicine. 2020; 46: 2168–2183.

[58] Manzano F, Fernández-Mondéjar E, Colmenero M, Poyatos ME, Rivera R, Machado J, *et al*. Positive-end expiratory pressure reduces incidence of ventilator-associated pneumonia in nonhypoxemic patients. Critical Care Medicine. 2008; 36: 2225–2231.

[59] Six S, Rouzé A, Pouly O, Poissy J, Wallet F, Preau S, *et al*. Impact of hyperoxemia on mortality in critically ill patients with ventilator-associated pneumonia. Annals of Translational Medicine. 2018; 6: 417.

[60] Povoa P, Martin-Loeches I, Nseir S. Secondary pneumonias in critically ill patients with COVID-19: risk factors and outcomes. Current Opinion in Critical Care. 2021; 27: 468–473.

[61] Fumagalli J, Panigada M, Klompas M, Berra L. Ventilator-associated pneumonia among SARS-CoV-2 acute respiratory distress syndrome patients. Current Opinion in Critical Care. 2022; 28: 74–82.

[62] Nseir S, Martin-Loeches I, Povoa P, Metzelard M, Du Cheyron D, Lambiotte F, *et al*. Relationship between ventilator-associated pneumonia and mortality in COVID-19 patients: a planned ancillary analysis of the coVAPid cohort. Critical Care. 2021; 25: 177.

[63] Martin-Loeches I, Motos A, Menéndez R, Gabarrús A, González J, Fernández-Barat L, *et al*. ICU-acquired pneumonia is associated with poor health post-COVID-19 sndrome. Journal of Cinical Mdicine. 2021; 11 224.

[64] Verweij PE, Rijnders BJA, Brüggemann RJM, Azoulay E, Bassetti M, Blot S, *et al*. Review of influenza-associated pulmonary aspergillosis in ICU patients and proposal for a case definition: an expert opinion. Intensive Care Medicine. 2020; 46: 1524–1535.

[65] Koehler P, Bassetti M, Kochanek M, Shimabukuro-Vornhagen A, Cornely OA. Intensive care management of influenza-associated pulmonary aspergillosis. Clinical Microbiology and Infection. 2019; 25: 1501–1509.

[66] Koehler P, Bassetti M, Chakrabarti A, Chen SCA, Colombo AL, Hoenigl M, *et al*. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. The Lancet Infectious Diseases. 2021; 21: e149–e162.

[67] Rouzé A, Lemaitre E, Martin-Loeches I, Povoa P, Diaz E, Nyga R, *et al*. Invasive pulmonary aspergillosis among intubated patients with SARS-CoV-2 or influenza pneumonia: a European multicenter comparative cohort study. Critical Care. 2022; 26: 11.

[68] Ranzani OT, Motos A, Chiurazzi C, Ceccato A, Rinaudo M, Li Bassi G, *et al*. Diagnostic accuracy of Gram staining when predicting staphylococcal hospital-acquired pneumonia and ventilator-associated pneumonia: a systematic review and meta-analysis. Clinical Microbiology and Infection. 2020; 26: 1456–1463.

[69] Peiffer-Smadja N, Bouadma L, Mathy V, Allouche K, Patrier J, Reboul M, *et al*. Performance and impact of a multiplex PCR in ICU patients with ventilator-associated pneumonia or ventilated hospital-acquired pneumonia. Critical Care. 2020; 24: 366.

[70] Monard C, Pehlivan J, Auger G, Alviset S, Tran Dinh A, Duquaire P, *et al*. Multicenter evaluation of a syndromic rapid multiplex PCR test for early adaptation of antimicrobial therapy in adult patients with pneumonia. Critical Care. 2020; 24: 434.

[71] Fagon J. Diagnosis and treatment of ventilator-associated pneumonia: fiberoptic bronchoscopy with bronchoalveolar lavage is essential. Seminars in Respiratory and Critical Care Medicine. 2006; 27: 034–044.

[72] Blot SI, Taccone FS, Van den Abeele A, Bulpa P, Meersseman W, Brusselaers N, *et al*. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. American Journal of Respiratory and Critical Care Medicine. 2012; 186: 56–64.

[73] Fan E, Del Sorbo L, Goligher EC, Hodgson CL, Munshi L, Walkey AJ, *et al*. An Official American Thoracic Society/European Society of Intensive Care Medicine/society of critical care medicine clinical practice guideline: mechanical ventilation in adult patients with acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. 2017; 195: 1253–1263.

[74] Gainnier M, Roch A, Forel J, Thirion X, Arnal J, Donati S, *et al*. Effect of neuromuscular blocking agents on gas exchange in patients presenting with acute respiratory distress syndrome. Critical Care Medicine. 2004; 32: 113–119.

[75] Papazian L, Forel J, Gacouin A, Penot-Ragon C, Perrin G, Loundou A, *et al*. Neuromuscular blockers in early acute respiratory distress syndrome. New England Journal of Medicine. 2010; 363: 1107–1116.

[76] Moss M, Huang DT, Brower RG, Ferguson ND, Ginde AA, Gong MN, *et al*. Early neuromuscular blockade in the acute respiratory distress syndrome. New England Journal of Medicine. 2019; 380: 1997–2008.

[77] Chang W, Sun Q, Peng F, Xie J, Qiu H, Yang Y. Validation of neuromuscular blocking agent use in acute respiratory distress syndrome: a meta-analysis of randomized trials. Critical Care. 2020; 24: 54.

[78] Watling SM, Dasta JF. Prolonged paralysis in intensive care unit patients after the use of neuromuscular blocking agents: a review of the literature. Critical Care Medicine. 1994; 22: 884–893.

[79] Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, *et al*. Diagnosis and treatment of adults with community-acquired pneumonia. An official clinical practice guideline of the American Thoracic Society and Infectious Diseases Society of America. American

Journal of Respiratory and Critical Care Medicine. 2019; 200: e45–e67.
[80] Ekren PK, Ranzani OT, Ceccato A, Li Bassi G, Muñoz Conejero E, Ferrer M, *et al*. Evaluation of the 2016 Infectious Diseases Society of America/American Thoracic Society guideline criteria for risk of multidrug-resistant pathogens in patients with hospital-acquired and ventilator-associated pneumonia in the ICU. American Journal of Respiratory and Critical Care Medicine. 2018; 197: 826–830.
[81] Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. New England Journal of Medicine. 2005; 353: 1711–1723.
[82] Bernard GR, Luce JM, Sprung CL, Rinaldo JE, Tate RM, Sibbald WJ, *et al*. High-dose corticosteroids in patients with the adult respiratory distress syndrome. New England Journal of Medicine. 1987; 317: 1565–1570.
[83] Steinberg KP, Hudson LD, Goodman RB, Hough CL, Lanken PN, Hyzy R, *et al*. Efficacy and safety of corticosteroids for persistent acute respiratory distress syndrome. The New England Journal of Medicine. 2006; 354: 1671–1684.
[84] Villar J, Ferrando C, Martínez D, Ambrós A, Muñoz T, Soler JA, *et al*. Dexamethasone treatment for the acute respiratory distress syndrome: a multicentre, randomised controlled trial. The Lancet Respiratory Medicine. 2020; 8: 267–276.
[85] ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, *et al*. Acute respiratory distress syndrome: the Berlin definition. The Journal of the American Medical Association. 2012; 307: 2526–2533.
[86] Prina E, Ceccato A, Torres A. New aspects in the management of pneumonia. Critical Care. 2016; 20: 267.
[87] Ceccato A, Ferrer M, Barbeta E, Torres A. Adjunctive therapies for community-acquired pneumonia. Clinics in Chest Medicine. 2018; 39: 753–764.
[88] Torres A, Sibila O, Ferrer M, Polverino E, Menendez R, Mensa J, *et al*. Effect of corticosteroids on treatment failure among hospitalized patients with severe community-acquired pneumonia and high inflammatory response: a randomized clinical trial. The Journal of the American Medical Association. 2015; 313: 677–686.
[89] Blum CA, Nigro N, Briel M, Schuetz P, Ullmer E, Suter-Widmer I, *et al*. Adjunct prednisone therapy for patients with community-acquired pneumonia: a multicentre, double-blind, randomised, placebo-controlled trial. The Lancet. 2015; 385: 1511–1518.
[90] Confalonieri M, Urbino R, Potena A, Piattella M, Parigi P, Puccio G, *et al*. Hydrocortisone infusion for severe community-acquired pneumonia: a preliminary randomized study. American Journal of Respiratory and Critical Care Medicine. 2005; 171: 242–248.
[91] Snijders D, Daniels JMA, de Graaff CS, van der Werf TS, Boersma WG. Efficacy of corticosteroids in community-acquired pneumonia: a randomized double-blinded clinical trial. American Journal of Respiratory and Critical Care Medicine. 2010; 181: 975–982.
[92] Marti C, Grosgurin O, Harbarth S, Combescure C, Abbas M, Rutschmann O, *et al*. Adjunctive corticotherapy for community acquired pneumonia: a systematic review and meta-analysis. PLoS One. 2015, 10: e0144032.
[93] Siempos II, Vardakas KZ, Kopterides P, Falagas ME. Adjunctive therapies for community-acquired pneumonia: a systematic review. Journal of Antimicrobial Chemotherapy. 2008; 62: 661–668.
[94] Nie W, Zhang Y, Cheng J, Xiu Q. Corticosteroids in the treatment of community-acquired pneumonia in adults: a meta-analysis. PLoS One. 2012; 7: e47926.
[95] Siemieniuk RAC, Meade MO, Alonso-Coello P, Briel M, Evaniew N, Prasad M, *et al*. Corticosteroid therapy for patients hospitalized with community-acquired pneumonia: a systematic review and meta-analysis. Annals of Internal Medicine. 2015; 163: 519–528.
[96] Horita N, Otsuka T, Haranaga S, Namkoong H, Miki M, Miyashita N, *et al*. Adjunctive systemic corticosteroids for hospitalized community-acquired pneumonia: systematic review and meta-analysis 2015 update. Scientific Reports. 2015; 5: 14061.
[97] Wan Y, Sun T, Liu Z, Zhang S, Wang L, Kan Q. Efficacy and safety of corticosteroids for community-acquired pneumonia: a systematic review and meta-analysis. Chest. 2016; 149: 209–219.
[98] Briel M, Spoorenberg SMC, Snijders D, Torres A, Fernandez-Serrano S, Meduri GU, *et al*. Corticosteroids in patients hospitalized with community-acquired pneumonia: systematic review and individual patient data metaanalysis. Clinical Infectious Diseases. 2018; 66: 346–354.
[99] Stern A, Skalsky K, Avni T, Carrara E, Leibovici L, Paul M. Corticosteroids for pneumonia. Cochrane Database of Systematic Reviews. 2017; 12: CD007720.
[100] Wu W, Fang Q, He G. Efficacy of corticosteroid treatment for severe community-acquired pneumonia: a meta-analysis. The American Journal of Emergency Medicine. 2018; 36: 179–184.
[101] Bi J, Yang J, Wang Y, Yao C, Mei J, Liu Y, *et al*. Efficacy and safety of adjunctive corticosteroids therapy for severe community-acquired pneumonia in adults: an updated systematic review and meta-analysis. PLoS One. 2016; 11: e0165942.
[102] Ceccato A, Russo A, Barbeta E, Oscanoa P, Tiseo G, Gabarrus A, *et al*. Real-world corticosteroid use in severe pneumonia: a propensity-score-matched study. Critical Care. 2021; 25: 432.
[103] Moreno G, Rodríguez A, Reyes LF, Gomez J, Sole-Violan J, Díaz E, *et al*. Corticosteroid treatment in critically ill patients with severe influenza pneumonia: a propensity score matching study. Intensive Care Medicine. 2018; 44: 1470–1482.
[104] Lansbury LE, Rodrigo C, Leonardi-Bee J, Nguyen-Van-Tam J, Shen Lim W. Corticosteroids as adjunctive therapy in the treatment of influenza: an updated Cochrane systematic review and meta-analysis. Critical Care Medicine. 2020; 48: e98–e106.
[105] Arabi YM, Mandourah Y, Al-Hameed F, Sindi AA, Almekhlafi GA, Hussein MA, *et al*. Corticosteroid therapy for critically ill patients with Middle East respiratory syndrome. American Journal of Respiratory and Critical Care Medicine. 2018; 197: 757–767.
[106] Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, *et al*. Dexamethasone in hospitalized patients with Covid-19. New England Journal of Medicine. 2021; 384: 693–704.
[107] Sterne JAC, Murthy S, Diaz JV, Slutsky AS, Villar J, Angus DC, *et al*. Association between administration of systemic corticosteroids and mortality among critically ill patients with COVID-19: a meta-analysis. The Journal of the American Medical Association. 2020; 324: 1330–1341.
[108] Reyes LF, Rodriguez A, Bastidas A, Parra-Tanoux D, Fuentes YV, García-Gallo E, *et al*. Dexamethasone as risk-factor for ICU-acquired respiratory tract infections in severe COVID-19. Journal of Critical Care. 2022; 69: 154014.
[109] Ranzani OT, Ferrer M, Esperatti M, Giunta V, Li Bassi G, Carvalho CRR, *et al*. Association between systemic corticosteroids and outcomes of intensive care unit-acquired pneumonia. Critical Care Medicine. 2012; 40: 2552–2561.
[110] Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA. Latent class analysis of ARDS subphenotypes: analysis of data from two randomized controlled trials. The Lancet Respiratory Medicine. 2014; 2: 611–620.
[111] Gautam S, Sharma L, Dela Cruz CS. Personalizing the management of pneumonia. Clinics in Chest Medicine. 2018; 39: 871–900.
[112] Matthay MA, Arabi YM, Siegel ER, Ware LB, Bos LDJ, Sinha P, *et al*. Phenotypes and personalized medicine in the acute respiratory distress syndrome. Intensive Care Medicine. 2020; 46: 2136–2152.
[113] Ware LB, Matthay MA, Mebazaa A. Designing an ARDS trial for 2020 and beyond: focus on enrichment strategies. Intensive Care Medicine, 2020; 46: 2153–2156.

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SYSTEMATIC REVIEW

Precision medicine in Acute Respiratory Distress Syndrome

Nanon F.L. Heijnen[1,*], Dennis C.J.J. Bergmans[1,2], Marcus J. Schultz[3,4,5,6], Lieuwe D.J. Bos[3,7]

[1]Department of Intensive Care Medicine, Maastricht University Medical Center+, 6229 HX Maastricht, The Netherlands
[2]NUTRIM school of Nutrition and Translational Research, Maastricht University, 6229 HX Maastricht, The Netherlands
[3]Department of Intensive Care Medicine, Amsterdam University Medical Centers, location Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands
[4]Laboratory of Experimental Intensive Care and Anesthesiology (L·E·I·C·A), Academic Medical Centers, location Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands
[5]Mahidol-Oxford Tropical Medicine Research Unit (MORU), Mahidol University, 10330 Bangkok, Thailand
[6]Nuffield Department of Medicine, University of Oxford, OX3 Oxford, UK
[7]Department of Respiratory Medicine, Amsterdam University Medical Centers, location Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands

***Correspondence**
nanon.heijnen@mumc.nl
(Nanon F.L. Heijnen)

Abstract

Many patients with acute respiratory failure fulfill the diagnosis of Acute Respiratory Distress Syndrome (ARDS), forming a very heterogeneous population. Clinical trials have not yielded beneficial treatment effects in ARDS, possibly caused by this heterogeneity. Dividing patients with ARDS into subgroups, each with similar characteristics, may result in improved treatment strategies as part of a precision medicine approach. In this systematic review, we summarize the subphenotypes identified so far, the current state, and future directions for precision medicine in ARDS. Multiple data-driven subphenotypes have been identified based on a wide range of variables. These subphenotypes are associated with differences in clinical outcomes, which could be used for prognostic- and predictive enrichment of future interventional studies. True treatable traits have not been identified yet, deeper phenotyping will hopefully reveal these along with mechanistic differences.

Keywords

Precision medicine; Phenotypes; ARDS

1. Introduction

Around 10% of critically ill receiving invasive ventilation fulfill the Berlin definition for Acute Respiratory Distress Syndrome (ARDS), approximately 1.5 cases per 100,000 person-years in Europe alone. It is associated with a high mortality and considerable morbidity [1, 2]. The Berlin definition specifies ARDS as acute onset hypoxemia, bilateral opacities on chest radiography, not fully explained by effusion, collapse or nodules, which is not due to cardiac dysfunction or volume overload [1, 3]. It is important to realize that this syndrome comprises a heterogeneous patient population with a multiplicity of underlying pathophysiological processes resulting in alveolar epithelial and lung endothelial injury, increased lung vascular permeability, and protein-rich alveolar oedema [1]. Randomized clinical trials (RCTs) with drugs targeting specific pathways that have been implicated in the pathophysiology of ARDS, like oxidative stress and endothelial injury, failed to improve outcomes. Therefore, supportive therapy remains the cornerstone of care for ARDS [4].

One reason for this failure could be the heterogeneity of the syndrome, which makes a "one-size fits all" approach insufficient [4, 5]. Precision medicine is defined as "*treatments targeted to the need of individual patients on the basis of genetic, biomarker, phenotypic, or psychosocial characteristics that distinguish a given patient from other patients with similar clinical presentations*" [6]. Human epidermal growth factor receptor 2 (HER-2) targeted therapy in breast cancer and type 2 (eosinophilic) asthma endotype-specific treatment are examples of precision medicine approaches that have revolutionized the treatment of syndrome diagnoses [7–9]. These examples illustrate that dividing a group of patients with the same syndrome into subgroups, each with similar characteristics, can result in improved treatment strategies. This has led the researchers in critical care to speculate that a precision medicine approach would be appropriate for a heterogeneous syndrome like ARDS as well [10].

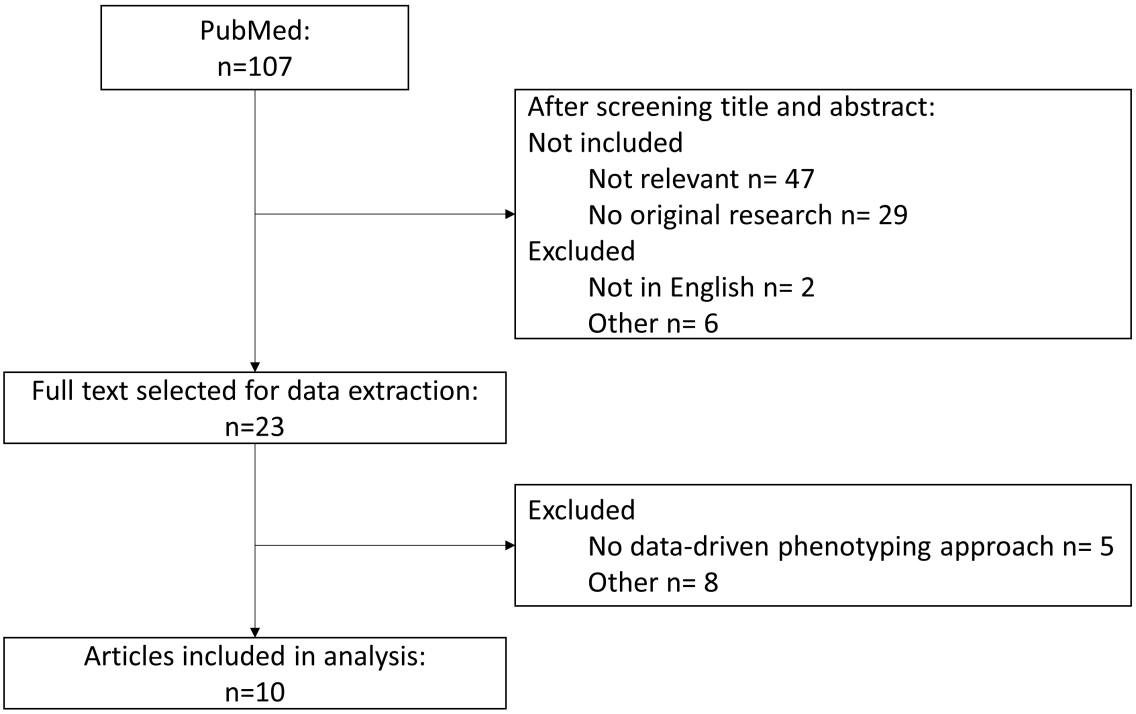

FIGURE 1. Flowchart of article selection.

In this systematic review, we present the current understanding of precision medicine in ARDS. We provide an overview of the currently identified subgroups in ARDS based on data-driven approaches, evaluate the evidence for heterogeneity of treatment effect in patients with distinct subphenotypes and speculate on the future directions for precision medicine in ARDS.

2. Search strategy and selection criteria

Relevant articles were identified by a search on PubMed for articles up to May 17, 2021, with the terms: "ARDS", "acute lung injury (ALI)", "Critical Care", "Intensive Care", "Critical illness", "Phenotype", "Subphenotype", "Subgroups", "Endotypes", and "Cluster". Inclusion criteria were (1) original research in (2) adult critically ill patients with ARDS (3) identifying subphenotypes based on patient data (4) using clustering analysis algorithms and (5) providing prognostic or predictive value. Studies using pre-defined not data-driven subgroups or studies on cell phenotypes, animal or preclinical work were excluded. Only articles published in English were considered. See Fig. 1 for flowchart of article selection. After reading, ten original articles remained which fulfilled the selection criteria for this review.

3. Definitions in precision medicine

Recently, definitions have been proposed to standardize the terminology used in the search for targetable (sub) phenotypes in the critically ill and associated broad defined syndromes, like ARDS.

In this review, we use the following: (1) Phenotype — "A set of clinical features in a group of patients who share a common syndrome or condition", (2) Subphenotype — "A set of features in a group of patients who share a phenotype, such as shared risk factor, trait, diagnostic feature, expression marker, mortality risk, or outcome in response to treatment, that distinguishes the group from other groups of patients with the same phenotype", (3) Endotype — "A distinct biological mechanism of disease, often associated with an anticipated response to treatment, that is shared by a subgroup of patients and might be indicated by shared mortality risk, clinical course, or treatment responsiveness", and (4) Treatable trait — "A subgroup characteristic that can be successfully targeted by an intervention" [10, 11].

It should be noted that a subphenotype does not necessarily comprise an endotype. For a subphenotype to have an endotype there must be a mechanistic difference between the subphenotypes, which can be identified by certain markers. Similarly, it should be noted that an endotype does not mean there is a treatable trait. Only if a mechanistic difference can be successfully targeted by plausible treatment, a treatable trait has been identified. This is the ultimate goal of precision medicine.

In addition to identification of treatable traits, (sub) phenotyping can also be used as a tool for prognostic- and predictive enrichment strategies in RCTs. Enrichment is a core tenet of precision medicine. Using prognostic enrichment, patients with a higher risk at a worse outcome or disease-related endpoint are selected, thereby increasing the absolute effect difference between groups [12]. Predictive enrichment entails selecting patients more likely to respond to a given therapy, increasing both absolute and relative effect, possibly resulting in a smaller required study population [12]. These strategies stimulate development of new drug therapies and

FIGURE 2. Heatmap of all included class-defining variables and identified predictive variables in ARDS subphenotyping models. Only variables significant in at least one study are depicted. Class-defining variables were used for identifying subphenotypes. Predictive variables were used to classify the subphenotypes using fewer variables. The increased class-defining variables: (1) gender implicates a higher percentage of males and (2) ethnicity implicates a higher percentage of white people present in that subphenotype, (3) source of infection is pre-dominantly the thorax, and (4) ARDS risk factor is pre-dominantly pneumonia. Subphenotypes depicted on the left side of the graph were associated with worse clinical outcomes compared to the subphenotypes depicted on the right side of the graph. The numbers above the subphenotypes refer to the original study and correspond to the reference bibliography number. BMI, body mass index; CRP, C-reactive protein; WBC, white blood cell count; ANG-2, angiopoietin-2; ANG2/1, angiopoietin 2 and 1 ratio; ICAM-1, intercellular adhesion molecule-1; IFNy, interferon gamma; IL, interleukin; PAI-1, plasminogen activator inhibitor 1; RAGE, receptor for advanced glycation end products; TNFR1, tumor necrosis factor receptor 1; PF ratio, PaO_2/FiO_2 ratio.

TABLE 1. Overview of prevalence and clinical outcome parameters per subphenotype identified in ARDS.

		Prevalence (%)	ICU-mortality (%)	Ventilator-free days (n)	28-day mortality (%)	30-day mortality (%)	60-day mortality (%)	90-day mortality (%)
Ranjeva *et al.* (2021) [13]	Phenotype 1	193 (73%)	-	-	23.3%	-	-	-
	Phenotype 2	70 (27%)	-	-	40.0%	-	-	-
Garcia *et al.* (2021) [14]	Non-recruitable	106 (45%)	27 (23%)	-	-	-	-	-
	Recruitable	132 (55%)	69 (52%)	-	-	-	-	-
Puybassat *et al.* (2000) [15]	Non-focal	45 (63%)	24 (53%)	-	-	-	-	-
	Focal	26 (37%)	11 (42%)	-	-	-	-	-
Bos *et al.* (2017) [22]	Uninflamed	218 (48%)	34 (15.6%)	21 (11–25)	-	47 (21.6%)	-	-
	Reactive	236 (52%)	86 (36.4%)	9 (0–21)	-	89 (37.7%)	-	-
Calfee *et al.* (2014) [21]	Hypoinflammatory	318 (67%)	-	17.8	-	-	-	23%
	Hyperinflammatory	155 (33%)	-	7.7	-	-	-	44%
Calfee *et al.* (2014) [21]	Hypoinflammatory	404 (74%)	-	18.4	-	-	-	19%
	Hyperinflammatory	145 (26%)	-	8.3	-	-	-	51%
Famous *et al.* (2017) [20]	Hypoinflammatory	727 (73%)	-	19	-	-	21%	22%
	Hyperinflammatory	273 (27%)	-	3	-	-	44%	45%
Sinha *et al.* (2018) [19]	Hypoinflammatory	468 (60%)	-	23 (6–26)	-	-	98 (20.9%)	100 (21.4%)
	Hyperinflammatory	277 (40%)	-	15 (1–23)	-	-	101 (36.5%)	104 (37.6%)
Calfee *et al.* (2018) [18]	Hypoinflammatory	353 (65%)	-	18 (0–23)	59 (17%)	-	-	78 (22%)
	Hyperinflammatory	186 (35%)	-	2 (0–17)	73 (39%)	-	-	87 (47%)
Sinha *et al.* (2021) [16]	Hypoinflammatory	457 (73%)	-	20 (11–25)	-	-	-	-
	Hyperinflammatory	167 (27%)	-	5 (0–20)	-	-	-	-
Sinha *et al.* (2021) [16]	Hypoinflammatory	211 (63%)	-	24 (0–28)	-	-	-	-
	Hyperinflammatory	124 (37%)	-	0 (0–23)	-	-	-	-
Kitsios *et al.* (2019) [17]	Hypoinflammatory	65 (62%)	-	ns	ns	-	-	ns
	Hyperinflammatory	39 (38%)	-	ns	ns	-	-	ns

All presented data is significant, except for ns (not significant). A dash represents an uninvestigated parameter. ARDS, Acute Respiratory Distress Syndrome; ICU, Intensive Care Unit.

tailoring treatments to patients most likely to benefit from them. Combined, prognostic- and predictive enrichment allow for optimal progress towards precision medicine.

4. Identified ARDS subphenotypes

A variety of strategies have been applied in order to identify subphenotypes in ARDS, covering aspects of etiology, physiology and morphology, and biology. Fig. 2 provides an overview of the identified subphenotypes, including the used class-defining variables and predictive variables. Table 1 (Ref. [13–22]) presents an overview of the subphenotypes with their prevalence and associated clinical outcomes. All described subphenotypes are based on clustering algorithms using a set of variables that did not include clinical outcomes.

4.1 Clinically-derived subphenotypes

Thus far, two subphenotypes have been identified (1 & 2) using readily available clinical data from a cohort of ARDS patients that had acute respiratory failure related to COVID-19 (Fig. 2). One subphenotype, named 'Phenotype 2', showed increased markers of coagulopathy, like D-dimer, prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen, compared to the other subphenotype, named 'phenotype 1'. White blood cell count and interleukin-6 (IL-6) were higher in 'phenotype 2', but plasma IL-6 concentration was much lower than has been observed in patients with ARDS due to other causes than COVID-19. There was no difference in parameters related to respiratory physiology, such as PaO_2:FiO_2, driving pressure, minute ventilation, and $PaCO_2$. There was strong evidence for prognostic enrichment as patients with 'phenotype 2' had double the odds for 28-day mortality than patients with 'phenotype 1' (odds ratio (OR) 2.2; 95% confidence interval (CI) 1.2–3.9; Table 1) [13].

4.2 Physiology and morphology-derived subphenotypes

A 'non-recruitable' and 'recruitable' subphenotype have been identified in patients with ARDS not related to COVID-19 using latent class analysis on a broad set of parameters related to respiratory mechanics, gas-exchange and Computer Tomography(CT)-derived gas- and tissue volume (Fig. 2). The non-recruitable subphenotype was associated with a non-pulmonary cause of ARDS, fewer moderate-severe ARDS cases, a lower respiratory system elastance, a decreased alveolar dead space, less potentially recruitable lung volume, and less inhomogeneous lungs compared to the recruitable subphenotype. The recruitable subphenotype could be used for prognostic enrichment as it was associated with an increased risk of ICU-mortality (HR 2.9, 95% CI 1.7–2.7) (Table 1) [14].

Three radiological subphenotypes of ARDS were described: lobar attenuations ('LA'); diffuse attenuations ('DA') and patchy attenuations ('PA'). These were later redefined as 'focal' ARDS (LA subphenotype) and 'non-focal' ARDS (DA and PA subphenotype) [15, 23]. It is important to note that these subphenotypes were not the result of data-driven evaluation of the CT images, but rather the result of systematic evaluation of these scans by human operators. Non-focal lung morphology is characterized by diffuse and patchy lung aeration loss (increased inhomogeneity) and distinct lung mechanics including decreased total lung gas volume, a lower compliance of the respiratory system and a higher amount of recruitable lung compared with focal ARDS. This subphenotype has also been associated with an increased ICU-mortality in a more recent study [23].

4.3 Biology-derived subphenotypes

Biological data, such as plasma biomarkers, have also been used to identify subphenotypes in ARDS. Two subphenotypes, named "reactive" and "uninflamed", were identified based on 20 plasma biomarkers of inflammation, coagulation, and endothelial activation (Fig. 2). The "reactive" subphenotype could be characterized by high plasma levels of inflammation, coagulation, and endothelial activation. Patients with the "reactive" subphenotype more frequently had a non-pulmonary cause for ARDS. Patients with the "reactive" subphenotype showed prognostic enrichment as it was associated with a higher ICU- and 30-day mortality and less ventilator-free days (Table 1) [22].

4.4 Subphenotypes based on combined variables

The majority of publications report on analyses based on combinations of clinical and biological variables. Two subphenotypes, named the "hypoinflammatory" and "hyperinflammatory", have been consistently identified throughout multiple datasets using latent class analysis. The hyperinflammatory subphenotype has been characterized by higher plasma concentrations of IL-6, IL-8, soluble tumor necrosis factor receptor-1 (sTNFR1), and plasminogen activator inhibitor-1 (PAI-1), higher heart rate and total minute ventilation. This subphenotype also had a lower systolic blood pressure, bicarbonate, and protein C compared to the hypoinflammatory subphenotype. In other words, the hyperinflammatory subphenotype reflects a more severe inflammation, shock, and metabolic acidosis. There was prognostic enrichment for mortality and duration of mechanical ventilation [21]. In several subsequent secondary analyses of RCTs in ARDS, similar subphenotype profiles were identified, which validated the original finding [18–20]. Even the use of a less comprehensive dataset revealed two subphenotypes with comparable characteristics and clinical outcomes [18]. This is indicative of the robustness of these subphenotypes in a highly selected patient population of ARDS. Importantly, the "hypo-" and "hyperinflammatory" subphenotypes were also identified in prospective observational cohort studies using a more comprehensive set of variables. These studies confirmed the potential for prognostic enrichment of the hyperinflammatory subphenotype in an unselected population of consecutive ARDS patients [16, 17].

5. Evidence for heterogeneity of treatment effect

Each of the above described subphenotype approaches revealed a subgroup with an increased risk of mortality and

selection of this subgroup could be used for prognostic enrichment. Differences in baseline risk of death could introduce non-random variation in treatment effect (heterogeneity of treatment effect, HTE), which might explain some indeterminate results of previous RCTs [24–26]. However, predictive enrichment of future intervention studies could provide more considerable HTE and this is most important for the design of future precision medicine studies.

Secondary analyses of three RCTs in ARDS patients showed potential HTE when using identified subphenotypes for risk stratification. Firstly, the multicenter Assessment of Low Tidal Volume and Elevated End-Expiratory Pressure to Obviate Lung Injury (ALVEOLI) trial compared the effect of mechanical ventilation with higher versus lower positive end-expiratory pressure (PEEP) within 36h of ARDS onset on mortality. The original analysis showed similar clinical outcomes regardless of the PEEP levels used [27]. A secondary analysis of this trial showed a subphenotype-dependent treatment effect. Patients with the hyperinflammatory subphenotype who received the high PEEP strategy had improved clinical outcomes (reduced mortality, more ventilator-free days and organ failure free-days) compared to the low PEEP strategy. Patients with the "hypoinflammatory" subphenotype showed strikingly opposite results with improved clinical outcomes using a low PEEP strategy compared to a high PEEP strategy [21]. Secondly, the Fluid and Catheter Treatment (FACTT) trial compared the effect of conservative versus liberal fluid management within 48h of ARDS onset on mortality. Conservative fluid management shortened the duration of mechanical ventilation, without showing a difference in 60-day mortality [28]. In a secondary analysis, hyperinflammatory patients had improved clinical outcomes (reduced 60- and 90-day mortality) when randomized to the liberal fluid strategy as compared to the conservative fluid strategy, while the hypoinflammatory patients showed the inverse association. However, no subphenotype-dependent significant difference in ventilator-free days was observed [20]. Thirdly, the multicenter Hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in Acute lung injury to Reduce Pulmonary dysfunction (HARP-2) trial compared the effect of simvastatin versus placebo within 48h of ARDS onset on ventilator-free days. No differences in clinical outcomes were found (ventilator-free days, non-pulmonary organ failure, and 28-day mortality) [29]. However, differences were observed across patients stratified by treatment and subphenotype in a secondary analysis. Specifically, patients with the hyperinflammatory subphenotype had a higher 28-day survival using simvastatin compared to placebo [19]. In addition, potential HTE for simvastatin in ARDS was also observed in another secondary analysis using the APACHE II score as risk modifier [25]. Combined, these secondary analyses support the idea that indeterminate trial results can be the result of heterogeneity in trial populations. Subphenotyping could play a role in predictive enrichment trial strategies by reducing some of the heterogeneity within the larger ARDS population.

The first and currently only prospective evaluation of a precision medicine by subphenotypes in a RCT is the LIVE-trial: Lung Imaging for Ventilator Settings in ARDS [30]. They tested whether personalized mechanical ventilation strategies based on morphology subphenotypes (non-focal and focal) improved the overall survival of ARDS patients compared to standard care. Personalized mechanical ventilation strategies entailed tailored tidal volumes, PEEP levels, recruitment manoeuvres, and prone positioning per group (PP). The primary analysis of the LIVE-trial did not show survival benefit in favour of the precision medicine approach (HR: 1.01; 95% CI 0.61–1.66, $p = 0.98$). Further analysis showed that in 21% (85 out of 400) of all included patients the lung morphology was misclassified based on chest imaging. For the classification of non-focal and focal ARDS both CT-scan and chest radiography was allowed, but CT scans were performed only in 29% (56 patients) of the patients randomized to the precision medicine approach. Despite the high agreement about lung morphology classification between experts (k = 0.94), only moderate agreement was found between local investigators who allocated patients to the precision medicine approach (k = 0.52). The high likelihood of misclassification can be explained by the limited availability of CT-scans and misinterpretation of chest radiography. Interestingly, subgroup analyses revealed that: (1) correctly classified patients receiving personalized mechanical ventilation had lower 90-day mortality compared to the control group and (2) that expert classification revealed the same beneficial effect, but (3) misclassified patients had higher 90-day mortality when receiving personalized mechanical ventilation compared to the control group. So, due to the possible influence of misclassification, the contribution of using morphology subphenotypes for precision mechanical ventilation remains uncertain. These trial results emphasize (1) the requirement of subphenotypes to be robust and not subject to individual interpretation and (2) that misclassification can harm patients.

5.1 Subphenotype classification using parsimonious models and time-related changes

Most of the cluster and latent class analyses algorithms are not suitable for clinical classification of patients at the bedside due to the number of variables required as input. Therefore, predictive models containing fewer variables have been identified to classify patients with high accuracy (Fig. 2; Table 2, Ref. [14, 16, 17, 19–21, 31]). This also provides guidance in developing classifying tests suitable for clinical practice, like IL-6 and TNFR1 point-of-care tests (ClinicalTrials.gov Identifier: NCT04009330). Awaiting these point-of-care tests for specific plasma markers, researchers were recently able to classify patients in hypo- and hyperinflammatory subphenotypes using readily available clinical data including demographics variables (e.g., age, sex, ARDS risk factor), respiratory variables (e.g., PaO_2/FiO_2 ratio, $PaCO_2$), vital signs (e.g., temperature, heart rate, respiratory rate), and laboratory variables (e.g., hematocrit, white cell count, platelets, sodium) with high accuracy (AUC: 0.95; 95% CI 0.94–0.96; Table 2) [31]. Although this classification was performed in highly selected study populations, this finding is very promising. Together with previous results, this provides multiple opportunities to enable classification in clinical practice.

TABLE 2. Predictive models classifying ARDS subphenotypes.

	Subphenotypes	Predictive model variables	AUC
Calfee *et al.* (2014) [21]	Hypoinflammatory vs. Hyperinflammatory (ARMA)	IL-6, sTNFR1, vasopressor use	0.94
Calfee *et al.* (2014) [21]	Hypoinflammatory vs. Hyperinflammatory (ALVEOLI)	IL-6, sTNFR1, vasopressor use	0.93
Famous *et al.* (2017) [20]	Hypoinflammatory vs. Hyperinflammatory (FACTT)	IL-8, sTNFR1, bicarbonate	0.95
Sinha *et al.* (2018) [19]	Hypoinflammatory vs. Hyperinflammatory (SAILS)	IL-8, sTNFR1, bicarbonate	0.95
Kitsios *et al.* (2019) [17]	Hypoinflammatory vs. Hyperinflammatory	Not defined	0.93
Sinha *et al.* (2020) [31]	Hypoinflammatory vs. Hyperinflammatory (ARMA, ALVEOLI, SAILS, FACTT)	IL-8, protein C, bicarbonate, vasopressor use.	0.96
Sinha *et al.* (2020) [31]	Hypoinflammatory vs. Hyperinflammatory (ARMA, ALVEOLI, SAILS, FACTT)	Clinical classifier model (demographic, respiratory, vital signs, laboratory data)	0.95
Sinha *et al.* (2021) [16]	Hypoinflammatory vs. Hyperinflammatory (VALID, EARLI)	IL-8, protein C, bicarbonate, vasopressor use	0.94
Bos *et al.* (2017) [22]	Uninflamed vs. reactive	Il-6, IFN-gamma, ANG2/1, PAI-1	0.98
Garcia *et al.* (2021) [14]	Non-recruitable vs. Recruitable	Dead space, respiratory system elastance, lung inhomogeneity, proportion of non-aerated lung tissue	0.99

ARMA, ALVEOLI, SAILS, and FACTT are different randomized controlled trial cohorts in patients with ARDS. VALID and EARLI are prospective observational cohort studies in patients with ARDS. Abbreviations: IL, Interleukin; IFN-gamma, interferon gamma; ANG, angiopoietin; PAI-1, plasminogen activator inhibitor 1; sTNFR1, soluble tumor necrosis factor receptor 1.

All above-described subphenotypes have been identified using data obtained at ICU admission or at enrollment in clinical trials. The hypo- and hyperinflammatory subphenotype have shown to be largely stable over the first 3 days [32]. As it remains uncertain whether subphenotypes reflect different temporal stages in ARDS, it is important for the usability of subphenotype classification in clinical trials to evaluate the subphenotype stability over the evolution of ARDS. Baseline levels of innate immunity biomarkers (TNFR1, fractalkine, and ST-2) and procalcitonin were higher in the hyperinflammatory patients and showed similar trajectory overtime compared to hypoinflammatory patients. However, angiopoietin-2 (ANG-2) (endothelial injury) and receptor for advanced glycation end products (RAGE; marker of epithelial injury) attenuated over time [17]. Hypothetically, if this host-response trajectory also occurs in the reactive subphenotype (which is plausible as the reactive and hyperinflammatory subphenotype have similar characteristics), this could influence the classification, since ANG-2 is used in the prediction model for the reactive and uninflamed subphenotype [22].

In a secondary analysis of the Evaluating Health Outcomes and QOL After ALI Among Participants of the ALTA, OMEGA, EDEN, and SAILS ARDS Network Trials (SAILS-ALTOS) with a long term follow-up (up to 12 months), the physical, mental health, and cognitive outcomes were not different between patients who were classified as having the hypo- or hyperinflammatory subphenotype at study enrollment [33]. This might suggest that these subphenotypes reflect an acute phase of critical illness, resolve at some point and that other factors attribute to long term dysfunction.

5.2 Underlying processes captured by subphenotypes

The identified subphenotypes have not been linked directly to pathophysiological mechanisms leading to ARDS. It is noteworthy that in studies (which included biological data) the most important contributing class-defining variables are linked to the innate immune response (i.e., TNFR1, IL-6, IL-8) [17, 18, 20, 21]. It could be speculated that these subphenotypes reflect a more general underlying inflammatory reaction, as these markers are not ARDS-specific. This is supported by the identification and validation of resembling subphenotypes (hypo-/hyperinflammatory and unreactive/reactive) in both patients at risk for ARDS and mechanically ventilated patients without ARDS with similar characteristics, blood leukocyte gene expression profiles, and clinical outcomes [17, 34–37].

