Personalised Treatment of Acute Respiratory Distress Syndrome

Philip Alexander van der Zee

ISBN 978-94-6361-886-1

Layout and printing by Optima Grafische Communicatie (www.ogc.nl)

Personalised Treatment of Acute Respiratory Distress Syndrome

Personalisering van de behandeling van acute respiratory distress syndrome

Thesis

to obtain the degree of Doctor from the
Erasmus University Rotterdam
by command of the
rector magnificus

Prof. dr. A.L. Bredenoord

and in accordance with the decision of the Doctorate Board

The public defence shall be held on

Thursday 12 October 2023
at 13:00 hrs

By

Philip Alexander van der Zee
Born in Rotterdam, The Netherlands

Erasmus University Rotterdam

Doctoral Committee:

Promotor:	Prof. dr. D.A.M.P.J. Gommers
Other members:	Prof. dr. J.G.J.V. Aerts
	Prof. dr. N.P. Juffermans
	Prof. dr. R.P. Pickkers
Copromotor:	Dr. H. Endeman

TABLE OF CONTENTS

1

General introduction and outline of this thesis

GENERAL INTRODUCTION

Acute Respiratory Distress Syndrome

The Acute Respiratory Distress Syndrome (ARDS) is a syndrome characterized by alveolar-capillary membrane injury following a clinical risk factor as sepsis or trauma.[1] Alveolar-capillary membrane injury results in increased pulmonary vascular permeability, pulmonary oedema, surfactant dysfunction, and decreased lung aeration. Alveolar-capillary membrane injury and subsequent pulmonary oedema are not routinely measured in clinical practice. Therefore, the Berlin definition of ARDS uses clinical surrogates for pulmonary oedema: bilateral opacities on chest x-ray and arterial hypoxemia (partial pressure of arterial oxygen/fraction of inspired oxygen, PaO_2/FiO_2 ratio <300mmHg).[2] In ARDS, cardiac failure or fluid overload should not be the sole reason for pulmonary oedema.

ARDS is a major problem in the intensive care unit (ICU). Approximately 10% of ICU patients fulfil the Berlin criteria of ARDS and an average in-hospital mortality rate of 40% has been reported.[3] Since the first description of ARDS in 1967, the cornerstones of ARDS treatment have remained unchanged: treatment of the underlying cause and supportive therapy consisting of invasive mechanical ventilation.[4] Especially the implementation of protective mechanical ventilation with low tidal volumes and a plateau airway pressure limited to 30 cmH_2O improved patient survival significantly, and is considered standard of care since 2000.[5]

Subsequently, multiple randomized controlled trials investigated the effectiveness of other supportive therapies in patients with ARDS. Only a minority of interventions further improved clinical outcomes, including prone positioning, conservative fluid management, and neuromuscular blockade.[6-8] In contrast, multiple randomized controlled trials on positive end-expiratory pressure (PEEP) titration or recruitment, and multiple clinical trials testing pharmacological compounds did not improve survival in patients with ARDS.[9-13]

Some treatments are actually ineffective in patients with ARDS, and other treatments – including protective mechanical ventilation – are effective in most patients with ARDS. However, some treatments might have been effective in a subgroup of patients with ARDS and harmful or ineffective in another subgroup, resulting in no significant treatment effect in the entire study population. This is known as the heterogeneity of treatment effect.[14] Heterogeneity is a major problem in studies that include patients with ARDS, as the ARDS definition itself contributes to significant heterogeneity.

Heterogeneity in Acute Respiratory Distress Syndrome

The Berlin definition and its predecessor (the American European Consensus Conference definition, AECC) identified ARDS in a broad range of patients with hypoxemia.[2, 15] These definitions allowed efficient patient recruitment in clinical trials. However, as the cause of ARDS is often not specified and patients with varying disease severity could all be included, there is significant heterogeneity among patients with ARDS. ARDS heterogeneity is evident upon histological examination, as only 43-69% of patients meeting the clinical criteria of ARDS have 'true ARDS': diffuse alveolar damage.[16-21] Vice versa, some patients with histological evidence of diffuse alveolar damage do not meet the clinical criteria of ARDS.[18] Thus, a substantial subgroup in patients with ARDS represent a different histopathological substrate. These patients with ARDS are likely to respond differently to a similar treatment, i.e. heterogeneity of treatment effect.