COVID-19 has added another frequent cause for ARDS. Patients with COVID-19-associated ARDS did not show the extensive systemic inflammatory response seen in non-COVID-19 related ARDS. Patients with COVID-19 also much more frequently had single organ failure [13]. Remarkably, an exploratory analysis revealed a lower prevalence of the hyperinflammatory subphenotype in COVID-19-associated ARDS compared to the other ARDS cohorts, and surprisingly higher 28-day mortality rates for both subphenotypes in COVID-19-associated ARDS [38]. This highlights that clustering algorithms might not be sufficient when leaving fundamental differences, like etiology and risk factors, out of the scope even when a wide range of variables were used in the derivation phase.

Given the multiple subphenotypes described in this review, it is possible that we end up with a multi-layered system just like in asthma, where stratification is based on age of symptom onset, lung function (FEV1), allergic status and type of airway inflammation [9]. In ARDS, the following layers could be considered: etiology, lung morphology, abnormalities in gas exchange, and biology. Integrating these aspects into intervention studies and clinical care is one of the key challenges for future research.

6. Towards precision medicine in ARDS

While there are promising results with regards to identified subphenotypes, the goal of precision medicine -identifying treatable traits- has not yet been reached. To generate treatable traits, it is pivotal to increase our knowledge about the underlying pathophysiological mechanisms reflected by the identified subphenotypes, allowing us to link biological differences and determine whether a marker doesn't only differentiate but also acts as a mediator. The current subphenotypes are mainly derived from clinical and blood biomarker data, omitting the pulmonary biology. The clinical pulmonary parameters included provide superficial insight into the pulmonary status but do not show a consistent difference between both subphenotypes [18, 19, 21]. The majority of the subphenotypes are identified in secondary analyses of datasets from RCTs with ARDS patients, which could be an explanation for the lack of consistent difference and advocates for studies with an unselected population. Furthermore, preliminary results with a small sample size showed no profound differences in a selected set of alveolar inflammatory mediators, emphasizing the importance of elucidating the pulmonary biology within the identified subphenotypes [39]. Despite the challenges associated with mapping the lung compartment, the link between the biological progression or resolution of the identified subphenotypes, and the phases in the pathogenesis of ARDS should be explored. Increasing our understanding of these subphenotypes in several areas is pivotal in order to understand the beneficial and harmful aspects of the host response within each subphenotype which could reveal the next steps towards precision medicine in ARDS.

As shown in Fig. 2, there is a broad range of class-defining variables that differ between subphenotypes resulting in unique sets of predictive variables. However, there is also considerable overlap and this allows us to integrate the available evidence into a bigger picture (Fig. 3). For example: the hyperinflammatory subphenotype is associated with worse clinical outcomes, more likely to have a non-pulmonary primary risk factor, and patients have increased levels of circulating RAGE compared to the hypoinflammatory subphenotype [18, 20, 21]. Subphenotypes based on lung morphology showed that the non-focal subphenotype was associated with worse clinical outcomes, alveolar fluid clearance (AFC) impairment, and increased RAGE levels [40]. Strikingly, RAGE itself seems to be inversely correlated to AFC rates [41]. One could therefore postulate that the non-focal subphenotype and hyperinflammatory subphenotype overlap to a large extent. If so, the RAGE

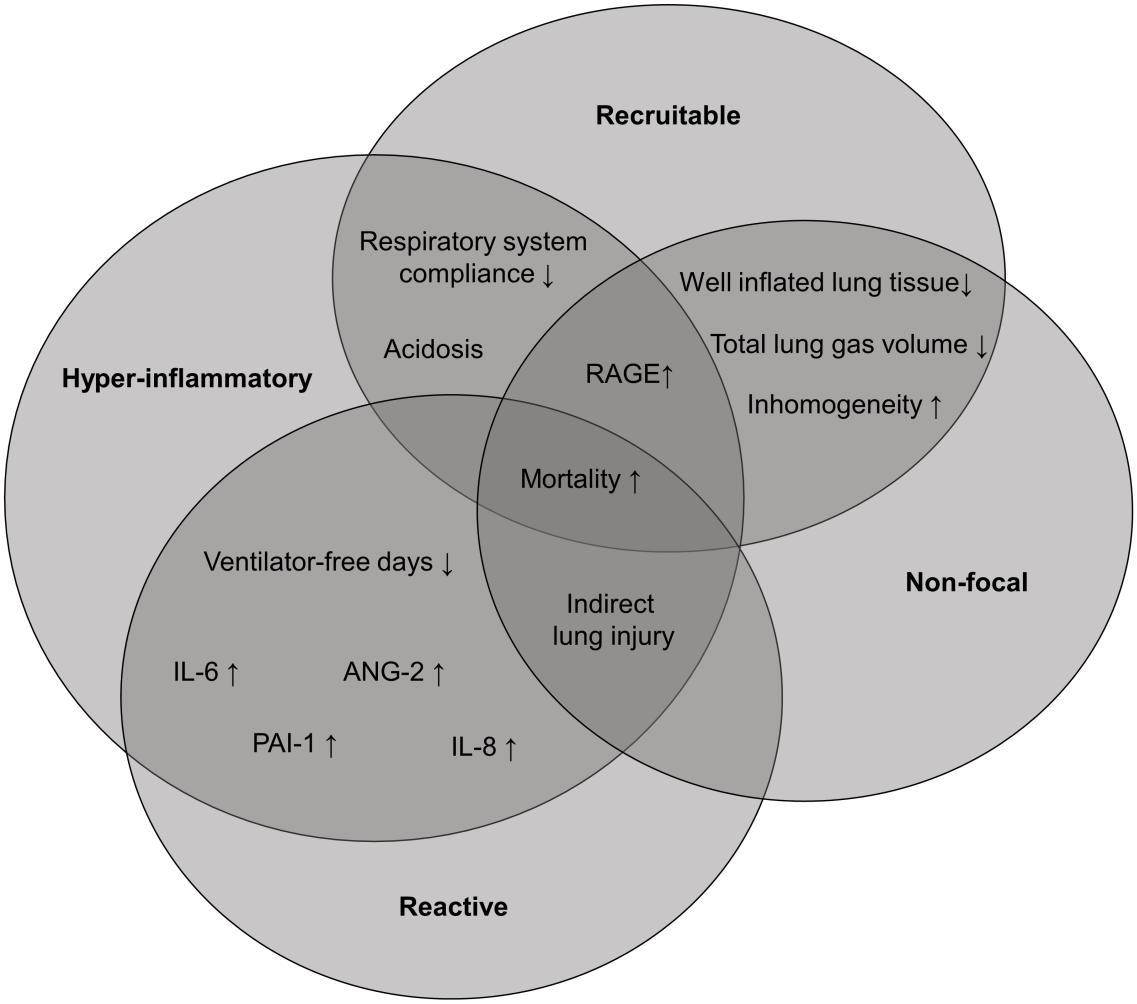

FIGURE 3. Venn diagram depicting an example of overlapping established associations between selected variables and the hyperinflammatory, reactive, recruitable, and non-focal subphenotype. RAGE, receptor for advanced glycation end products.

pathway could be of interest as possible treatable trait. Since these subphenotypes were derived from completely disjointed variables, overlap might be a key indicator of possible pathways to target for researching treatable traits.

7. Conclusions

The recognition of ARDS heterogeneity has created an opportunity to identify various subphenotypes, associated with different clinical outcomes. Key challenges will be (1) the characterization of the lung compartment and (2) integrating our subphenotypes related to clinical variables, lung morphology, gas-exchange abnormalities and biology in pre-clinical models and clinical trials. Deeper phenotyping, with parallel use of prognostic- and predictive enrichment strategies, will hopefully reveal mechanistic differences and treatable traits, marking the beginning of precision medicine in ARDS.

AUTHOR CONTRIBUTIONS

NFLH, DCJJB, MJS, LDJB contributed to the study concept and design. NFLH performed the data collection and wrote the first draft of the manuscript. DCJJB, MJS, LDJB commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Thompson BT, Chambers RC, Liu KD. Acute Respiratory Distress Syndrome. New England Journal of Medicine. 2017; 377: 562–572.

[2] Confalonieri M, Salton F, Fabiano F. Acute respiratory distress syndrome. European Respiratory Review : an Official Journal of the European Respiratory Society. 2017; 26: 160116.

[3] Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, *et al*. Acute respiratory distress syndrome: the Berlin Definition. Journal of the American Medical Association. 2012; 307: 2526–2533.

[4] Matthay MA, McAuley DF, Ware LB. Clinical trials in acute respiratory distress syndrome: challenges and opportunities. The Lancet Respiratory Medicine. 2017; 5: 524–534.

[5] Bos LDJ, Artigas A, Constantin J, Hagens LA, Heijnen N, Laffey JG, *et al*. Precision medicine in acute respiratory distress syndrome: workshop report and recommendations for future research. European Respiratory Review. 2021; 30: 200317.

[6] Jameson JL, Longo DL. Precision medicine–personalized, problematic, and promising. The New England Journal of Medicine. 2015; 372: 2229–2234.

[7] Loibl S, Gianni L. Her2-positive breast cancer. Lancet. 2017; 389: 2415–2429.

[8] Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, *et al*. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. American Journal of Respiratory and Critical Care Medicine. 1999; 160: 1001–1008.

[9] Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nature Medicine. 2012; 18: 716–725.

[10] Prescott HC, Calfee CS, Thompson BT, Angus DC, Liu VX. Toward Smarter Lumping and Smarter Splitting: Rethinking Strategies for Sepsis and Acute Respiratory Distress Syndrome Clinical Trial Design. American Journal of Respiratory and Critical Care Medicine. 2016; 194: 147–155.

[11] Reddy K, Sinha P, O'Kane CM, Gordon AC, Calfee CS, McAuley DF. Subphenotypes in critical care: translation into clinical practice. The Lancet Respiratory Medicine. 2020; 8: 631–643.

[12] FDA. Enrichment Strategies for Clinical Trials to Support Determination of Effectiveness of Human Drugs and Biological Products. Guidance for Industry. 2019. Available at: https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm (Accessed: 20 May 2021).

[13] Ranjeva S, Pinciroli R, Hodell E, Mueller A, Hardin CC, Thompson BT, *et al*. Identifying clinical and biochemical phenotypes in acute respiratory distress syndrome secondary to coronavirus disease-2019. EClinicalMedicine. 2021; 34: 100829.

[14] Wendel Garcia PD, Caccioppola A, Coppola S, Pozzi T, Ciabattoni A, Cenci S, *et al*. Latent class analysis to predict intensive care outcomes in Acute Respiratory Distress Syndrome: a proposal of two pulmonary phenotypes. Critical Care. 2021; 25: 154.

[15] Puybasset L, Cluzel P, Gusman P, Grenier P, Preteux F, Rouby J-. Regional distribution of gas and tissue in acute respiratory distress syndrome. i. Consequences for lung morphology. Intensive Care Medicine. 2000; 26: 857–869.

[16] Sinha P, Delucchi KL, Chen Y, Zhuo H, Abbott J, Wang C, *et al*. Latent class analysis-derived subphenotypes are generalisable to observational cohorts of acute respiratory distress syndrome: a prospective study. Thorax. 2021. (in press)

[17] Kitsios GD, Yang L, Manatakis DV, Nouraie M, Evankovich J, Bain W, *et al*. Host-Response Subphenotypes Offer Prognostic Enrichment in Patients with or at Risk for Acute Respiratory Distress Syndrome. Critical Care Medicine. 2019; 47: 1724–1734.

[18] Calfee CS, Delucchi KL, Sinha P, Matthay MA, Hackett J, Shankar-Hari M, *et al*. Acute respiratory distress syndrome subphenotypes and differential response to simvastatin: secondary analysis of a randomised controlled trial. The Lancet Respiratory Medicine. 2018; 6: 691–698.

[19] Sinha P, Delucchi KL, Thompson BT, McAuley DF, Matthay MA, Calfee CS. Latent class analysis of ARDS subphenotypes: a secondary analysis of the statins for acutely injured lungs from sepsis (SAILS) study. Intensive Care Medicine. 2018; 44: 1859–1869.

[20] Famous KR, Delucchi K, Ware LB, Kangelaris KN, Liu KD, Thompson BT, *et al*. Acute Respiratory Distress Syndrome Subphenotypes Respond Differently to Randomized Fluid Management Strategy. American Journal of Respiratory and Critical Care Medicine. 2017; 195: 331–338.

[21] Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. The Lancet Respiratory Medicine. 2014; 2: 611–620.

[22] Bos LD, Schouten LR, van Vught LA, Wiewel MA, Ong DSY, Cremer O, *et al*. Identification and validation of distinct biological phenotypes in patients with acute respiratory distress syndrome by cluster analysis. Thorax. 2017; 72: 876–883.

[23] Rouby J-, Puybasset L, Cluzel P, Richecoeur J, Lu Q, Grenier P. Regional distribution of gas and tissue in acute respiratory distress syndrome. II. Physiological correlations and definition of an ARDS Severity Score. Intensive Care Medicine. 2000; 26: 1046–1056.

[24] Senn S. Mastering variation: variance components and personalised medicine. Statistics in Medicine. 2016; 35: 966–977.

[25] Santhakumaran S, Gordon A, Prevost AT, O'Kane C, McAuley DF, Shankar-Hari M. Heterogeneity of treatment effect by baseline risk of mortality in critically ill patients: re-analysis of three recent sepsis and ARDS randomised controlled trials. Critical Care. 2019; 23: 156.

[26] Kent DM, Rothwell PM, Ioannidis JPA, Altman DG, Hayward RA. Assessing and reporting heterogeneity in treatment effects in clinical trials: a proposal. Trials. 2010; 11: 85.

[27] Brower RG, Lanken PN, MacIntyre N, Matthay MA, Morris A, Ancukiewicz M, *et al*. Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome. The New England Journal of Medicine. 2004; 351: 327–336.

[28] Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, deBoisblanc B, *et al*. Comparison of two fluid-management strategies in acute lung injury. The New England Journal of Medicine. 2006; 354: 2564–2575.

[29] McAuley DF, Laffey JG, O'Kane CM, Perkins GD, Mullan B, Trinder TJ, *et al*. Simvastatin in the acute respiratory distress syndrome. The New England Journal of Medicine. 2014; 371: 1695–1703.

[30] Constantin J, Jabaudon M, Lefrant J, Jaber S, Quenot J, Langeron O, *et al*. Personalised mechanical ventilation tailored to lung morphology versus low positive end-expiratory pressure for patients with acute respiratory distress syndrome in France (the LIVE study): a multicentre, single-blind, randomised controlled trial. The Lancet Respiratory Medicine. 2019; 7: 870–880.

[31] Sinha P, Churpek MM, Calfee CS. Machine Learning Classifier Models can Identify Acute Respiratory Distress Syndrome Phenotypes Using Readily Available Clinical Data. American Journal of Respiratory and Critical Care Medicine. 2020; 202: 996–1004.

[32] Delucchi K, Famous KR, Ware LB, Parsons PE, Thompson BT, Calfee CS. Stability of ARDS subphenotypes over time in two randomised controlled trials. Thorax. 2018; 73: 439–445.

[33] Hashem MD, Hopkins RO, Colantuoni E, Dinglas VD, Sinha P, Aronson Friedman L, *et al*. Six-month and 12-month patient outcomes based on inflammatory subphenotypes in sepsis-associated ARDS: secondary analysis of SAILS-ALTOS trial. Thorax. 2021. (in press)

[34] Bos LDJ, Scicluna BP, Ong DSY, Cremer O, van der Poll T, Schultz MJ. Understanding Heterogeneity in Biologic Phenotypes of Acute Respiratory Distress Syndrome by Leukocyte Expression Profiles. American Journal of Respiratory and Critical Care Medicine. 2019; 200: 42–50.

[35] Heijnen NFL, Hagens LA, Smit MR, Cremer OL, Ong DSY, van der Poll T, *et al*. Biological Subphenotypes of Acute Respiratory Distress Syndrome Show Prognostic Enrichment in Mechanically Ventilated Patients without Acute Respiratory Distress Syndrome. American Journal of Respiratory and Critical Care Medicine. 2021; 203: 1503–1511.

[36] Drohan CM, Nouraie SM, Bain W, Shah FA, Evankovich J, Zhang Y,

et al. Biomarker-Based Classification of Patients with Acute Respiratory Failure into Inflammatory Subphenotypes: a Single-Center Exploratory Study. Critical Care Explorations. 2021; 3: e0518.

[37] Sathe NA, Zelnick LR, Mikacenic C, Morrell ED, Bhatraju PK, McNeil JB, *et al*. Identification of persistent and resolving subphenotypes of acute hypoxemic respiratory failure in two independent cohorts. Critical Care. 2021; 25: 336.

[38] Sinha P, Calfee CS, Cherian S, Brealey D, Cutler S, King C, *et al*. Prevalence of phenotypes of acute respiratory distress syndrome in critically ill patients with COVID-19: a prospective observational study. The Lancet Respiratory Medicine. 2020; 8: 1209–1218.

[39] Heijnen NFL, Hagens LA, Smit MR, Schultz MJ, Poll T, Schnabel RM, *et al*. Biological subphenotypes of acute respiratory distress syndrome may not reflect differences in alveolar inflammation. Physiological Reports. 2021; 9: e14693.

[40] Jabaudon M, Blondonnet R, Lutz J, Roszyk L, Bouvier D, Guérin R, *et al*. Net alveolar fluid clearance is associated with lung morphology phenotypes in acute respiratory distress syndrome. Anaesthesia, Critical Care & Pain Medicine. 2016; 35: 81–86.

[41] Jabaudon M, Blondonnet R, Roszyk L, Bouvier D, Audard J, Clairefond G, *et al*. Soluble Receptor for Advanced Glycation End-Products Predicts Impaired Alveolar Fluid Clearance in Acute Respiratory Distress Syndrome. American Journal of Respiratory and Critical Care Medicine. 2015; 192: 191–199.

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REVIEW

From preclinical to clinical models of acute respiratory distress syndrome

Ruoyang Zhai[1], Woodys Lenga Ma Bonda[1], Gustavo Matute-Bello[2,3], Matthieu Jabaudon[1,4,*]

[1]GReD, Université Clermont Auvergne, CNRS, INSERM, 63000 Clermont-Ferrand, France
[2]Center for Lung Biology, Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, University of Washington, Seattle, WA 98105, USA
[3]Medical Research Service, VA Puget Sound Healthcare System, Seattle, WA 98105, USA
[4]Department of Perioperative Medicine, CHU Clermont-Ferrand, 63000 Clermont-Ferrand, France

***Correspondence**
mjabaudon@chu-clermontferrand.fr
(Matthieu Jabaudon)

Abstract

Various preclinical models that mimic the clinical causes of acute respiratory distress syndrome (ARDS) have been used to better understand the mechanisms of acute lung injury and its repair and to investigate novel therapies targeting such mechanisms. Despite important preclinical and clinical research efforts in recent decades, few candidate therapies with promising preclinical effects have been successfully translated into the clinical scenario, which could be attributable to the intrinsic limitations of the models as well as to the incorrect identification of appropriate phenotypes of patients to target with novel therapies that have proven beneficial in select preclinical models. However, current translational research strategies based on the use of multiple complementary preclinical and clinical models hold the promise of revolutionizing intensive care by using granular knowledge that should allow for a better diagnosis, greater predictability of the disease course, and the development of targeted therapies while ensuring patient safety through reduced adverse effects. Our goal was to summarize the strengths and limitations of the available models of ARDS, including animal, *in vitro*, and clinical models, and to discuss the current challenges and perspectives for research.

Keywords

Acute respiratory distress syndrome; Acute lung injury; Preclinical models; Translational research

1. Introduction

The acute respiratory distress syndrome (ARDS) is a severe form of acute lung injury characterized by the onset of hypoxemic respiratory failure associated with noncardiogenic pulmonary edema and dysregulated inflammatory responses [1–3]. The incidence of the syndrome is high, representing approximately 10% of patients admitted to the intensive care unit (ICU) and, despite important preclinical and clinical research efforts since its first description in 1967 [4], the syndrome is still associated with high mortality rates and long-term impacts on survivors [5]. Substantial progress has been made in improving supportive intensive care, such as the application of lung-protective mechanical ventilation, but specific pharmacological therapy is still lacking. Although this may be due to the incorrect identification of appropriate subsets of patients to target with novel therapies that have proven beneficial in select preclinical models, it may also be explained by the poor clinical translation of promising therapies from preclinical models, which can be attributable to the intrinsic limitations of the models [6, 7].

Research efforts have been held back in part by the difficulty of modeling human ARDS in animals, mainly due its heterogeneity, with many clinical or biological/functional variations among patients, in addition to its distinct causative factors, such as pulmonary or extrapulmonary sepsis, gastric fluid aspiration, transfusions, severe trauma, injurious mechanical ventilation, and/or reperfusion of ischemic tissues, among other causes [3, 8–11]. In this perspective, various preclinical models of "acute lung injury" that mimic the causes of clinical "ARDS" have been used to better understand the mechanisms of injury and its repair, and to develop novel therapies targeting these mechanisms [12].

Ideally, a comprehensive model of acute lung injury should be able to reproduce all the relevant features of ARDS pathophysiology, including all the physiological, functional, biological, and pathological symptoms related to injury and their consequences. However, such an ideal model mimicking the clinical scenario does not exist, and all the preclinical models have intrinsic limitations and strengths [13, 14]. Importantly, the "ideal" model may not always be the one that best reproduces human ARDS, but the one that is best suited to answer a specific scientific question. For example, despite all their limitations, mouse models remain key for mechanistic studies because of the ease of genetic manipulations, the ability to generate a large cohort in a short time, etc. Large animal models have more translational value but are less well suited to mechanistic studies. This leads to a proposed stepwise approach for animal studies, with reductionistic, targeted ro-

dent models as an initial step to identify potential mechanisms or therapeutic targets, and increasingly translational models (e.g., large animals) as recommended pre-clinical steps as the research gets closer to the bedside (e.g., testing of novel therapeutics). Although most preclinical studies on ARDS have been performed using animal models, other preclinical *in vitro* models are available and, more recently, the use of clinical models of ARDS has also broadened our ability to decipher injury or repair mechanisms and to identify novel targets for therapy development.

In this narrative review, our goal was to summarize the strengths and limitations of the available models of acute lung injury, including animal, *in vitro*, and clinical models, and to discuss the current challenges and prospects for research.

2. *In vivo* models of ARDS

Live animal models of ARDS play an important role as a bridge between clinical and laboratory studies in research translation. The consensus criteria of an *in vivo* model include acute onset of injury, altered alveolar-capillary membrane, alveolar inflammation, and lung histopathological changes that, together, lead to physiological impairment, such as arterial hypoxemia or impaired alveolar fluid clearance (Table 1, Ref. [14]) [14, 15]. Despite the many anatomical and physiological differences between animals and humans influencing the response of the lung to an acute injurious stimulus and affecting the evaluation of lung injury, *in vivo* models are frequently used as a reliable tool to test hypotheses with variably controlled parameters [14]. The latest updates on what constitutes an animal model of ARDS have focused on the clinical presentation, highlighted the importance of some degree of pre-existing lung injury, suggested the use of mechanical ventilation (to better coincide with the most frequent clinical scenario), and recommended the assessment of physiological outcomes to test potential therapeutic candidates [13, 14, 16]. Since no single animal model can fully replicate all the pathophysiological features of ARDS, multiple animal models have been developed, with the goal of replicating, sometimes in a very caricatural way, the clinical risk factors for ARDS, such as aspiration, pulmonary/extra-pulmonary infections, and mechanical ventilation-induced lung injury, among others [2, 3]. Schematically, preclinical ARDS can be caused *in vivo* through direct lung injury (such as after pneumonia or acid installation) or indirect lung injury (such as after peritonitis). "Double-hit" models have also been developed, which are intended to mimic clinical scenarios combining a specific risk factor (such as pneumonia) and a superimposed injury (such as hyperoxia or injurious ventilation) [17].

Different animal species have been used in the models, from large animals, such as non-human primates, pigs, dogs, cattle, sheep, and rabbits, to smaller animals, such as rats and mice. Larger animals are believed to better replicate human conditions, but these models are expensive and require specialized animal facilities. Models using smaller animals, such as mice, are more widely accessible and are a very powerful research tool, as the animals can be genetically modified in multiple ways to facilitate the detailed mechanistic study of complex pathways [13, 18–20].

2.1 Lipopolysaccharide-induced sepsis models

Lipopolysaccharide (LPS), often named endotoxin, is composed of a polar lipid head group (lipid A) and a chain of repeating disaccharides. It is present on the outer membrane of Gram-negative bacteria such as *escherichia coli* and *haemophilus influenzae*. The host response to LPS plays an important role as a mediator of bacterial sepsis via its binding with toll-like receptor 4 and the subsequent secretion of inflammatory mediators [21, 22]. LPS-induced lung injury caused by pulmonary or extra-pulmonary sepsis is one of the most commonly used ARDS models [13]. LPS can be administered into the lungs by intratracheal instillation or inhalation to produce direct lung injury in which the alveolar epithelium is the primary structure that is damaged. LPS can also be administered intraperitoneally or intravenously to reproduce peritonitis or blood infection, respectively, with marked systemic inflammatory response. Interestingly, repeated or continuous LPS exposure has been shown to exacerbate lung injury in models of extrapulmonary ARDS [23]. The LPS model ideally mimics a neutrophilic inflammatory response with increases in intrapulmonary cytokines and is, therefore, typically suitable for studies of inflammatory processes [13, 18]. However, it has significant disadvantages. First, the responses to LPS are highly variable among animal species, depending on the presence or absence of specific lung intravenous macrophages; for example, rodents are more tolerant to endotoxin exposure than pigs or sheep. Rodent models have been widely used to study LPS-induced lung injury due to their availability, easy accommodation, and relatively low cost. However, rodents are small animals with limited blood volume available for serial sampling [13, 24]. The endotoxin preparations used in animal studies may also vary in serotype and purity and can be contaminated with bacterial lipoproteins and other bacterial materials [25]. The duration of LPS exposure may also introduce some variability in the published results. In addition, the LPS model is often associated with mild changes in alveolar-capillary permeability and degrees of endothelial and epithelial injury, thus limiting clinical translation.

2.2 Live bacteria-induced sepsis models

Intrapulmonary or intravenous administration of live bacteria is another option to induce sepsis in animal models. Intratracheal instillation or inhalation of live bacteria, such as *streptococcus pneumoniae* or *pseudomonas aeruginosa*, can cause ARDS and, depending on the importance of the bacterial inoculum, systemic manifestations of sepsis [26–28]. The intravenous administration of live bacteria is followed within an hour by an initial phase of hypotension and leukopenia, which can progress to septic shock, intravascular coagulation, and death [13, 14]. Typically, live bacteria-induced sepsis models induce increased permeability, interstitial edema, and neutrophilic alveolitis. They are often used for studies of bacterial sepsis-induced lung injury. The intratracheal administration of live bacteria often results in localized pneumonia (rather than ARDS) in histological studies; however, the unilateral administration of bacteria can result in lung injury in the

TABLE 1. Features of experimental acute respiratory distress syndrome in animals and their main relevant measures in animals, as proposed by the official American Thoracic Society workshop report published in 2011 [14].

Features of experimental ARDS	Very relevant measures
Histological evidence of tissue injury	• Accumulation of neutrophils in the alveolus or the interstitium
	• Presence of hyaline membranes
	• Presence of proteinaceous debris in the alveolus
	• Thickening of the alveolar wall
	• Enhanced injury as measured by a standardized histology score
Alteration of the alveolar-capillary barrier	• Increase in extravascular lung water content
	• Accumulation of an exogenous tracer in the alveolar spaces or the extravascular compartment
	• Increase in total bronchoalveolar protein concentration
	• Increase in concentration of high molecular weight proteins in bronchoalveolar fluid (such as albumin, immunoglobulin M (IgM))
	• Increase in the microvascular filtration coefficient
Inflammatory response	• Increase in the absolute number of neutrophils in bronchoalveolar fluid
	• Increase in lung myeloperoxidase activity or protein concentration
	• Increase in the concentrations of proinflammatory cytokines in lung tissue or bronchoalveolar fluid
Physiological dysfunction	• Hypoxemia
	• Increased alveolar–arterial oxygen difference

It is recommended that at least three of the four "main" features are present in animal models of acute respiratory distress syndrome, and that at least one of the "very relevant" measures is performed for each feature of interest. This list of measures is indicative and may not be exhaustive, meaning that other measures may be relevant.

contralateral lung, depending on the bacterial dose. Therefore, the main technical issue with this model resides in the potential variability in the doses of live bacteria being administered.

Although viral infections are less frequent clinical causes of ARDS than bacterial infections outside of some specific pandemics (e.g., the coronavirus disease 2019 pandemic) [3, 5], animal models of viral pneumonia-induced lung injury are also being used to study the specific responses to or test the potential therapies for pathogens, such as influenza viruses or coronaviruses [29, 30].

2.3 Acid aspiration model

Gastric aspiration is one of the common causes leading to the development of ARDS in patients [31, 32]. This neutrophil-dependent form of lung injury is characterized by damage to the alveolar epithelium, alveolar hemorrhage, and intra-alveolar and interstitial edema. One of the most important features of this toxic process is the low pH of the gastric content, and hydrochloric acid (HCl) intratracheal instillation is the most used method to mimic gastric aspiration in animals, in particular in mice or larger animals such as pigs. This model induces the pathophysiological hallmarks of ARDS, with neutrophil recruitment and moderate effects on mortality [13]. The acid aspiration model is particularly useful for studying mechanisms of disruption of the alveolar-capillary barrier and of neutrophil recruitment. In addition, this reproducible model can be used to study the resolution phase of ARDS over multiple days after injury [33–35]. However, the narrow margin between injurious and noninjurious acid concentrations remains a limitation.

2.4 Abdominal sepsis models

Multiple models of peritonitis-induced lung injury have been described. In the cecal ligation and puncture (CLP) model, the cecum is ligated and punctured with a needle. The severity of the injury depends on the number of holes in the cecum and the size of the needle. In contrast to models using LPS and live bacteria, in which the effects are almost immediate, the effects of CLP develop over days, and the onset is less abrupt and consistent [13]. The main features of CLP-induced lung injury are mild hypoxemia, neutrophilic inflammation, and interstitial and alveolar edema, thus providing a complex representation of clinical extra-pulmonary sepsis [13]. Abdominal sepsis models can, therefore, be useful to study mechanisms of indirect lung injury due to sepsis. However, mortality is high, ranging from 25% at 18 h to 70–90% at 30 h, and it requires invasive surgery, although alternative surgical methods have been reported, such as colon ascendens stent peritonitis and laparoscopic cecal ligation [36, 37]. Other investigators have used intraperitoneal inoculation of fibrin clots containing controlled inoculum of bacteria, such as *escherichia coli*, to reproduce peritonitis-induced lung injury in mice and rabbits; in this model, lung injury more likely occurred at high doses, with overwhelmed host response, while lower doses only caused mild lung injury, such as in CLP model [38]. A more reliable and titratable model of peritonitis by the intraperitoneal injection of cecal slurry has been recently used to induce indirect ARDS [39, 40]. This model was first adapted from a neonatal necrotizing enterocolitis model [41]. Briefly, cecal contents are collected from euthanized donor mice, resuspended, and filtered before intraperitoneal injection.

2.5 Ventilator-induced lung injury

The use of ventilator-induced lung injury models has largely contributed to our understanding of the clinical benefits of lung-protective strategies of mechanical ventilation [42, 43]. Ventilation with high tidal volumes, especially without positive end-expiratory pressure, is associated with alveolar recruitment of inflammatory cells, changes in water and protein permeability, and histological injury, and, in general, severe hypoxemia develops within several hours [13, 14]. Although increased alveolar cytokine release has been reported in isolated lung preparations from mice and rats, it may not be present in all species. In addition, these models generally use very high tidal volumes (20–30 mL/kg body weight), which are not necessarily very relevant for clinical translation. However, they are very useful to study mechanical stretch and the activation of specific intracellular pathways involved in mechanotransduction. The effects of moderate increases in tidal volumes or of other changes in ventilator settings are best investigated in a "double-hit" model, following another primary insult.

2.6 Hyperoxia model

Prolonged exposure to hyperoxia may cause hyperoxia-induced lung injury in humans, with alveolar edema and endothelial and epithelial injury [44]. Animal models of hyperoxia have been used as direct lung injury models, sometimes as a secondary hit after peritonitis or LPS [45, 46]. The mechanism of hyperoxia-induced lung injury remains unclear and may be mediated by reactive oxygen species. The hyperoxia model provides an excellent model to study lung repair after lung injury. However, the major limitations are that this model requires specific equipment and prolonged exposure (for 72 h in many studies) [13].

2.7 Ischemia/Reperfusion model

Ischemia and reperfusion following lung transplantation or at other nonpulmonary sites can lead to a wide range of effects, including lung injury. This injury is associated with alveolar edema, epithelial and endothelial injury, inflammatory responses, massive production of free reactive oxygen species, and hypoxemia [13, 47]. Direct lung ischemia is generally induced by clamping the pulmonary artery, followed by reperfusion of the pulmonary and bronchial circulations. This model reproduces the development of acute lung injury after lung, intestinal or peripheral ischemia and reperfusion in humans and is probably more clinically relevant to transplantation studies than to ARDS. Of note, innovative approaches have been developed to allow non-invasive and repetitive in vivo microscopy of ectopic lung tissue using dorsal skinfold chambers in transplantation studies [48]. The main limitation of the ischemia/reperfusion model is that it requires specific surgical skills and equipment [13, 49, 50].

2.8 Models of viral infections

Live animal models of acute lung injury can be used to study the mechanisms of ARDS due to viruses, such as influenza viruses or, more recently, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [51–54]. Results from these models have emphasized the major role of the inflammatory host immune response to infection as a major contributor to lung injury. Although most airborne viruses initially affect the respiratory epithelium, the role of endothelial dysfunction has not been well established, and pathogen-specific pathways may contribute to diffuse alveolar damage [55]. Small animal models are widely used to study viral infections; however, translation may require genetic modifications (to the animal and/or the virus) to make the model susceptible, for example to SARS-CoV-2 [54]. Animal models can be rapidly mobilized to better understand the mechanisms of emerging viruses, such as during the recent coronavirus disease 2019 (COVID-19) pandemic, and to test new diagnostic, preventive, and/or therapeutic approaches [30].

2.9 Other models

The oleic acid model was first used to mimic clinical ARDS, although it is primarily based on the induction of fat embolism [56]. The intravenous administration of oleic acid leads to direct lung endothelial injury caused by necrosis and microvascular thrombosis. This model is rather reproducible, rapidly reproduces the most basic features of experimental ARDS in large animal models (pigs/piglets, sheep, dogs) [57–59], and is particularly useful to study lung mechanics, ventilatory strategies, and ventilation/perfusion distribution during acute lung injury. However, it is now seldom performed; its main limitations include a high mortality rate, a difficult application in smaller animals, and unclear effects on alveolar inflammation [13, 60].

Alveolar surfactant proteins regulate surface tension during breathing, and surfactant deficiency and dysfunction are frequent during ARDS [3], primarily due to decreased secretion by injured alveolar type II epithelial cells [61]. Surfactant depletion can be modeled by repeated saline lavage of the lungs and this model has good value to study surfactant functions and to assess the effects of ventilation strategies in animals. It induces rapid and reproducible, yet transient, hypoxemia and alveolar recruitment but only modest lung injury per se and very little neutrophil recruitment [13, 60, 62, 63].

Whether the bleomycin model is a good acute lung injury model is still discussed by many researchers, as it is one the few that leads to an acute inflammatory phase followed by a fibrotic phase that eventually resolves [13, 14].

3. *In vitro* models of ARDS

In vitro cell culture models can provide a direct link to lung cell responses in a simplified way and represent valuable methods to investigate basic biological and functional mechanisms and roles for specific cell types, receptors or pathways. They allow the manipulation of one or multiple variables through rigorously controlled, bias-free experiments to investigate the variation in quantitative protein markers, physiological functions, and/or gene expression in response to multiple conditions, including candidate therapies targeting precise mechanisms of injury or repair.

A monoculture of either alveolar epithelial, lung endothelial, or alveolar macrophages, among other cell types, can be performed to test mechanistic hypotheses or optimize the experimental parameters in subsequent *in vivo* or clinical research. *In vitro* monocultures can also be used to study important cellular functions, such as wound healing after a scratch assay [64] or transepithelial fluid transport by alveolar epithelial cells (often called “alveolar fluid clearance” *in vivo*), using transwell experiments [65, 66]. For example, monolayer cultures of commercialized, immortalized or primary isolated alveolar epithelial type I (AT I) or type II (AT II) cells have been used to mimic the alveolar epithelium and its barrier function [67]). Non-sterile inflammation was first studied in 2D monoculture or classical culture models exposed to LPS *in vitro*, mimicking the clinical infection with Gram-negative bacteria [68–72]. In contrast, the setting of sterile inflammation can be studied *in vitro* by exposing cultured cells to a mixture of cytokines, such as interleukin-1 beta, tumor necrosis factor alpha, and interferon gamma [65, 73, 74]. In addition, some biological mechanisms of mechanical ventilation-induced lung injury have been investigated *in vitro* through exposure to cyclic mechanical stretch, hypercapnia or hyperoxia [75–79]. Interestingly, alveolar epithelial cells or alveolar progenitor cells (such as induced pluripotent stem cells-derived AT2-like cells) can be differentiated at the air-liquid interface, inducing cell polarization, epithelial barrier formation through the establishment of intercellular junctions, and surfactant production.

However, monocultures are unable to reproduce the complexity of the alveolar-capillary environment, and, for example, a traditional submersion culture model does not reproduce the air-liquid environment of human alveoli. The main advances have, therefore, come from modeling the human airway at the air-liquid interface, building co-culture models (such as of epithelial cells and endothelial cells or macrophages), and developing 3D-engineered lung cellular environments. *In vitro* co-culture or multicellular models can better reproduce the *in vivo* environment, compared to 2D monocultures [80]. Unlike 2D cultures, co-culture or multicellular systems can model complex interactions between different cell types in a more relevant environment, such as a model of alveolar-capillary barrier using epithelial and endothelial cells [81, 82]. For example, a 3D multicellular model composed of an alveolar epithelial cell layer cultured in interaction with alveolar macrophages on one side and monocyte-derived dendritic cells on the other has been recently described [67, 81, 83]. *Ex vivo* organoid cultures have also been proposed to better model the multiple features of ARDS. These are 3D models assembled from cultured human alveolar stem cells to reproduce all the characteristics of a functional human alveolus *in vitro* [84]. These cultures are long-term, feeder-free, and chemically defined systems that represent a very powerful model to investigate complex mechanisms, such as those involved in severe acute respiratory syndrome coronavirus 2 infection [85–87]. Ultimately, the combination of microfluidic bioengineering and 3D cell culture has led

to the development of "lung-on-a-chip" models comprising a full alveolar-capillary interface that can be exposed to cyclic ventilation and perfusion [88–90]. This model requires long-term cultures of human cell lines, and it is only very recently that the use of primary human alveolar epithelial cells has been reported [91].

4. Human models of ARDS

4.1 Human in vivo models of ARDS

Because a recognized shortcoming in human ARDS research is the difficulty in translating the findings from bench to bedside, novel *in vivo* models have been successfully developed to investigate the mechanisms of lung injury or therapies for ARDS. These models are major breakthroughs in translational ARDS research and have clear advantages in allowing potentially effective therapies to be readily investigated *in vivo* in humans and to inform subsequent clinical trials in patients.

Seminal studies included intravenous administrations of LPS to human volunteers [92, 93]; however, in some studies, direct lung instillations of LPS [94] or other agents, such as leukotriene B4 (produced by human alveolar macrophages, with potent chemotactic activity for neutrophils) [95], were performed using bronchoscopy. More recently, the inhalation of low-dose LPS by healthy humans was successfully used to replicate alveolar epithelial cell activation, alveolar inflammation, and systemic inflammatory response without causing significant adverse effects [96–99]. In this recently developed human *in vivo* model, lung injury is only transient, and inflammation has mainly been investigated within a few hours after LPS exposure.

4.2 Human ex vivo models of ARDS

An *ex vivo* human lung preparation has recently been proposed to better reflect the *in vivo* settings of experimental ARDS [100]. In this model, donor human lungs that have been rejected for transplantation are ventilated and perfused *ex vivo* and used to study the mechanisms of lung injury, isolate multiple primary lung cell types, and test potential therapies before clinical translation into trials. The model allows for analyses of physiological indices, such as oxygenation and alveolar fluid clearance, and the sampling of multiple tissues and fluids up to 6–10 hours in most experiments [101]. Although the *ex vivo* human lung preparation is rather convenient, inexpensive, and the model closest to clinical conditions, ethical and practical issues in obtaining human lungs for research may exist depending on the country. The main limitation of *ex vivo* models resides in the heterogeneity in human lungs due to donor-specific and pre-procurement variables that limit baseline comparisons of measures among experimental lungs. Notably, in addition to its use in ARDS research, the *ex vivo* human lung preparation is being largely used in conditioning or therapeutic studies of donor lungs before transplantation [102].

5. Perspectives and challenges

Multiple models of acute lung injury induced by the main clinical risk factors for ARDS have been developed *in vitro* (from monocultures to more complex constructions), *in vivo* in animals, *ex vivo* (using human or animal lung preparations), and *in vivo* in human volunteers (Table 2). While no single model can truly reproduce the complexity and heterogeneity of clinical ARDS, combining multiple preclinical approaches with *in vivo* and clinical investigations is probably the most promising strategy for future mechanistic and therapeutic research (Fig. 1). Each experimental model has its limitations. For example, despite the recent developments of 3D cultures and lung organoids, *in vitro* models may probably never reproduce the clinical setting, but they are still very useful to inform mechanistic and drug discovery studies [6]. Recent animal studies are able to better reflect the multiple-hit hypothesis for ARDS pathogenesis, and they can be used to investigate the different phases of ARDS, from onset (such as in the hydrochloric acid and the oleic acid models) to recovery (such as in the hyperoxia acid and the bleomycin models), thus allowing studies of preventive ARDS therapies and the combined effects of pre-existing lung injury and exposure to high lung stress through mechanical ventilation use in the intensive care unit [14, 17, 103]. Animal studies typically use young and healthy animals, and older animals should also be investigated to better reflect the clinical picture of ARDS as a disease of aging. In addition, they do not reproduce the common clinical ARDS settings of patient comorbidities and multiorgan failure, with a prolonged need for intensive care over multiple days, if not weeks, and most studies do not take into consideration the impact of ventilatory settings (e.g., the level of positive end-expiratory pressure) on oxygenation. To distinguish models of acute lung injury from conditions that reflect more subacute or chronic lung injury, maximal lung injury should be evident within 24 hours of exposure to the inciting stimulus [14]. However, although some animal models allow studies of lung injury over multiple days and can capture the different phases of ARDS over time [33], most of them are limited to the first few hours after injury, thus limiting clinical translation and the ability to explore the nonlinearity of biological processes. Another limitation that is particularly relevant in mice is the profound impact of strain variability in murine models of injury; not only a particular observation may not be translatable to humans, but it may not even be translatable to other strains of mice. This highlights the need to restrict mice to mechanistic questions and use more translational models for preclinical studies.

Human models, *in vivo* or *ex vivo*, represent major progress toward the clinical translation of basic findings. In addition to the development of novel preclinical and clinical ARDS models, such as the lung-on-a-chip *in vitro* and the *ex vivo* human lung preparation, recent evolutions in the field of translational ARDS research have been made possible by the refinement of experimental techniques. In particular, the field of genome editing now offers a broad range of opportunities to develop investigational or therapeutic strategies by downregulating or upregulating the expression of a specific gene *in vitro* and *in vivo*, such as with the gene knockout or knockdown techniques based on ribonucleic acid interference or the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) system (capable of site-specific deoxyribonucleic acid

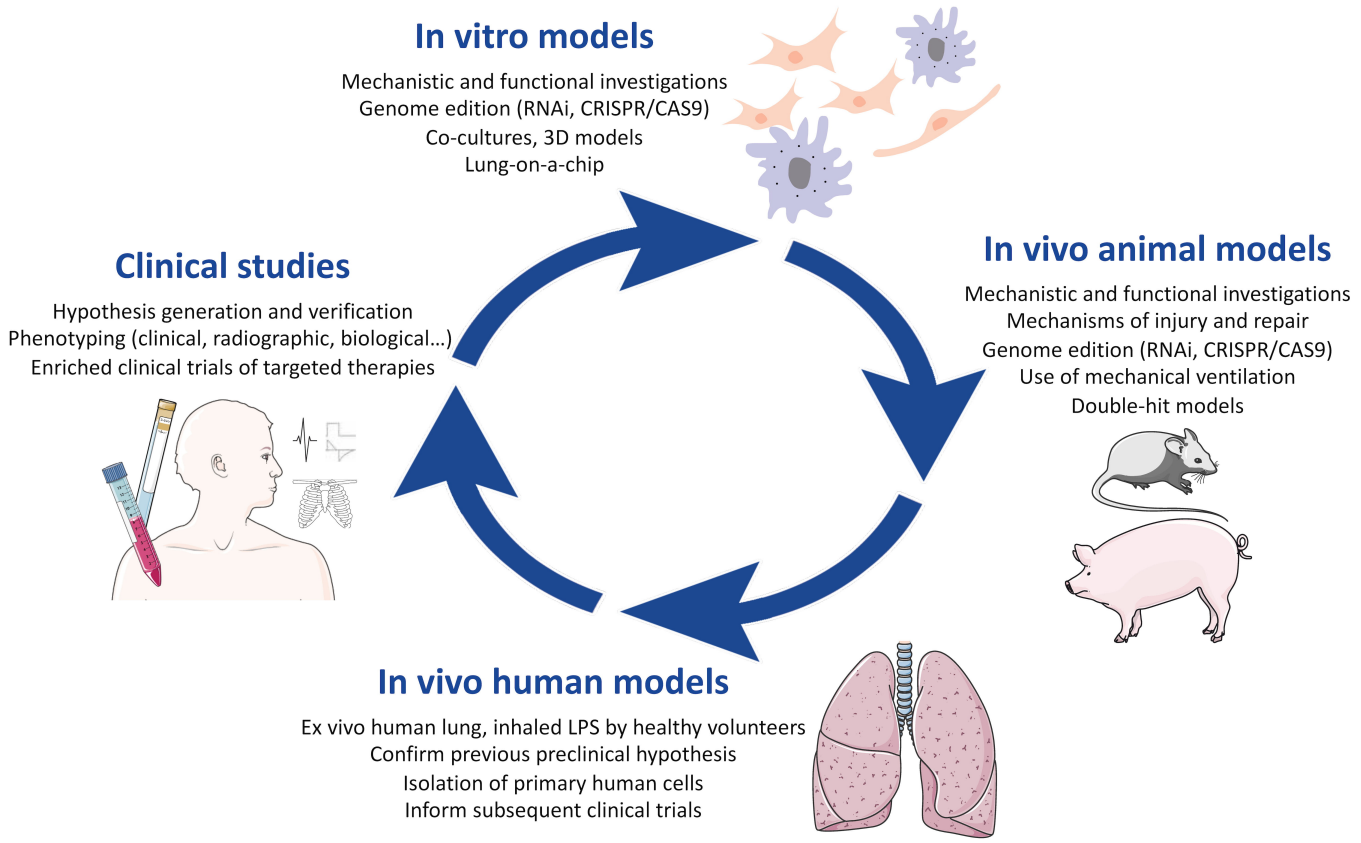

FIGURE 1. Schematic view of a translational research framework based on multiple experimental preclinical approaches and clinical studies. RNAi, interference ribonucleic acid; CRISPR, clustered regularly interspaced short palindromic repeats; CAS9, CRISPR-associated protein 9; LPS, lipopolysaccharide.

cleavage) [89, 104]. Novel methods have also improved our ability to understand the functional significance of polymorphisms or genes identified through genome-wide studies or transcriptomic screens in models and patients, to investigate the potential pathogenic causality for markers or mediators and the potential value of targeting treatment in specific genetic backgrounds. Such innovative approaches have been successfully deployed in COVID-19 research [30].

These traditional and novel experimental designs are important pieces, along with the acquisition of granular clinical, physiological, and biological data (e.g., from bronchoalveolar fluid and blood samples to study mechanisms of lung and systemic responses, respectively, to injury) within clinical studies, in identifying new drug targets, developing targeted therapies, and ultimately selecting patients most likely to benefit. Such strategies of trial enrichment, in which patients are selected who are more likely to develop an outcome, such as mortality (prognostic enrichment), and/or when they are more likely to respond to a targeted therapy (predictive enrichment), hold the promise of precision approaches for ARDS [105–107]. In such a translational framework, preclinical models may reveal that a biological/functional trait or marker has a causal role in the severity of ARDS and suggest that measuring this marker could have value for endotype identification [34, 105, 108, 109]. Ideally, such markers (e.g., biological or radiographic indices) could help in selecting patients to enroll in future precision trials and monitoring their individual responses to the intervention being tested [103, 110–112]. This would require markers that are rapidly available to inform potential trial eligibility. Of note, the first clinical study evaluating a point-of-care assay to prospectively identify hyper- and hypo-inflammatory phenotypes in patients with ARDS and hypoxemic acute respiratory failure is currently enrolling patients (ClinicalTrials.gov Identifier: NCT04009330), and the preliminary results in patients with COVID-19 have been published [113]. However, it remains uncertain to date whether available preclinical models may be representative of known clinical ARDS phenotypes/endotypes or may be helpful to identify phenotype-specific therapy responsive traits [114]. The performance of candidate markers over time during the natural course of ARDS and their kinetics under candidate therapies should also be rigorously evaluated [10]. In addition, even when a diagnostic or therapeutic precision approach is consistently supported by findings across combined preclinical and clinical ARDS models, it should not be associated with adverse effects that may preclude its application to improve patient outcomes.

In conclusion, multiple experimental models have been developed in the last few decades, with major recent developments in the fields of *in vitro*, *ex vivo*, and *in vivo* experimental ARDS. While some of these experiments failed, others succeeded in advancing our knowledge of the complex mechanisms of ARDS pathophysiology and the clinical translation of a few therapeutic interventions, such as lung-protective

TABLE 2. Non-exhaustive list of preclinical and clinical models of acute respiratory distress syndrome.

Setting	Model	Injury feature	Technical notes
Live animal models	Lipopolysaccharide-induced sepsis	Pulmonary or systemic sepsis	Variable response to injury across species
	Live bacteria-induced sepsis		Variability in the doses of live bacteria administered
	Acid aspiration	Direct lung injury	Mimics aspiration of gastric contents
			Narrow margin between injury and absence of injury
	Abdominal sepsis	Peritonitis-induced indirect lung injury	Invasive surgery required with high mortality
			Intraperitoneal injection of cecal slurry more reliable and titratable
	Ventilator-induced lung injury	Direct alveolar injury with severe hypoxemia	High tidal volumes not relevant for clinical translation
	Hyperoxia	Hyperoxia-induced injury	Requires prolonged exposure and specific equipment
	Ischemia-reperfusion	Induced by clamping the pulmonary artery lung or other nonpulmonary arteries	Requires specific surgical skills and equipment
	Viral infection	Acute lung injury of viral causes (mostly airborne viruses)	Can be rapidly mobilized to better understand the mechanisms of emerging viruses
			Translation may require genetic modifications to the animal and/or the virus
In vitro models	Submerged monoculture	Cell injury of sterile or non-sterile causes	Reproducible for testing and establishing experimental conditions
			Submerged cultures or cultures at the air-liquid interface
	Multicellular co-culture	Cell injury of sterile or non-sterile causes in a more relevant multicellular environment	Can model complex interactions between different cell types
	Organoid culture		
	Lung-on-a-chip	Cell injury of sterile or non-sterile causes in a human lung-like environment	Allows multicellular co-culture
			Reproduces all characteristics of a functional human alveolus unit
Human *in vivo* models	Lipopolysaccharide-induced sepsis in human volunteers	Intravenous administration or direct lung instillation of high-dose lipopolysaccharide	Seminal models
		Inhalation of low-dose lipopolysaccharide	Transient lung injury and rapid investigation of inflammation
Human *ex vivo* models	*Ex vivo* human lung preparation	Donor human lungs rejected for transplantation are ventilated and perfused *ex vivo* before exposure to sterile or non-sterile injuries	Most translatable model, allows for analyses of physiological indices
			Rather convenient and inexpensive but potential ethical and practical issues

mechanical ventilation, neuromuscular blockade, and corticoid therapy [115–117]. Therefore, the judicious and imaginative use of a broad range of experimental and analytical approaches is of paramount importance in developing translational discovery research, with the goal of developing prediction medicine strategies to ultimately improve patient outcomes.

AUTHOR CONTRIBUTIONS

RZ, WLMB, and GMB were involved in the conception and design of the review, in writing the paper, and in its revision prior to submission. MJ takes responsibility for the content of the paper and was involved in the conception and design of the review, in writing the paper, and in its revision prior to submission.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, *et al*. Acute respiratory distress syndrome: the Berlin Definition. Journal of the American Medical Association. 2012; 307: 2526–33.

[2] Thompson BT, Chambers RC, Liu KD. Acute Respiratory Distress Syndrome. The New England Journal of Medicine. 2017; 377: 562–572.

[3] Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, *et al*. Acute respiratory distress syndrome. Nature Reviews Disease Primers. 2019; 5: 18.

[4] Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. Lancet. 1967; 2: 319–323.

[5] Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, *et al*. Epidemiology, Patterns of Care, and Mortality for Patients with Acute Respiratory Distress Syndrome in Intensive Care Units in 50 Countries. Journal of the American Medical Association. 2016; 315: 788–800.

[6] Matthay MA, Zimmerman GA, Esmon C, Bhattacharya J, Coller B, Doerschuk CM, *et al*. Future research directions in acute lung injury: summary of a National Heart, Lung, and Blood Institute working group. American Journal of Respiratory and Critical Care Medicine. 2003; 167: 1027–1035.

[7] Matthay MA, McAuley DF, Ware LB. Clinical trials in acute respiratory distress syndrome: challenges and opportunities. The Lancet Respiratory Medicine. 2018; 5: 524–534.

[8] Calfee CS, Eisner MD, Ware LB, Thompson BT, Parsons PE, Wheeler AP, *et al*. Trauma-associated lung injury differs clinically and biologically from acute lung injury due to other clinical disorders. Critical Care Medicine. 2007; 35: 2243–2250.

[9] Tejera P, Meyer NJ, Chen F, Feng R, Zhao Y, O'Mahony DS, *et al*. Distinct and replicable genetic risk factors for acute respiratory distress syndrome of pulmonary or extrapulmonary origin. Journal of Medical Genetics. 2012; 49: 671–680.

[10] Delucchi K, Famous KR, Ware LB, Parsons PE, Thompson BT, Calfee CS. Stability of ARDS subphenotypes over time in two randomised controlled trials. Thorax. 2018; 73: 439–445.

[11] Wilson JG, Calfee CS. ARDS Subphenotypes: Understanding a Heterogeneous Syndrome. Critical Care. 2020; 24: 102.

[12] Juffermans NP, Radermacher P, Laffey JG. The importance of discovery science in the development of therapies for the critically ill. Intensive Care Medicine Experimental. 2020; 8: 17.

[13] Matute-Bello G, Frevert CW, Martin TR. Animal models of acute lung injury. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2008; 295: L379–L399.

[14] Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, *et al*. An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. American Journal of Respiratory Cell and Molecular Biology. 2011; 44: 725–738.

[15] Matthay MA. Resolution of pulmonary edema. Thirty years of progress. American Journal of Respiratory and Critical Care Medicine. 2014; 189: 1301–1308.

[16] Oakley C, Koh M, Baldi R, Soni S, O'Dea K, Takata M, *et al*. Ventilation following established ARDS: a preclinical model framework to improve predictive power. Thorax. 2019; 74: 1120–1129.

[17] Laffey JG, Kavanagh BP. Fifty Years of Research in ARDS. Insight into Acute Respiratory Distress Syndrome. From Models to Patients. American Journal of Respiratory and Critical Care Medicine. 2017; 196: 18–28.

[18] Bastarache JA, Blackwell TS. Development of animal models for the acute respiratory distress syndrome. Disease Models & Mechanisms. 2009; 2: 218–223.

[19] Aeffner F, Bolon B, Davis IC. Mouse Models of Acute Respiratory Distress Syndrome: a Review of Analytical Approaches, Pathologic Features, and Common Measurements. Toxicologic Pathology. 2015; 43: 1074–1092.

[20] Cockrell AS, Yount BL, Scobey T, Jensen K, Douglas M, Beall A, *et al*. A mouse model for MERS coronavirus-induced acute respiratory distress syndrome. Nature Microbiology. 2016; 2: 16226.

[21] Lu Y, Yeh W, Ohashi PS. LPS/TLR4 signal transduction pathway. Cytokine. 2008; 42: 145–151.

[22] Ciesielska A, Matyjek M, Kwiatkowska K. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cellular and Molecular Life Sciences. 2021; 78: 1233–1261.

[23] Cheng D, Han W, Chen SM, Sherrill TP, Chont M, Park G, *et al*. Airway epithelium controls lung inflammation and injury through the NF-kappa B pathway. Journal of Immunology. 2007; 178: 6504–6513.

[24] Kohman RA, Crowell B, Kusnecov AW. Differential sensitivity to endotoxin exposure in young and middle-age mice. Brain, Behavior, and Immunity. 2010; 24: 486–492.

[25] Tapping RI, Akashi S, Miyake K, Godowski PJ, Tobias PS. Toll-like receptor 4, but not toll-like receptor 2, is a signaling receptor for Escherichia and Salmonella lipopolysaccharides. Journal of Immunology. 2000; 165: 5780–5787.

[26] Su X, Looney MR, Gupta N, Matthay MA. Receptor for advanced glycation end-products (RAGE) is an indicator of direct lung injury in models of experimental lung injury. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2009; 297: L1–L5.

[27] Matthay MA, Goolaerts A, Howard JP, Lee JW. Mesenchymal stem cells for acute lung injury: preclinical evidence. Critical Care Medicine. 2010; 38: S569–S573.

[28] Asmussen S, Ito H, Traber DL, Lee JW, Cox RA, Hawkins HK, *et al*. Human mesenchymal stem cells reduce the severity of acute lung injury in a sheep model of bacterial pneumonia. Thorax. 2014; 69: 819–825.

[29] Rosas LE, Doolittle LM, Joseph LM, El-Musa H, Novotny MV, Hickman-Davis JM, *et al*. Postexposure Liponucleotide Prophylaxis and Treatment Attenuates Acute Respiratory Distress Syndrome in Influenza-

infected Mice. American Journal of Respiratory Cell and Molecular Biology. 2021; 64: 677–686.

[30] Cleary SJ, Pitchford SC, Amison RT, Carrington R, Robaina Cabrera CL, Magnen M, *et al*. Animal models of mechanisms of SARS-CoV-2 infection and COVID-19 pathology. British Journal of Pharmacology. 2020; 177: 4851–65.

[31] Marik PE. Aspiration pneumonitis and aspiration pneumonia. The New England Journal of Medicine. 2001; 344: 665–671.

[32] Raghavendran K, Nemzek J, Napolitano LM, Knight PR. Aspiration-induced lung injury. Critical Care Medicine. 2011; 39: 818–826.

[33] Patel BV, Wilson MR, Takata M. Resolution of acute lung injury and inflammation: a translational mouse model. The European Respiratory Journal. 2012; 39: 1162–1170.

[34] Jabaudon M, Blondonnet R, Roszyk L, Bouvier D, Audard J, Clairefond G, *et al*. Soluble Receptor for Advanced Glycation End-Products Predicts Impaired Alveolar Fluid Clearance in Acute Respiratory Distress Syndrome. American Journal of Respiratory and Critical Care Medicine. 2015; 192: 191–199.

[35] Chimenti L, Morales-Quinteros L, Puig F, Camprubi-Rimblas M, Guillamat-Prats R, Gómez MN, *et al*. Comparison of direct and indirect models of early induced acute lung injury. Intensive Care Medicine Experimental. 2020; 8: 62.

[36] Traeger T, Koerner P, Kessler W, Cziupka K, Diedrich S, Busemann A, *et al*. Colon ascendens stent peritonitis (CASP)–a standardized model for polymicrobial abdominal sepsis. Journal of Visualized Experiments. 2010; 46: 2299.

[37] Kuhn R, Schubert D, Tautenhahn J, Nestler G, Schulz H, Bartelmann M, *et al*. Effect of intraperitoneal application of an endotoxin inhibitor on survival time in a laparoscopic model of peritonitis in rats. World Journal of Surgery. 2005; 29: 766–770.

[38] Matute-Bello G, Frevert CW, Kajikawa O, Skerrett SJ, Goodman RB, Park DR, *et al*. Septic shock and acute lung injury in rabbits with peritonitis: failure of the neutrophil response to localized infection. American Journal of Respiratory and Critical Care Medicine. 2001; 163: 234–243.

[39] Shaver CM, Paul MG, Putz ND, Landstreet SR, Kuck JL, Scarfe L, *et al*. Cell-free hemoglobin augments acute kidney injury during experimental sepsis. American Journal of Physiology-Renal Physiology. 2019; 317: F922–F929.

[40] Meegan JE, Shaver CM, Putz ND, Jesse JJ, Landstreet SR, Lee HNR, *et al*. Cell-free hemoglobin increases inflammation, lung apoptosis, and microvascular permeability in murine polymicrobial sepsis. PLoS ONE. 2020; 15: e0228727.

[41] Wynn JL, Scumpia PO, Delano MJ, O'Malley KA, Ungaro R, Abouhamze A, *et al*. Increased mortality and altered immunity in neonatal sepsis produced by generalized peritonitis. Shock. 2007; 28: 675–683.

[42] Webb HH, Tierney DF. Experimental pulmonary edema due to intermittent positive pressure ventilation with high inflation pressures. Protection by positive end-expiratory pressure. American Review of Respiratory Disease. 1974; 110: 556–565.

[43] Dreyfuss D, Saumon G. Ventilator-induced lung injury: lessons from experimental studies. American Journal of Respiratory and Critical Care Medicine. 1998; 157: 294–323.

[44] Kallet RH, Matthay MA. Hyperoxic Acute Lung Injury. Respiratory Care. 2013; 58: 123–141.

[45] Vuichard D, Ganter MT, Schimmer RC, Suter D, Booy C, Reyes L, *et al*. Hypoxia aggravates lipopolysaccharide-induced lung injury. Clinical and Experimental Immunology. 2005; 141: 248–260.

[46] Hauser B, Barth E, Bassi G, Simon F, Gröger M, Öter S, *et al*. Hemodynamic, metabolic, and organ function effects of pure oxygen ventilation during established fecal peritonitis-induced septic shock. Critical Care Medicine. 2009; 37: 2465–2469.

[47] Eltzschig HK, Eckle T. Ischemia and reperfusion–from mechanism to translation. Nature Medicine. 2011; 17: 1391–1401.

[48] Regelin N, Heyder S, Laschke MW, Hadizamani Y, Borgmann M, Moehrlen U, *et al*. A murine model to study vasoreactivity and intravascular flow in lung isograft microvessels. Scientific Reports. 2019; 9: 5170.

[49] Fard N, Saffari A, Emami G, Hofer S, Kauczor H, Mehrabi A. Acute respiratory distress syndrome induction by pulmonary ischemia–reperfusion injury in large animal models. Journal of Surgical Research. 2014; 189: 274–284.

[50] Rocco PRM, Nieman GF. ARDS: what experimental models have taught us. Intensive Care Medicine. 2016; 42: 806–810.

[51] Su HC, Nguyen KB, Salazar-Mather TP, Ruzek MC, Dalod MY, Biron CA. NK cell functions restrain T cell responses during viral infections. European Journal of Immunology. 2001; 31: 3048–3055.

[52] Lang PA, Lang KS, Xu HC, Grusdat M, Parish IA, Recher M, *et al*. Natural killer cell activation enhances immune pathology and promotes chronic infection by limiting CD8+ T-cell immunity. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109: 1210–1215.

[53] Zhou G, Juang SWW, Kane KP. NK cells exacerbate the pathology of influenza virus infection in mice. European Journal of Immunology. 2013; 43: 929–938.

[54] Johansen MD, Irving A, Montagutelli X, Tate MD, Rudloff I, Nold MF, *et al*. Animal and translational models of SARS-CoV-2 infection and COVID-19. Mucosal Immunology. 2020; 13: 877–891.

[55] Hendrickson CM, Matthay MA. Viral pathogens and acute lung injury: investigations inspired by the SARS epidemic and the 2009 H1N1 influenza pandemic. Seminars in Respiratory and Critical Care Medicine. 2013; 34: 475–486.

[56] Schuster DP. ARDS: clinical lessons from the oleic acid model of acute lung injury. American Journal of Respiratory and Critical Care Medicine. 1994; 149: 245–260.

[57] Hartmann EK, Bentley A, Duenges B, Klein KU, Boehme S, Markstaller K, *et al*. TIP peptide inhalation in oleic acid-induced experimental lung injury: a post-hoc comparison. BMC Research Notes. 2013; 6: 385.

[58] Prat NJ, Meyer AD, Langer T, Montgomery RK, Parida BK, Batchinsky AI, *et al*. Low-Dose Heparin Anticoagulation during Extracorporeal Life Support for Acute Respiratory Distress Syndrome in Conscious Sheep. Shock. 2015; 44: 560–568.

[59] Du G, Wang S, Li Z, Liu J. Sevoflurane Posttreatment Attenuates Lung Injury Induced by Oleic Acid in Dogs. Anesthesia and Analgesia. 2017; 124: 1555–1563.

[60] Ballard-Croft C, Wang D, Sumpter LR, Zhou X, Zwischenberger JB. Large-Animal Models of Acute Respiratory Distress Syndrome. The Annals of Thoracic Surgery. 2012; 93: 1331–1339.

[61] Raghavendran K, Willson D, Notter RH. Surfactant therapy for acute lung injury and acute respiratory distress syndrome. Critical Care Clinics. 2011; 27: 525–559.

[62] Muellenbach RM, Kredel M, Bernd Z, Johannes A, Kuestermann J, Schuster F, *et al*. Acute respiratory distress induced by repeated saline lavage provides stable experimental conditions for 24 hours in pigs. Experimental Lung Research. 2009; 35: 222–233.

[63] Russ M, Boerger E, von Platen P, Francis RCE, Taher M, Boemke W, *et al*. Surfactant Depletion Combined with Injurious Ventilation Results in a Reproducible Model of the Acute Respiratory Distress Syndrome (ARDS). Journal of Visualized Experiments. 2021.

[64] Zhai R, Blondonnet R, Ebrahimi E, Belville C, Audard J, Gross C, *et al*. The receptor for advanced glycation end-products enhances lung epithelial wound repair: an in vitro study. Experimental Cell Research. 2020; 391: 112030.

[65] Lee JW, Fang X, Dolganov G, Fremont RD, Bastarache JA, Ware LB, *et al*. Acute lung injury edema fluid decreases net fluid transport across human alveolar epithelial type II cells. The Journal of Biological Chemistry. 2007; 282: 24109–24119.

[66] Fang X, Matthay MA. Measurement of Protein Permeability and Fluid Transport of Human Alveolar Epithelial Type II Cells under Pathological Conditions. Methods in Molecular Biology. 2018; 122: 121–128.

[67] Drasler B, Karakocak BB, Tankus EB, Barosova H, Abe J, Sousa de Almeida M, *et al*. An Inflamed Human Alveolar Model for Testing the Efficiency of Anti-inflammatory Drugs in vitro. Frontiers in Bioengineering and Biotechnology. 2020; 8: 987.

[68] King P. Haemophilus influenzae and the lung (Haemophilus and the lung). Clinical and Translational Medicine. 2012; 1: 10.

[69] Camprubí–Rimblas M, Guillamat-Prats R, Lebouvier T, Bringué J, Chimenti L, Iglesias M, *et al*. Role of heparin in pulmonary cell populations in an in-vitro model of acute lung injury. Respiratory Research. 2017; 18: 89.

[70] Tojo K, Tamada N, Nagamine Y, Yazawa T, Ota S, Goto T. Enhancement of glycolysis by inhibition of oxygen-sensing prolyl hydroxylases protects alveolar epithelial cells from acute lung injury. The FASEB Journal. 2018; 32: 2258–2268.

[71] Pooladanda V, Thatikonda S, Bale S, Pattnaik B, Sigalapalli DK, Bathini NB, *et al*. Nimbolide protects against endotoxin-induced acute respiratory distress syndrome by inhibiting TNF-α mediated NF-κB and HDAC-3 nuclear translocation. Cell Death & Disease. 2019; 10: 81.

[72] Wu D, Fu X, Zhang Y, Li Q, Ye L, Han S, *et al*. The protective effects of C16 peptide and angiopoietin-1 compound in lipopolysaccharide-induced acute respiratory distress syndrome. Experimental Biology and Medicine. 2020; 245: 1683–1696.

[73] Bastarache JA, Wang L, Geiser T, Wang Z, Albertine KH, Matthay MA, *et al*. The alveolar epithelium can initiate the extrinsic coagulation cascade through expression of tissue factor. Thorax. 2007; 62: 608–616.

[74] Metz JK, Wiegand B, Schnur S, Knoth K, Schneider-Daum N, Groß H, *et al*. Modulating the Barrier Function of Human Alveolar Epithelial (hAELVi) Cell Monolayers as a Model of Inflammation. Alternatives to Laboratory Animals. 2020; 48: 252–267.

[75] Otulakowski G, Engelberts D, Gusarova GA, Bhattacharya J, Post M, Kavanagh BP. Hypercapnia attenuates ventilator-induced lung injury via a disintegrin and metalloprotease-17. The Journal of Physiology. 2016; 592: 4507–4521.

[76] Wang T, Gross C, Desai A, Zemskov E, Wu X, Garcia AN, *et al*. Endothelial Cell Signaling and Ventilator-Induced Lung Injury (VILI): Molecular Mechanisms, Genomic Analyses & Therapeutic Targets. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2017; 312: L452–L476.

[77] Joelsson JP, Myszor IT, Arason AJ, Ingthorsson S, Cherek P, Windels GS, *et al*. Innovative in vitro method to study ventilator induced lung injury. ALTEX. 2019; 36: 634–642.

[78] Monjezi M, Jamaati H, Noorbakhsh F. Attenuation of ventilator-induced lung injury through suppressing the pro-inflammatory signaling pathways: a review on preclinical studies. Molecular Immunology. 2021; 135: 127–136.

[79] Pao H-P, Liao W-I, Tang S-E, Wu S-Y, Huang K-L, Chu S-J. Suppression of Endoplasmic Reticulum Stress by 4-PBA Protects Against Hyperoxia-Induced Acute Lung Injury via Up-Regulating Claudin-4 Expression. Frontiers in Immunology. 2021; 12: 674316.

[80] Fitzgerald KA, Malhotra M, Curtin CM, O' Brien FJ, O' Driscoll CM. Life in 3D is never flat: 3D models to optimise drug delivery. Journal of Controlled Release. 2015; 215: 39–54.

[81] Rothen-Rutishauser BM, Kiama SG, Gehr P. A three-dimensional cellular model of the human respiratory tract to study the interaction with particles. American Journal of Respiratory Cell and Molecular Biology. 2005; 32: 281–289.

[82] Blank F, Rothen-Rutishauser B, Gehr P. Dendritic cells and macrophages form a transepithelial network against foreign particulate antigens. American Journal of Respiratory Cell and Molecular Biology. 2007; 36: 669–677.

[83] Viola H, Chang J, Grunwell JR, Hecker L, Tirouvanziam R, Grotberg JB, *et al*. Microphysiological systems modeling acute respiratory distress syndrome that capture mechanical force-induced injury-inflammation-repair. APL Bioengineering. 2019; 3: 041503.

[84] Nikolić MZ, Rawlins EL. Lung Organoids and their Use to Study Cell-Cell Interaction. Current Pathobiology Reports. 2017; 5: 223–231.

[85] Katsura H, Sontake V, Tata A, Kobayashi Y, Edwards CE, Heaton BE, *et al*. Human Lung Stem Cell-Based Alveolospheres Provide Insights into SARS-CoV-2-Mediated Interferon Responses and Pneumocyte Dysfunction. Cell Stem Cell. 2020; 27: 890–904.e8.

[86] Salahudeen AA, Choi SS, Rustagi A, Zhu J, van Unen V, de la O SM, *et al*. Progenitor identification and SARS-CoV-2 infection in human distal lung organoids. Nature. 2020; 588: 670–675.

[87] Youk J, Kim T, Evans KV, Jeong Y, Hur Y, Hong SP, *et al*. Three-Dimensional Human Alveolar Stem Cell Culture Models Reveal Infection Response to SARS-CoV-2. Cell Stem Cell. 2020; 27: 905–919.e10.

[88] Huh D, Leslie DC, Matthews BD, Fraser JP, Jurek S, Hamilton GA, *et al*. A human disease model of drug toxicity-induced pulmonary edema in a lung-on-a-chip microdevice. Science Translational Medicine. 2012; 4: 159ra147.

[89] Meyer NJ, Calfee CS. Novel translational approaches to the search for precision therapies for acute respiratory distress syndrome. the Lancet. Respiratory Medicine. 2017; 5: 512–523.

[90] da Silva da Costa FA, Soares MR, Malagutti-Ferreira MJ, da Silva GR, Lívero FADR, Ribeiro-Paes JT. Three-Dimensional Cell Cultures as a Research Platform in Lung Diseases and COVID-19. Tissue Engineering and Regenerative Medicine. 2021; 18: 735–745.

[91] Huang D, Liu T, Liao J, Maharjan S, Xie X, Pérez M, *et al*. Reversed-engineered human alveolar lung-on-a-chip model. Proceedings of the National Academy of Sciences. 2021; 118: e2016146118.

[92] Wyshock EG, Suffredini AF, Parrillo JE, Colman RW. Cofactors V and VIII after endotoxin administration to human volunteers. Thrombosis Research. 1995; 80: 377–389.

[93] DeLa Cadena RA, Majluf-Cruz A, Stadnicki A, Agosti JM, Colman RW, Suffredini AF. Activation of the contact and fibrinolytic systems after intravenous administration of endotoxin to normal human volunteers: correlation with the cytokine profile. Immunopharmacology. 1996; 33: 231–237.

[94] Luca R, Lijnen HR, Suffredini AF, Pepper MS, Steinberg KP, Martin TR, *et al*. Increased angiostatin levels in bronchoalveolar lavage fluids from ARDS patients and from human volunteers after lung instillation of endotoxin. Thrombosis and Haemostasis. 2002; 87: 966–971.

[95] Martin TR, Pistorese BP, Chi EY, Goodman RB, Matthay MA. Effects of leukotriene B4 in the human lung. Recruitment of neutrophils into the alveolar spaces without a change in protein permeability. The Journal of Clinical Investigation. 1989; 84: 1609–1619.

[96] Shyamsundar M, McKeown STW, O'Kane CM, Craig TR, Brown V, Thickett DR, *et al*. Simvastatin decreases lipopolysaccharide-induced pulmonary inflammation in healthy volunteers. American Journal of Respiratory and Critical Care Medicine. 2009; 179: 1107–1114.

[97] Shyamsundar M, McAuley DF, Ingram RJ, Gibson DS, O'Kane D, McKeown ST, *et al*. Keratinocyte growth factor promotes epithelial survival and resolution in a human model of lung injury. American Journal of Respiratory and Critical Care Medicine. 2014; 189: 1520–1529.

[98] Moazed F, Burnham EL, Vandivier RW, O'Kane CM, Shyamsundar M, Hamid U, *et al*. Cigarette smokers have exaggerated alveolar barrier disruption in response to lipopolysaccharide inhalation. Thorax. 2016; 71: 1130–1136.

[99] Hamid U, Krasnodembskaya A, Fitzgerald M, Shyamsundar M, Kissenpfennig A, Scott C, *et al*. Aspirin reduces lipopolysaccharide-induced pulmonary inflammation in human models of ARDS. Thorax. 2017; 72: 971–980.

[100] Lee JW, Fang X, Gupta N, Serikov V, Matthay MA. Allogeneic human mesenchymal stem cells for treatment of E. coli endotoxin-induced acute lung injury in the ex vivo perfused human lung. Proceedings of the National Academy of Sciences. 2009; 106: 16357–16362.

[101] Ross JT, Nesseler N, Lee J, Ware LB, Matthay MA. The ex vivo human lung: research value for translational science. JCI Insight. 2019; 4: e128833.

[102] Shaver CM, Ware LB. Primary graft dysfunction: pathophysiology to guide new preventive therapies. Expert Review of Respiratory Medicine. 2017; 11: 119–128.

[103] Beitler JR, Goligher EC, Schmidt M, Spieth PM, Zanella A, Martin-Loeches I, *et al*. Personalized medicine for ARDS: the 2035 research agenda. Intensive Care Medicine. 2016; 42: 756–767.

[104] Jagrosse ML, Dean DA, Rahman A, Nilsson BL. RNAi therapeutic strategies for acute respiratory distress syndrome. Translational Research. 2019; 214: 30–49.

[105] Jabaudon M, Blondonnet R, Audard J, Fournet M, Godet T, Sapin V, *et al*. Recent directions in personalised acute respiratory distress syndrome medicine. Anaesthesia Critical Care & Pain Medicine. 2018; 37: 251–258.

[106] Matthay MA, Arabi YM, Siegel ER, Ware LB, Bos LDJ, Sinha P, *et al*. Phenotypes and personalized medicine in the acute respiratory distress syndrome. Intensive Care Medicine. 2020; 46: 2136–2152.

[107] Jabaudon M, Blondonnet R, Ware LB. Biomarkers in acute respiratory distress syndrome. Current Opinion in Critical Care. 2021; 27: 46–54.

[108] Jabaudon M, Futier E, Roszyk L, Chalus E, Guerin R, Petit A, *et al*. Soluble form of the receptor for advanced glycation end products is a

marker of acute lung injury but not of severe sepsis in critically ill patients. Critical Care Medicine. 2011; 39: 480–488.

[109] Mrozek S, Jabaudon M, Jaber S, Paugam-Burtz C, Lefrant J, Rouby J, *et al*. Elevated Plasma Levels of sRAGE are Associated with Nonfocal CT-Based Lung Imaging in Patients with ARDS: a Prospective Multicenter Study. Chest. 2016; 150: 998–1007.

[110] Sinha P, Calfee CS. Phenotypes in acute respiratory distress syndrome: moving towards precision medicine. Current Opinion in Critical Care. 2019; 25: 12–20.

[111] Jabaudon M, Audard J, Pereira B, Jaber S, Lefrant J-Y, Blondonnet R, *et al*. Early Changes Over Time in the Radiographic Assessment of Lung Edema Score Are Associated With Survival in ARDS. Chest. 2020; 158: 2394–2403.

[112] Jabaudon M, Pereira B, Laroche E, Roszyk L, Blondonnet R, Audard J, *et al*. Changes in Plasma Soluble Receptor for Advanced Glycation End-Products Are Associated with Survival in Patients with Acute Respiratory Distress Syndrome. Journal of Clinical Medicine Research. 2021; 10: 2076.

[113] Sinha P, Calfee CS, Cherian S, Brealey D, Cutler S, King C, *et al*. Prevalence of phenotypes of acute respiratory distress syndrome in critically ill patients with COVID-19: a prospective observational study. The Lancet Respiratory Medicine. 2020; 8: 1209–1218.

[114] Carla A, Pereira B, Boukail H, Audard J, Pinol-Domenech N, De Carvalho M, *et al*. Acute respiratory distress syndrome subphenotypes and therapy responsive traits among preclinical models: protocol for a systematic review and meta-analysis. Respiratory Research. 2020; 21: 81.

[115] Papazian L, Forel J, Gacouin A, Penot-Ragon C, Perrin G, Loundou A, *et al*. Neuromuscular blockers in early acute respiratory distress syndrome. The New England Journal of Medicine. 2010; 363: 1107–1116.

[116] Acute Respiratory Distress Syndrome Network, Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, *et al*. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. The New England Journal of Medicine. 2000; 342: 1301–1308.

[117] Villar J, Ferrando C, Martínez D, Ambrós A, Muñoz T, Soler JA, *et al*. Dexamethasone treatment for the acute respiratory distress syndrome: a multicentre, randomised controlled trial. Lancet Respiratory Medicine. 2020; 8: 267–276.

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REVIEW

New insights in mechanical ventilation and adjunctive therapies in ARDS

Denise Battaglini[1,2,*], Patricia Rieken Macedo Rocco[3,†], Paolo Pelosi[1,4,*,†]

[1]Anesthesia and Intensive Care, San Martino Policlinico Hospital, 16132 Genoa, Italy
[2]Department of Medicine, University of Barcelona (UB), 08001 Barcelona, Spain
[3]Laboratory of Pulmonary Investigation, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro, 21941-902 Rio de Janeiro, Brazil
[4]Department of Surgical Sciences and Integrated Diagnostic (DISC), University of Genoa, M5R 1C1 Genoa, Italy

***Correspondence**
ppelosi@hotmail.com
(Paolo Pelosi);
battaglini.denise@gmail.com
(Denise Battaglini)

† These authors contributed equally.

Abstract

Patients with acute respiratory distress syndrome (ARDS) often require mechanical ventilation (MV) and may experience high morbidity and mortality. The ventilatory management of ARDS patients has changed over the years to mitigate the risk of ventilator-induced lung injury (VILI) and improve outcomes. Current recommended MV strategies include the use of low tidal volume (V_T) at 4–6 mL/kg of predicted body weight (PBW) and plateau pressure (P_{PLAT}) below 27 cmH_2O. Some patients achieve better outcomes with low V_T than others, and several strategies have been proposed to individualize V_T, including standardization for end-expiratory lung volume or inspiratory capacity. To date, no strategy for individualizing positive-end expiratory pressure (PEEP) based on oxygenation, recruitment, respiratory mechanics, or hemodynamics has proven superior for improving survival. Driving pressure, transpulmonary pressure, and mechanical power have been proposed as markers to quantify risk of VILI and optimize ventilator settings. Several rescue therapies, including neuromuscular blockade, prone positioning, recruitment maneuvers (RMs), vasodilators, and extracorporeal membrane oxygenation (ECMO), may be considered in severe ARDS. New ventilator strategies such as airway pressure release ventilation (APRV) and time-controlled adaptive ventilation (TCAV) have demonstrated potential benefits to reduce VILI, but further studies are required to evaluate their clinical relevance. This review aims to discuss the cornerstones of MV and new insights in ARDS ventilatory management, as well as their rationales, to guide the physician in an individually tailored rather than a fixed, sub-physiological approach. We recommend that MV be individualized based on physiological targets to achieve optimal ventilatory settings for each patient.

Keywords

Mechanical ventilation; ARDS; COVID-19; Mechanical power; APRV

1. Background

The definition of acute respiratory distress syndrome (ARDS) dates back to 1967 [1]. Despite 55 years of research and clinical experience, ARDS management remains challenging, and the syndrome is associated with a high mortality rate [2], requiring intensive care unit (ICU) admission and mechanical ventilation (MV) [3]. In recent decades, a huge effort has been made to investigate the impact of lung-protective ventilation on ARDS outcome and to modify ventilatory management strategies to reduce the risk of ventilator induced lung injury (VILI). Although several ventilatory strategies are now recognized as the standard of care in the management of ARDS patients, an individualized approach, which takes into account the limits of physiological gain and the uncertainty concerning ventilatory manipulation on outcome, is now under consideration [4] (Fig. 1). This review aims to discuss the cornerstones of MV and new insights in ARDS ventilatory management, as well as their rationale, to guide the physician in an individually tailored rather than a fixed, less physiological approach.

2. Standard of care

2.1 Low tidal volume

The current standard of care of MV in ARDS includes lung-protective ventilation targeting a low tidal volume (V_T) of 4–6 mL/kg of predicted body weight (PBW), and plateau pressure (P_{PLAT}) below 27 cmH_2O [5]. The introduction of these targets dates back to the 2000 ARMA trial, where a traditional approach of V_T = 12 mL/kg of PBW with a P_{PLAT} less than 50 cmH_2O was compared with a lung protective approach of V_T = 6 mL/kg with a P_{PLAT} below 27 cmH_2O, showing that ARDS patients with low V_T had significant reductions in mortality [6]. Although these large trials established that lung-protective ventilation using low V_T should be pursued in ARDS, research

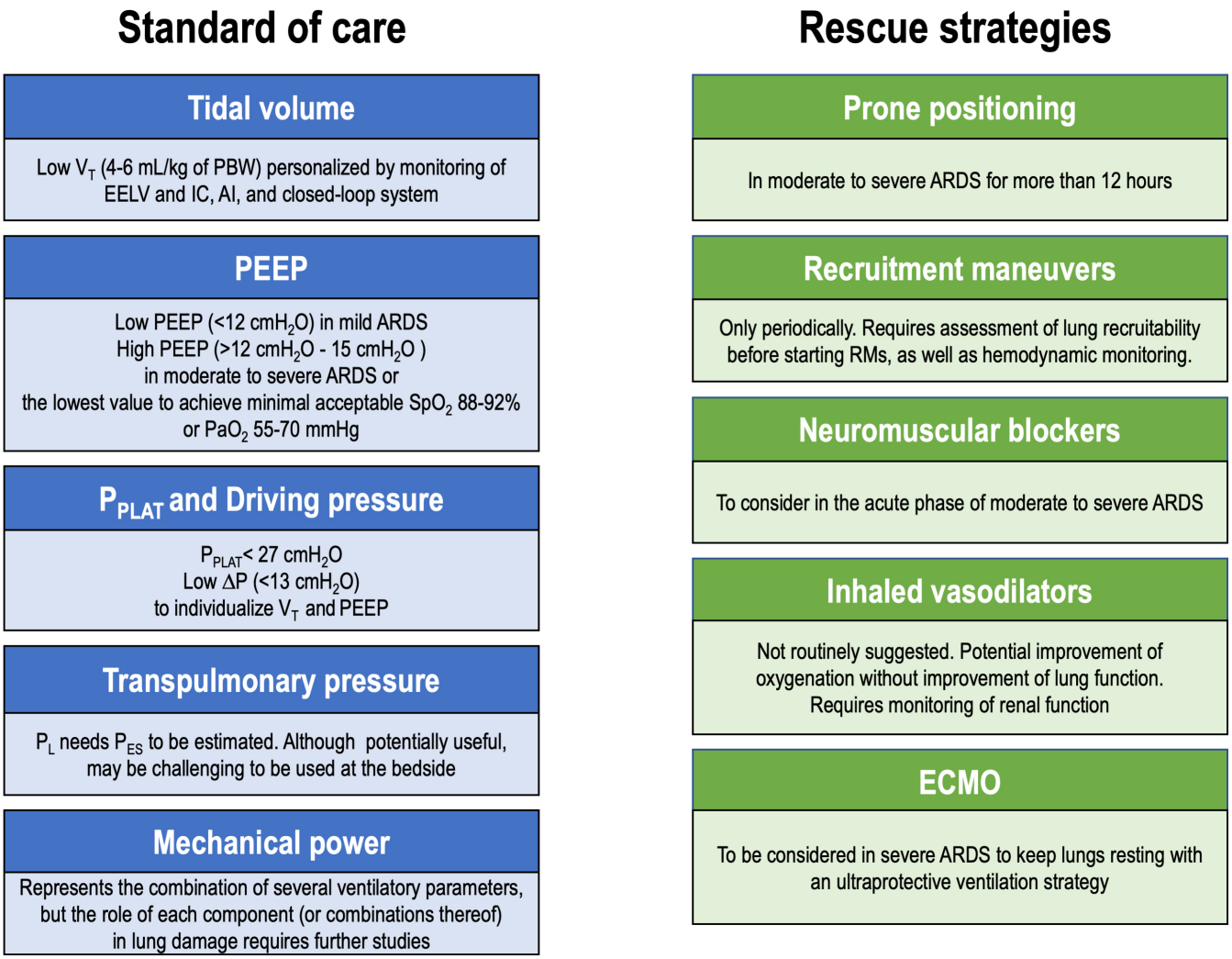

FIGURE 1. Mechanical ventilation in ARDS: standard of care and rescue strategies. On the left, the cornerstones of mechanical ventilation in acute respiratory distress syndrome (ARDS). On the right, possible rescue strategies in case of moderate to severe ARDS refractory to conventional strategies. V_T, tidal volume; PBW, predicted body weight; EELV, end-expiratory lung volume; IC, inspiratory capacity; AI, artificial intelligence; PEEP, positive end-expiratory pressure; PaO_2, arterial partial pressure of oxygen; P_{PLAT}, plateau pressure; ΔP, driving pressure; P_L, pleural pressure; P_{ES}, esophageal pressure; RMs, recruitment maneuvers.

regarding the use of low V_T in ARDS continued over the next 20 years [7]. A large multinational prospective cohort study, LUNG SAFE, identified a frequent underdiagnosis of ARDS at ICU admission and noncompliance with lung-protective ventilation strategies, resulting in a strong association with mortality [8]. The detrimental sequelae of MV with high V_T have been clearly demonstrated [9]. Current approaches suggest individualizing MV according to patient and disease characteristics [4]. Given that V_T has been strongly associated with mortality in patients with lower respiratory system compliance (C_{RS}) [10], it should ideally be set according to the amount of aeration, using inspiratory capacity (IC), or end-expiratory lung volume (EELV) measured at 30 cmH_2O. This could be considered the approach of choice since, in heterogenous ARDS-affected lungs, lung volumes do not correlate well with PBW. However, V_T can be set according to EELV only if positive end-expiratory pressure (PEEP) is reduced, since it may change with C_{RS} [11]. Therefore, IC seems to be a more reliable technique at bedside, V_T being easily achieved with automated systems and artificial intelligence (AI) support [4].

2.2 Positive end-expiratory pressure

PEEP represents an essential component in ARDS management. PEEP allows alveolar recruitment to potentially open collapsed or edematous and inhomogeneously distributed areas of the ARDS "baby lung" [12]. A recruitment maneuver (RM) to open the collapsed alveoli is commonly followed by the application of PEEP to keep recruited alveoli open and improve gas exchange [4]. The use of high PEEP levels and RMs has been questioned, however. Two meta-analyses of randomized controlled trials (RCTs) concluded that low V_T combined with high PEEP improves survival in patients with ARDS [13, 14]. A secondary analysis of the Open Lung Ventilation Study showed improvement in oxygenation with high PEEP, associated with lower risk of death [15]. On the contrary, in the

ART trial, a PEEP higher than 15 cmH_2O was associated with increased risk of mortality in patients who were hemodynamically unstable [16], while in the PHARLAP trial an aggressive recruitment strategy was associated with cardiac arrhythmias [17]. In a third scenario, three RCTs of lung-protective ventilation in ARDS patients found no differences in mortality with high and moderate PEEP levels [18–20]. Benefits of PEEP application include alveolar recruitment, reduction of intrapulmonary shunting, and improvement of oxygenation, while harms include increased EELV, possible volutrauma, and VILI [3, 21]. High PEEP is associated with increased static stress, even though a meta-analysis concluded that neither RMs nor higher PEEP affect mortality in ARDS patients [22]. Current recommendations suggest adopting high PEEP (>12 cmH_2O) only for patients with moderate or severe ARDS [23]. However, individualization of PEEP according to the potential for alveolar recruitment should be considered [24]. Indeed, it is important to distinguish recruitable and non-recruitable ARDS patients. In the latter, the airway pressure tends to increase, causing hemodynamic impairment and lung overdistension, whereas when the collapsed areas are recruitable, the lung can benefit from reduction of pressures. Unfortunately, monitoring alveolar recruitment at the bedside remains challenging and, to date, no definitive recommendations on how to set PEEP are available. A possible strategy could be to set PEEP according to transpulmonary pressure (P_L) or a low PEEP/arterial partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) table, which does not seem to influence mortality [16, 25]. A possible, relatively new strategy to estimate PEEP at bedside expects to appraise the recruitment volume by performing two pressure/volume (P/V) curves (at high and low PEEP) and measuring the difference between the expired volume and the volume predicted by the compliance of the respiratory system above the airway opening pressure: $\frac{\Delta Vrec}{\Delta Prec}$ where $\Delta Prec = PEEP_{high} - PEEP_{low}$.

The compliance of the recruited lung can be estimated by the ratio: $\frac{\frac{\Delta Vrec}{(PEEP_{high} - PEEP_{low})}}{Crs\ above\ airway\ opening\ pressure\ or\ at\ PEEP_{low}}$ [26]. When this ratio is equal to or greater than 0.5, patients are more likely to be recruitable, and might need higher levels of PEEP. In any case, from a clinical point of view, PEEP should be set at the lowest level to achieve a minimal acceptable peripheral saturation of oxygen (SpO_2) (88–92%) or PaO_2 (55–70 mmHg) [27, 28], but keeping in mind possible detrimental clinical effects on right ventricular function, cardiac output, and lymphatic flow drainage [29, 30]. In addition to the aforementioned methods for PEEP titration, electrical impedance tomography (EIT), lung ultrasound (LUS), and computed tomography (CT) should be mentioned. As compared with pressure/volume curve, PEEP titration using EIT was associated with improved oxygenation, compliance, driving pressure, and weaning success rate [31]. However, "optimal" PEEP levels determined by EIT may differ significantly among ARDS patients (of around 10%) due to the presence of non-recruitable lungs and heterogeneity of ventilation. The advantage of using EIT at the bedside to individualize PEEP is the possibility of identifying lung heterogeneity, thus avoiding alveolar cycling and regional overdistension and minimizing the risk of VILI in a personalized manner. Despite this potential advantage, the literature on possible optimization of mechanical ventilation using EIT in ARDS is still scarce, and further implementation is needed [32]. LUS demonstrated good estimation of lung recruitment at the bedside, with the limitation of not assessing PEEP-induced lung hyperinflation [33], but ability to distinguish between different ARDS morphologies (focal *vs.* non-focal) [34]. The use of LUS to individualize PEEP in patients with ARDS has several advantages, including bedside availability, low cost, no ionizing radiation, and relatively little dependence on operator skills. LUS provides the possibility of observing changes in ultrasound patterns during PEEP implementation and successfully selecting an appropriate level of PEEP, and can detect response to the application of RMs, helping the clinician distinguish between recruiters and non-recruiters [35]. Other methods such as CT could help in titration of PEEP in case of limitations of noninvasive methods [36], allowing a visual, anatomical analysis of lung recruitability [37]. However, CT has potential disadvantages, including the impossibility to be performed routinely and repeated due to the limitations of patient transportability and ionizing radiations exposure, as well as the need for possible increased sedation and neuromuscular blockade. For this reason, CT cannot be considered for routine use in individualizing PEEP at the bedside [35].

2.3 Driving pressure

Driving pressure (ΔP) represents the ratio between V_T and C_{RS} or the airway plateau pressure minus PEEP (P_{PLAT}–PEEP). In other terms, since C_{RS} correlates with aeration of the lung, ΔP represents an easy estimator of strain (V_T/aeration of the lung at end expiration) for that particular V_T. ΔP was first considered a component of lung protective ventilation in 1998 by Amato et al. in a small RCT [38]. Since then, ΔP has been adopted as a method to set PEEP, but the benefits of this strategy are counterbalanced by potential harms, including the fact that ΔP depends on the different V_T used as well as C_{RS}. At high C_{RS}, lower ΔP may help achieve higher PEEP. ΔP may also be affected by changes in chest wall compliance, and airway closure may confound the relationship between PEEP and ΔP [4]. Decreases in ΔP have been associated with survival benefit even when the patient received protective plateau pressure and V_T [39], while ΔP higher than 13 cmH_2O was associated with mortality [40]. A meta-analysis of 7 RCTs and 2 observational studies also confirmed that ΔP above 15 cmH_2O is associated with significantly higher mortality [41]. In short, maintaining ΔP below 13 cmH_2O and P_{PLAT} below 27 cmH_2O is the best suggested approach, although an individualized tailored strategy according to V_T and PEEP is preferable [42]. It is our opinion that the beneficial effects of reduced ΔP on outcome are because of lower V_T, and not to the reduction of ΔP with higher PEEP, mostly associated with increased P_{PLAT}.

2.4 Transpulmonary pressure

Transpulmonary pressure (P_L) represents the distending force of the lung determined by the equation $P_{AW} - P_{PL}$ (where P_{AW} is airway pressure and P_{PL} is the pleural pressure), and

it is estimated by esophageal pressure (P_{ES}) [43]. In ARDS, both lung and chest wall elastance (E_{CW}) are often impaired. To induce alveolar recruitment, PEEP needs to overcome P_L [44, 45]. P_{AW} is not injurious at tidal ventilation, provided E_{CW} is increased. P_{PL} allows differentiation of lungs *vs.* chest-wall mechanics [43]. In the supine position, P_L acts as the pressure that works on alveoli and airways due to the pressure gradient between nondependent and dependent areas [46]. Using P_{ES} to interpret P_L, the difference between P_{AW} − P_{ES} at end-expiration or end-inspiration can reflect the P_L in the middle lung, while the difference in P_L (ΔP_L) between end-inspiration and end-expiration estimates the ΔP_{ES} [4]. Further, one should consider that P_{ES} overestimates the pleural pressure by + 5 cmH_2O in nondependent lung regions (near the sternum), while underestimating by −5 cmH_2O the pleural pressure in dependent lung regions (near the vertebrae). For these reasons, the absolute P_L in the dependent lung regions at end-expiration should be calculated as PEEP − P_{ES} − 5 cmH_2O, while in the nondependent lung regions at end-inspiration, it should be calculated as P_{PLAT} − P_{ES} + 5 cmH_2O. Several trials targeting mechanical ventilation by using P_L have found no beneficial effects on outcome [45, 47]. However, none of them appropriately corrected for appropriate absolute P_L. Preliminary data regarding the use of transpulmonary pressure to tailor ventilator settings are encouraging, but further, adequately powered studies are warranted. Therefore, although this technique represents an appealing "precision medicine" approach to individualized mechanical ventilation parameters, the routine use of transpulmonary pressure is limited and should be reserved only for selected cases (*e.g.* obese patients, to assess the impact of the chest wall; patients in whom ventilatory pressures are too high to be explained by other, easier methods). Indeed, the assessment of transpulmonary pressure with continuous monitoring of P_{ES} at the bedside is often challenging because of the need to insert an esophageal catheter connected to a computer running dedicated software [43, 45, 47]. Furthermore, as explained elsewhere in this review, other, more suitable, and accessible methods to personalize mechanical ventilation in ARDS are available.

2.5 Mechanical power

Mechanical power (MP) is the product of mechanical energy and respiratory rate [48], also defined as the amount of energy per unit of time. Lung damage can be directly explained by using some parameters that are set on the ventilator by the clinician (V_T, ΔP, airflow, respiratory rate, and PEEP). The mechanisms associated with these variables alone or different combinations thereof cause direct damage to epithelial/endothelial cells and extracellular matrix [48]. MP calculation is based on the following formulas, according to the type of ventilation that is applied:

$$MP_{VCV} = 0.098 \times V_T \times (P_{PEAK} - \frac{\Delta P}{2}) \times RR$$

$$MP_{PCV} = 0.098 \times V_T \times (\Delta P + PEEP) \times RR$$

where VCV is volume-controlled mode, PCV is pressure-controlled mode [49, 50], and RR represents the respiratory rate in breaths per minute. In general, these MP formulas are based on the basic equation of motion, $P_{RS} = E_{RS} \times V_T + V'_{INSP} \times R_{AW}$, which considers changes in pressure as well as elastic and resistive components (V'_{INSP} is the inspiratory flow and R_{AW} is the airway resistance). The same equation can be computed for the "absolute" level of respiratory system energy as $P_{RS} = E_{RS} \times V_T + V'_{INSP} \times R_{AW} + PEEP$. However, to date, controversies remain regarding the best equation to evaluate MP at bedside [51]. MP has been associated with increased mortality and worse oxygenation in ARDS and non-ARDS populations [52, 53], although in another report this was true only if normalized to compliance as well as to aerated tissue [54]. More studies are needed to better understand the association between MP and survival in ARDS patients. For this reason, although MP represents an appealing and easily available method that integrates several ventilatory parameter in a unique equation which can be calculated at the bedside, the lack of literature confirming the impact of this parameter on hard outcomes limits its routine use as a potential target to individualize mechanical ventilation in ARDS [4].

3. Other ventilation modes

3.1 Airway pressure release ventilation and time-controlled adaptive ventilation

Airway pressure release ventilation (APRV) is a ventilatory strategy first developed by Downs *et al.* [55] for patients with reduced compliance. This ventilatory mode uses a continuous positive airway pressure combined with a partial and short release phase for ventilation, allowing the patient to breathe spontaneously. A high pressure (P_{high}) around 20–30 cmH_2O is applied and maintained for a certain time (T1) during which the patient can breathe spontaneously. At the end of T1, the pressure decreases to low pressure (P_{low}) according to lung elastic recoil. T2 is obtained with an expiratory flow around 25–50% of the maximum value. However, $P_{high}$and P_{low} should be set according to the higher and lower inflection points of the P/V loop [56]. The efficacy of APRV in ARDS has been recently demonstrated in a meta-analysis of 6 clinical trials and 375 patients, showing an improvement in oxygenation with shorter ICU stay [57]. Regarding hemodynamic stability, another meta-analysis found an increase in the mean arterial pressure and reductions in peak pressure and 28-day mortality [58]. APRV, compared to lung-protective ventilation, increased compliance and oxygenation and improved hemodynamics, thus resulting in reduced mortality, duration of MV, and ICU stay [59, 60]. The use of time-controlled adaptive ventilation (TCAV) during APRV showed improvement of lung recruitment, more homogeneous ventilation, and reduction in alveolar strain and stress [61]. In experimental ARDS, TCAV, compared to lung-protective MV, reduced lung damage and inflammation [62], making this strategy a possible valuable alternative to classic APRV. These two ventilatory techniques are implemented for the management of patients with ARDS for all the above-mentioned reasons. However, when targeting patients who might benefit from this techniques to individualize therapy, several potential situations should be

considered, including the fact that spontaneous breathing effort can result in increased oxygen consumption by the respiratory muscles; that vigorous breathing efforts may increase the transcapillary pressure gradient, enhancing pulmonary edema formation; and large tidal volumes and transpulmonary pressure swings can be achieved because APRV is also a type of pressure-controlled ventilation, thus potentially contributing to volutrauma [63].

3.2 High-frequency oscillatory ventilation

High-frequency oscillatory ventilation (HFOV) is a conceptually appealing method of MV to reduce VILI in ARDS patients, using V_T equal or lower than dead space (0.1–3 mL/kg) but respiratory rates >150 breaths/min or 3–15 Hz and a bias flow of gas set at 5–60 L/min [64]. The equation of Fredberg explains how alveolar ventilation is obtained with HFOV: $(f)^x \times (V_T)^y$, where x is between 0.5 and 1 and y between 1.5 and 2.2, which can be written as follows: $(f) \times (V_T)^2$. Based on this equation, it can be noted that V_T has a greater influence than respiratory rate in determining alveolar ventilation. HFOV maintains a continuous distending pressure and facilitates elimination of carbon dioxide, mainly by accelerating the molecular diffusion process [64]. In experimental ARDS, HFOV reduced lung injury, hyaline membrane formation, airway epithelial cell damage, and biomarkers of inflammation (interleukin (IL)-1β, IL-6, IL-8, IL-10, transforming growth factor and adhesion molecules, as well as tumor necrosis factor (TNF)) when compared to conventional MV [64, 65]. In ARDS patients, HFOV, when used as a rescue therapy, improved oxygenation [66]. However, other studies found it resulted in higher mortality rates in patients whose oxygenation failed to improve [67], or a nonsignificant trend towards reduced 30-day mortality when compared to conventional MV [68]. In 2017, a meta-analysis by Meade *et al.* [69] reported that HFOV increases mortality in patients with ARDS, but not in case of severe hypoxemia on conventional MV. A previous Cochrane review concluded that there is not enough evidence to demonstrate superiority of HFOV in adult ARDS patients when compared with lung-protective conventional MV, but benefits of HFOV were seen regarding survival and treatment failure (*i.e.*, refractory hypoxemia, hypercapnia, hypotension, or barotrauma) [70]. In summary, the use of HFOV in adult ARDS remains controversial, especially regarding survival outcomes. HFOV can be considered as rescue therapy in ARDS if potential harms (higher intrathoracic pressure, interference with right ventricular preload, pneumothorax, displacement of the endotracheal tube, airway obstruction from mucus plug, refractory acidosis, cellular injury) and benefits (improved oxygenation, reduced VILI, failed conventional ventilation, lower V_T, lungs inflation avoiding repeated opening and closing of alveoli) are weighed carefully with respect to individual patient characteristics and needs [71]. Patients who can benefit from HFOV as a rescue strategy are those with severe ARDS whose lungs cannot tolerate high tidal distending pressure.

4. Adjunctive therapies

4.1 Prone positioning

Prone positioning represents a rescue therapy in severe ARDS. In ARDS lungs, dependent areas are commonly more perfused than the nondependent due to gravitational gradient, resulting in hypoxia associated with ventilation/perfusion mismatch. Prone positioning allows a more homogenous distribution of ventilation/perfusion with diminished intrapulmonary shunt [72]. Nevertheless, some conflicting results were published in the clinical setting regarding ARDS patient outcomes. The prone-supine RCT found no differences in survival when comparing prone with supine positioning, but more complications [73]; in contrast, the PROSEVA trial showed reduced mortality in prone compared to supine groups, and similar rates of complications [74]. A meta-analysis of RCTs confirmed the benefits of reduced mortality using prone positioning [75]. Particularly, in a sub-analysis, mortality rate was further reduced when prone positioning was applied for more than 12 hours [76]. Finally, two recent meta-analyses supported the use of prone positioning and venous-venous extracorporeal membrane oxygenation (VV-ECMO) in adjunction to lung-protective ventilation in ARDS patients, demonstrating survival benefits [77, 78]. Prone positioning has also become one of the cornerstones of mechanical ventilation in COVID-19 patients with ARDS, as briefly explained in the appropriate section below “5. Mechanical ventilation in COVID-19”.

4.2 Recruitment maneuvers

Recruitment maneuvers (RMs) are considered part of the “open lung approach”, reducing repeated opening and closing of collapsed alveoli and intrapulmonary shunt, thus improving oxygenation [79]. However, RMs may lead to VILI and hemodynamic impairment. The ART trial reported that high-pressure stepwise lung RMs (up to P_{PLAT} of 50–60 cmH_2O) combined with higher PEEP titration increased patient mortality [16], while the PHARLAP trial [17], assessing RMs up to a P_{PLAT} of 28 cmH_2O, was interrupted as several patients experienced hemodynamic issues. Meta-analyses of RCTs, despite supporting the use of RMs in combination with PEEP or alone, did not describe which type of RMs was performed in each trial, thus leading to poor accuracy. RMs are usually adopted in cases of severe hypoxemia, but there is no evidence regarding their optimal frequency or exact timing. Some studies report systematic application of RMs, while others report the application of RMs when the lung is de-recruited, as a rescue measure. Regardless, RMs appear to be safe if used periodically (*i.e.*, not systematically), since they improve oxygenation and seem not to lead to barotrauma or hemodynamic compromise [22, 80, 81]. Additionally, it is important to identify lung recruitability at bedside to individualize the use of RM strategies in ARDS patients. An approach which targets at the need of the patient by assessing lung recruitability at the bedside before applying potentially harmful maneuvers is suggested. A potentially recruitable lung consists of some areas of open alveoli and others of collapsed alveoli, which can be opened, thus decreasing shunt, pulmonary vascular resistance, and edema, as well as improving oxygenation. Conversely, a potentially non-recruitable or poorly recruitable lung is mainly constituted of already open alveoli, carrying a high risk of

VILI from excessive stress and strain, increased dead space, shunting, and potentially high pulmonary vascular resistances [82]. Methods to assess lung recruitability have been explained in paragraph 2.2 "Positive end-expiratory pressure".

4.3 Sedation, analgesia and neuromuscular blockers

In the acute phase, patients with severe ARDS remain deeply sedated and require the use of neuromuscular blocking agents (NMBAs) to improve gas exchange. On the other hand, early active breathing has the advantage of reducing respiratory muscle wasting, improving oxygenation, and increasing compliance [83]. Analgesia and sedation with or without the use of NMBAs is challenging in patients with ARDS. The primary objective of analgesia and sedation in patients with ARDS is to provide safety and comfort, to help the patient interact with the ventilator and the staff, to facilitate critical interventions, and to promote physical and cognitive recovery to minimize the risk of delirium and agitation [84]. Sedation and analgesia should be set according to individual patient requirements, without rigid adherence to a single strategy—*i.e.*, accepting short intervals of moderate sedation to reduce patient-ventilator asynchronies and discomfort, occasional deep sedation (especially in case of need for invasive mechanical ventilation with high pressures and neuromuscular blockade), or mild sedation with adequate analgesia, such as during ventilator weaning. In any case, sedation and analgesia should be individualized to patient requirements and ventilation needs [84]. Monitoring of sedation and pain levels with validated tools (*i.e.*, Richmond Agitation Sedation Scale (RASS), Sedation Agitation Scale (SAS), Behavioral Pain Scale (BPS), etcetera) should be encouraged. Analgesic and sedative infusions should be continued unless NMBAs are stopped [84]. It is important to distinguish which ARDS patients will benefit from the use of NMBAs, including those with higher The Acute Physiology and Chronic Health Evaluation (APACHE) II score, alveolar-arterial oxygen gradient, and P_{PLAT}, or those who are critical and require rescue therapies like VV-ECMO or prone positioning [85]. In 2010, Papazian *et al.* [86] found that a strategy of early administration of NMBAs improved 90-day survival and liberation from MV without increasing muscle weakness from disuse. In the ROSE trial, which included patients with moderate to severe ARDS, no significant differences in mortality were found between patients who received an early and continuous infusion of NMBAs *vs.* those who received usual care and lighter sedation [87]. A recent meta-analysis excluding the ROSE trial concluded that NMBAs did not reduce the overall risk of death at 28 days and 90 days, while ICU mortality was significantly reduced [88]. The reasons for excluding the ROSE trial were (1) the use of different PEEP titration strategies and (2) different degrees of sedation (light sedation compared to deep sedation strategy used in the other trials) [88]. Considering the differing results obtained from RCTs including severe ARDS patients, NMBAs appear to improve oxygenation and reduce the risk of barotrauma, but do not decrease mortality risk, ventilator-free days, or duration of MV.

4.4 Vasodilators

Selective pulmonary vasodilators, like inhaled nitric oxide (iNO), are an another rescue therapy for ARDS patients unresponsive to conventional therapies [89]. iNO improves oxygenation through a selective vasodilatation of capillary vessels in well-aerated alveoli, thus reducing ventilation/perfusion mismatch and pulmonary vascular resistance as well as increasing right ventricular output [89]. However, a meta-analysis of RCTs did not support routine use of iNO in ARDS, since no significant changes in survival were observed and a risk of renal dysfunction was detected [90]. As an alternative to iNO, inhaled epoprostenol has been suggested. The advantages of inhaled epoprostenol compared to iNO are (1) reduced potential side effects, (2) easier administration, and (3) lower costs. However, there are few studies regarding the use of inhaled epoprostenol in ARDS targeting mortality as a primary outcome [91].

4.5 Venous-venous (VV)-ECMO

VV-ECMO is often adopted as a rescue strategy for severe ARDS patients. The risk of VILI is reduced as an ultra-protective ventilatory strategy is provided [92]. The suggested criteria for VV-ECMO initiation in ARDS are: (1) mortality risk $>50\%$ and PaO_2/FiO_2 <150 with FiO_2 $>90\%$ and/or a Murray score of 2–3, an Age-Adjusted Oxygenation Index (AOI) score of 60; (2) mortality risk $\geq 80\%$ and PaO_2/FiO_2 <100 with FiO_2 $>90\%$, and/or Murray score 3–4, AOI score >80 or Acute Physiology of Stroke Score (APSS) (Age, PaO_2/FiO_2, Plateau Pressure) of 8; (3) hypercapnia despite protective mechanical ventilation and rescue therapies (*e.g.* prone positioning, recruitment maneuver); (4) severe air leak syndrome; (5) need for lung transplantation; or (6) acute severe heart or pulmonary failure that is potentially reversible but unresponsive to conventional management [93–95]. A meta-analysis of 2 RCTs and 5 observational studies concluded that ARDS patients undergoing VV-ECMO and MV exhibited a significantly lower mortality rate than those receiving MV alone at 30, 60, and 90 days [96]. However, a recent reanalysis of the data presented by Munshi *et al.* [97] using both traditional and Bayesian models to estimate the treatment effect concluded no certainty regarding the efficacy of VV-ECMO in ARDS on mortality. Compared with conventional MV, VV-ECMO showed lower 60-day, 90-day, and 1-year mortality in patients with ARDS, as demonstrated by both conventional and individual-patient-data meta-analyses [98, 99]. Hence, the latest evidence does not clearly support the use of VV-ECMO for patients who are critical and cannot obtain other benefits from conventional therapies. Therefore, patients with ARDS who might benefit from VV-ECMO are those needing complete pulmonary support to allow adequate oxygenation and carbon dioxide removal, while limiting the risk of VILI due to conventional ventilator strategies. However, given that VV-ECMO is commonly adopted as a rescue strategy, the decision to start VV-ECMO is difficult to place into the context of personalized ARDS therapy. The decision to initiate ECMO should also weigh the patients' possibility of recovery, family expectations, odds of survival, potential life-threatening complications, and ethical considerations [100].

5. Mechanical ventilation in COVID-19

The coronavirus disease 2019 (COVID-19) pandemic has called into question several cornerstones of MV in ARDS, mainly because at the onset of the pandemic ARDS and COVID-19 were considered very similar; thus, there was an attempt to employ the same MV strategies for both conditions. The main driver of MV strategies in COVID-19 ARDS is actually the identification of pathophysiological differences and similarities between COVID-19 ARDS and non-COVID-19 ARDS, although both are characterized by severe refractory hypoxemia and high mortality [101]. Severe COVID-19 and typical ARDS are usually characterized by respiratory compromise and multiorgan failure. Biological markers have been identified as exacerbating factors for severe disease in both cases [102]. Particularly, variations in the immune and inflammatory response, including cytokine release (*e.g.* interleukin-6 and 10), endothelial dysfunction, microthrombus formation with an altered coagulation cascade, have led to the identification of several serum biomarkers (lactate dehydrogenase, D-dimer, among others) able to provide early detection of progression to severe disease, although their potential association with outcomes is unclear [103]. This concept has been previously raised in non-COVID-19 ARDS, with the identification of sub-phenotypes (*i.e.*, hyperinflammatory and hypoinflammatory), which may represent a shift toward a more targeted "precision medicine" approach [104]. In COVID-19 ARDS, unlike in typical ARDS, nondependent aerated regions show mostly perfusion over ventilation, with a certain degree of hypoxic vasoconstriction in the dependent lung regions that results in a non-gravitational distribution of regional blood flow [105]. The identification of COVID-19 phenotypes (1 or L and 2 or H) through chest CT could be a valid strategy to select patients who would benefit from early intubation and those who would not [106]. In COVID-19 phenotype 1, lung compliance typically is not markedly affected, whereas gas exchange and hypoxia deteriorate rapidly due to microthrombosis, with increased wasted ventilation, and reduced ventilation/perfusion mismatch, while lung weight is lower [107]. On the other hand, in COVID-19 phenotype 2, lung weight is increased, with reduced compliance, increased wasted ventilation, and true shunting, whereas ventilation/perfusion mismatch is less compromised [106]. Indeed, COVID-19 patients receiving invasive MV show a decrease in lung volume and increase in poorly aerated or non-aerated lung tissue areas compared to patients receiving noninvasive respiratory support (NIRS) [108].

Therefore, the use of NIRS as a first-line strategy should be put within the context of COVID-19 phenotypes and considered especially for COVID-19 phenotype 1. In general, current recommendations moved from an early intubation approach at the onset of the pandemic to a more conservative one [109], distinguishing between COVID-19 phenotypes 1 and 2 in order to intubate early only those patients who clearly present with COVID-19 phenotype 2 or deterioration of phenotype 1 after NIRS. The recognition of patients who are at higher risk of NIRS failure is challenging [109] and should consider possible patient self-inflicted lung injury (P-SILI). NIRS methods include high-flow nasal oxygen (HFNO), noninvasive continuous positive airway pressure (CPAP), and noninvasive ventilation (NIV). An initial strategy using non-invasive CPAP was found to reduce the risk of tracheal intubation or mortality compared to conventional oxygen therapy, while this was not confirmed for HFNO [110]. A brief period of awake prone positioning during NIRS can also be considered before moving forward to intubation [111]. In the presence of clinical deterioration or if patients already present with phenotype 2 (or H) on admission, intubation and invasive mechanical ventilation can be considered. This mode of ventilation should be set using a low VT of 4–6 mL/kg of PBW, low plateau pressure $<$28–30 cmH_2O, and moderate levels of PEEP (10 to 15 cmH_2O) according to individual patient response and requirements [111]. When lung compliance is preserved and areas of atelectasis are few, low to moderate rather than high PEEP levels might be indicated [111]. Hence, a strategy for the early phase (with predominance of low ventilation/perfusion areas) would comprise higher oxygen fraction and moderate levels of PEEP, while in the late stage (predominance of shunt), higher PEEP levels (but not exceeding 15 cmH_2O) might be suggested, given that poor response to oxygen is expected [111]. Regarding the use of prone positioning during invasive mechanical ventilation in patients with COVID-19 ARDS, there is no agreement in the literature as to which patients may benefit from this strategy. In general, more severe patients with COVID-19 phenotype 2 are considered eligible. The main rationale is that the improvement in oxygenation achieved with prone positioning allows a more homogeneous distribution of ventilation and perfusion, reducing the risk of VILI. This improvement in oxygenation is often associated with redistribution of perfusion (anti-gravitational as compared with non-COVID-19 ARDS) rather than effective alveolar recruitment in COVID-19 [28, 112]. Although prone positioning led to an improvement in oxygenation, this improvement was not always associated with better survival [113–115]. Moreover, the identification of "responders" to prone positioning among patients with COVID-19 is highly heterogeneous by definition [113, 115, 116], due to such factors as the use of different thresholds for defining an improvement in oxygenation. Some studies also identified a higher mortality in "non-responders" [114]. APRV and RMs could be considered in patients with COVID-19 and ARDS who do not improve despite optimization of mechanical ventilation. The use of VV-ECMO in patients with COVID-19 ARDS should be considered individually, based on a careful evaluation of risks, benefits, and available resources (*i.e.*, ECMO center, ICU beds and staff). Indications for initiation of VV-ECMO in COVID-19 overlap with those for non-COVID-19 ARDS. The main difference between these two entities of ARDS is represented by the constrained availability of resources within the context of a pandemic, as patients with COVID-19 exhibit mortality rates similar to those of historical VV-ECMO cohorts [117].

6. Summary

Mechanical ventilation in patients with ARDS has changed markedly over the last decades. A recommended approach is that of keeping V_T, P_{PLAT}, ΔP, and MP low. Several rescue

therapies, including neuromuscular blocking agents, vasodilators, prone positioning, RMs, and VV-ECMO, may be used in severe ARDS. An individually tailored mechanical ventilation strategy based on each patient's characteristics might be the cornerstone of future enhancement of MV in ARDS and may represent a promising approach for respiratory diseases with presentations like ARDS, such as COVID-19.

AUTHOR CONTRIBUTIONS

DB designed and wrote the manuscript; PRMR designed, wrote, edited, and approved the manuscript; PP edited and approved the manuscript. All authors read and approved the submitted manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Ashbaugh D, Boyd Bigelow D, Petty T, Levine B. Acute respiratory distress in adults. Lancet. 1967; 290: 319–323.

[2] Cochi SE, Kempker JA, Annangi S, Kramer MR, Martin GS. Mortality trends of acute respiratory distress syndrome in the United States from 1999 to 2013. Annals of the American Thoracic Society. 2016; 13: 1742–1751.

[3] Laffey JG, Bellani G, Pham T, Fan E, Madotto F, Bajwa EK, *et al*. Potentially modifiable factors contributing to outcome from acute respiratory distress syndrome: the LUNG SAFE study. Intensive Care Medicine. 2016; 42: 1865–1876.

[4] Pelosi P, Ball L, Barbas CSV, Bellomo R, Burns KEA, Einav S, *et al*. Personalized mechanical ventilation in acute respiratory distress syndrome. Critical Care. 2021; 25: 250.

[5] Putensen C, Theuerkauf N, Zinserling J, Wrigge H, Pelosi P. Meta-analysis: ventilation strategies and outcomes of the acute respiratory distress syndrome and acute lung injury. Annals of Internal Medicine. 2009; 151: 566.

[6] Brower R, Matthay M, Morris A, Schoenfeld D, Thompson BT, Wheeler A. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The New England Journal of Medicine. 2000; 342: 1301–1308.

[7] Fan E, Del Sorbo L, Goligher EC, Hodgson CL, Munshi L, Walkey AJ, *et al*. An official american thoracic Society/European society of intensive care medicine/society of critical care medicine clinical practice guideline: mechanical ventilation in adult patients with acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. 2017; 195: 1253–1263.

[8] Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, *et al.* Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. JAMA. 2016; 315: 788.

[9] Slutsky AS. History of mechanical ventilation. from vesalius to ventilator-induced lung injury. American Journal of Respiratory and Critical Care Medicine. 2015; 191: 1106–1115.

[10] Goligher EC, Costa ELV, Yarnell CJ, Brochard LJ, Stewart TE, Tomlinson G, *et al*. Effect of lowering Vt on mortality in acute respiratory distress syndrome varies with respiratory system elastance. American Journal of Respiratory and Critical Care Medicine. 2021; 203: 1378–1385.

[11] Hubmayr RD. Point: Is low tidal volume mechanical ventilation preferred for all patients on ventilation? Yes. Chest. 2011; 140: 9–11.

[12] Gattinoni L, Marini JJ, Pesenti A, Quintel M, Mancebo J, Brochard L. The "baby lung" became an adult. Intensive Care Medicine. 2016; 42: 663–673.

[13] Walkey AJ, Goligher EC, Del Sorbo L, Hodgson CL, Adhikari NKJ, Wunsch H, *et al*. Low tidal volume versus non–volume-limited strategies for patients with acute respiratory distress syndrome. A systematic review and meta-analysis. Annals of the American Thoracic Society. 2017; 14: S271–S279.

[14] Briel M, Meade M, Mercat A, Brower RG, Talmor D, Walter SD, *et al*. Higher *vs.* lower positive end-expiratory pressure in patients with acute lung injury and acute respiratory distress syndrome. JAMA. 2010; 303: 865.

[15] Goligher EC, Kavanagh BP, Rubenfeld GD, Adhikari NK, Pinto R, Fan E, *et al.* Oxygenation response to positive end-expiratory pressure predicts mortality in acute respiratory distress syndrome. A secondary analysis of the lovs and express trials. American Journal of Respiratory and Critical Care Medicine. 2014; 190: 70–76.

[16] Cavalcanti AB, Suzumura ÉA, Laranjeira LN, Paisani DM, Damiani LP, Guimarães HP, *et al*. Effect of lung recruitment and titrated positive end-expiratory pressure (PEEP) *vs.* low PEEP on mortality in patients with acute respiratory distress syndrome. JAMA. 2017; 318: 1335.

[17] Hodgson CL, Cooper DJ, Arabi Y, King V, Bersten A, Bihari S, *et al*. Maximal recruitment open lung ventilation in acute respiratory distress syndrome (PHARLAP). A Phase II, multicenter randomized controlled clinical trial. American Journal of Respiratory and Critical Care Medicine. 2019; 200: 1363–1372.

[18] Mercat A, Richard J-CM, Vielle B, Jaber S, Osman D, Diehl JL, *et al*. Positive end-expiratory pressure setting in adults with acute lung injury and acute respiratory distress syndrome. JAMA. 2008; 299: 646.

[19] Brower RG, Lanken PN, MacIntyre N, Matthay MA, Morris A, Ancukiewicz M, *et al*. Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome. The New England Journal of Medicine. 2004; 351: 327–336.

[20] Meade MO, Cook DJ, Guyatt GH, Slutsky AS, Arabi YM, Cooper DJ, *et al*. Ventilation strategy using low tidal volumes, recruitment maneuvers, and high positive end-expiratory pressure for acute lung injury and acute respiratory distress syndrome. JAMA. 2008; 299: 637.

[21] Sahetya SK, Goligher EC, Brower RG. Fifty years of research in ards. setting positive end-expiratory pressure in acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. 2017; 195: 1429–1438.

[22] Ball L, Serpa Neto A, Trifiletti V, Mandelli M, Firpo I, Robba C, *et al*. Effects of higher PEEP and recruitment manoeuvres on mortality in patients with ARDS: a systematic review, meta-analysis, meta-regression and trial sequential analysis of randomized controlled trials. Intensive Care Medicine Experimental. 2020; 8: 39.

[23] Papazian L, Aubron C, Brochard L, Chiche JD, Combes A, Dreyfuss D, *et al*. Formal guidelines: management of acute respiratory distress syndrome. Annals of Intensive Care. 2019; 9: 69.

[24] Dianti J, Tisminetzky M, Ferreyro BL, Englesakis M, Del Sorbo L, Sud S, *et al*. Association of PEEP and lung recruitment selection strategies

with mortality in acute respiratory distress syndrome: a systematic review and network meta-analysis. To be published in American Journal of Respiratory and Critical Care Medicine. 2022. [Preprint].

[25] Chiumello D, Cressoni M, Carlesso E, Caspani ML, Marino A, Gallazzi E, *et al*. Bedside selection of positive end-expiratory pressure in mild, moderate, and severe acute respiratory distress syndrome. Critical Care Medicine. 2014; 42: 252–264.

[26] Chen L, Del Sorbo L, Grieco DL, Junhasavasdikul D, Rittayamai N, Soliman I, *et al*. Potential for lung recruitment estimated by the recruitment-to-inflation ratio in acute respiratory distress syndrome. A clinical trial. American Journal of Respiratory and Critical Care Medicine. 2020; 201: 178–187.

[27] Barrot L, Asfar P, Mauny F, Winiszewski H, Montini F, Badie J, *et al*. Liberal or conservative oxygen therapy for acute respiratory distress syndrome. The New England Journal of Medicine. 2020; 382: 999–1008.

[28] Battaglini D, Sottano M, Ball L, Robba C, Rocco PRM, Pelosi P. Ten golden rules for individualized mechanical ventilation in acute respiratory distress syndrome. Journal of Intensive Medicine. 2021; 1: 42–51.

[29] Battaglini D, Brunetti I, Anania P, Fiaschi P, Zona G, Ball L, *et al*. Neurological manifestations of severe SARS-CoV-2 infection: potential mechanisms and implications of individualized mechanical ventilation settings. Frontiers in Neurology. 2020; 11: 845.

[30] Bouferrache K, Vieillard-Baron A. Acute respiratory distress syndrome, mechanical ventilation, and right ventricular function. Current Opinion Critical Care. 2011; 17: 30–35.

[31] Zhao Z, Chang MY, Chang MY, Gow CH, Zhang JH, Hsu YL, *et al*. Positive end-expiratory pressure titration with electrical impedance tomography and pressure-volume curve in severe acute respiratory distress syndrome. Annals of Intensive Care. 2019; 9: 7.

[32] Becher T, Buchholz V, Hassel D, Meinel T, Schädler D, Frerichs I, *et al*. Individualization of PEEP and tidal volume in ARDS patients with electrical impedance tomography: a pilot feasibility study. Annals of Intensive Care. 2021; 11: 89.

[33] Bouhemad B, Brisson H, Le-Guen M. Bedside ultrasound assessment of positive end-expiratory pressure–induced lung recruitment. American Journal of Respiratory and Critical Care Medicine. 2011; 183: 341–347.

[34] Costamagna A, Pivetta E, Goffi A, Steinberg I, Arina P, Mazzeo AT, *et al*. Clinical performance of lung ultrasound in predicting ARDS morphology. Annals of Intensive Care. 2021; 11: 51.

[35] Singh A, Gupta A, Sen MK, Suri JC, Chakrabarti S, Bhattacharya D. Utility of bedside lung ultrasound for assessment of lung recruitment in a case of acute respiratory distress syndrome. Lung India. 2019; 36: 451.

[36] Ball L, Robba C, Maiello L, Herrmann J, Gerard SE, Xin Y, *et al.* Computed tomography assessment of PEEP-induced alveolar recruitment in patients with severe COVID-19 pneumonia. Critical Care. 2021; 25: 81.

[37] Chiumello D, Marino A, Brioni M, Menga F, Cigada I, Lazzerini M, *et al*. Visual anatomical lung CT scan assessment of lung recruitability. Intensive Care Medicine. 2013; 39: 66–73.

[38] Amato MBP, Barbas CSV, Medeiros DM, Magaldi RB, Schettino GP, Lorenzi-Filho G, *et al*. Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. The New England Journal of Medicine. 1998; 338: 347–354.

[39] Amato MBP, Meade MO, Slutsky AS, Brochard L, Costa EL, Schoenfeld DA, *et al*. Driving pressure and survival in the acute respiratory distress syndrome. The New England Journal of Medicine. 2015; 372: 747–755.

[40] Guérin C, Papazian L, Reignier J, Ayzac L, Loundou A, Forel JM. Effect of driving pressure on mortality in ARDS patients during lung protective mechanical ventilation in two randomized controlled trials. Critical Care. 2016; 20: 384.

[41] Aoyama H, Pettenuzzo T, Aoyama K, Pinto R, Englesakis M, Fan E. Association of driving pressure with mortality among ventilated patients with acute respiratory distress syndrome. Critical Care Medicine. 2018; 46: 300–306.

[42] Costa ELV, Slutsky AS, Brochard LJ, Brower R, Serpa-Neto A, Cavalcanti AB, *et al*. Ventilatory variables and mechanical power in patients with acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. 2021; 204: 303–311.

[43] Grieco DL, Chen L, Brochard L. Transpulmonary pressure: importance and limits. Annals of Translational Medicine. 2017; 5: 285–285.

[44] Talmor D, Sarge T, O'Donnell CR, Ritz R, Malhotra A, Lisbon A, *et al*. Esophageal and transpulmonary pressures in acute respiratory failure. Critical Care Medicine. 2006; 34: 1389–1394.

[45] Talmor D, Sarge T, Malhotra A, O'Donnell CR, Ritz R, Lisbon A, *et al*. Mechanical ventilation guided by esophageal pressure in acute lung injury. The New England Journal of Medicine. 2008; 359: 2095–2104.

[46] Pelosi P, Goldner M, McKibben A, Adams A, Eccher G, Caironi P, *et al*. Recruitment and derecruitment during acute respiratory failure: an experimental study. American Journal of Respiratory and Critical Care Medicine. 2001; 164: 122–130.

[47] Beitler JR, Sarge T, Banner-Goodspeed VM, Gong MN, Cook D, Novack V, *et al*. Effect of titrating positive end-expiratory pressure (PEEP) with an esophageal pressure-guided strategy *vs.* an empirical high PEEP-Fio_2 strategy on death and days free from mechanical ventilation among patients with acute respiratory distress syndrome: a randomized clinical trial. JAMA. 2019; 321: 846.

[48] Silva PL, Ball L, Rocco PRM, Pelosi P. Power to mechanical power to minimize ventilator-induced lung injury? Intensive Care Medicine Experimental. 2019; 7: 38.

[49] Becher T, van der Staay M, Schädler D, Frerichs I, Weiler N. Calculation of mechanical power for pressure-controlled ventilation. Intensive Care Medicine. 2019; 45: 1321–1323.

[50] Giosa L, Busana M, Pasticci I, Bonifazi M, Macrì MM, Romitti F, *et al*. Mechanical power at a glance: a simple surrogate for volume-controlled ventilation. Intensive Care Medicine Expermental. 2019; 7: 61.

[51] Marini JJ, Gattinoni L, Rocco PR. Estimating the damaging power of high-stress ventilation. Respiratory Care. 2020; 65: 1046–1052.

[52] Mahmoud O. Mechanical power is associated with increased mortality and worsened oxygenation in ARDS. Chest. 2020; 158: A679.

[53] Serpa Neto A, Deliberato RO, Johnson AEW, Bos LD, Amorim P, Pereira SM, *et al*. Mechanical power of ventilation is associated with mortality in critically ill patients: an analysis of patients in two observational cohorts. Intensive Care Medicine. 2018; 44: 1914–1922.

[54] Coppola S, Caccioppola A, Froio S, Formenti P, De Giorgis V, Galanti V, *et al*. Effect of mechanical power on intensive care mortality in ARDS patients. Critical Care. 2020; 24: 246.

[55] Downs JB, Stock MC. Airway pressure release ventilation: a new concept in ventilatory support. Critical Care Medicine. 1987; 15: 459–461.

[56] Jain SV, Kollisch-Singule M, Sadowitz B, Dombert L, Satalin J, Andrews P, *et al*. The 30-year evolution of airway pressure release ventilation (APRV). Intensive Care Medicine Experimental. 2016; 4: 11.

[57] Othman F, Alsagami N, Alharbi R, Almuammer Y, Alshahrani S, Ismaeil T. The efficacy of airway pressure release ventilation in acute respiratory distress syndrome adult patients: a meta-analysis of clinical trials. Annals of Thoracic Medicine. 2021; 16: 245.

[58] Chen C, Zhen J, Gong S, Yan J, Li L. Efficacy of airway pressure release ventilation for acute respiratory distress syndrome: a systematic review with meta-analysis. Annals of Palliative Medicine. 2021; 10: 10349–10359.

[59] Zhong X, Wu Q, Yang H, Dong W, Wang B, Zhang Z, *et al*. Airway pressure release ventilation versus low tidal volume ventilation for patients with acute respiratory distress syndrome/acute lung injury: a meta-analysis of randomized clinical trials. Annals of Translational Medicine. 2020; 8: 1641–1641.

[60] Sun X, Liu Y, Li N, You D, Zhao Y. The safety and efficacy of airway pressure release ventilation in acute respiratory distress syndrome patients : a PRISMA-compliant systematic review and meta-analysis. Medicine (Baltimore). 2020; 99: e18586.

[61] Kollisch-Singule M, Andrews P, Satalin J, Gatto LA, Nieman GF, Habashi NM. The time-controlled adaptive ventilation protocol: mechanistic approach to reducing ventilator-induced lung injury. European Respiratory Review. 2019; 28: 180126.

[62] Silva PL, Cruz FF, Samary CDS, Moraes L, de Magalhães RF, Fernandes MVS, *et al*. Biological response to time-controlled adaptive ventilation depends on acute respiratory distress syndrome etiology. Critical Care Medicine. 2018; 46: e609–e617.

[63] Daoud EG, Farag HL, Chatburn RL. Airway pressure release ventilation: what do we know? Respiratory Care. 2011; 57: 282–292.

[64] Downar J, Mehta S. Bench-to-bedside review: high-frequency oscillatory ventilation in adults with acute respiratory distress syndrome. Critical

Care. 2006; 10: 240.
[65] Liu S, Yi Y, Wang M, Chen Q, Huang Y, Liu L, *et al*. Higher frequency ventilation attenuates lung injury during high-frequency oscillatory ventilation in sheep models of acute respiratory distress syndrome. Anesthesiology. 2013; 119: 398–411.
[66] Bollen CW, van Well GTJ, Sherry T, Beale RJ, Shah S, Findlay G, *et al*. High frequency oscillatory ventilation compared with conventional mechanical ventilation in adult respiratory distress syndrome: a randomized controlled trial [ISRCTN24242669]. Critical Care. 2005; 9: R430–R439.
[67] David M, Weiler N, Heinrichs W, Neumann M, Joost T, Markstaller K, *et al*. High-frequency oscillatory ventilation in adult acute respiratory distress syndrome. Intensive Care Medicine. 2003; 29: 1656–1665.
[68] Derdak S, Mehta S, Stewart TE, Sminth T, Rogers M, Buchman TG, *et al*. High-frequency oscillatory ventilation for acute respiratory distress syndrome in adults. American Journal of Respiratory Critical Care Medicine. 2002; 166: 801–808.
[69] Meade MO, Young D, Hanna S, Zhou Q, Bachman TE, Bollen C, *et al.* Severity of hypoxemia and effect of high-frequency oscillatory ventilation in acute respiratory distress syndrome. American Journal of Respiratory Critical Care Medicine. 2017; 196: 727–733.
[70] Sud S, Sud M, Friedrich JO, Wunsch H, Meade MO, Ferguson ND, *et al*. High-frequency ventilation versus conventional ventilation for treatment of acute lung injury and acute respiratory distress syndrome. Cochrane Database of Systematic Reviews. 2013; 28: CD004085.
[71] Myers M, Rodrigues N, Ari A. High-frequency oscillatory ventilation: a narrative review. Canadian Journal of Respiratory Therapy. 2019; 55: 40–46.
[72] Santana MCE, Garcia CSNB, Xisto DG, Nagato LK, Lassance RM, Prota LF, *et al*. Prone position prevents regional alveolar hyperinflation and mechanical stress and strain in mild experimental acute lung injury. Respiratory Physiology & Neurobiology. 2009; 167: 181–188.
[73] Taccone P, Pesenti A, Latini R, Polli F, Vagginelli F, Mietto C, *et al*. Prone positioning in patients with moderate and severe acute respiratory distress syndrome: a randomized controlled trial. JAMA. 2009; 302: 1977.
[74] Guérin C, Reignier J, Richard JC, Beuret P, Gacouin A, Boulain T, *et al*. Prone positioning in severe acute respiratory distress syndrome. The New England Journal of Medicine. 2013; 368: 2159–2168.
[75] Moran JL, Graham PL. Multivariate meta-analysis of the mortality effect of prone positioning in the acute respiratory distress syndrome. Journal of Intensive Care Medicine. 2021; 36: 1323–1330.
[76] Munshi L, Del Sorbo L, Adhikari NKJ, Hodgson CL, Wunsch H, Meade MO, *et al*. Prone position for acute respiratory distress syndrome. A systematic review and meta-analysis. Annals of the American Thoracic Society. 2017; 14: S280–S288.
[77] Aoyama H, Uchida K, Aoyama K, Pechlivanoglou P, Englesakis M, Yamada Y, *et al*. Assessment of therapeutic interventions and lung protective ventilation in patients with moderate to severe acute respiratory distress syndrome: a systematic review and network meta-analysis. JAMA Network Open. 2019; 2: e198116.
[78] Sud S, Friedrich JO, Adhikari NKJ, Fan E, Ferguson ND, Guyatt G, *et al*. Comparative effectiveness of protective ventilation strategies for moderate and severe acute respiratory distress syndrome. A network meta-analysis. American Journal of Respiratory and Critical Care Medicine. 2021; 203: 1366–1377.
[79] Muscedere JG, Mullen JB, Gan K, Slutsky AS. Tidal ventilation at low airway pressures can augment lung injury. American Journal of Respiratory and Critical Care Medicine. 1994; 149: 1327–1334.
[80] Goligher EC, Hodgson CL, Adhikari NKJ, Meade MO, Wunsch H, Uleryk E, *et al*. Lung recruitment maneuvers for adult patients with acute respiratory distress syndrome. A systematic review and meta-analysis. Annals of the American Thoracic Society. 2017; 14: S304–S311.
[81] Rocco PR, Pelosi P, de Abreu MG. Pros and cons of recruitment maneuvers in acute lung injury and acute respiratory distress syndrome. Expert Review of Respiratory Medicine. 2010; 4: 479–489.
[82] Del Sorbo L, Tonetti T, Ranieri VM. Alveolar recruitment in acute respiratory distress syndrome: should we open the lung (no matter what) or may accept (part of) the lung closed? Intensive Care Medicine. 2019; 45: 1436–1439.
[83] Carvalho NC, Güldner A, Beda A, Rentzsch I, Uhlig C, Dittrich S, *et al*. Higher levels of spontaneous breathing reduce lung injury in experimental moderate acute respiratory distress syndrome. Critical Care Medicine. 2014; 42: e702–e715.
[84] Chanques G, Constantin JM, Devlin JW, Ely EW, Fraser GL, Gélinas C, *et al*. Analgesia and sedation in patients with ARDS. Intensive Care Medicine. 2020; 46: 2342–2356.
[85] Guérin C, Mancebo J. Prone positioning and neuromuscular blocking agents are part of standard care in severe ARDS patients: yes. Intensive Care Medicine. 2015; 41: 2195–2197.
[86] Papazian L, Forel JM, Gacouin A, Penot-Ragon C, Perrin G, Loundou A, *et al*. Neuromuscular blockers in early acute respiratory distress syndrome. The New England Journal of Medicine. 2010; 363: 1107–1116.
[87] Moss M, Huang DT, Brower RG, Ferguson ND, Ginde AA, Gong MN, *et al.* Early neuromuscular blockade in the acute respiratory distress syndrome. The New England Journal of Medicine. 2019; 380: 1997–2008.
[88] Ho ATN, Patolia S, Guervilly C. Neuromuscular blockade in acute respiratory distress syndrome: a systematic review and meta-analysis of randomized controlled trials. Journal of Intensive Care. 2020; 8: 12.
[89] Griffiths MJD, Evans TW. Inhaled nitric oxide therapy in adults. The New England Journal of Medicine. 2005; 353: 2683–2695.
[90] Gebistorf F, Karam O, Wetterslev J, Afshari A. Inhaled nitric oxide for acute respiratory distress syndrome (ARDS) in children and adults. The Cochrane Database of Systematic Reviews. 2016; 6: CD002787.
[91] Alessandri F, Pugliese F, Ranieri VM. The role of rescue therapies in the treatment of severe ARDS. Respiratory Care. 2018; 63: 92–101.
[92] Abrams D, Schmidt M, Pham T, Beitler JR, Fan E, Goligher EC, *et al*. Mechanical ventilation for acute respiratory distress syndrome during extracorporeal life support. research and practice. American Journal of Respiratory and Critical Care Medicine. 2020; 201: 514–525.
[93] Murray JF, Matthay MA, Luce JM, Flick MR. An expanded definition of the adult respiratory distress syndrome. American Review of Respiratory Disease. 1988; 138: 720–723.
[94] Dechert RE, Park PK, Bartlett RH. Evaluation of the oxygenation index in adult respiratory failure. Journal of Trauma and Acute Care Surgery. 2014; 76: 469–473.
[95] Villar J, Ambrós A, Soler JA, Martínez D, Ferrando C, Solano R, *et al*. Age, PaO2/FIO2, and plateau pressure score: a proposal for a simple outcome score in patients with the acute respiratory distress syndrome. Critical Care Medicine. 2016; 44: 1361–1369.
[96] Zhu Y, Zhang M, Zhang R, Ye X, Wei J. Extracorporeal membrane oxygenation versus mechanical ventilation alone in adults with severe acute respiratory distress syndrome: a systematic review and meta-analysis. International Journal of Clinical Practice. 2021; 75: e14046.
[97] Graham PL, Moran JL. ECMO, ARDS and meta-analyses: bayes to the rescue? Journal of Critical Care. 2020; 59: 49–54.
[98] Wang J, Wang Y, Wang T, Xing X, Zhang G. Is extracorporeal membrane oxygenation the standard care for acute respiratory distress syndrome: a systematic review and meta-analysis. Hear, Lung & Circulation. 2021; 30: 631–641.
[99] Combes A, Peek GJ, Hajage D, Hardy P, Abrams D, Schmidt M, *et al*. ECMO for severe ARDS: systematic review and individual patient data meta-analysis. Intensive Care Medicine. 2020; 46: 2048–2057.
[100] Dao B, Savulescu J, Suen JY, Fraser JF, Wilkinson DJC. Ethical factors determining ECMO allocation during the COVID-19 pandemic. BMC Medical Ethics. 2021; 22: 70.
[101] Ball L, Barisione E, Mastracci L, Campora M, Costa D, Robba C, *et al*. Extension of collagen deposition in COVID-19 post mortem lung samples and computed tomography analysis findings. Internation Journal of Molecular Sciences. 2021; 22: 7498.
[102] Fajgenbaum DC, June CH. Cytokine storm. The New England Journal of Medicine. 2020; 383: 2255–2273.
[103] Li X, To KKW. Biomarkers for severe COVID-19. EBioMedicine. 2021; 68: 103405.
[104] Sinha P, Calfee CS. Phenotypes in acute respiratory distress syndrome: moving towards precision medicine. Current Opinion in Critical Care. 2019; 25: 12–20.
[105] Dakin J, Jones AT, Hansell DM, Hoffman EA, Evans TW. Changes in lung composition and regional perfusion and tissue distribution in patients

with ARDS. Respirology. 2011; 16: 1265–1272.

[106] Robba C, Battaglini D, Ball L, Patroniti N, Loconte M, Brunetti I, *et al*. Distinct phenotypes require distinct respiratory management strategies in severe COVID-19. Respiratory Physiology & Neurobiology. 2020; 279: 103455.

[107] Chiumello D, Busana M, Coppola S, Romitti F, Formenti P, Bonifazi M, *et al*. Physiological and quantitative CT-scan characterization of COVID-19 and typical ARDS: a matched cohort study. Intensive Care Medicine. 2020; 46: 2187–2196.

[108] Ball L, Robba C, Herrmann J, Gerard SE, Xin Y, Mandelli M, *et al*. Lung distribution of gas and blood volume in critically ill COVID-19 patients: a quantitative dual-energy computed tomography study. Critical Care. 2021; 25: 214.

[109] Papoutsi E, Giannakoulis VG, Xourgia E, Routsi C, Kotanidou A, Siempos II. Effect of timing of intubation on clinical outcomes of critically ill patients with COVID-19: a systematic review and meta-analysis of non-randomized cohort studies. Critical Care. 2021; 25: 121.

[110] Perkins GD, Ji C, Connolly BA, Couper K, Lall R, Baillie JK, *et al.* Effect of noninvasive respiratory strategies on intubation or mortality among patients with acute hypoxemic respiratory failure and COVID-19: the RECOVERY-RS randomized clinical trial. Journal of the American Medical Association. 2022; 327: 546–558.

[111] Robba C, Battaglini D, Ball L, Pelosi P, Rocco PRM. Ten things you need to know about intensive care unit management of mechanically ventilated patients with COVID-19. Expert Review of Respiratory Medicine. 2021; 15: 1293–1302.

[112] Rossi S, Palumbo MM, Sverzellati N, Busana M, Malchiodi L, Bresciani P, *et al*. Mechanisms of oxygenation responses to proning and recruitment in COVID-19 pneumonia. Intensive Care Medicine. 2021; 48: 1–11.

[113] Langer T, Brioni M, Guzzardella A, Carlesso E, Cabrini L, Castelli G, *et al*. Prone position in intubated, mechanically ventilated patients with COVID-19: a multi-centric study of more than 1000 patients. Critical Care. 2021; 25: 128.

[114] Cunha M, Schardong J, Camponogara Righi N, Claudia Lunardi A , Nery de Sant'Anna G, Padrão Isensee L, *et al*. Impact of prone positioning on patients with COVID-19 and ARDS on invasive mechanical ventilation: a multicenter cohort study. Brazilian Journal of Pulmonology. 2022; 48: e20210374.

[115] Scaramuzzo G, Gamberini L, Tonetti T, Zani G, Ottaviani I, Mazzoli CA, *et al*. Sustained oxygenation improvement after first prone positioning is associated with liberation from mechanical ventilation and mortality in critically ill COVID-19 patients: a cohort study. Annals of Intensive Care. 2021; 11: 63.

[116] Park SY, Kim HJ, Yoo KH, Park YB, Kim SW, Lee SJ, *et al*. The efficacy and safety of prone positioning in adults patients with acute respiratory distress syndrome: a meta-analysis of randomized controlled trials. Journal of Thoracic Disease. 2015; 7: 356–367.

[117] Badulak J, Antonini MV, Stead CM, Shekerdemian L, Raman L, Paden ML, *et al*. Extracorporeal membrane oxygenation for COVID-19: updated 2021 guidelines from the extracorporeal life support organization. ASAIO Journal. 2021; 67: 485–495.

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REVIEW

New insights in ARDS pathogenesis

Paloma González-Rodríguez[1], Raquel Herrero[1,2,3,*], Gema Sánchez[1,4], Jose Angel Lorente[1,2,3,5,6]

[1]Foundation for Biomedical Research of the Getafe University Hospital, 28905 Getafe, Madrid, Spain
[2]Department of Critical Care Medicine, Getafe University Hospital, 28905 Getafe, Madrid, Spain
[3]CIBER of respiratory diseases (CIBERES), Carlos III Health Institute, 28029 Madrid, Spain
[4]Department of Clinical Analysis, Getafe University Hospital, 28905 Getafe, Madrid, Spain
[5]Clinical Department, European University of Madrid, 28670 Villaviciosa de Odón, Madrid, Spain
[6]Department of Bioengineering, Carlos III University, 28911 Leganés, Madrid, Spain

***Correspondence**
raquelher@hotmail.com
(Raquel Herrero)

Abstract

Acute respiratory distress syndrome (ARDS) is a life-threatening condition in critically ill patients characterized by hypoxemia and non-compliant lung. In this review, we discuss the pathophysiology of ARDS, including the mechanisms involved in the formation of pulmonary edema, the dysregulated inflammatory and immune responses, the activation of procoagulant events, the cellular communication by extracellular vesicles (EVs) between different types of cells and the interaction of the lung with other organs. Activation of inflammation, coagulation, and cell death processes result in the disruption of the alveolar-capillary membrane and the consequent protein-rich edema formation in the alveoli, in which structural and functional alterations of the alveolar epithelium play an essential role. Inflammation and activated endothelial cells trigger coagulation cascades and platelets that generate a procoagulant state in both the airspace and the intravascular compartment with the formation of fibrin in airspaces and thrombi in the microvasculature that aggravate alveolar injury and gas exchange. The crosstalk between epithelial/endothelial cells, platelets, and immune cells is mediated by EVs, whose role in the pathogenesis of ARDS is not known. Finally, the interaction of the lung with other organs has become an important determinant in the development and resolution of ARDS. Understanding the pathophysiological mechanisms involved in ARDS is crucial to developing new therapeutic strategies.

Keywords

Acute respiratory distress syndrome; Mechanisms; Pulmonary edema; Inflammation; Coagulation; Extracellular vesicles (EVs); Organ interaction

1. Introduction

Acute respiratory distress (ARDS) is a life-threatening condition in critically ill patients defined as the rapid onset of pulmonary edema not fully explained by cardiac failure or fluid overload, resulting in respiratory failure and hypoxemia. ARDS is an inflammatory lung injury characterized by acute onset, bilateral pulmonary infiltrates, poor oxygenation, and diffuse alveolar damage [1]. The leading causes of ARDS are pneumonia and non-pulmonary sepsis. Other causes of ARDS are the aspiration of gastric contents, major trauma (including burns or penetrating injuries), acute pancreatitis, hemorrhagic shock, ischemic insults, reperfusion injury, drug overdose, and transfusions [2, 3]. The infection by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), responsible for the current pandemic of coronavirus disease 2019 (COVID-19), has dramatically risen the ARDS incidence, which reaches nearly 40% of hospitalized patients and 75% of Intensive Care Unit (ICU) patients with COVID-19 pneumonia [4, 5].

The histological hallmark of ARDS is the diffuse alveolar damage (DAD), characterized by protein-rich edema, neutrophil accumulation into alveolar spaces, alveolar hemorrhage, fibrin deposition (due to the enhanced pro-coagulation), and hyaline membrane formation [2] (Fig. 1). However, not all patients clinically diagnosed of ARDS have the histological manifestation of DAD in the lung. Indeed, clinical reports before the SARS-CoV-2 pandemic indicate that DAD is only found in 56.4% of ARDS patients [6]. On the other hand, in postmortem studies in patients with ARDS, the presence of DAD has been associated with a different clinical profile compared to patients without DAD [2, 3, 5, 7].

ARDS is a common cause of death in critically ill patients, with a high mortality rate of 30–40% before the pandemic of SARS-CoV-2, reaching a mortality rate of 69–73% in COVID-19 patients with ARDS in some countries [8]. Although improvements in supportive care have been achieved during the last 30 years, no effective pharmacological treatment has been developed yet [9]. Moreover, ARDS often occurs in the setting of multiple organ failure, which in turn aggravates lung damage [10]. In addition, many studies have reported that ARDS survivors have a reduced quality of life as indicated by restrictive ventilatory deficits, significant exercise limitation, fatigue, muscle weakness, and neurocognitive and mood disorders [2, 11, 12]. Therefore, understanding the pathophysiological mechanisms responsible for ARDS development is crucial to developing new therapeutic strategies.

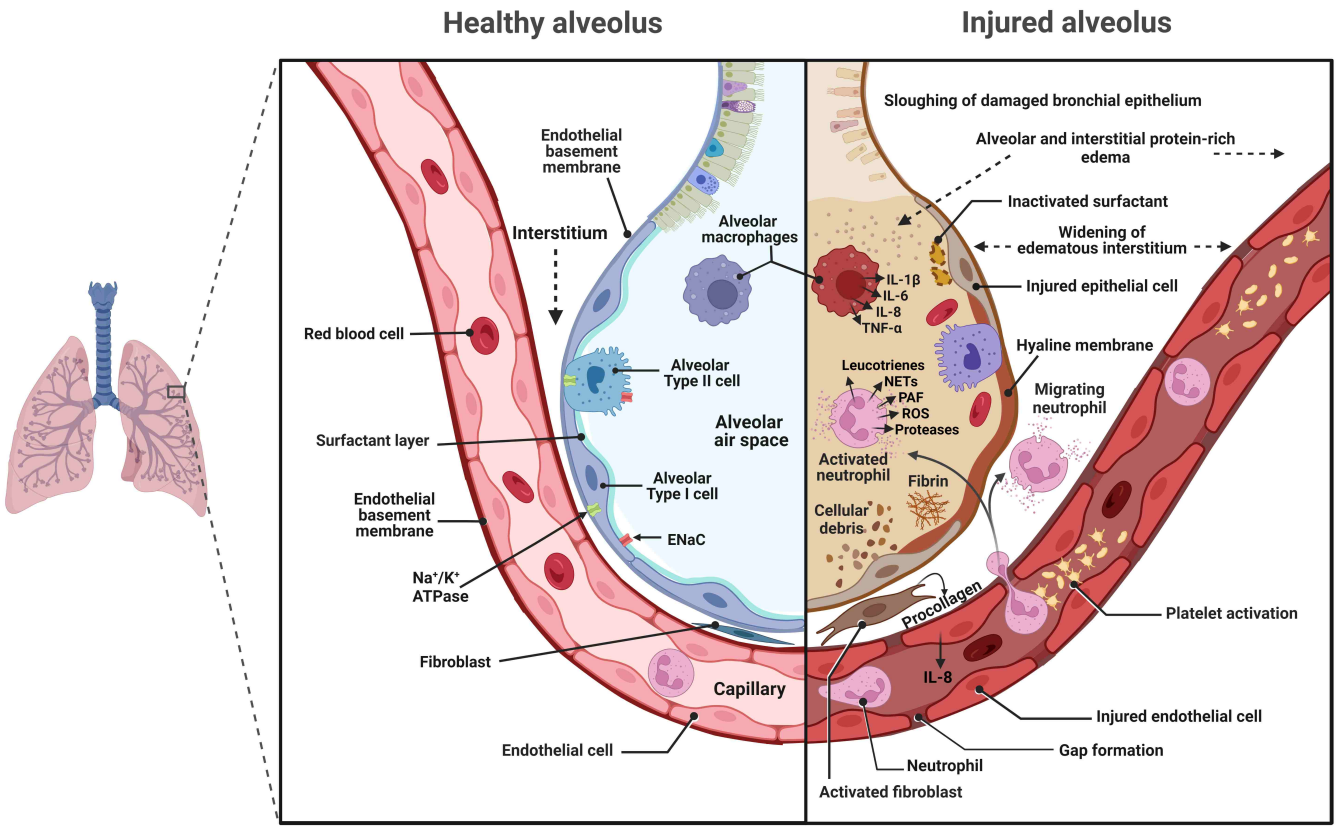

FIGURE 1. Characteristics of diffuse alveolar damage (DAD). The acute phase of DAD is characterized by alveolar epithelial and endothelial cell damage, an early increased permeability of the alveolar-capillary membrane, and flooding the airspace with protein-rich pulmonary edema fluid. Activation of resident alveolar macrophages and enhanced neutrophil migration and activation provide host defense, but they also release pro-inflammatory chemokines, cytokines, and other products (proteases, ROS, NETs) that can be deleterious. Platelet activation and release of vasoactive-procoagulant products lead to thrombi formation in the microvasculature and fibrin deposition in the alveolar airspaces, which contributes to the formation of hyaline membrane (mainly formed by deposition of proteins, fibrin, and cellular debris) on the denuded epithelial membrane. Activation of fibroblast leads to collagen deposition in the interstitium. Also, alveolar hemorrhage can occur, and the extravasated red blood cells can release cell-free hemoglobin, exacerbating injury via oxidant-dependent mechanisms. Figure created with BioRender.com. Na^+/K^+-ATPase, sodium/potassium adenosine triphosphatase; ROS, reactive oxygen species; NETs, neutrophil extracellular traps; PAF, platelet-activating factor; IL, interleukin; TNF, tumor necrosis factor.

2. Pathology of ARDS

The pathological findings in the lungs of patients with ARDS change over time, and the disease progression is variable. Although several phases have been described, they can occur concurrently [13]. The early exudative phase includes diffuse alveolar damage with disruption and loss of epithelial and endothelial cells, interstitial and alveolar flooding by protein-rich edema fluid, neutrophil and macrophage influx, and hemorrhage into the alveolar space. In the alveolar epithelium, type I cells can be irreversibly damaged, and the denuded space is replaced by hyaline membranes, while injury to the surfactant-producing type II cells contributes to alveolar collapse [14]. Because of endothelial damage and a procoagulant state, microthrombi form. In the subacute phase (the next 7–14 days), some of the edema has usually been reabsorbed, and proliferation of alveolar epithelial type II cells can take place associated with squamous metaplasia as a repairment mechanism of the alveolar epithelium. Although ARDS may resolve entirely in some patients at this point, in others it progresses to a fibroproliferative phase (after 14 days), in which there is infiltration of fibroblasts and more evidence of collagen deposition and remodeling of the interstitial and alveolar spaces [13, 15, 16]. In this chronic phase, there is a resolution of the acute neutrophilic infiltrate and more evidence of fibrosis, while alveolar epithelial proliferation can still progress. The persistence of fibroblast activation and collagen deposition can lead to lung fibrosis, which in some cases is irreversible.

3. Pathophysiological mechanisms of ARDS

ARDS is characterized by a dysregulated inflammatory response resulting in enhanced leukocyte infiltration into the alveolar space, a procoagulant state, and epithelial and endothelial cell damage that results in an enhanced permeability of the alveolar-capillary membrane. This increased perme-

ability leads to protein-rich edema formation in the alveolar and interstitial spaces in contrast to the low protein pulmonary edema that results from hydrostatic causes such as congestive heart failure [17–19].

4. Pulmonary epithelial/endothelial injury and edema in ARDS

In ARDS, a pulmonary protein-rich edema is an early event that markedly contributes to hypoxemia in these patients. Alterations in alveolar fluid transport and clearance and the increase in endothelial/epithelial permeability lead to alveolar proteinaceous edema. Multiple factors, including dysregulated inflammation, intense leukocyte infiltration, activation of procoagulant processes, cell death, and mechanical stretch, contribute to the disruption and dysfunction of both epithelial and endothelial barriers [17–19].

4.1 Pulmonary epithelial injury

In healthy conditions, the alveolar-epithelial barrier is intact and maintains its capability of alveolar fluid clearance, allowing the reabsorption of excess alveolar fluid. This absorption of alveolar fluid from the airspaces to the interstitium is carried on by a vectorial ion transport, mainly mediated by the apical epithelial sodium channels (ENaC) and the basolateral sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase) pumps (Fig. 2) [20]. Some factors such as influenza infection, hypoxia, or hypercapnia can diminish the function of these sodium channels and Na^+/K^+-ATPase pumps, resulting in a reduced capacity of fluid clearance in the lung of patients with ARDS [2, 21, 22].

Activation of cell death mechanisms during ARDS, such as FasL (Fas Ligand)-mediated apoptosis or pyroptosis (highly inflammatory type of programmed cell death), are responsible for the loss of alveolar epithelial cells, thus contributing to barrier hyperpermeability [23–25]. Cell death can be triggered on epithelial cells by direct injury on the epithelium or activation of the pattern recognition receptors (PRRs). These PRRs are cell-surface or cytosolic proteins activated by the pathogen-associated molecular patterns (PAMPs) and/or damage-associated molecular patter (DAMPs). PAMPS are extrinsic molecules derived from various microorganisms, while DAMPs are intrinsic molecules derived from injured cells or extracellular molecules. Toll-like receptors and the receptor for advanced glycation end products (RAGE) are examples of PRRs. Besides apoptosis, activation of alveolar epithelial PRRs activates inflammatory cascades that alter the alveolar epithelial and endothelial barriers. Internalization of the PRRs upon pathogen binding, for instance, releases new particles of pathogens, inflammatory molecules (*e.g.*, cytokines), DAMPs, and PAMPs into the alveolar space that can exert a deleterious effect on the epithelial integrity and function [26, 27].

Alterations in cell-cell adhesion in the alveolar epithelium and its interaction with extracellular matrix (ECM) have also been reported in ARDS. Intercellular junctions of epithelial cells are mediated by tight junctions (TJs) complexes, which consist of some transmembrane proteins such as junctional adhesion molecules (JAMs), occludin, and claudins that interact with the adaptor protein zonula occludens (ZO), which, in turn, binds to the actin fibers of the cytoskeleton (Fig. 2). These TJ-actin complexes are essential structures in alveolar epithelial permeability since they control cell tension and contraction and the paracellular transport of fluid and solutes into the airspace [19] (Fig. 2). Therefore, dysfunction of the TJs results in increased permeability to water and proteins and deterioration of the capacity of alveolar fluid clearance of the epithelium, leading to the formation and perpetuation of lung edema. Studies in experimental models of acute lung injury indicate massive changes in the expression and localization of ZO and claudins with the consequent increase in epithelial permeability [25, 28, 29]. The ECM represents the scaffold of alveolar epithelium and capillary endothelium that participates in cell-cell adhesion, and in the fluid trafficking into the airspace and cell signaling. In ARDS, the oxidative stress and the dysregulated inflammation in the lung induce the expression of some enzymes, such as elastases or matrix metalloproteinases (MMPs), that change the structure and stiffness of the ECM and, consequently, modify the expression of the TJ proteins and barrier function, contributing to lung edema formation [19, 30–32].

Alveolar inflammation is characterized by marked neutrophil influx, activation of alveolar macrophages, and release into the airspaces of cytokines (tumor necrosis factor-α (TNF-α), tumor necrosis factor receptor (TNFR), interleukin-1β (IL-1β), interleukin-1 receptor antagonist (IL1RA), IL-6, interferon-γ (INF-γ), granulocyte colony-stimulating factor (G-CSF), transforming growth factor-β (TGF-β)) and chemokines ((IL-8, epithelial neutrophil-activating protein 78 (ENA-78), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2)) by alveolar endothelial, epithelial, and immune cells [19, 33]. Among them, TGF-β is a key mediator in ARDS that can be detected in bronchoalveolar lavage (BAL) fluid from patients with ARDS in the first 24 h of diagnosis and has an essential role in its onset and progression. In the early phase, TGF-β causes apoptosis in alveolar epithelial cells and contributes to lung edema by increasing permeability and decreasing the alveolar fluid clearance of the alveolar epithelium [34]. This decrease in the alveolar fluid clearance is due to changes in the expression of apical epithelial sodium channels (ENaC) and the basolateral Na^+/K^+-ATPase pumps [14, 19, 35]. In a later stage, TGF-β exerts an essential role in regulating inflammation, immunity, tissue repair, and fibrosis. In this line, TGF-β contributes to lung fibrosis via activation of lung fibroblasts and indirectly via inducing apoptosis of alveolar epithelial cells [34, 36].

4.2 Pulmonary endothelial injury

Alteration of the vascular endothelial function and extensive alveolar-capillary leak also occurs in the lung of patients with ARDS. Endothelial injury can be caused by the adhesion and migration of neutrophil granulocytes on and through the endothelium [37] and by the direct effects of cytotoxic factors present in the intravascular and the intra-alveolar compartments. This contact between intra-alveolar factors and the

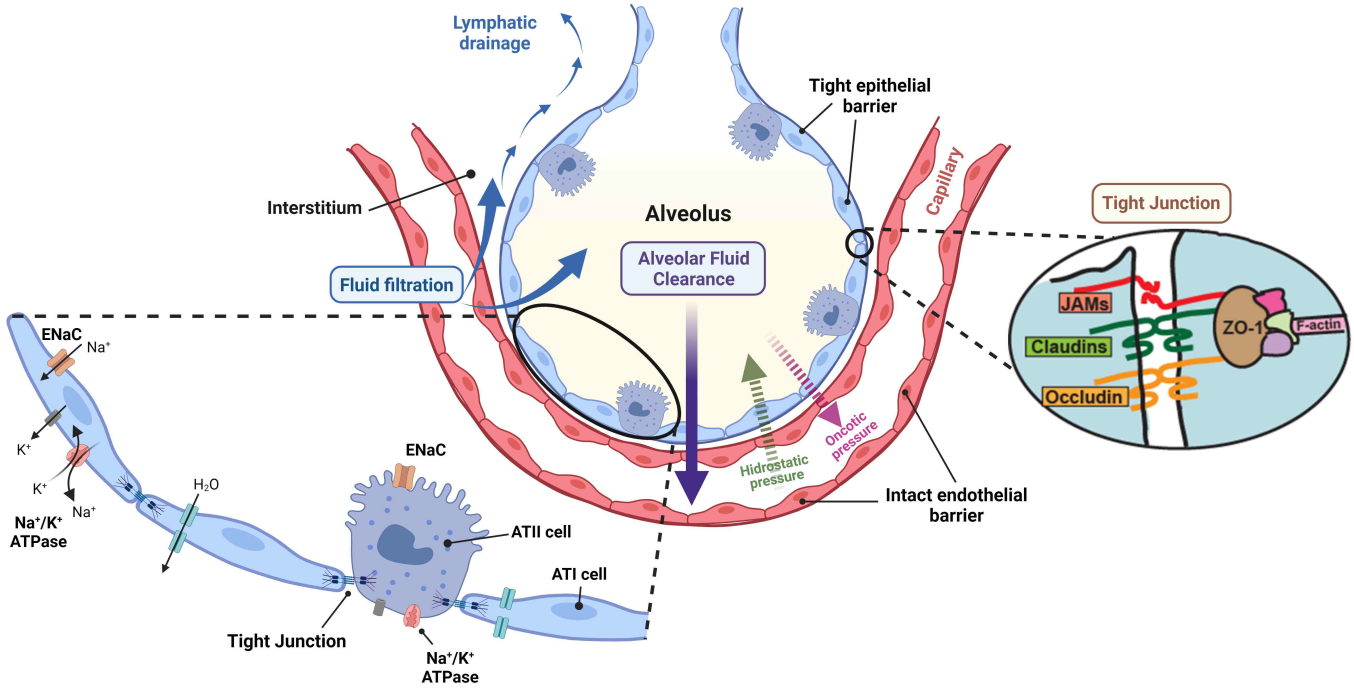

FIGURE 2. Role of alveolar epithelium in lung edema. The alveolar epithelium is a continuous and tight monolayer of alveolar type I (ATI) and alveolar type II (ATII) cells. ATI cells are very thin and permit gas exchange, and ATII cells produce surfactant to enable lung expansion with low surface tension. The intact alveolar epithelium is linked by intercellular tight junctions that restrict the passage of water, electrolytes, and small hydrophilic solutes to the airspaces. In the normal lung, the transvascular flux of fluid out of the capillary moves water and low-molecular-weight solutes into the interstitial space depending on the permeability of the capillary membrane and the net difference between hydrostatic and protein osmotic pressure. In health, this fluid does not cross the epithelial barrier and moves into the lymphatics. When alveolar edema occurs, this edema fluid accumulating in airspaces is absorbed by the epithelium following a transepithelial osmotic gradient created by an active sodium transport. This sodium gradient is created and maintained by the apical membrane epithelial Na^+ channels (ENaC) and the basolateral sodium/potassium adenosine triphosphatase (Na^+/K^+-ATPase) in both ATI and ATII cells, causing excess water to move passively from the airspaces to the interstitium. Figure created with BioRender.com.
JAMs, junctional adhesion molecules; ZO-1, Zonula occludens-1.

endothelium occurs because of the disruption of the alveolar epithelial barrier. Many intravascular and intra-alveolar factors activate cell death mechanisms on endothelial cells, such as apoptosis and pyroptosis, and contribute to the breakdown of endothelial intercellular junctions [38, 39], leading to an increase in vascular permeability that contributes to lung edema and respiratory failure in these patients [2].

Like alveolar epithelial cells, endothelial cells can be activated by PAMPs and DAMPs, some of them derived from alveolar epithelial cells and resident macrophages, as well as from circulating leukocytes and platelets, such as TNF-α, IL-1β, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), platelet-activating factor (PAF), TGF-β1 and angiostatin (a cleavage product from plasminogen with an intense antiangiogenic activity) that are increased in the BAL fluid of patients with ARDS [34, 40, 41]. Among these factors, TNF-α, TGF-β, and angiostatin contribute to endothelial injury by inducing apoptosis [42–44]. In addition, TGF-β contributes to increased endothelial permeability via phosphorylation of adherent junction proteins and the formation of stress actin fiber in endothelial cells *in vitro* [45]. TNF-α also disrupts tight junction proteins (ZO-1, claudin 2-4-5) and β-catenin in pulmonary endothelial and epithelial cell layers, which can be exacerbated by IFN-γ [28, 46, 47]. Il-1β increases alveolar endothelial and epithelial permeability via Ras homolog family member A (RhoA)/integrins-mediated epithelial TGF-β release [48].

Activated endothelial cells trigger a cascade of events, including activation of coagulation cascades, activation and aggregation of platelets, formation of platelet-leukocyte aggregates, and up-regulation of cell adhesion molecules, such as P-selectin, E-selectin, ICAM (intercellular adhesion molecule) and VCAM (vascular cell adhesion molecule), that mediate leukocyte adhesion and transmigration across the endothelium (Fig. 3). This transmigration of leukocytes, the deposition of platelets and neutrophils on endothelium, or the formation of platelet-neutrophil aggregates play a synergic role in increasing vascular permeability in the lung [39, 49] (Fig. 3).

Elevated levels of angiotensin II (AngII) have been found in the lung of patients with ARDS. AngII interacts with Ang II receptor type 1 (AT1R), mainly expressed in the endothelium, and induces the production of several mediators (inflammatory cytokines, eicosanoids, and VEGF) that trigger proinflammatory responses and elevate the pulmonary vascular permeabil-

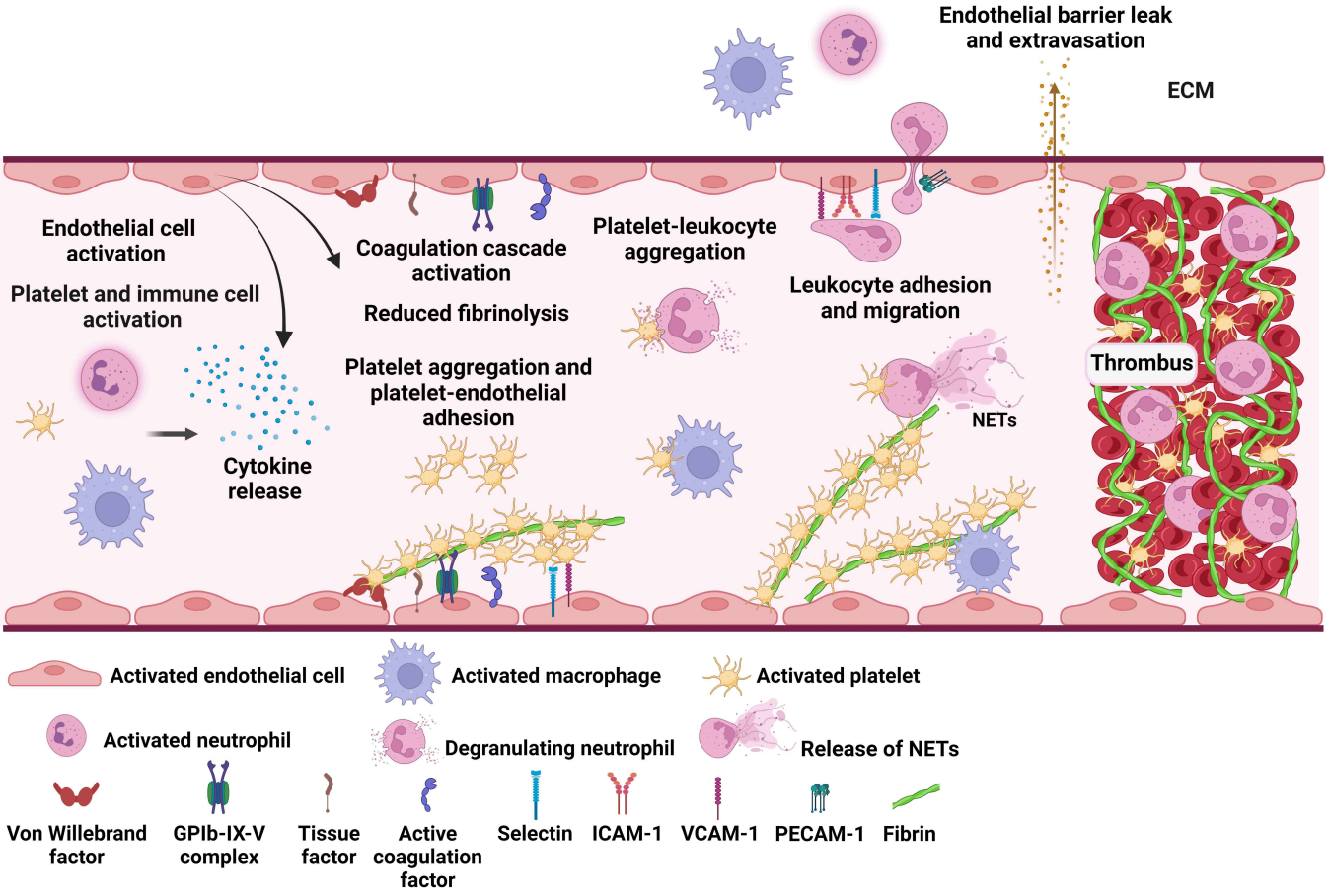

FIGURE 3. The inflammation and coagulation interaction in ARDS. Activated endothelial cells trigger various events that include activation of the coagulation cascade (with reduction of anticoagulant and fibrinolytic factors), the release of cytokines, and up-regulation of leukocyte and endothelial cell adhesion molecules (P-selectin, E-selectin, ICAM, VCAM, PECAM). These events, in turn, promote activation of leukocytes and platelets that leads to platelet aggregation and formation of platelet-leukocyte aggregates, facilitating the adhesion and migration of leukocytes to the interstitium and alveolar airspaces. In addition, the generation of tissue factor (from exposed subendothelium or released by activated macrophages/monocytes and platelets) and von Willebrand factor (vWF) (released by activated endothelial cells and platelets) mediates further platelet adhesion and aggregation. Activated neutrophils release pro-inflammatory mediators (chemokines, cytokines) along with ROS, enzymes (MMPs, elastase, myeloperoxidase), and neutrophil extracellular traps (NETs) that have an essential role in host defense but cause endothelial and epithelial injury under overwhelming pathological conditions. Coagulation and activated platelets and leukocytes augment microvascular endothelial damage leading to the disruption and increased permeability of the endothelial barrier. All these events facilitate further movement of inflammatory cells and protein-rich fluid into the interstitium and alveoli. In the intravascular compartment, activation of coagulation, platelets, leukocytes, and endothelial cells, along with the generation of thrombin and fibrin, leads to the formation of thrombi in the pulmonary microvasculature. Figure created with BioRender.com.
ECM, extracellular matrix; ROS, Reactive Oxygen Species; MMPs, Matrix Metalloproteinases; NETs, Neutrophil Extracellular Traps; GPIb-IX-V, glicoprotein Ib-IX-V; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion protein 1; PECAM-1, platelet-endothelial cell adhesion molecule-1.

ity, contributing to pulmonary edema [50, 51]. Interestingly, SARS-CoV-2 is internalized by alveolar epithelial cells via binding to angiotensin-converting enzyme 2 (ACE2) receptor, resulting in an ACE2 downregulation and the subsequent up-regulation of angiotensin II (Ang II) that contribute to lung endothelial vascular dysfunction in COVID-19 pneumonia [52].

Besides TJs, endothelial cells are also connected by adherens junctions, which contain vascular endothelial cadherin (VE-cadherin) that links to the actin cytoskeleton. The weakening of endothelial junctions induced by inflammation also relies on the destabilization of VE-cadherin contacts and alterations in the endothelial actin-myosin cytoskeleton [39].

Altogether, the increase in endothelial and epithelial permeability leads to protein-rich edema formation in the lung, resulting in the alteration of gas exchange and the subsequent hypoxemia in ARDS. Extensive alveolar epithelium damage has been observed in non-surviving ARDS patients, whereby the degree of alveolar epithelial damage seems to determine the ARDS severity and prognosis [2, 53].

5. Humoral and cellular immune system in ARDS

5.1 Activation of pattern recognition receptors

In the lung, activation and regulation of innate and adaptive immunity are mediated by patter recognition receptors (PRRs), also present in alveolar epithelial and endothelial cells as mentioned above [27]. Activation of these PRRs by PAMPs and DAMPs leads to nuclear translocation of transcription factors such as nuclear factor (NF)-κB, predominantly through a myeloid differentiation primary response gene 88 (MyD88)-dependent mechanism. This is followed by the transcription of proinflammatory cytokines such as TNF-α, IL-1β, and IL-8, which can activate immune cells and alter alveolar epithelial and endothelial functions in the lung. This early humoral and cellular immune activation contributes to lung injury and widespread lung inflammation to other organs, promoting multi-organ damage [54].

5.2 Innate immune cells

5.2.1 Activation of M1 macrophages

Two types of activated macrophages have been described, the M1 proinflammatory phenotype and the M2 anti-inflammatory. The M1 polarization of resident alveolar macrophages represents one of the local defenses against pathogens. In this stage, M1 alveolar macrophages trigger pathogen clearance mechanisms and release reactive oxygen species (ROS) and proinflammatory cytokines (IL-1β, IL-6, IL-18, MCP-1, MIP-2, and TNF-α) that activate and recruit immune cells to the site of infection, including natural killer cells, cytotoxic T cells, and innate lymphoid cells. These cells are primed and increase their cytotoxicity activity against the pathogen, partially mediated by the production of IFN-γ. These immune cells release a second wave of cytokines, acting as chemoattracts of circulating monocytes and neutrophils [55, 56] that can be cytotoxic for the alveolar-capillary membrane. To assist in the clearance of viral, bacterial, or fungal pathogens, resident and recruited neutrophils release molecules, such as myeloperoxidase (MPO), metalloproteinases (MMPs), elastase, and neutrophil extracellular traps (NETs), that can alter the structure and function of the alveolar epithelial/endothelial barrier and ECM [57].

In ARDS, the immune response is dysregulated, and M1 alveolar macrophages release high levels of proinflammatory cytokines (IL-1β, IL-6, IL-2, IL-7, and IL-8, TNF-α) and chemokines (MCP-1, MIP-1α), resulting in infiltration of immune cells (mainly circulating monocytes and neutrophils) [58, 59]. Although the early polarization of macrophages to M1 has an initial protective function against pathogen infection, growing evidence demonstrates that both resident and recruited M1 macrophages play a relevant role in ARDS pathogenesis. Indeed, experimental models of acute lung injury have reported reduced mortality when inhibitors of M1 polarization were administered [60–63].

5.2.2 Activation of M2 macrophages

The polarization of M1 macrophages to M2 phenotype is induced to counteract the proinflammatory stage. M2 macrophages play a relevant role in inflammation resolution and lung tissue repair by clearance of cellular debris and apoptotic cells, limiting proinflammatory cytokine release and inducing the expression of anti-inflammatory mediators (IL-10, fibronectin 1, TGF-β), which reduce levels of nitric oxide synthase and nitric oxide species via arginase 1 induction [64]. In this regard, the administration of M2 alveolar macrophages to mice with lipopolysaccharide (LPS)-induced acute lung injury depletes circulating monocytes, reduces neutrophil infiltration and oxidative stress, and decreases the levels of inflammatory molecules (TNF-α, IL-1β, and IL-6), elevates the expression of anti-inflammatory mediators (IL-17, MCP-1, IL-10), and increases levels of regulatory T-cells (Treg) [65]. Apart from balancing the pro- and anti-inflammatory cytokines levels, M2 macrophages also recognize and phagocytize neutrophils, reducing and preventing their cytotoxic effects on the lung [64].

Finally, the late and complicated phase of ARDS, known as the fibro-proliferative phase, is characterized by excessive fibroblast proliferation and increased ECM deposition. Numerous studies have reported that persistently activated M2-macrophages also participate in this phase of ARDS [64, 66]. In this regard, persistently activated M2-macrophages release TGF-β, fibronectin, proline, and tissue inhibitors of metalloproteinase (TIMP) that promote fibroblast proliferation and hamper the removal of excessive ECM. On the other hand, M1-macrophages have been reported to play an anti-fibrotic role in this phase by producing antifibrotic cytokines (*e.g.*, CXCL10) and MMPs capable of degrading the excessive ECM [67]. Therefore, in this late phase of ARDS, the M1/M2 macrophage balance in the microenvironment of the injured lung seems crucial for ARDS resolution.

5.2.3 Neutrophil activation

The activation of neutrophils into alveolar space releases ROS that triggers oxidative stress and intracellular enzymes (MMPs, elastase) that degrade the ECM, contributing to alveolar epithelial barrier disruption [31, 68]. In addition, neutrophils can undergo NETosis, a type of cell death by which neutrophils extrude NETs. NETs are composed of DNA fibers, histones, and antimicrobial proteins, in which pathogens are immobilized and exposed to a local high and lethal concentration of effector proteins [69]. An excessive NET formation enhances a proinflammatory response that alters endothelial and epithelial barriers mainly by decreasing ZO-1, VE-cadherin, and β-catenin [70, 71]. Moreover, *in vitro* assays have shown the role of NETs as a scaffold for platelets, red blood cells, and procoagulant factors (such as von Willebrand factor and tissue factor), contributing to thrombus formation and propagation [72, 73]. Indeed, elevated plasma levels of NETs in humans have been associated with ARDS severity and mortality [74].

5.3 Adaptive immune cells

During infection, the adaptive immune response is rapidly initiated. Pathogen particles are presented through major his-

tocompatibility complex class I (MHC I) of activated dendritic cells (DC) to CD8+ T cells. The latter cells are cytotoxic and induce apoptosis on infected cells by producing perforin and granzymes. Activated CD8+ T cells also become pathogen-specific effectors and memory T cells. The major histocompatibility complex class II (MHCII) is presented by DCs to CD4+ T cells. Then, CD4+ T cells may differentiate into one of several T helper (Th) cell lineages, including Th1, Th2, Th17, and T follicular cells, as defined by their pattern of cytokine production and function. Th1, Th2, and Th17 cells contribute to pathogen clearance, whereas T follicular cells assist B cells in the production of neutralizing antibodies [56].

Alterations in adaptive immune response have been broadly described in ARDS. For example, levels of CD4+ T cells are dramatically reduced in patients with sepsis-induced ARDS, as well as the Th1 and Th2-associated cytokine production and pathogen clearance [75]. In contrast, Th17 cells are elevated in the lung [76], BAL fluid [77], and blood [78] of ARDS patients and experimental animals after acute lung injury. Differentiation to Th17 is mainly induced by IL-6, which is elevated in ARDS. Th17 cells activate macrophages, DCs, and neutrophils, triggering the release of proinflammatory cytokines (IL-1, IL-6, IL-8, IL-21, IL-17A, TNF-α, and MCP-1) by these cells [78], which is accompanied by increased alveolar epithelial permeability [76] and greater severity of illness [77].

Another subtype of CD4+ T cells, the regulatory T (Treg) cells, are also altered during ARDS. Treg cells exert an anti-inflammatory effect that is essential in resolving lung injury. They suppress the effector T+ cell responses (mainly from Th17 cells) and maintain the tolerance to self-antigens, avoiding autoimmune responses [79]. During acute lung injury, the increased levels of IL-6 enhance Th17 cell activation and Treg suppression, altering Th17/Treg balance [80]. The Th17/Treg ratio increases in mild to severe ARDS patients and has been proposed as a predictor biomarker of mortality in ARDS, correlating with increased APACHE (Acute Physiology and Chronic Health disease Classification System), SOFA (Sequential Organ Failure Assessment) and lung injury scores [81]. Interestingly, increasing Treg levels via stimulation of the cAMP/FOXP3 (cyclic adenosine monophosphate/ forkhead box P3) signal reduces the number of Th17 cells protecting against lung injury and mortality in mice with acute lung injury [82].

6. Hemostatic and immune system interaction

ARDS is characterized by an imbalance between coagulation and the immune system. Activation of procoagulant factors along with an impaired anticoagulant system leads to reduced fibrinolysis, a massive production of thrombin, and, consequently, an intra-alveolar and lung intravascular fibrin formation. A cross-talk between coagulation and the innate immune system initiates the complex process of immunothrombosis, which exerts a vital role as a host defense mechanism [83]. In ARDS, immunothrombosis is dysregulated, leading to excess formation of immunologically mediated thrombi that affect the lung microvasculature (Fig. 3). Therefore, the restoration of the alveolar and intravascular hemostasis and the adequate control of immune responses are crucial in the pathogenesis of ARDS [84–87].

Immunothrombosis is the consequence of endothelial, platelet, and innate immune cell activation, excessive coagulation, and decreased fibrinolysis (Fig. 3) [84]. It has been shown that patients with ARDS have increased levels of fibrinopeptide A, a direct marker of thrombin generation, and soluble thrombomodulin (probably degraded from alveolar epithelial thrombomodulin), and decreased levels of the anticoagulant activated protein C (APC) in their alveolar airspaces and plasma. On the other hand, there is suppression of the fibrinolysis in the alveoli caused by increased levels of plasminogen activator inhibitor-1 (PAI-1) produced by endothelial cells and mediated by inflammation. Increased levels of PAI-1 are present in bronchoalveolar fluid and plasma from patients with ARDS. PAI-1 suppresses tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) from converting plasminogen to plasmin, which ultimately leads to reduced fibrin degradation [86, 88–93].

6.1 Endothelial damage

In injured alveoli, endothelial damage activates innate host responses and coagulation, promoting platelet activation and aggregation (Fig. 3). After endothelial damage, disruption of the intercellular junctions exposes the subendothelial extracellular matrix containing the procoagulant tissue factor (TF). TF is also produced by endothelial and epithelial cells in the alveoli and by activated macrophages/monocytes and platelets. Then, TF binds to Factor VII to initiate TF-dependent coagulation, resulting in thrombin generation, platelet aggregation, conversion of fibrinogen to fibrin, and, consequently, formation of blood clots (Fig. 3) and fibrin deposition in the alveolar airspaces [94, 95]. The von Willebrand factor (VWF), produced by activated endothelial cells, platelets, or exposed subendothelium, mediates further platelet adhesion and aggregation [92]. Under activation, vascular endothelial cells express cell adhesion proteins such as P-selectin, E-selectin, ICAM, and VCAM that enable the recruitment of platelets and leukocytes, which also have a pivotal role in hemostasis and thrombosis [96] (Fig. 3). In addition, the renin-angiotensin pathway plays an essential role in ARDS promoting coagulation. Accumulation of angiotensin II (AngII) has been observed in the lung of patients with ARDS. The binding of AngII to angiotensin II receptor type 1 (AT1) augments pulmonary vasoconstriction and contributes to TF and PAI-1 expression on platelets and endothelial cells [97].

6.2 Platelet activation and interaction with immune cells

Platelets are an essential component of ARDS pathogenesis. In a small human study, platelet activation was greater in ARDS patients than in healthy controls [98]. Also, it has been shown that the severity of lung injury is tightly correlated with platelet-derived α-granule mediators in BAL fluid [99]. Platelets have an essential function in coagulation and the innate immune system, participating

in neutrophil and monocyte activation and recruitment (Fig. 3). During endothelial damage, the exposure to subendothelial collagen leads to platelet activation and subsequent release of cell membrane proteins and granular contents, including chemokines, cytokines (IL-1, TNF-α), coagulation proteases, adhesive molecules, growth factors, and mediators of angiogenesis that cause further platelet activation and amplification of the innate immune responses [100–102]. Activated platelets bind to leukocytes, such as neutrophils and monocytes, promoting their activation, adhesion, and migration at the site of injured endothelium via expression of adhesion molecules, such as ICAM-1 and VACM-1 (Fig. 3). Also, leukocyte rolling on vascular endothelium is facilitated by platelet-derived P-selectin and thromboxane-A2, facilitating leukocyte migration into injured tissue (Fig. 3). This platelet-neutrophil binding is mediated by toll-like receptor 4 (TLR4) engagement and participates in neutrophil activation and release of NETs [103]. Although NETs show antimicrobial properties by trapping and inactivating microorganisms in blood vessels, they also have procoagulant properties and might cause collateral tissue damage, in which the neutrophil-derived proteases have an important role. Furthermore, NETs cause platelet activation and aggregation, and activates coagulation pathway, contributing to fibrin formation [103–105]. Also, activated platelets exert an important function in immune defense by releasing antimicrobial peptides (*e.g.*, AMPs) and enhancing the phagocytosis capacity of leukocytes, however, an increased formation of the platelet-leukocyte complex contributes to acute lung injury and other organ failure [106]. Actually, preclinical studies of acute lung injury (ALI) show that the depletion of platelet leads to a significant reduction of neutrophil recruitment in the lung, and that the inhibition of platelet-neutrophil complex improves gas exchange and prolongs animal survival [107].

6.3 Molecular link between hemostatic and immune systems

Immune cells as well as platelets and endothelial cells can be activated by factors of the coagulation cascade. The molecular link between hemostatic and immune systems is mainly based on the protease activated receptors (PARs) on immune cells, platelets, and endothelial cells. Complexes of TF/Factor VIIa (TF/FVIIa), TF/FVIIa/Factor Xa (TF/FVIIa/FXa) and Factor Xa and thrombin trigger PARs, which activate innate immune cells and the expression of cytokine and adhesion molecules. These effects enhance inflammatory processes in the lung, including P-selectin-mediated leukocyte migration, that cause disruption of endothelial barrier by altering endothelial cytoskeleton and further platelet activation [83, 92, 108, 109]. In addition, fibrinogen and fibrin can directly initiate the activation of neutrophils [110].

On the other hand, inflammation facilitates and enhances coagulation. In this regard, proinflammatory cytokines activate coagulation system and also play an important role in the down-regulation of physiological anticoagulant pathways [111]. Inflammatory cytokines, such as IL-1, TNF-α, IFN-γ, and lipopolysaccharide (LPS) of gram-negative bacteria, known to be elevates in ARDS patients, induce TF expression on macrophages and platelets [84]. IL-2, also elevated in these patients, decreases fibrinolysis by upregulating of the antifibrinolytic PAI-1. Interestingly, it has been shown that platelets have receptors for IL-1β, IL-6 and IL-8. These cytokines that are one of the most reported elevated cytokines in ARDS have the capability of activating and spreading platelets [112]. IL-6 and IFN-γ also increases the expression of TF on endothelial cells and monocytes and can impair vascular endothelium function. The chemokines, such as IL-8, have an indirect prothrombotic effect via attracting neutrophils to the site of infection [84, 101]. As mentioned before, the release of NETs by neutrophils can contribute to tissue damage by exacerbating local inflammation and enhancing microvascular thrombosis in the lung. This organized recruitment of innate cells and platelets at the site of endothelial injury, in turn, leads to the release of pro-inflammatory mediators contributing to further activation of intravascular immune responses [84, 113].

6.4 Hypoxia enhances immunothrombosis

Finally, hypoxia occurs in moderate-to-severe ARDS and this can lead to endothelial dysfunction, including disruption of vascular tone and hypercoagulability. Hypoxia-induced expression of adhesion molecules P-selectin, E-selectin, ICAM-1 and VCAM-1 that results in platelet and leukocyte recruitment and more expression of TF, causing hypercoagulability [114, 115]. Under hypoxia, endothelial and immune cells release hypoxia-induced factors (HIFs), a transcription factors that promote thrombosis by increasing endothelial release of PAI-1 and inflammatory cytokines (TNF-α, IL-2) and by downregulating thrombomodulin [114–116]. In macrophages, HIFs promote their activation and local aggregation, with the consequent release of proinflammatory cytokines, including IL-6 and TNF-α [116].

Altogether, immunothrombosis in the lung results in the formation of an intravascular scaffold, enhancing the recognition and destruction of pathogens and supporting endothelial integrity. However, uncontrolled immunothrombosis might induce collateral tissue damage and contribute to ARDS and multiorgan dysfunction.

7. Intercellular communication mediated by extracellular vesicles (EVs)

Extracellular vesicles (EVs) are membrane-bound vesicles that mediate intercellular communication by transferring proteins, genetic material, and organelles between cells in both physiological and pathological conditions [117]. A growing body of evidence demonstrates the role of EVs in the pathogenesis of ARDS by modulating the onset and the progression of alveolar inflammation, coagulation, and epithelial/endothelial barrier dysfunction.

During lung injury, EVs derived from structural (endothelial and epithelial cells) and immune cells carry proinflammatory cytokines and chemokines (such as TNF-α, IL-6, IL-8, IL-1β, CXCL1, CXCL-10, MCP-1, MIP-2) capable of activating and recruiting immune cells into alveolar space, which exacerbate

alveolar damage [118]. Moreover, these pro-inflammatory EVs can reach epithelial and endothelial cells and contribute to direct alteration of the alveolar-capillary membrane, increasing lung permeability by mechanisms involving apoptosis and weakening intercellular TJ complexes [119–121]. During lung injury, EVs derived from activated monocytes, neutrophils and platelets upregulate the adhesion molecules VCAM, ICAM, or/and CCL5 (C-C motif chemokine ligand 5) on endothelial cells, promoting and enhancing the adhesiveness of these immune cells to the endothelium [39]. The proinflammatory stimulus on platelets, endothelial cells, and alveolar epithelial cells also induced the release of EVs enriched on TF, which initiates the coagulation cascade and results in thrombin generation, fibrin deposition, and clot formation [122–124].

After the injury, alveolar epithelial cells release EVs enriched in IL-6 and MMP-1 that can be uptaken by nearby epithelial cells, contributing to pulmonary inflammation, degradation of ECM, and epithelial barrier disruption [125, 126]. Alveolar epithelial cell-derived EVs also transfer their cargo to immune cells un lung injury. Specifically, EV-mediated transfer of caspase-3 from epithelial cells to macrophages has been reported, resulting in the activation of macrophages and their secretion of proinflammatory molecules such as TNF-α, IL-6, and MIP-2 [127]. Epithelial cell-derived EVs can also activate NF-κB signaling on alveolar macrophages upon upregulation of TLR2, Myd88, TNF-α, and IL-6. Experimental models of acute lung injury also reveal the role of epithelial cell-derived EVs in triggering the migration of macrophages into the lung. In this regard, epithelial cell-derived EVs transfer several microRNAs such as miR-17, miR-221, miR-320a, miR-22, and miR-342 to macrophages, resulting in the expression of integrin β1 onto macrophage surface, which promotes macrophage adhesion and migration. This miRNA transfer also mediates macrophage secretion of TNF-α and NF-κB activation, further exacerbating lung inflammation in ARDS [128]. Importantly, it has been found that EVs derived from alveolar epithelial cells in ARDS patients are enriched in TF, highlighting the contribution of epithelial cells to the coagulation disorder occurring in these patients [123].

Experimental models of ALI/ARDS have also demonstrated the contribution of EVs derived from activated macrophages to lung injury by triggering inflammation. Under proinflammatory stimuli, macrophages release EVs enriched in TNF-α, IL-1β, and IL-6, which can be uptaken by alveolar epithelial cells and upregulates ICAM-1, IL-8, and MCP-1 expression. Macrophage-derived EVs also contain miRNAs, such as miR-223, capable of triggering monocyte differentiation into macrophages [129]. Activated macrophages also communicate with endothelial cells, activating ERK1/2 (extracellular signal-regulated kinase 1/2) and NF-κB signaling pathways and expressing the endothelial-leukocyte adhesion proteins VCAM-1, ICAM-1, and E-selectin, which promote leukocyte adhesion to endothelium and increase endothelial barrier permeability [130–132]. Importantly, this EV-mediated interaction between monocytes and endothelial cells also promotes intravascular activation of coagulation while reducing the anticoagulant properties of the vascular luminal surface of the endothelium. These events mainly occur by increasing TF and decreasing the levels of anticoagulant tissue factor pathway inhibitor (TFPI) and thrombomodulin in endothelial cells [130].

Several studies on experimental models of acute lung injury have shown that endothelial cell derived EVs induce changes in vascular permeability and modulate immune cells responses. Endothelial derived-EVs can transfer nitrated sphingosine-1-phosphate receptor 3, a critical molecule involved in vascular permeability, and Src kinase that impairs adherens junction and cytoskeleton integrity of targeted endothelial cells by mechanisms involving phosphorylation of myosin light chains and vascular endothelial-cadherin (VE-cadherin) [133–137]. Endothelial cell-derived EVs also target and activate macrophages, inducing the macrophage production of proinflammatory molecules (CXCL10, CCL4, CCL5, IL-6, IL-8, MCP-1) and increasing macrophage adhesiveness to the endothelium [138]. Endothelial cell-derived EVs have also been reported to target neutrophils and induce NET formation in mice with abdominal sepsis [136, 137]. In addition, increasing evidence reveals that endothelial cell-derived EVs have a relevant role in coagulopathies. Elevated plasma levels of pro-coagulant endothelial-cell derived EVs containing TF have been found in patients with sepsis [139], influenza A infection [140], and COVID-19 [141], which are associated with severity and mortality. In addition, in patients with sepsis, these elevated levels of TF on circulating endothelial-derived EVs correlate with the severity of sepsis and disseminated intravascular coagulation (DIC) [139].

As occurs in other types of cells, neutrophils release EVs whose content varies depending on the stimuli received, exerting distinct properties such as anti-inflammatory, proinflammatory, antibacterial, or procoagulant effects [142, 143]. Regarding the protective effects, neutrophil-derived EVs have been reported to specifically transfer miR-223 to alveolar epithelial cells, reducing the alveolar permeability and inflammatory cytokines (IL-6, IL-1β, CXCL1) in a mouse model of ventilator-induced lung injury [121]. Neutrophil-derived EVs can also reach macrophages and induce M1 or M2 macrophage polarization. In this regard, a study of acute lung injury in mice has shown that neutrophil-derived EVs contain proinflammatory molecules (such as miR-1260, miR-1285, miR-4454, and miR-7975) that induce proinflammatory M1 macrophage polarization. On the contrary, these EVs can contain anti-inflammatory miRNAs (miR-126, miR-150, and miR-451a) that promote the macrophage polarization to the M2-anti-inflammatory phenotype [144]. Neutrophil-derived EVs also exert proinflammatory effects on endothelial cells, enhancing the adhesiveness to leukocytes and platelets [145, 146], and increasing vascular permeability due to their content of cathepsin G, S100A-8, and S100A-9 [147]. In addition, proinflammatory neutrophil-derived EVs transfer arachidonic acid to platelets, resulting in increased production of thromboxane that contributes to platelet activation and aggregation [148]. Because of their content in enzymes, such as elastase and MMPs, neutrophil-derived EVs also mediate the degradation of ECM, resulting in endothelial and epithelial barrier disruption [31, 68].

The role of EVs derived from activated platelets on the pathogenesis of ARDS has also been demonstrated in ex-

perimental models of acute lung injury. Specifically, they dysregulate coagulation, enhance inflammation and contribute to alveolar-capillary membrane disruption [103, 136, 137]. Platelet-derived EVs activate monocyte and endothelial cells, on which they trigger the release of proinflammatory cytokines and increase their adhesiveness [149, 150]. Moreover, circulating platelet-derived EVs play an important role in vascular endothelial permeability; their levels have been considered promising biomarkers of endothelial dysfunction [151–153]. Indeed, they can transfer IL-1β to endothelial cells, augmenting vascular permeability via activation of the NLR family pyrin domain containing-3 (NLRP3)-inflammasome pathway [151–154]. They also can induce apoptosis in endothelial cells via miR-142-3p transfer [151–153, 155]. Elevated levels of TF have been found in circulating platelet-derived EVs in experimental models of acute lung injury and ARDS patients [156–158]. In general, platelet-derived EVs have been proposed to act as relevant clotting initiation agents, contributing to the severity of ARDS.

Finally, the release of lung-derived EVs into the systemic circulation following lung injury might spread the damage to distant organs. New knowledge of the implication of the EVs in mediating intercellular communication between structural—endothelial and epithelial cells—and immune cells during ARDS offers an extraordinary opportunity to understand specific pathological mechanisms fully and develop novel therapeutic strategies.

8. Multiorgan-lung interaction in ARDS

In ARDS, the exacerbated lung inflammatory response and the dysregulation of immune defense along with hypoxemia and coagulopathy alter other distant organs [54, 159–161]. At ARDS onset, 80 and 90% of the patients have at least one dysfunctional nonpulmonary organ system. The most prevalent is cardiovascular (73%), followed by hematologic (46%), renal (20%), and hepatic (19%) dysfunction. Nonpulmonary organ dysfunction is significantly greater in severe ARDS (reaching 90%) compared with mild and moderate ARDS. The number of the associated dysfunctional organs also increases with ARDS severity. On the other hand, patients with prior nonpulmonary organ dysfunction are at higher risk of developing ARDS, with worse evolution and increased mortality [162]. Therefore, it is becoming more apparent that the communication between the lung and other organs is a crucial determinant for the development and resolution of ARDS, leading toward an integrative approach in the management of critical patients.

8.1 Liver-lung interaction

The liver has multiple functions, such as the clearance of pathogens and their products and cellular debris, the metabolism of toxins and drugs, the synthesis of proteins, and the modulation of systemic inflammatory response and host defense [163]. Critical patients with previous cirrhosis and other chronic liver diseases have a higher risk of developing ARDS and worse clinical outcomes than patients with no liver diseases [164, 165]. On the other hand, hepatic dysfunction during the first 48-h period of moderate-to-severe ARDS is strongly associated with a worse outcome. It has been shown that early liver dysfunction and not kidney dysfunction is independently associated with death in ARDS patients ventilated according to a protective ventilation strategy [166]. Therefore, the bidirectional liver-lung communication seems to play a significant role in the development, progression, and resolution of ARDS [167].

8.1.1 Reticuloendothelial system in the liver

The liver harbors resident macrophages, known as Kupffer cells, which account for approximately 85% of the tissue macrophages in the body. These macrophages are involved in the clearance of pathogens and their products through phagocytosis and secretion of some mediators [168]. The dysfunction of the reticuloendothelial system of the liver facilitates the release of pathogens and PAMPs to the circulatory system, reaching other organs, such as the lung, in which they activate pulmonary and systemic inflammatory responses [169]. The liver also protects the lung due to the inactivation and detoxification of some molecules from the systemic circulation, including pro-inflammatory cytokines, vasoactive mediators, and eicosanoids [163]. The defective clearance of these products by the liver can cause damage to the alveolar endothelial-capillary barrier, activate immune cells, and promote platelet aggregation in the lung, contributing to the development of diffuse alveolar damage [170, 171]. During liver injury, hepatic immune cells release proinflammatory cytokines (IL-1β, IL-6, TNF-α), PAF, and leukotrienes to the systemic circulation [172]. In several acute inflammatory diseases, such as sepsis, it has been shown that those inflammatory mediators released by the injured liver can activate alveolar macrophages and impair lung function [173, 174]. In addition, increased oxidative stress markers and cytokines, such as TNF-α and IL-1β, have been found in the lungs of rats with tetrachloride (CCl4)-induced cirrhosis. Gas exchange and the size of pulmonary vessels are also altered in experimental models of cirrhosis [175, 176].

8.1.2 Acute-phase proteins, bilirubin, and extracellular vesicles

During infection or tissue injury, the organism initiates a systemic response, known as the acute-phase response, to restore homeostasis. This response is mainly mediated by the liver and includes relevant changes in the levels of acute-phase proteins (APPs) in plasma [177]. APPs include many molecules involved in pathogen clearance, immune cell recruitment, or antioxidant processes. Interestingly, in the lung of patients with ARDS, the activation of local alveolar inflammation triggers the acute-phase response in the liver. Moreover, in patients with ARDS induced by pneumonia, the pro-inflammatory cytokines (IL-1, IL-6, and TNF-α) released by pulmonary immune cells lead to the synthesis of APPs by the liver, mainly via NF-κB activation [178, 179]. These liver-derived APPs, which include reactive protein C, SAA (serum amyloid A), or SAP (serum amyloid P), activate alveolar macrophages and trigger more cytokine release (CXCL1, IL-6), which lead to an enhancement of the neutrophil recruitment and oxidative stress in the alveolar spaces, contributing to lung damage [180, 181]. In addition, elevated levels of bilirubin produced in liver

diseases reach the alveolar space and alter the surface tension properties of the alveolar surfactant [182]. Growing evidence demonstrates that hyperbilirubinemia may contribute to the development of ARDS via activation of apoptosis, oxidative stress, and inflammation in different cell types [183, 184]. On the other hand, circulating EVs are increased during lung injury and in liver diseases [157, 185]. The potential role of these circulating EVs in liver-lung communication remains unknown and is an exciting field for future investigation.

Altogether, these observations evidence that the crosstalk between liver and lung seems to have a relevant role in the pathogenesis of ARDS.

8.2 Brain-lung interaction

The brain-lung interactions have also been reported in critically ill patients in both directions. It is well known that patients with brain injury can develop ARDS. In contrast, patients with ARDS frequently associate some neurocognitive deficiencies such as alterations in language, memory, and/or disorientation that can even persist a long time after discharge [54, 186, 187].

The neurological damages after lung injury remain unclear but may include the combination of hypoxemia, the effect of an activated systemic inflammatory response, and the circulatory changes caused by mechanical ventilation, including the effects of the positive end-expiratory pressure on cerebral microcirculation and intracranial pressure. The brain is an organ extremely sensitive to oxygen deprivation; thus, the hypoxemia resulting from ARDS seems to be a contributing but not the unique factor to brain dysfunction [187]. A recent systemic review has found an association between mechanical ventilation and acute cognitive impairment, describing greater neuroinflammation and lower cognitive scores in subjects with long-term mechanical ventilation [188].

In an experimental model of acute lung injury in pigs, Fries *et al.* [189] demonstrated elevated levels of the proinflammatory protein S-100B in serum and significant neuronal damage in the Cornu Ammonis, a subregion of the hippocampus that is especially vulnerable to a variety of pathologic conditions, such as ischemia, inflammation, and hypoxia. This hippocampal damage could explain the cognitive impairment associated with lung injury in patients.

A growing body of evidence indicates that the blood-brain barrier (BBB) permeability can be altered during systemic inflammation and/or infection because of the effect of circulating proinflammatory mediators (*i.e.*, IL-6, IL-1β, or TNF-α). These mediators activate cerebral endothelial cells, alter tight junction proteins and promote leukocyte transendothelial migration through the BBB, enhancing the local brain inflammatory responses. In this line, it has been shown that massive recruitment of monocytes into the brain initiates a complex neuroinflammatory response driving microglia polarization towards the M1 phenotype, which aggravates the cerebral inflammatory state, increases the BBB permeability, and activates different types of cell death by mechanisms involving the release of MMP9 and proinflammatory cytokine [190, 191].

The autonomic nervous system also plays an important role in the neuroimmune crosstalk between the brain and lung. The cholinergic pathway exerts an antiinflammatory effect that controls the systemic inflammatory response. In an experimental study of acute lung injury, Dos Santos *et al.* [192] showed that while vagus nerve inhibition enhances ALI, stimulation of the antiinflammatory cholinergic reflex exerts a protective effect in the lung.

Brain-lung interactions have received little attention in the literature, but a growing body of evidence suggests that both the lungs and brain establish a relevant cross-talk that modulates local and systemic inflammatory responses through common mediators.

8.3 Kidney-lung interaction

Acute kidney injury is also a life-threatening condition commonly presented in critically ill patients with systemic inflammatory response syndrome (SIRS), septic shock, or multiorgan dysfunction [193, 194]. Hemodynamic alterations induced by mechanical ventilation alter kidney perfusion and function by reducing the cardiac output, which leads to a redistribution of the renal blood flow with a reduction of the glomerular filtration rate and free water clearance [195, 196]. Mechanical ventilation also stimulates renin-angiotensin and sympathetic pathways, resulting in suppression of the atrial natriuretic peptide release. These changes lead to renal blood flow reduction and fluid retention [197, 198]. In addition, some preclinical studies suggest an essential role of several inflammatory mediators secondary to ventilator-induced lung injury (VILI) in developing acute kidney injury (known as ventilator-induced kidney injury). In experimental models of VILI, there is an increase of nitric oxide synthase (NOS) in both lung and kidney and of VEGF in serum along with systemic microvascular leak [199]. NOS enhances vascular permeability upon VEGF-ERK1/2 activation [200]. Increased levels of IL-6 and VEGF in the kidney have been shown in an experimental model of acid-induced lung injury in animals ventilated with high tidal volume (17 mL/kg) [201]. Apoptosis is also induced in kidney epithelial cells in animals with VILI. In ARDS patients, there is a correlation between elevated proapoptotic soluble Fas ligand and creatinine levels in the serum of ARDS patients [202].

In addition, hypoxemia has also been reported to alter kidney function, reducing renal blood flow by activating vasoactive factors such as angiotensin II, endothelin, and a decrease in nitric oxide that result in elevated renal vascular resistance [203, 204]. Moreover, in vitro models have demonstrated that low oxygen (O_2) and high carbon dioxide (CO_2) induced apoptosis in renal tubular cells [205].

On the other hand, detrimental effects of acute kidney injury on the lung have also been observed. In this line, acute kidney injury induced in animals results in a downregulation of epithelial sodium channel (eNaC), Na^+/K^+-ATPase, and aquaporin-5 in the lungs [206]. These proteins play relevant roles in fluid clearance and permeability of the alveolar epithelium; thus, dysregulation of these processes may lead to ARDS development. Acute kidney injury also elevates proinflammatory cytokines (IL-1β, IL-6, and IL-12) in serum leading to a secondary ALI characterized by pulmonary vascular congestion and neutrophil infiltration [207]. Another preclinical study in

mice shows that acute kidney injury is followed 4 h later by neutrophil infiltration, increased myeloperoxidase activation, and high levels of the neutrophil chemokines KC (keratinocyte chemoattractant) and MIP-2 along with capillary leak in the lung [208].

Altogether, kidneys could become damaged by mediators of inflammation or immuno-mediated factors related to ARDS, including the ventilator-related systemic and renal circulatory changes. On the contrary, it could be the renal disease determining consecutive pulmonary damage in critically ill patients.

9. Summary and conclusions

In summary, activation of PAMP and DAMP-mediated cell signals, dysregulated inflammatory response with pulmonary leukocyte infiltration, a procoagulant state, and the activation of cell death processes result in the disruption of the alveolar-capillary membrane and consequently in the protein-riched edema formation, in which weaknesses of the TJ complexes and alterations of the ECM in the alveolar epithelium play a key role. Inflammation and activated endothelial cells trigger coagulation cascades and platelet activation and aggregation. Activated platelets directly interact with neutrophils, facilitating their extravasation and recruitment into the lung and enhancing the systemic inflammatory responses. All these events generate a procoagulant state with the formation of fibrin in the airspaces and thrombosis in the microvasculature that aggravate alveolar injury and gas exchange. The crosstalk between alveolar epithelial cells, immune cells, platelets, and endothelial cells is mediated at least in part by EVs, which also mediate interorgan communication. Interaction of the lung with other organs has been revealed as an essential determinant in the development and resolution of ARDS.

Altogether, the pathophysiology of ARDS comprises many interconnected mechanisms responsible for modulating the onset and progression of lung injury. A complete understanding of the cross-talk between the different types of cells involved and the interaction of the lung with other organs will improve our knowledge of the physiopathogenesis of ARDS and offer an excellent opportunity to discover new biomarkers and novel therapeutic strategies in this and other clinical conditions.

AUTHOR CONTRIBUTIONS

PGR—conception and design, collection and assembly of information, manuscript writing, RH—conception and design, collection and assembly of information, manuscript writing, GS—collection and assembly of data, JAL—manuscript revision and approval of the final version of the manuscript. All authors contributed to the article and approved the submitted version.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, *et al*. Acute respiratory distress syndrome: the Berlin definition. JAMA. 2012; 307: 2526–2533.

[2] Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, *et al*. Acute respiratory distress syndrome. Nature Reviews Disease Primers. 2019; 5: 18.

[3] Diamond M, Peniston HL, Sanghavi D, Mahapatra S. Acute respiratory distress syndrome. StatPearls Publishing: Treasure Island (FL). 2022.

[4] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, *et al*. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet. 2020; 395: 1054–1062.

[5] Williams GW, Berg NK, Reskallah A, Yuan X, Eltzschig HK. Acute respiratory distress syndrome. Anesthesiology. 2021; 134: 270–282.

[6] Kao K, Hu H, Chang C, Hung C, Chiu L, Li S, *et al*. Diffuse alveolar damage associated mortality in selected acute respiratory distress syndrome patients with open lung biopsy. Critical Care. 2015; 19: 228.

[7] Lorente JA, Ballén-Barragán A, Herrero R, Esteban A. Acute respiratory distress syndrome: does histology matter? Critical Care. 2015; 19: 337.

[8] Hasan SS, Capstick T, Ahmed R, Kow CS, Mazhar F, Merchant HA, *et al*. Mortality in COVID-19 patients with acute respiratory distress syndrome and corticosteroids use: a systematic review and meta-analysis. Expert Review of Respiratory Medicine. 2020; 14: 1149–1163.

[9] Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, *et al*. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. JAMA. 2016; 315: 788.

[10] Gumbert SD, Kork F, Jackson ML, Vanga N, Ghebremichael SJ, Wang CY, *et al*. Perioperative acute kidney injury. Anesthesiology. 2020; 132: 180–204.

[11] de Oliveira RP, Teixeira C, Rosa RG. Acute respiratory distress syndrome: how do patients fare after the intensive care unit? Brazilian Journal of Intensive Therapy. 2019; 31: 555–560.

[12] Mart MF, Ware LB. The long-lasting effects of the acute respiratory distress syndrome. Expert Review of Respiratory Medicine. 2020; 14: 577–586.

[13] Matthay MA, Zemans RL. The acute respiratory distress syndrome: pathogenesis and treatment. Annual Review of Pathology: Mechanisms of Disease. 2011; 6: 147–163.

[14] Zemans RL, Matthay MA. Bench-to-bedside review: the role of the alveolar epithelium in the resolution of pulmonary edema in acute lung injury. Critical Care. 2004; 8: 469–477.

[15] Bachofen M, Weibel ER. Structural alterations of lung parenchyma in the adult respiratory distress syndrome. Clinics in Chest Medicine. 1982; 3: 35–56.

[16] Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR,

Mercat A, *et al*. Acute respiratory distress syndrome. Nature Reviews Disease Primers. 2019; 5: 18.

[17] Holter JF, Weiland JE, Pacht ER, Gadek JE, Davis WB. Protein permeability in the adult respiratory distress syndrome. Loss of size selectivity of the alveolar epithelium. Journal of Clinical Investigation. 1986; 78: 1513–1522.

[18] Ware LB, Fremont RD, Bastarache JA, Calfee CS, Matthay MA. Determining the aetiology of pulmonary oedema by the oedema fluid-to-plasma protein ratio. European Respiratory Journal. 2010; 35: 331–337.

[19] Herrero R, Sanchez G, Lorente JA. New insights into the mechanisms of pulmonary edema in acute lung injury. Annals of Translational Medicine. 2018; 6: 32–32.

[20] Matthay MA. Resolution of pulmonary edema. Thirty years of progress. American Journal of Respiratory and Critical Care Medicine. 2014; 189: 1301–1308.

[21] Gwoździńska P, Buchbinder BA, Mayer K, Herold S, Morty RE, Seeger W, *et al*. Hypercapnia impairs ENaC cell surface stability by promoting phosphorylation, polyubiquitination and endocytosis of β-ENac in a human alveolar epithelial cell line. Frontiers in Immunology. 2017; 8: 591.

[22] Vadász I, Sznajder JI. Gas exchange disturbances regulate alveolar fluid clearance during acute lung injury. Frontiers in Immunology. 2017; 8: 757.

[23] Herrero R, Tanino M, Smith LS, Kajikawa O, Wong VA, Mongovin S, *et al*. The Fas/FasL pathway impairs the alveolar fluid clearance in mouse lungs. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2013; 305: L377–L388.

[24] Herrero R, Kajikawa O, Matute-Bello G, Wang Y, Hagimoto N, Mongovin S, *et al*. The biological activity of FasL in human and mouse lungs is determined by the structure of its stalk region. Journal of Clinical Investigation. 2011; 121: 1174–1190.

[25] Herrero R, Prados L, Ferruelo A, Puig F, Pandolfi R, Guillamat-Prats R, *et al*. Fas activation alters tight junction proteins in acute lung injury. Thorax. 2019; 74: 69–82.

[26] Fujishima S. Pathophysiology and biomarkers of acute respiratory distress syndrome. Journal of Intensive Care. 2014; 2: 32.

[27] Xiang M, Fan J. Pattern recognition receptor-dependent mechanisms of acute lung injury. Molecular Medicine. 2010; 16: 69–82.

[28] Mazzon E, Cuzzocrea S. Role of TNF-α in lung tight junction alteration in mouse model of acute lung inflammation. Respiratory Research. 2007; 8: 75.

[29] Lin X, Barravecchia M, Kothari P, Young JL, Dean DA. B1-Na+, K+-ATPase gene therapy upregulates tight junctions to rescue lipopolysaccharide-induced acute lung injury. Gene Therapy. 2016; 23: 489–499.

[30] Davey A, McAuley DF, O'Kane CM. Matrix metalloproteinases in acute lung injury: mediators of injury and drivers of repair. European Respiratory Journal. 2011; 38: 959–970.

[31] Mammoto A, Mammoto T, Kanapathipillai M, Wing Yung C, Jiang E, Jiang A, *et al*. Control of lung vascular permeability and endotoxin-induced pulmonary oedema by changes in extracellular matrix mechanics. Nature Communications. 2013; 4: 1759.

[32] Watson WH, Ritzenthaler JD, Roman J. Lung extracellular matrix and redox regulation. Redox Biology. 2016; 8: 305–315.

[33] Pugin J, Verghese G, Widmer MC, Matthay MA. The alveolar space is the site of intense inflammatory and profibrotic reactions in the early phase of acute respiratory distress syndrome. Critical Care Medicine. 1999; 27: 304–312.

[34] Pittet J, Griffiths MJD, Geiser T, Kaminski N, Dalton SL, Huang X, *et al*. TGF-β is a critical mediator of acute lung injury. Journal of Clinical Investigation. 2001; 107: 1537–1544.

[35] Eaton DC, Helms MN, Koval M, Bao HF, Jain L. The contribution of epithelial sodium channels to alveolar function in health and disease. Annual Review of Physiology. 2009; 71: 403–423.

[36] Drakopanagiotakis F, Xifteri A, Polychronopoulos V, Bouros D. Apoptosis in lung injury and fibrosis. European Respiratory Journal. 2008; 32: 1631–1638.

[37] Moss M, Gillespie MK, Ackerson L, Moore FA, Moore EE, Parsons PE. Endothelial cell activity varies in patients at risk for the adult respiratory distress syndrome. Critical Care Medicine. 1996; 24: 1782–1786.

[38] Cheng KT, Xiong S, Ye Z, Hong Z, Di A, Tsang KM, *et al*. Caspase-11—mediated endothelial pyroptosis underlies endotoxemia-induced lung injury. Journal of Clinical Investigation. 2017; 127: 4124–4135.

[39] Vassiliou AG, Kotanidou A, Dimopoulou I, Orfanos SE. Endothelial damage in acute respiratory distress syndrome. International Journal of Molecular Sciences. 2020; 21: 8793.

[40] Lijnen H, Suffredini A, Pepper M, Steinberg K, Martin T, Pugin J, *et al*. Increased angiostatin levels in bronchoalveolar lavage fluids from ARDS patients and from human volunteers after lung instillation of endotoxin. Thrombosis and Haemostasis. 2002; 87: 966–971.

[41] Fahy RJ, Lichtenberger F, McKeegan CB, Nuovo GJ, Marsh CB, Wewers MD. The acute respiratory distress syndrome. American Journal of Respiratory Cell and Molecular Biology. 2003; 28: 499–503.

[42] Wang Y, Wang H, Zhang C, Zhang C, Yang H, Gao R, *et al*. Lung fluid biomarkers for acute respiratory distress syndrome: a systematic review and meta-analysis. Critical Care. 2019; 23: 43.

[43] Leksa V, Godar S, Schiller HB, Fuertbauer E, Muhammad A, Slezakova K, *et al*. TGF-β-induced apoptosis in endothelial cells mediated by M6P/IGFII-R and mini-plasminogen. Journal of Cell Science. 2005; 118: 4577–4586.

[44] Polunovsky VA, Wendt CH, Ingbar DH, Peterson MS, Bitterman PB. Induction of endothelial cell apoptosis by TNF α: modulation by inhibitors of protein synthesis. Experimental Cell Research. 1994; 214: 584–594.

[45] Lee YH, Kayyali US, Sousa AM, Rajan T, Lechleider RJ, Day RM. Transforming growth factor-β1 effects on endothelial monolayer permeability involve focal adhesion kinase/Src. American Journal of Respiratory Cell and Molecular Biology. 2007; 37: 485–493.

[46] Bruewer M, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, *et al*. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. The Journal of Immunology. 2003; 171: 6164–6172.

[47] Li Q, Zhang Q, Wang M, Zhao S, Ma J, Luo N, *et al*. Interferon-gamma and tumor necrosis factor-alpha disrupt epithelial barrier function by altering lipid composition in membrane microdomains of tight junction. Clinical Immunology 2008; 126: 67–80.

[48] Ganter MT, Roux J, Miyazawa B, Howard M, Frank JA, Su G, *et al*. Interleukin-1β causes acute lung injury via αvβ5 and αvβ6 integrin-dependent mechanisms. 2008; 102: 804–812.

[49] Millar FR, Summers C, Griffiths MJ, Toshner MR, Proudfoot AG. The pulmonary endothelium in acute respiratory distress syndrome: insights and therapeutic opportunities. Thorax. 2016; 71: 462–473.

[50] Imai Y, Kuba K, Penninger JM. The renin-angiotensin system in acute respiratory distress syndrome. Drug Discovery Today: Disease Mechanisms. 2006; 3: 225–229.

[51] Revercomb L, Hanmandlu A, Wareing N, Akkanti B, Karmouty-Quintana H. Mechanisms of pulmonary hypertension in acute respiratory distress syndrome (ARDS). Frontiers in Molecular Biosciences. 2021; 7: 624093.

[52] Kuba K, Yamaguchi T, Penninger JM. Angiotensin-converting enzyme 2 (ACE2) in the pathogenesis of ARDS in COVID-19. Frontiers in Immunology. 2021; 12: 732690.

[53] Yanagi S, Tsubouchi H, Miura A, Matsumoto N, Nakazato M. Breakdown of epithelial barrier integrity and overdrive activation of alveolar epithelial cells in the pathogenesis of acute respiratory distress syndrome and lung fibrosis. BioMed Research International. 2015; 2015: 1–12.

[54] Quílez ME, López-Aguilar J, Blanch L. Organ crosstalk during acute lung injury, acute respiratory distress syndrome, and mechanical ventilation. Current Opinion in Critical Care. 2012; 18: 23–28.

[55] Iwasaki A, Foxman EF, Molony RD. Early local immune defences in the respiratory tract. Nature Reviews Immunology. 2017; 17: 7–20.

[56] Wong JJM, Leong JY, Lee JH, Albani S, Yeo JG. Insights into the immuno-pathogenesis of acute respiratory distress syndrome. Annals of Translational Medicine. 2019; 7: 504–504.

[57] Grommes J, Soehnlein O. Contribution of Neutrophils to Acute Lung Injury. Molecular Medicine. 2011; 17: 293–307.

[58] Johnston LK, Rims CR, Gill SE, McGuire JK, Manicone AM. Pulmonary macrophage subpopulations in the induction and resolution of acute lung injury. American Journal of Respiratory Cell and Molecular Biology. 2012; 47: 417–426.

[59] Rosseau S, Hammerl P, Maus U, Walmrath H, Schütte H, Grimminger

F, *et al*. Phenotypic characterization of alveolar monocyte recruitment in acute respiratory distress syndrome. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2000; 279: L25–L35.

[60] Bittencourt-Mernak MI, Pinheiro NM, Santana FPR, Guerreiro MP, Saraiva-Romanholo BM, Grecco SS, *et al*. Prophylactic and therapeutic treatment with the flavonone sakuranetin ameliorates LPS-induced acute lung injury. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2017; 312: L217–L230.

[61] Pinheiro NM, Santana FPR, Almeida RR, Guerreiro M, Martins MA, Caperuto LC, *et al*. Acute lung injury is reduced by the a7nAChR agonist PNU-282987 through changes in the macrophage profile. FASEB Journal. 2017; 31: 320–332.

[62] Wang Y, Xu Y, Zhang P, Ruan W, Zhang L, Yuan S, *et al*. Smiglaside a ameliorates LPS-induced acute lung injury by modulating macrophage polarization via AMPK-PPARγ pathway. Biochemical Pharmacology. 2018; 156: 385–395.

[63] Zhuo Y, Li D, Cui L, Li C, Zhang S, Zhang Q, *et al*. Treatment with 3,4-dihydroxyphenylethyl alcohol glycoside ameliorates sepsis-induced ALI in mice by reducing inflammation and regulating M1 polarization. Biomedicine & Pharmacotherapy. 2019; 116: 109012.

[64] Mishra P, Pandey N, Pandey R, Tripathi YB. Role of macrophage polarization in acute respiratory distress syndrome. Journal of Respiration. 2021; 1: 260–272.

[65] Wang F, Fu X, Wu X, Zhang J, Zhu J, Zou Y, *et al*. Bone marrow derived M 2 macrophages protected against lipopolysaccharide-induced acute lung injury through inhibiting oxidative stress and inflammation by modulating neutrophils and T lymphocytes responses. International Immunopharmacology. 2018; 61: 162–168.

[66] Chen X, Tang J, Shuai W, Meng J, Feng J, Han Z. Macrophage polarization and its role in the pathogenesis of acute lung injury/acute respiratory distress syndrome. Inflammation Research. 2020; 69: 883–895.

[67] Yang CY, Chen CS, Yiang GT, Cheng YL, Yong SB, Wu MY, *et al*. New insights into the immune molecular regulation of the pathogenesis of acute respiratory distress syndrome. International Journal of Molecular Sciences. 2018; 19: 588.

[68] Genschmer KR, Russell DW, Lal C, Szul T, Bratcher PE, Noerager BD, *et al*. Activated PMN exosomes: pathogenic entities causing matrix destruction and disease in the lung. Cell. 2019; 176: 113–126.e15.

[69] Vorobjeva NV, Chernyak BV. NETosis: molecular mechanisms, role in physiology and pathology. Biochemistry. 2020; 85: 1178–1190.

[70] Meegan JE, Yang X, Coleman DC, Jannaway M, Yuan SY. Neutrophil-mediated vascular barrier injury: role of neutrophil extracellular traps. Microcirculation. 2017; 24: e12352.

[71] Ma Y, Yang X, Chatterjee V, Meegan JE, Beard RS, Yuan SY. Role of neutrophil extracellular traps and vesicles in regulating vascular endothelial permeability. Frontiers in Immunology. 2019; 10: 1037.

[72] Thålin C, Hisada Y, Lundström S, Mackman N, Wallén H. Neutrophil extracellular traps. Arteriosclerosis, Thrombosis, and Vascular Biology. 2019; 39: 1724–1738.

[73] Zhang H, Zhou Y, Qu M, Yu Y, Chen Z, Zhu S, *et al*. Tissue factor-enriched neutrophil extracellular traps promote immunothrombosis and disease progression in sepsis-induced lung injury. Frontiers in Cellular and Infection Microbiology. 2021; 11: 677902.

[74] Lefrançais E, Mallavia B, Zhuo H, Calfee CS, Looney MR. Maladaptive role of neutrophil extracellular traps in pathogen-induced lung injury. JCI Insight. 2018; 3: e98178.

[75] Delano MJ, Ward PA. Sepsis-induced immune dysfunction: can immune therapies reduce mortality? Journal of Clinical Investigation. 2016; 126: 23–31.

[76] Li JT, Melton AC, Su G, Hamm DE, LaFemina M, Howard J, *et al*. Unexpected role for adaptive $\alpha\beta$Th17 cells in acute respiratory distress syndrome. The Journal of Immunology. 2015; 195: 87–95.

[77] Yi X, Jian W, Di W, Jun Z, Mei Y. Prognostic values of Th17 cells level in bronchoalveolar lavage fluid in children of sepsis with acute lung injury. Chinese Journal of Contemporary Pediatrics. 2015; 17: 942–945.

[78] Holloway TL, Rani M, Cap AP, Stewart RM, Schwacha MG. The association between the Th-17 immune response and pulmonary complications in a trauma ICU population. Cytokine. 2015; 76: 328–333.

[79] Liston A, Gray DHD. Homeostatic control of regulatory T cell diversity. Nature Reviews Immunology. 2014; 14: 154–165.

[80] Lin S, Wu H, Wang C, Xiao Z, Xu F. Regulatory T cells and acute lung injury: cytokines, uncontrolled inflammation, and therapeutic implications. Frontiers in Immunology. 2018; 9: 1545.

[81] Yu Z, Ji M, Yan J, Cai Y, Liu J, Yang H, *et al*. The ratio of Th17/Treg cells as a risk indicator in early acute respiratory distress syndrome. Critical Care. 2015; 19: 82.

[82] Li Q, Hu X, Sun R, Tu Y, Gong F, Ni Y. Resolution acute respiratory distress syndrome through reversing the imbalance of Treg/Th17 by targeting the cAMP signaling pathway. Molecular Medicine Reports. 2016; 14: 343–348.

[83] Strukova S. Blood coagulation-dependent inflammation. Coagulation-dependent inflammation and inflammation-dependent thrombosis. Frontiers in Bioscience. 2006; 11: 59.

[84] Frantzeskaki F, Armaganidis A, Orfanos SE. Immunothrombosis in acute respiratory distress syndrome: cross talks between inflammation and coagulation. Respiration. 2017; 93: 212–225.

[85] Glas GJ, Van Der Sluijs KF, Schultz MJ, Hofstra J-H, Van Der Poll T, Levi M. Bronchoalveolar hemostasis in lung injury and acute respiratory distress syndrome. Journal of Thrombosis and Haemostasis. 2013; 11: 17–25.

[86] Taylor, Jr. FB. Response of anticoagulant pathways in disseminated intravascular coagulation. Seminars in Thrombosis and Hemostasis. 2001; 27: 619–632.

[87] Camprubí-Rimblas M, Tantinyà N, Bringué J, Guillamat-Prats R, Artigas A. Anticoagulant therapy in acute respiratory distress syndrome. Annals of Translational Medicine. 2018; 6: 36.

[88] Bernard GR, Vincent J, Laterre P, LaRosa SP, Dhainaut J, Lopez-Rodriguez A, *et al*. Efficacy and safety of recombinant human activated protein c for severe sepsis. New England Journal of Medicine. 2001; 344: 699–709.

[89] Ozolina A, Sarkele M, Sabelnikovs O, Skesters A, Jaunalksne I, Serova J, *et al*. Activation of coagulation and fibrinolysis in acute respiratory distress syndrome: a prospective pilot study. Frontiers in Medicine. 2016; 3: 64.

[90] Jiang J, Chou H, Wang L, Lang Y, Chen C. Effects of activated protein C on ventilator-induced lung injury in rats. Respiration. 2010; 80: 246–253.

[91] Ware LB, Fang X, Matthay MA. Protein C and thrombomodulin in human acute lung injury. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2003; 285: L514–L521.

[92] Asokananthan N, Graham PT, Fink J, Knight DA, Bakker AJ, McWilliam AS, *et al*. Activation of protease-activated receptor (PAR)-1, PAR-2, and PAR-4 stimulates IL-6, IL-8, and prostaglandin E 2 release from human respiratory epithelial cells. The Journal of Immunology. 2002; 168: 3577–3585.

[93] Camprubí-Rimblas M, Tantinyà N, Guillamat-Prats R, Bringué J, Puig F, Gómez MN, *et al*. Effects of nebulized antithrombin and heparin on inflammatory and coagulation alterations in an acute lung injury model in rats. Journal of Thrombosis and Haemostasis. 2020; 18: 571–583.

[94] Sabharwal AK, Bajaj SP, Ameri A, Tricomi SM, Hyers TM, Dahms TE, *et al*. Tissue factor pathway inhibitor and von Willebrand factor antigen levels in adult respiratory distress syndrome and in a primate model of sepsis. American Journal of Respiratory and Critical Care Medicine. 1995; 151: 758–767.

[95] Gando S, Otomo Y. Local hemostasis, immunothrombosis, and systemic disseminated intravascular coagulation in trauma and traumatic shock. Critical Care. 2015; 19: 72.

[96] Haitsma JJ, Schultz MJ, Hofstra JJH, Kuiper JW, Juco J, Vaschetto R, *et al*. Ventilator-induced coagulopathy in experimental Streptococcus pneumoniae pneumonia. European Respiratory Journal. 2008; 32: 1599–1606.

[97] Hrenak J, Simko F. Renin-angiotensin system: an important player in the pathogenesis of acute respiratory distress syndrome. Int International Journal of Molecular Sciences. 2020; 21: 8038.

[98] Carvalho AC, Quinn DA, Demarinis SM, Beitz JG, Zapol WM. Platelet function in acute respiratory failure. American Journal of Hematology. 1987; 25: 377–388.

[99] Idell S, Maunder R, Fein AM, Switalska HI, Tuszynski GF, McLarty J, *et al*. Platelet-specific α-granule proteins and thrombospondin in bronchoalveolar lavage in the adult respiratory distress syndrome. Chest.

1989; 96: 1125–1132.

[100] Wijten P, van Holten T, Woo LL, Bleijerveld OB, Roest M, Heck AJR, *et al*. High precision platelet releasate definition by quantitative reversed protein profiling—brief report. Arteriosclerosis, Thrombosis, and Vascular Biology. 2013; 33: 1635–1638.

[101] Puneet P, Moochhala S, Bhatia M. Chemokines in acute respiratory distress syndrome. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2005; 288: L3–L15.

[102] Golebiewska EM, Poole AW. Platelet secretion: from haemostasis to wound healing and beyond. Blood Reviews. 2015; 29: 153–162.

[103] Yadav H, Kor DJ. Platelets in the pathogenesis of acute respiratory distress syndrome. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2015; 309: L915–L923.

[104] de Bont CM, Boelens WC, Pruijn GJM. NETosis, complement, and coagulation: a triangular relationship. Cellular & Molecular Immunology. 2019; 16: 19–27.

[105] Kaplan MJ, Radic M. Neutrophil extracellular traps: double-edged swords of innate immunity. The Journal of Immunology. 2012; 189: 2689–2695.

[106] Song C, Li H, Li Y, Dai M, Zhang L, Liu S, *et al*. NETs promote ALI/ARDS inflammation by regulating alveolar macrophage polarization. Experimental Cell Research. 2019; 382: 111486.

[107] Zarbock A, Singbartl K, Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. Journal of Clinical Investigation. 2006; 116: 3211–3219.

[108] Camerer E, Huang W, Coughlin SR. Tissue factor- and factor X-dependent activation of protease-activated receptor 2 by factor VIIa. Proceedings of the National Academy of Sciences. 2000; 97: 5255–5260.

[109] Ruf W, Dorfleutner A, Riewald M. Specificity of coagulation factor signaling. Journal of Thrombosis and Haemostasis. 2003; 1: 1495–1503.

[110] Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. Nature Reviews Immunology. 2013; 13: 34–45.

[111] Choi G, Schultz MJ, Levi M, van der Poll T. The relationship between inflammation and the coagulation system. Swiss Medical Weekly. 2006; 136: 139–144.

[112] Bester J, Pretorius E. Effects of IL-1β, IL-6 and IL-8 on erythrocytes, platelets and clot viscoelasticity. Scientific Reports. 2016; 6: 32188.

[113] Livingstone SA, Wildi KS, Dalton HJ, Usman A, Ki KK, Passmore MR, *et al*. Coagulation dysfunction in acute respiratory distress syndrome and its potential impact in inflammatory subphenotypes. Frontiers in Medicine. 2021; 8: 723217.

[114] Gupta N, Zhao YY, Evans CE. The stimulation of thrombosis by hypoxia. Thrombosis Research. 2019; 181: 77–83.

[115] Chan CK, Vanhoutte PM. Hypoxia, vascular smooth muscles and endothelium. Acta Pharmaceutica Sinica B. 2013; 3: 1–7.

[116] Palazon A, Goldrath A, Nizet V, Johnson R. HIF transcription factors, inflammation, and immunity. Immunity. 2014; 41: 518–528.

[117] Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. Journal of Cell Biology. 2013; 200: 373–383.

[118] Esquivel-Ruiz S, González-Rodríguez P, Lorente JA, Pérez-Vizcaíno F, Herrero R, Moreno L. Extracellular Vesicles and alveolar epithelial-capillary barrier disruption in acute respiratory distress syndrome: pathophysiological role and therapeutic potential. Frontiers in Physiology. 2021; 12: 752287.

[119] Wang L, Luo H, Chen X, Jiang Y, Huang Q. Functional characterization of S100A8 and S100A9 in altering monolayer permeability of human umbilical endothelial cells. PLoS ONE. 2014; 9: e90472.

[120] Mitra S, Wewers MD, Sarkar A. Mononuclear phagocyte-derived microparticulate caspase-1 induces pulmonary vascular endothelial cell injury. PLoS ONE. 2015; 10: e0145607.

[121] Neudecker V, Brodsky KS, Clambey ET, Schmidt EP, Packard TA, Davenport B, *et al*. Neutrophil transfer of miR-223 to lung epithelial cells dampens acute lung injury in mice. Science Translational Medicine. 2017; 9: eaah5360.

[122] Combes V, Simon A, Grau G, Arnoux D, Camoin L, Sabatier F, *et al*. In vitro generation of endothelial microparticles and possible prothrombotic activity in patients with lupus anticoagulant. Journal of Clinical Investigation. 1999; 104: 93–102.

[123] Kulshreshtha A, Ahmad T, Agrawal A, Ghosh B. Proinflammatory role of epithelial cell–derived exosomes in allergic airway inflammation. Journal of Allergy and Clinical Immunology. 2013; 131: 1194–1203.e14.

[124] Iba T, Ogura H. Role of extracellular vesicles in the development of sepsis-induced coagulopathy. Journal of Intensive Care. 2018; 6: 68.

[125] Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harbor Perspectives in Biology. 2011; 3: a005058–a005058.

[126] Moon H, Kim S, Gao J, Quan T, Qin Z, Osorio JC, *et al*. CCN1 secretion and cleavage regulate the lung epithelial cell functions after cigarette smoke. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2014; 307: L326–L337.

[127] Moon H, Cao Y, Yang J, Lee JH, Choi HS, Jin Y. Lung epithelial cell-derived extracellular vesicles activate macrophage-mediated inflammatory responses via ROCK1 pathway. Cell Death & Disease. 2015; 6: e2016–e2016.

[128] Lee H, Zhang D, Wu J, Otterbein LE, Jin Y. Lung epithelial cell-derived microvesicles regulate macrophage migration via MicroRNA-17/221-induced integrin β 1 recycling. Journal of Immunology. 2017; 199: 1453–1464.

[129] Ismail N, Wang Y, Dakhlallah D, Moldovan L, Agarwal K, Batte K, *et al*. Macrophage microvesicles induce macrophage differentiation and miR-223 transfer. Blood. 2013; 121: 984–995.

[130] Tamari T, Brenner B, Aharon A. Monocyte-derived microparticles and exosomes induce procoagulant and apoptotic effects on endothelial cells. Thrombosis and Haemostasis. 2008; 100: 878–885.

[131] Li L, Cheng Y, Emrich S, Schorey J. Activation of endothelial cells by extracellular vesicles derived from Mycobacterium tuberculosis infected macrophages or mice. PLoS ONE. 2018; 13: e0198337.

[132] Wang J, Williams JC, Davis BK, Jacobson K, Doerschuk CM, Ting JP-, *et al*. Monocytic microparticles activate endothelial cells in an IL-1β–dependent manner. Blood. 2011; 118: 2366–2374.

[133] Buesing KL, Densmore JC, Kaul S, Pritchard KA, Jarzembowski JA, Gourlay DM, *et al*. Endothelial microparticles induce inflammation in acute lung injury. Journal of Surgical Research. 2011; 166: 32–39.

[134] Sun X, Singleton PA, Letsiou E, Zhao J, Belvitch P, Sammani S, *et al*. Sphingosine-1–phosphate receptor-3 is a novel biomarker in acute lung injury. American Journal of Respiratory Cell and Molecular Biology. 2012; 47: 628–636.

[135] Li H, Meng X, Liang X, Gao Y, Cai S. Administration of microparticles from blood of the lipopolysaccharide-treated rats serves to induce pathologic changes of acute respiratory distress syndrome. Experimental Biology and Medicine. 2015; 240: 1735–1741.

[136] Chatterjee V, Yang X, Ma Y, Wu MH, Yuan SY. Extracellular vesicles: new players in regulating vascular barrier function. American Journal of Physiology-Heart and Circulatory Physiology. 2020; 319: H1181–H1196.

[137] Chatterjee V, Yang X, Ma Y, Cha B, Meegan JE, Wu M, *et al*. Endothelial microvesicles carrying Src-rich cargo impair adherens junction integrity and cytoskeleton homeostasis. Cardiovascular Research. 2020; 116: 1525–1538.

[138] Hosseinkhani B, Kuypers S, van den Akker NMS, Molin DGM, Michiels L. Extracellular vesicles work as a functional inflammatory mediator between vascular endothelial cells and immune cells. Frontiers in Immunology. 2018; 9: 1789.

[139] Matsumoto H, Yamakawa K, Ogura H, Koh T, Matsumoto N, Shimazu T. Enhanced expression of cell-specific surface antigens on endothelial microparticles in sepsis-induced disseminated intravascular coagulation. Shock. 2015; 43: 443–449.

[140] Rondina MT, Tatsumi K, Bastarache JA, Mackman N. Microvesicle tissue factor activity and interleukin-8 levels are associated with mortality in patients with influenza a/H1N1 infection. Critical Care Medicine. 2016; 44: e574–e578.

[141] Rosell A, Havervall S, von Meijenfeldt F, Hisada Y, Aguilera K, Grover SP, *et al*. Patients with COVID-19 have elevated levels of circulating extracellular vesicle tissue factor activity that is associated with severity and mortality—brief report. Arteriosclerosis, Thrombosis, and Vascular Biology. 2021; 41: 878–882.

[142] Hong CW. Extracellular vesicles of neutrophils. Immune Network. 2018; 18: e43.

[143] Kolonics F, Szeifert V, Timár CI, Ligeti E, Lőrincz ÁM. The functional heterogeneity of neutrophil-derived extracellular vesicles reflects the

status of the parent cell. Cells. 2020; 9: 2718.

[144] Youn Y, Shrestha S, Lee Y, Kim J, Lee JH, Hur K, *et al*. Neutrophil-derived trail is a proinflammatory subtype of neutrophil-derived extracellular vesicles. Theranostics. 2021; 11: 2770–2787.

[145] Mesri M, Altieri DC. Endothelial cell activation by leukocyte microparticles. Journal of Immunology. 1998; 161: 4382–4387.

[146] Mesri M, Altieri DC. Leukocyte microparticles stimulate endothelial cell cytokine release and tissue factor induction in a jnk1 signaling pathway. Journal of Biological Chemistry. 1999; 274: 23111–23118.

[147] Dalli J, Montero-Melendez T, Norling LV, Yin X, Hinds C, Haskard D, *et al*. Heterogeneity in neutrophil microparticles reveals distinct proteome and functional properties. Molecular & Cellular Proteomics. 2013; 12: 2205–2219.

[148] Rossaint J, Kühne K, Skupski J, Van Aken H, Looney MR, Hidalgo A, *et al*. Directed transport of neutrophil-derived extracellular vesicles enables platelet-mediated innate immune response. Nature Communications. 2016; 7: 13464.

[149] Barry OP, Pratico D, Lawson JA, FitzGerald GA. Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. Journal of Clinical Investigation. 1997; 99: 2118–2127.

[150] Nomura S, Tandon NN, Nakamura T, Cone J, Fukuhara S, Kambayashi J. High-shear-stress-induced activation of platelets and microparticles enhances expression of cell adhesion molecules in THP-1 and endothelial cells. Atherosclerosis. 2001; 158: 277–287.

[151] Chiva-Blanch G, Padró T, Alonso R, Crespo J, Perez de Isla L, Mata P, *et al*. Liquid biopsy of extracellular microvesicles maps coronary calcification and atherosclerotic plaque in asymptomatic patients with familial hypercholesterolemia. Arteriosclerosis, Thrombosis, and Vascular Biology. 2019; 39: 945–955.

[152] Oggero S, Austin-Williams S, Norling LV. The contrasting role of extracellular vesicles in vascular inflammation and tissue repair. Frontiers in Pharmacology. 2019; 10: 1479.

[153] de Freitas RCC, Hirata RDC, Hirata MH, Aikawa E. Circulating extracellular vesicles as biomarkers and drug delivery vehicles in cardiovascular diseases. Biomolecules. 2021; 11: 388.

[154] Hottz ED, Lopes JF, Freitas C, Valls-De-Souza R, Oliveira MF, Bozza MT, *et al*. Platelets mediate increased endothelium permeability in dengue through NLRP3-inflammasome activation. Blood. 2013; 122: 3405–3414.

[155] Bao H, Yao QP, Huang K, Chen XH, Han Y, Jiang ZL, *et al*. Platelet-derived miR-142-3p induces apoptosis of endothelial cells in hypertension. Cellular and Molecular Biology. 2017; 63: 3–9.

[156] Nieuwland R, Berckmans RJ, McGregor S, Böing AN, Th. M. Romijn FPH, Westendorp RGJ, *et al*. Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. Blood. 2000; 95: 930–935.

[157] Tripisciano C, Weiss R, Eichhorn T, Spittler A, Heuser T, Fischer MB, *et al*. Different potential of extracellular vesicles to support thrombin generation: contributions of phosphatidylserine, tissue factor, and cellular origin. Scientific Reports. 2017; 7: 6522.

[158] Puhm F, Boilard E, MacHlus KR. Platelet extracellular vesicles; beyond the blood. Arteriosclerosis, Thrombosis, and Vascular Biology. 2021; 41: 87–96.

[159] Darmon M, Clec'h C, Adrie C, Argaud L, Allaouchiche B, Azoulay E, *et al*. Acute respiratory distress syndrome and risk of aki among critically ill patients. Clinical Journal of the American Society of Nephrology. 2014; 9: 1347–1353.

[160] Del Sorbo L, Slutsky AS. Acute respiratory distress syndrome and multiple organ failure. Current Opinion in Critical Care. 2011; 17: 1–6.

[161] Matuschak GM. Liver-lung interactions in critical illness. Update in Intensive Care and Emergency Medicine. 1995; 256: 73–93.

[162] Kallet RH, Lipnick MS, Zhuo H, Pangilinan LP, Gomez A. Characteristics of nonpulmonary organ dysfunction at onset of ARDS based on the berlin definition. Respiratory Care. 2019; 64: 493–501.

[163] Trefts E, Gannon M, Wasserman DH. The liver. Current Biology. 2017; 27: R1147–R1151.

[164] Yang P, Formanek P, Scaglione S, Afshar M. Risk factors and outcomes of acute respiratory distress syndrome in critically ill patients with cirrhosis. Hepatology Research. 2019; 49: 335–343.

[165] Dong V, Sun K, Gottfried M, Cardoso FS, McPhail MJ, Stravitz RT, *et al*. Significant lung injury and its prognostic significance in acute liver failure: a cohort analysis. Liver International. 2020; 40: 654–663.

[166] Dizier S, Forel JM, Ayzac L, Richard JC, Hraiech S, Lehingue S, *et al*. Early hepatic dysfunction is associated with a worse outcome in patients presenting with acute respiratory distress syndrome: a post-hoc analysis of the ACURASYS and PROSEVA studies. PLoS ONE. 2015; 10: e0144278.

[167] Herrero R, Sánchez G, Asensio I, López E, Ferruelo A, Vaquero J, *et al*. Liver–lung interactions in acute respiratory distress syndrome. Intensive Care Medicine Experimental. 2020; 8: 48.

[168] Bilzer M, Roggel F, Gerbes AL. Role of Kupffer cells in host defense and liver disease. Liver International. 2006; 26: 1175–1186.

[169] Triantafyllou E, Woollard KJ, McPhail MJW, Antoniades CG, Possamai LA. The role of monocytes and macrophages in acute and acute-on-chronic liver failure. Frontiers in Immunology. 2018; 9: 2948.

[170] Wiedermann FJ, Mayr AJ, Kaneider NC, Fuchs D, Mutz NJ, Schobersberger W. Alveolar granulocyte colony-stimulating factor and alpha-chemokines in relation to serum levels, pulmonary neutrophilia, and severity of lung injury in ARDS. Chest. 2004; 125: 212–219.

[171] Kobayashi K, Horikami D, Omori K, Nakamura T, Yamazaki A, Maeda S, *et al*. Thromboxane a2 exacerbates acute lung injury via promoting edema formation. Scientific Reports. 2016; 6: 32109.

[172] Panos RJ, Baker SK. Mediators, cytokines, and growth factors in liver-lung interactions. Clinics in Chest Medicine. 1996; 17: 151–169.

[173] Callery MP. Organ Interactions in Sepsis. Archives of Surgery. 1991; 126: 28.

[174] Matuschak GM. Lung-liver interactions in sepsis and multiple organ failure syndrome. Clinics in Chest Medicine. 1996; 17: 83–98.

[175] Salatti Ferrari R, da Rosa DP, Forgiarini LF, Bona S, Simões Dias A, Marroni NP. Oxidative stress and pulmonary changes in experimental liver cirrhosis. Oxidative Medicine and Cellular Longevity. 2012; 2012: 1–8.

[176] Ferrari RS, Tieppo M, Rosa DPD, Forgiarini JR LA, Dias AS, Marroni NP. Lung and liver changes due to the induction of cirrhosis in two experimental models. Archives of Gastroenterology. 2013; 50: 208–213.

[177] Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. New England Journal of Medicine. 1999; 340: 448–454.

[178] Quinton LJ, Jones MR, Robson BE, Mizgerd JP. Mechanisms of the hepatic acute-phase response during bacterial pneumonia. Infection and Immunity. 2009; 77: 2417–2426.

[179] Quinton LJ, Blahna MT, Jones MR, Allen E, Ferrari JD, Hilliard KL, *et al*. Hepatocyte-specific mutation of both NF-κB RelA and STAT3 abrogates the acute phase response in mice. Journal of Clinical Investigation. 2012; 122: 1758–1763.

[180] Hilliard KL, Allen E, Traber KE, Yamamoto K, Stauffer NM, Wasserman GA, *et al*. The lung-liver axis: a requirement for maximal innate immunity and hepatoprotection during pneumonia. American Journal of Respiratory Cell and Molecular Biology. 2015; 53: 378–390.

[181] Frank JA, Wray CM, McAuley DF, Schwendener R, Matthay MA. Alveolar macrophages contribute to alveolar barrier dysfunction in ventilator-induced lung injury. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2006; 291: L1191–L1198.

[182] Dani C, Martelli E, Tronchin M, Buonocore G, Longini M, Di Filippo A, *et al*. Bilirubin influence on oxidative lung damage and surfactant surface tension properties. Pediatric Pulmonology. 2004; 38: 179–185.

[183] Rodrigues CMP, Solá S, Brito MA, Brites D, Moura JJG. Bilirubin directly disrupts membrane lipid polarity and fluidity, protein order, and redox status in rat mitochondria. Journal of Hepatology. 2002; 36: 335–341.

[184] Fernandes A, Falcão AS, Silva RFM, Gordo AC, Gama MJ, Brito MA, *et al*. Inflammatory signalling pathways involved in astroglial activation by unconjugated bilirubin. Journal of Neurochemistry. 2006; 96: 1667–1679.

[185] Mohan A, Agarwal S, Clauss M, Britt NS, Dhillon NK. Extracellular vesicles: novel communicators in lung diseases. Respiratory Research. 2020; 21: 175.

[186] Chacón-Aponte AA, Durán-Vargas ÉA, Arévalo-Carrillo JA, Lozada-Martínez ID, Bolaño-Romero MP, Moscote-Salazar LR, *et al*. Brain-lung interaction: a vicious cycle in traumatic brain injury. Acute and Critical Care. 2022; 37: 35–44.

[187] Mikkelsen ME, Christie JD, Lanken PN, Biester RC, Thompson BT,

Bellamy SL, *et al*. The adult respiratory distress syndrome cognitive outcomes study. American Journal of Respiratory and Critical Care Medicine. 2012; 185: 1307–1315.

[188] Bassi TG, Rohrs EC, Reynolds SC. Systematic review of cognitive impairment and brain insult after mechanical ventilation. Critical Care. 2021; 25: 99.

[189] Fries M, Bickenbach J, Henzler D, Beckers S, Dembinski R, Sellhaus B, *et al*. S-100 protein and neurohistopathologic changes in a porcine model of acute lung injury. Anesthesiology. 2005; 102: 761–767.

[190] Bernardo-Castro S, Sousa JA, Brás A, Cecília C, Rodrigues B, Almendra L, *et al*. Pathophysiology of blood-brain barrier permeability throughout the different stages of ischemic stroke and its implication on hemorrhagic transformation and recovery. Frontiers in Neurology. 2020; 11: 594672.

[191] Galea I. The blood–brain barrier in systemic infection and inflammation. Cellular & Molecular Immunology. 2021; 18: 2489–2501.

[192] Santos CCD, Shan Y, Akram A, Slutsky AS, Haitsma JJ. Neuroimmune regulation of ventilator-induced lung injury. American Journal of Respiratory and Critical Care Medicine. 2011; 183: 471–482.

[193] Ko GJ, Rabb H, Hassoun HT. Kidney-lung crosstalk in the critically ill patient. Blood Purification. 2009; 28: 75–83.

[194] Koyner JL, Murray PT. Mechanical ventilation and the kidney. Blood Purification. 2010; 29: 52–68.

[195] Murdaugh HV, Sieker HO, Manfredi F. Effect of altered intrathoracic pressure on renal hemodynamics, electrolyte excretion and water clearance. Journal of Clinical Investigation. 1959; 38: 834–842.

[196] Kuiper JW, Groeneveld ABJ, Slutsky AS, Plötz FB. Mechanical ventilation and acute renal failure. Critical Care Medicine. 2005; 33: 1408–1415.

[197] Moore ES, Galvez MB, Paton JB, Fisher DE, Behrman RE. Effects of positive pressure ventilation on intrarenal blood flow in infant primates. Pediatric Research. 1974; 8: 792–796.

[198] Priebe HJ, Heimann JC, Hedley-Whyte J. Mechanisms of renal dysfunction during positive end-expiratory pressure ventilation. Journal of Applied Physiology. 1981; 50: 643–649.

[199] Choi WI, Quinn DA, Park KM, Moufarrej RK, Jafari B, Syrkina O, *et al*. Systemic microvascular leak in an in vivo rat model of ventilator-induced lung injury. American Journal of Respiratory and Critical Care Medicine. 2003; 167: 1627–1632.

[200] Breslin JW, Pappas PJ, Cerveira JJ, Hobson RW, Durán WN. VEGF increases endothelial permeability by separate signaling pathways involving ERK-1/2 and nitric oxide. American Journal of Physiology-Heart and Circulatory Physiology. 2003; 284: H92–H100.

[201] Gurkan OU, O'Donnell C, Brower R, Ruckdeschel E, Becker PM. Differential effects of mechanical ventilatory strategy on lung injury and systemic organ inflammation in mice. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2003; 285: L710–L718.

[202] Imai Y. Injurious mechanical ventilation and end-organ epithelial cell apoptosis and organ dysfunction in an experimental model of acute respiratory distress syndrome. JAMA. 2003; 289: 2104.

[203] Semama DS, Thonney M, Guignard JP. Does endothelin-1 mediate the hypoxemia-induced renal dysfunction in newborn rabbits? Biology of the Neonate. 1995; 67: 216–222.

[204] Ballèvre L, Thonney M, Guignard J-. Role of nitric oxide in the hypoxemia-induced renal dysfunction of the newborn rabbit. Pediatric Research. 1996; 39: 725–730.

[205] Hotter G, Palacios L, Sola A. Low O_2 and high CO_2 in LLC-PK1 cells culture mimics renal ischemia-induced apoptosis. Laboratory Investigation. 2004; 84: 213–220.

[206] Rabb H, Wang Z, Nemoto T, Hotchkiss J, Yokota N, Soleimani M. Acute renal failure leads to dysregulation of lung salt and water channels. Kidney International. 2003; 63: 600–606.

[207] Hoke TS, Douglas IS, Klein CL, He Z, Fang W, Thurman JM, *et al*. Acute renal failure after bilateral nephrectomy is associated with cytokine-mediated pulmonary injury. Journal of the American Society of Nephrology. 2007; 18: 155–164.

[208] Klein CL, Hoke TS, Fang WF, Altmann CJ, Douglas IS, Faubel S. Interleukin-6 mediates lung injury following ischemic acute kidney injury or bilateral nephrectomy. Kidney International. 2008; 74: 901–909.

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REVIEW

Cellular therapies in ARDS

Aina Areny-Balagueró[1,2,*], Antonio Artigas[1,2,3,4], John G Laffey[5,6], Daniel Closa[7]

[1]Department of Respiratory Physiopathology, Parc Taulí Research and Innovation Institution Foundation (I3PT), 08208 Sabadell, Spain
[2]Autonomous University of Barcelona, 08193 Bellaterra, Spain
[3]Biomedical Research Network Centre of Respiratory Diseases (CIBERES), Institute of Health Carlos III, 28029 Madrid, Spain
[4]Department of Intensive Care Medicine, Parc Taulí Health Corporation, 08208 Sabadell, Spain
[5]Anaesthesia and Intensive Care Medicine, School of Medicine, Clinical Sciences Institute, National University of Ireland, H91 TK33 Galway, Ireland
[6]Department of Anaesthesia, SAOLTA University Health Group, Galway University Hospitals, H91 TK33Galway, Ireland
[7]Institute of Biomedical Research of Barcelona, Spanish National Research Council (IIBB-CSIC), 08036 Barcelona, Spain

***Correspondence**
aareny@tauli.cat
(Aina Areny-Balagueró)

Abstract

Acute respiratory distress syndrome (ARDS) is a critical illness characterized by a severe hypoxemic respiratory failure, caused by an inflammatory response which results in diffuse lung damage. Despite decades of research, the treatment of ARDS remains supportive. However, in recent years, cell-based therapies have been the subject of intensive ongoing research efforts, showing relevant therapeutic potential in preclinical ARDS models. Among all the different cells that have been identified as suitable candidates for use, mesenchymal stromal cells (MSCs) have been the most attractive candidates and have generated significant interest. MSCs are multipotent adult stem/stromal cells that can modulate the immune response and enhance repair of damaged tissue in multiple in vivo models. Their promising effect seems to be not primarily mediated by MSCs differentiation and engraftment but more by the paracrine release of different soluble mediators and cellular components such as extracellular vesicles (EVs). Preclinical experiments have provided encouraging evidence for the therapeutic potential of MSCs, leading to the launch of several phase I and II clinical trials that have shown safety of MSCs in ARDS, which became very common nowadays due to the Coronavirus disease (COVID-19) pandemic. However, some translational challenges have yet to be solved, such as the reproducibility of cell harvest, storage, reconstitution, and administration of cells/cell-products, before the therapeutic potential of stem cells therapies can be realized.

Keywords

ARDS; Cell therapy; MSCs; EVs

1. Introduction

Acute respiratory distress syndrome (ARDS) constitutes a condition of progressive acute hypoxic respiratory failure characterized by the dysfunction of alveolar-capillary barrier and by rapid onset of inflammation in the lungs, leading to diffuse alveolar damage [1]. In 2012, a panel of experts developed the Berlin definition for ARDS that comprised three severity levels (mild, moderate and severe) based on degree of hypoxemia that are associated with progressively increased mortality [2]. ARDS can be caused by a number of clinical disorders, predominantly bacterial and viral infection and/or sepsis, with other common causes including aspiration of gastric contents and major trauma, but it can be also triggered by less common events as severe acute pancreatitis, shock, drug overdose or devastating neurologic injury [3]. Recently, the Covid-19 pandemic added a new viral cause of ARDS with a huge impact on Intensive Care Units (ICUs) around the world [4, 5].

It is clear that ARDS is a complex clinical syndrome with distinct clinicopathological characteristics [6]. The reported incidence appears to vary widely, although this is likely due to differences in clinical recognition of the syndrome, and variable ICU bed availability [7]. Despite this, there is no doubt that ARDS is common in critically ill patients and represents one of the leading causes of death in intensive care units. It is important to note that, despite decades of study on the pathogenesis of ARDS, the transfer of this knowledge to discovering new therapies for ARDS has been disappointing. Currently treatment is still limited to assisted ventilation and other life support techniques such as fluid management, antimicrobial therapies and nutritional supplementation. Increases in survival rates in recent years are mainly related to improvements in these life support techniques [8–11]. Unfortunately, at present no effective pharmacological treatment is available for the treatment of ARDS. The consequence is that mortality remains unacceptably high, ranging from 35% in patients with mild ARDS to 46% in cases of severe ARDS [7].

This situation highlights the need to explore new therapeutic strategies for ARDS. In this regard, cell therapies have exhibited promising therapeutic potential in preclinical and clinical studies [12], but also they have a number of challenges to solve. The advantage of cell therapies is that their effects are exerted at different levels, from the regulation at molecular level to the structural regeneration of tissue. This offers remarkable therapeutic potential in conditions such as ARDS with a complex pathogenesis in which acting on individual pathways is often ineffective. Different cells [13] and cell products

have been used as potential therapeutic agents, including embryonic stem cells, induced pluripotent stem cells (iPSC), Endothelial progenitor cells (EnPC) or epithelial Progenitor cells (EpPC) stromal or mesenchymal stromal cells (MSC), and also products released by the cells [14], as conditioned media or extracellular vesicles [15], in particular exosomes. The ethical issues associated with embryonic stem cells as well as difficulties in obtaining and standardizing progenitor cells led most researchers to focus on adult stem cells, especially mesenchymal stem cells, which also have low immunogenicity and high capacity for expansion.

2. Mesenchymal Stromal Cells

Of all the options, the cells that have probably generated the most interest and in which there are the most studies underway are the MSCs [16, 17]. These multipotent adult stem cells can be obtained from the bone marrow, umbilical cord, or peripheral blood and can be maintained without losing their ability to differentiate into mesodermal lineages. In addition, they have low immunogenicity and possess anti-inflammatory, angiogenic, antifibrotic and immunomodulatory activities [18]. All these properties have potential to attenuate ARDS severity and/or promote recovery and tissue repair. Ideally, MSC administration may reprogram the immune response, decrease inflammation, and promote regeneration of damaged lung areas (Fig. 1). In addition, its antifibrotic potential could also prevent the appearance of foci of fibrosis that would compromise the proper exchange of gases [19]. Initially it was also considered that MSC grafting, differentiation and multiplication potential could facilitate the reconstruction of overly damaged tissue areas, but later it has been seen that this effect is very limited [20]. Finally, it has been observed that the therapeutic potential of MSC could be enhanced by stimulating them prior to administration. Exposure to hypoxia, lipopolysaccharide (LPS), different cytokine combinations and other stressful stimuli trigger survival genetic programs that strengthen the regenerative activity of MSCs [21].

2.1 Epithelial repair

Alveolar epithelial cell damage is one of the typical features of ARDS. In cases of severe ARDS, the damage can affect both type II and I alveolar cells, generating focal areas of destruction and exposing the basement membrane. All this increases lung permeability, triggers processes of fibrosis and coagulation and, obviously, dramatically affects lung function [22, 23]. Consequently, for the treatment of ARDS, it is essential to improve and accelerate the processes of epithelial regeneration to restore the functionality of the alveolar wall. Without this fundamental step, the effectiveness of supportive care, such as assisted ventilation, is relatively limited.

MSC administration had been demonstrated to enhance the regeneration of the pulmonary epithelium [24], via multiple mechanisms, including Keratinocyte Growth Factor (KGF) secretion [25], Matrix Metalloproteinase-8 (MMP-8) expression [26], β-catenin activation [27], NF-κB inhibition [28] and the induction of a reparative M2 phenotype in macrophages [29]. These effects are potentiated when MSCs are pre-treated with stimulating inflammatory agents as LPS or cytokines [21].

2.2 Alveolar fluid clearance

Fluid accumulation inside the alveoli is a consequence of the loss of endothelial integrity during ARDS and strongly contributes to lung edema and hypoxemia [30]. Several studies demonstrate that MSC treatments can enhance clearance of alveolar fluid reducing the amount of lung water contents in both *in vivo* and *ex vivo* models of lung injury [31, 32]. The mechanisms proposed includes the restoration of sodium equilibrium by acting on the sodium channels in a mechanism mediated by KGF [33] or by miRNA-34c [34]. Angiopoietin-1 appears to be also involved in the protective mechanism of MSC via stabilization of endothelial permeability [24].

2.3 Immune response modulation

MSCs have been reported to exert a number of effects in both adaptive and innate immune system [35]. The release of paracrine factors and extracellular vesicles modulate the phenotype and/or function of macrophages, neutrophils, T cells and B cells [29, 36–39]. Changes in the phenotype of these cells results in additional release of anti-inflammatory and immunosuppressive mediators, as Interleukin 10 (IL-10) or prostaglandin-E2, that reduces lung damage associated with the inflammatory response [40]. In particular, exposure of MSCs to an inflammatory microenvironment causes changes in the expression of genes that modulate the inflammatory response and the activation of different lymphocyte populations [41–43]. These effects of MSCs are of particular relevance given the role of the immune response in the pathogenesis of ARDS.

3. MSCs Engraftment

While engraftment and trans-differentiation of MSCs to replace damaged host tissue was initially considered an important potential mechanisms of action, it is now known that this is not the case. In fact, experimental data indicate that less than 1% of the administered cells will end up grafting into the damaged tissue [44, 45]. This amount is too small to justify the observed protective effects. This fact does not change the potential of MSC based therapies for controlling the progression of ARDS but open the door to additional treatments based on paracrine factors released by the MSCs. This is why, in addition to the administration of MSCs, studies have also been carried out to investigate the effect of treatment with conditioned medium, secretome and, in particular, extracellular vesicles (EVs) [33, 46–49].

4. MSC Secretome and EVs

The advantage of using elements of MSC secretome is that they avoid some of the potential problems associated with the use of whole MSCs as a therapy. This includes the difference in therapeutic efficacy between different batches of cells, the control of apoptosis and other ways of cell clearance including phagocytosis by macrophages, the potential toxicity of different agents required in the process of MSCs culture

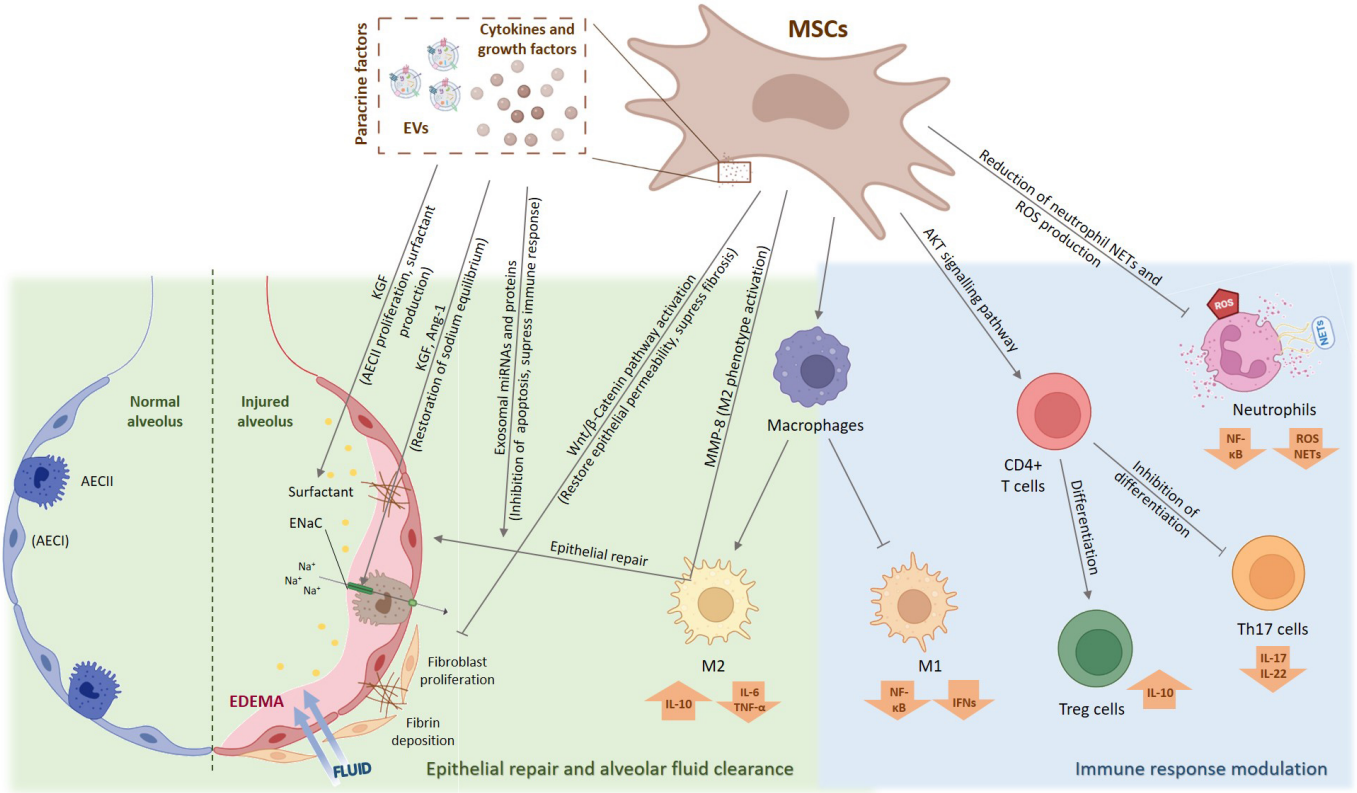

FIGURE 1. MSCs mechanisms for ARDS treatment. ROS: reactive oxygen species; NETs: neutrophil extracellular traps; Th17: T helper 17; Treg cells: regulatory T cells; M1: M1 macrophage phenotype; M2: M2 macrophage phenotype; KGF: keratinocyte growth factor; Ang-1: angiopoietin-1; AEC I: type I alveolar epithelial cells; AEC II: type II alveolar epithelial cells; ENaC: epithelial sodium channel; MMP-8: matrix metalloproteinase-8; EVs: extracellular vesicles; MSCs: mesenchymal stromal cells.

and preservation, the potential risk associated to the use of heterologous cells and the logistical problems linked to the use of cells in the clinical practice [50]. Although most of these drawbacks can be controlled or have not been found to be as significant as expected [51], the use of exosomes allows them to be avoided while maintaining much of the therapeutic potential of the cells themselves. In some ways exosomes can be seen as a delivery system for regenerative and anti-inflammatory proteins and microRNAs to damaged epithelial cells or, alternatively, activated inflammatory cells in lung.

However, some challenges have yet to be solved. For example, as with MSCs, there are also differences in the content, and therefore in the therapeutic activity, between the different batches of exosomes. Storage and reconstitution have been also challenging since exosomes could form aggregates during the process of freezing and thawing [52]. The standardization of methods for determining the therapeutic potential of exosomes in a homogeneous manner is also proving difficult to establish [53].

5. Route of Administration

5.1 MSC routes of Delivery

The optimal route of administration for MSCs remains under debate. It can be delivered either by intravenous or intratracheal routes, and for exosomes or paracrine mediators, delivered as an aerosol using a nebulizer. Intravenous use currently remains the preferred route due to its greater feasibility in clinical practice. However, this way makes it difficult to control the amount of MSCs that effectively reach the lung and are retained there [54]. Depending on the patient's condition, the administered cells may be retained in different organs. In experimental studies it has been suggested that in non-injured animals, large amounts of administered cells are trapped in the liver, spleen or kidney while in injured animals, cells accumulate in the lungs [55, 56]. This adds a degree of uncertainty to the dose of cells that will actually reach the lung, particularly where there are multiple sites of injury, *e.g.*, multiple organ injury. The effects that cells retained in other tissues and organs have on these tissues is also uncertain, which adds to the complexity of using this therapy.

The alternative is direct administration into the lung. The intratracheal route, based on the administration of fluid-suspended cells using an intratracheal tube, has been extensively used in experimental models [57], but has many disadvantages in clinical application. It is an invasive delivery approach, associated with an irregular distribution of cells and, above all, adding fluid to lungs which, given their already increased water content, might worsen the pre-existing pathology. The alternative is the use of aerosols or nebulizers, that convert the liquid into aerosols that can be easily inhaled. This approach offers higher efficiency than the alternative ways but there are differences depending on the type of

TABLE 1. Clinical trials: registered MSC-based treatment in Covid-19-associated ARDS.

Identifier (status)	Clinical trial phase	Cell source	Dosage	Route	Enrolled number	Primary outcomes
NCT04525378 (Recruiting)	1	BM-MSCs	2.5, 5, 10 $\times$ 10^7 cells/kg	I.V	20	Intrahospital mortality at day 28
NCT04456361(Active)	1	WJ-MSCs	1 $\times$ 10^8 cells/kg	I.V	9	Oxygen saturation
CHICTR2000029990 (Recruiting)	1–2	BM-MSCs	1 $\times$ 10^6 cells/kg	I.V	60	Oxygen saturation
NCT04355728 (Recruiting)	1–2	UC-MSCs	1 $\times$ 10^8 cells/kg (2 times)	I.V	24	Adverse events
NCT03042143 (Active)	1–2	UC-MSCs	1, 2, 4 $\times$ 10^8 cells/kg	I.V	75	Oxygenation index, adverse events
NCT04390139 (Recruiting)	1–2	WJ-MSCs	1 $\times$ 10^6 cells/kg	I.V	30	All-cause mortality at day 28
NCT04416139 (Recruiting)	2	UC-MSCs	1 $\times$ 10^6 cells/kg	I.V	10	PaO_2/FiO_2 ratio, heart and respiratory rate, changes in body temperature
NCT04865107 (Recruiting)	2	UC-MSCs	2, 7 $\times$ 10^8 cells/kg	I.V	54	Number of days free of oxygen mechanical ventilation at Day 28
NCT04366063 (Recruiting)	2–3	BM-MSCs	1 $\times$ 10^8 cells/kg (2 times)	I.V	80	Adverse events, blood oxygen saturation
NCT04371393 (Recruiting)	3	BM-MSCs	2 $\times$ 10^6 cells/kg (2 times)	I.V	300	All-cause mortality at day 30

BM-MSCs: bone marrow-derived mesenchymal stem cells; I.V.: intravenous; WJ-MSCs: Wharton-Jelly mesenchymal stromal cells; UC-MSCs: umbilical cord-derived mesenchymal stem cells.

nebulizer used and there is still much research that need to be done before cell product nebulization become routine in clinical practice. Specifically, the administration of intact cells by nebulizers needs to be optimized, although there is great potential for administering MSCs-derived EVs or the whole secretome this way [12].

5.2 Clinical Trials

Preclinical experiments have provided encouraging evidence for the therapeutic potential of MSCs in a variety of diseases, including ARDS [18]. This led to the launch of several phase I and II clinical trials which have demonstrated the safety and feasibility of these treatments [58–60]. Relevant issues that remain to be determined include the need to establish the appropriate dose of cells administered, and the most effective dose regimen. Lower doses could be ineffective while the administration of an excessive number of cells could result in complications associated to thromboembolic risk. It should be noted that the selected route of administration is in almost all cases intravenous. Only in a few Covid-19 trials the inhaled route of administration has been selected [61], showing that, despite its advantages, the aerosolized and nebulized routes require additional improvements before moving on to a clinical application.

5.3 COVID-19-related ARDS

The number of studies increased dramatically during 2020 due to the arrival of the Covid-19 pandemic. In just one year, a substantial number of phase I and II clinical trials focused on controlling Covid-19-associated ARDS were initiated, mostly in China [62] (Table 1). Predictably, there is a huge variation in the origin of MSCs, the number of patients recruited, or the administration protocols. There are also a number of studies administering MSCs-derived EVs [63]. Despite these differences, these studies consistently demonstrated that the administration of MSCs is, as expected, safe and. In some studies, patients have shown improvement in some clinical parameters. For instance, in a phase IIa clinical trial conducted in the USA, in which patients received a high dose level of allogeneic Bone Marrow-MSC (BM-MSC) (10 $\times$ 106 cells/kg), no predefined MSC-related haemodynamic or respiratory adverse effects were observed. Besides, infusioned patients showed an improvement in the oxygenation index and a reduced level of Angiopoietin 2 (ANG-2) in plasma, demonstrating that the MSC administration improved endothelial injury [64].

One of the factors that facilitated the application of MSCs in therapies for Covid-19-ARDS is the fact that these cells are highly resistant to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection, as they do not express either angiotensin-converting enzyme 2 (ACE2) or transmembrane protease serine subtype 2 (TMPRSS2) on its surface

[65]. Importantly, this low expression is observed also in inflammatory situations [66].

6. Conclusions

The development of new and effective therapies for ARDS is a key objective of biomedical research and the therapies based on MSCs are among the approaches with the greatest potential. The potential suggested by preclinical studies has been extended in clinical studies which have shown that, in the treatment of ARDS, MSCs were safe and well tolerated. This impression has been reinforced by the large number of studies initiated in response to the Covid-19 pandemic. However, mechanistic studies will still be needed to fully understand the mechanisms of action so that these therapies can be optimized.

AUTHOR CONTRIBUTIONS

AAB, DC contributed in the design of the review, searched the literature, designed and elaborated the figure and wrote the manuscript. JGL and AA critically revised the manuscript for important intellectual content and accurate English language. All authors approved the last version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. Antonio Artigas is serving as one of the Guest editors of this journal. We declare that Antonio Artigas had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to TCS.

REFERENCES

[1] Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. The Lancet. 1967; 2: 319–323.

[2] ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, *et al*. Acute respiratory distress syndrome: the Berlin definition. JAMA. 2012; 307: 2526–2533.

[3] Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, *et al*. Acute respiratory distress syndrome. Nature Reviews Disease Primers. 2019; 5: 18.

[4] COVID-ICU Group on behalf of the REVA Network and the COVID-ICU Investigators. Clinical characteristics and day-90 outcomes of 4244 critically ill adults with COVID-19: a prospective cohort study. Intensive Care Medicine. 2021; 47: 60–73.

[5] Daviet F, Guilloux P, Hraiech S, Tonon D, Velly L, Bourenne J, *et al*. Impact of obesity on survival in COVID-19 ARDS patients receiving ECMO: results from an ambispective observational cohort. Annals of Intensive Care. 2021; 11: 157.

[6] Cortegiani A, Madotto F, Gregoretti C, Bellani G, Laffey JG, Pham T, *et al*. Immunocompromised patients with acute respiratory distress syndrome: secondary analysis of the LUNG SAFE database. Critical Care. 2018; 22: 157.

[7] Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, *et al*. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in Intensive Care units in 50 countries. JAMA. 2016; 315: 788–800.

[8] Frat JP, Thille AW, Mercat A, Girault C, Ragot S, Perbet S, *et al*. High-flow oxygen through nasal cannula in acute hypoxemic respiratory failure. The New England Journal of Medicine. 2015; 372: 2185–2196.

[9] Lee J, Corl K, Levy MM. Fluid therapy and acute respiratory distress syndrome. Critical Care Clinics. 2021; 37: 867–875.

[10] Dulhunty JM, Roberts JA, Davis JS, Webb SAR, Bellomo R, Gomersall C, *et al*. A multicenter randomized trial of continuous versus intermittent β-lactam infusion in severe sepsis. American Journal of Respiratory and Critical Care Medicine. 2015; 192: 1298–1305.

[11] Kumar N, Kumar A, Kumar A, Pattanayak A, Singh K, Singh P. NUTRIC score as a predictor of outcome in COVID-19 ARDS patients: a retrospective observational study. Indian Journal of Anaesthesia. 2021; 65: 669.

[12] Brave H, MacLoughlin R. State of the art review of cell therapy in the treatment of lung disease, and the potential for aerosol delivery. International Journal of Molecular Sciences. 2020; 21: 6435.

[13] Guillamat-Prats R, Camprubí-Rimblas M, Bringué J, Tantinyà N, Artigas A. Cell therapy for the treatment of sepsis and acute respiratory distress syndrome. Annals of Translational Medicine. 2017; 5: 446.

[14] Silva JD, Krasnodembskaya AD. Investigation of the MSC paracrine effects on alveolar—capillary barrier integrity in the in vitro models of ARDS. Methods in Molecular Biology. 2021; 97: 63–81.

[15] Abraham A, Krasnodembskaya A. Mesenchymal stem cell-derived extracellular vesicles for the treatment of acute respiratory distress syndrome. Stem Cells Translational Medicine. 2020; 9: 28–38.

[16] Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Experimental Hematology. 1976; 4: 267–274

[17] Wilson JG, Liu KD, Zhuo H, Caballero L, McMillan M, Fang X, *et al*. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. The Lancet Respiratory Medicine. 2015; 3: 24–32.

[18] Byrnes D, Masterson CH, Artigas A, Laffey JG. Mesenchymal stem/stromal cells therapy for sepsis and acute respiratory distress syndrome. Seminars in Respiratory and Critical Care Medicine. 2021; 42: 020–039.

[19] Wu J, Song D, Li Z, Guo B, Xiao Y, Liu W, *et al*. Immunity-and-matrix-regulatory cells derived from human embryonic stem cells safely and effectively treat mouse lung injury and fibrosis. Cell Research. 2020; 30: 794–809.

[20] Kotton DN, Fabian AJ, Mulligan RC. Failure of bone marrow to reconstitute lung epithelium. American Journal of Respiratory Cell and Molecular Biology. 2005; 33: 328–334.

[21] Hu C, Li L. Preconditioning influences mesenchymal stem cell properties in vitro and in vivo. Journal of Cellular and Molecular Medicine. 2018; 22: 1428–1442.

[22] Budinger GRS, Mutlu GM, Urich D, Soberanes S, Buccellato LJ, Hawkins K, *et al*. Epithelial cell death is an important contributor to oxidant-mediated acute lung injury. American Journal of Respiratory and Critical Care Medicine. 2011; 183: 1043–1054.

[23] Short KR, Kasper J, van der Aa S, Andeweg AC, Zaaraoui-Boutahar F, Goeijenbier M, *et al*. Influenza virus damages the alveolar barrier by disrupting epithelial cell tight junctions. European Respiratory Journal. 2016; 47: 954–966.

[24] Fang X, Neyrinck AP, Matthay MA, Lee JW. Allogeneic human mesenchymal stem cells restore epithelial protein permeability in cultured

human alveolar type II cells by secretion of angiopoietin-1. Journal of Biological Chemistry. 2010; 285: 26211–26222.

[25] Curley GF, Hayes M, Ansari B, Shaw G, Ryan A, Barry F, *et al*. Mesenchymal stem cells enhance recovery and repair following ventilator-induced lung injury in the rat. Thorax. 2012; 67: 496–501.

[26] Maron-Gutierrez T, Silva JD, Asensi KD, Bakker-Abreu I, Shan Y, Diaz BL, *et al*. Effects of mesenchymal stem cell therapy on the time course of pulmonary remodeling depend on the etiology of lung injury in mice. Critical Care Medicine. 2013; 41: e319–e333.

[27] Cai SX, Liu AR, Chen S, He HL, Chen QH, Xu JY, *et al*. Activation of Wnt/β-catenin signalling promotes mesenchymal stem cells to repair injured alveolar epithelium induced by lipopolysaccharide in mice. Stem Cell Research & Therapy. 2015; 6: 65.

[28] Xiao K, He W, Guan W, Hou F, Yan P, Xu J, *et al*. Mesenchymal stem cells reverse EMT process through blocking the activation of NF-κB and Hedgehog pathways in LPS-induced acute lung injury. Cell Death & Disease. 2020; 11: 863.

[29] Tian J, Cui X, Sun J, Zhang J. Exosomal microRNA-16-5p from adipose mesenchymal stem cells promotes TLR4-mediated M2 macrophage polarization in septic lung injury. International Immunopharmacology. 2021; 98: 107835.

[30] Ware LB, Matthay MA. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. 2001; 163: 1376–1383.

[31] Hao Q, Zhu YG, Monsel A, Gennai S, Lee T, Xu F, *et al*. Study of bone marrow and embryonic stem cell-derived human mesenchymal stem cells for treatment of escherichia coli endotoxin-induced acute lung injury in mice. Stem Cells Translational Medicine. 2015; 4: 832–840.

[32] Ihara K, Fukuda S, Enkhtaivan B, Trujillo R, Perez-Bello D, Nelson C, *et al*. Adipose-derived stem cells attenuate pulmonary microvascular hyperpermeability after smoke inhalation. PLoS One. 2017; 12: e0185937.

[33] Goolaerts A, Pellan-Randrianarison N, Larghero J, Vanneaux V, Uzunhan Y, Gille T, *et al*. Conditioned media from mesenchymal stromal cells restore sodium transport and preserve epithelial permeability in an in vitro model of acute alveolar injury. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2014; 306: L975–L985.

[34] Zhou Z, Hua Y, Ding Y, Hou Y, Yu T, Cui Y, *et al*. Conditioned medium of bone marrow mesenchymal stem cells involved in acute lung injury by regulating epithelial sodium channels via miR-34c. Frontiers in Bioengineering and Biotechnology. 2021; 9: 640116.

[35] Ho MSH, Mei SHJ, Stewart DJ. The Immunomodulatory and therapeutic effects of mesenchymal stromal cells for acute lung injury and sepsis. Journal of Cellular Physiology. 2015; 230: 2606–2617.

[36] Yao M, Cui B, Zhang W, Ma W, Zhao G, Xing L. Exosomal miR-21 secreted by IL-1β-primed-mesenchymal stem cells induces macrophage M2 polarization and ameliorates sepsis. Life Sciences. 2021; 264: 118658.

[37] Kwon M, Ghanta S, Ng J, Tsoyi K, Lederer JA, Bronson RT, *et al*. Expression of stromal cell–derived factor-1 by mesenchymal stromal cells impacts neutrophil function during sepsis. Critical Care Medicine. 2020; 48: e409–e417.

[38] Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005; 105: 1815–1822.

[39] Ribeiro A, Laranjeira P, Mendes S, Velada I, Leite C, Andrade P, *et al*. Mesenchymal stem cells from umbilical cord matrix, adipose tissue and bone marrow exhibit different capability to suppress peripheral blood B, natural killer and T cells. Stem Cell Research & Therapy. 2013; 4: 125.

[40] Németh K, Leelahavanichkul A, Yuen PST, Mayer B, Parmelee A, Doi K, *et al*. Bone marrow stromal cells attenuate sepsis via prostaglandin E2—dependent reprogramming of host macrophages to increase their interleukin-10 production. Nature Medicine. 2009; 15: 42–49.

[41] dos Santos CC, Murthy S, Hu P, Shan Y, Haitsma JJ, Mei SHJ, *et al*. Network analysis of transcriptional responses induced by mesenchymal stem cell treatment of experimental sepsis. The American Journal of Pathology. 2012; 181: 1681–1692.

[42] Abreu SC, Rolandsson Enes S, Dearborn J, Goodwin M, Coffey A, Borg ZD, *et al*. Lung inflammatory environments differentially alter mesenchymal stromal cell behavior. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2019; 317: L823–L831.

[43] Rolandsson Enes S, Hampton TH, Barua J, McKenna DH, Dos Santos CC, Amiel E, *et al*. Healthy versus inflamed lung environments differentially affect mesenchymal stromal cells. The European Respiratory Journal. 2021; 58: 2004149.

[44] Bartlett RS, Guille JT, Chen X, Christensen MB, Wang SF, Thibeault SL. Mesenchymal stromal cell injection promotes vocal fold scar repair without long-term engraftment. Cytotherapy. 2016; 18: 1284–1296.

[45] Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, *et al*. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell. 2001; 105: 369–377.

[46] Hezam K, Mo R, Wang C, Liu Y, Li Z. Anti-inflammatory effects of mesenchymal stem cells and their secretomes in pneumonia. Current Pharmaceutical Biotechnology. 2022; 23: 1153–1167.

[47] Sholugu N, Scully M, Laffey JG, O'Toole D. Human mesenchymal stem cell secretome from bone marrow or adipose-derived tissue sources for treatment of hypoxia-induced pulmonary epithelial injury. International Journal of Molecular Sciences. 2018; 19: 2996.

[48] Porzionato A, Zaramella P, Dedja A, Guidolin D, Bonadies L, Macchi V, *et al*. Intratracheal administration of mesenchymal stem cell-derived extracellular vesicles reduces lung injuries in a chronic rat model of bronchopulmonary dysplasia. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2021; 320: L688–L704.

[49] Willis GR, Fernandez-Gonzalez A, Anastas J, Vitali SH, Liu X, Ericsson M, *et al*. Mesenchymal stromal cell exosomes ameliorate experimental bronchopulmonary dysplasia and restore lung function through macrophage immunomodulation. American Journal of Respiratory and Critical Care Medicine. 2018; 197: 104–116.

[50] Moll G, Alm JJ, Davies LC, von Bahr L, Heldring N, Stenbeck-Funke L, *et al*. Do cryopreserved mesenchymal stromal cells display impaired immunomodulatory and therapeutic properties? Stem Cells. 2014; 32: 2430–2442.

[51] Luetzkendorf J, Nerger K, Hering J, Moegel A, Hoffmann K, Hoefers C, *et al*. Cryopreservation does not alter main characteristics of good manufacturing process—grade human multipotent mesenchymal stromal cells including immunomodulating potential and lack of malignant transformation. Cytotherapy. 2015; 17: 186–198.

[52] Pinky, Gupta S, Krishnakumar V, Sharma Y, Dinda AK, Mohanty S. Mesenchymal stem cell derived exosomes: a nano platform for therapeutics and drug delivery in combating COVID-19. Stem Cell Reviews and Reports. 2021; 17: 33–43.

[53] Gimona M, Brizzi MF, Choo ABH, Dominici M, Davidson SM, Grillari J, *et al*. Critical considerations for the development of potency tests for therapeutic applications of mesenchymal stromal cell-derived small extracellular vesicles. Cytotherapy. 2021; 23: 373–380.

[54] Lu H, Cook T, Poirier C, Merfeld-Clauss S, Petrache I, March KL, *et al*. Pulmonary retention of adipose stromal cells following intravenous delivery is markedly altered in the presence of ARDS. Cell Transplantation. 2016; 25: 1635–1643.

[55] Kang SK, Shin IS, Ko MS, Jo JY, Ra JC. Journey of mesenchymal stem cells for homing: strategies to enhance efficacy and safety of stem cell therapy. Stem Cells International. 2012; 2012: 1–11.

[56] Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organs. 2001; 169: 12–20.

[57] Lian J, Lin J, Zakaria N, Yahaya BH. Acute lung injury: disease modelling and the therapeutic potential of stem cells. Cell Biology and Translational Medicine. 2020; 306: 149–166.

[58] Glassberg MK, Minkiewicz J, Toonkel RL, Simonet ES, Rubio GA, DiFede D, *et al*. Allogeneic human mesenchymal stem cells in patients with idiopathic pulmonary fibrosis via intravenous delivery (AETHER) chest. 2017; 151: 971–981.

[59] Wilson JG, Liu KD, Zhuo H, Caballero L, McMillan M, Fang X, *et al*. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. the Lancet Respiratory Medicine. 2015; 3: 24-32.

[60] Fernández-Francos S, Eiro N, González-Galiano N, Vizoso FJ. Mesenchymal stem cell-based therapy as an alternative to the treatment of acute respiratory distress syndrome: current evidence and future perspectives. International Journal of Molecular Sciences. 2021; 22: 7850.

[61] Abdelgawad M, Bakry NS, Farghali AA, Abdel-Latif A, Lotfy A.

Mesenchymal stem cell-based therapy and exosomes in COVID-19: current trends and prospects. Stem Cell Research & Therapy. 2021; 12: 469.

[62] Xu Z, Huang Y, Zhou J, Deng X, He W, Liu X, *et al*. Current status of cell-based therapies for COVID-19: evidence from mesenchymal stromal cells in sepsis and ARDS. Frontiers in Immunology. 2021; 12: 738697.

[63] Sengupta V, Sengupta S, Lazo A, Woods P, Nolan A, Bremer N. Exosomes derived from bone marrow mesenchymal stem cells as treatment for severe COVID-19. Stem Cells and Development. 2020; 29: 747–754.

[64] Matthay MA, Calfee CS, Zhuo H, Thompson BT, Wilson JG, Levitt JE, *et al*. Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial. The Lancet. Respiratory Medicine. 2019; 7: 154–162.

[65] Avanzini MA, Mura M, Percivalle E, Bastaroli F, Croce S, Valsecchi C, *et al*. Human mesenchymal stromal cells do not express ACE2 and TMPRSS2 and are not permissive to SARS-CoV-2 infection. Stem Cells Translational Medicine. 2021; 10: 636–642.

[66] Schäfer R, Spohn G, Bechtel M, Bojkova D, Baer PC, Kuçi S, *et al*. Human Mesenchymal Stromal Cells Are Resistant to SARS-CoV-2 Infection under Steady-State, Inflammatory Conditions and in the Presence of SARS-CoV-2-Infected Cells. Stem Cell Reports. 2021; 16: 419–427.

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