Researchers have explored strategies to reduce the amount of heterogeneity and turn the heterogeneous ARDS syndrome into more homogeneous subgroups. The identification of patients that are more likely to respond to a certain treatment is known as predictive enrichment.[22] Enrichment strategies have the potential to improve signal-to-noise ratio and reduce sample size, as benefit or harm of treatment is likely to be concentrated in a subgroup of patients.[23] Treatment of ARDS can be personalised based on clinically, biologically, and physiologically derived phenotypes.[24]

Clinically derived phenotypes in ARDS

Clinical phenotypes can be used to personalise treatment of patients with ARDS. Clinical phenotypes in ARDS can be based on similar risk factor for ARDS – including pulmonary or extra-pulmonary ARDS –, time course of ARDS, or radiological presentation (i.e. morphological ARDS).[25-27]

A large number of risk factors for ARDS have been described, including pneumonia, sepsis, aspiration pneumonia, trauma, or blood transfusion.[3] The underlying risk factor or trigger for ARDS influences disease severity, prognosis, and response to treatment.[26, 28] For example, aspiration pneumonia or trauma cause a short-termed trigger, whereas sepsis causes sustained exposure to the trigger for ARDS. Consequently, patients with trauma-related ARDS had less endothelial and epithelial injury as compared to patients with other risk factors for ARDS, and had a better prognosis.[29] The risk factor for ARDS also influences ARDS time course, as more than 10% of patients with ARDS at trial enrolment did no longer meet the criteria of ARDS within 24 hours.[30] Furthermore, patients that developed ARDS within 48 hours of hospital admission had a better prognosis as compared to patients that developed ARDS after 48 hours of hospital admission.[31, 32]

The increased mortality rate may be associated with the underlying risk factor for ARDS and with sustained exposure to a trigger resulting in late ARDS.[30]

Pulmonary ARDS and extra-pulmonary ARDS have different radiological presentation. In pulmonary ARDS (e.g. pneumonia, aspiration pneumonia, or inhalation injury), a pulmonary insult causes a direct disruption of the alveolar membrane and subsequently the alveolar-capillary membrane. In extra-pulmonary ARDS (e.g. sepsis, pancreatitis, or trauma), progressive systemic inflammation damages the endothelium and progresses to diffuse injury of the alveolar-capillary membrane.[33] On a chest CT scan, pulmonary ARDS is more focal and consolidative, whereas extra-pulmonary ARDS is diffuse and consists of more ground glass opacities (i.e. capillary leakage).[34, 35] Higher PEEP recruited more lung volume in patients with extra-pulmonary ARDS as compared to pulmonary ARDS.[34] However, it can be difficult to correctly identify the risk factor or lung morphology of ARDS, as in more than a third of patients with ARDS clinicians were uncertain about the cause of ARDS and lung morphology was misclassified in 21% of patients.[36, 37]

The value of personalised treatment of ARDS based on a similar risk factor was underscored by the COVID-19 pandemic. Randomized controlled trials performed in the COVID-19 related ARDS subgroup had more success in two years of research than two decades of research in ARDS, as corticosteroids improved patient survival in COVID-19 related ARDS.[38-40] However, heterogeneity of treatment effect was observed even in this relatively homogeneous subgroup of ARDS, as patients that received no respiratory support did not benefit from treatment with corticosteroids.

Biologically derived phenotypes in ARDS

Treatment of ARDS can be personalised based on the identification of a biologically derived phenotype. It has been hypothesized that the addition of a biomarker to the clinical definition of ARDS could identify biological subgroups and improve homogeneity in patients with ARDS.[41-44] A biomarker derived phenotype could be especially useful in clinical trials assessing the efficacy of a pharmacological compound.[12]

Biomarkers for ARDS have multiple purposes: to predict the development of ARDS, to stratify disease severity, to provide insights into ARDS pathogenesis, to predict outcome, or to monitor treatment effect or disease progression.[41] The majority of ARDS biomarkers are a surrogate for systemic inflammation, alveolar epithelial injury, endothelial injury, or coagulation.[45] However, most biomarkers are not specific for ARDS, and rather reflect systemic inflammation or disease severity.[46]

Measurement of biomarkers in the pulmonary compartment could be more specific for ARDS and better reflect pulmonary disease severity instead of systemic disease severity.[47] Bronchoalveolar lavage fluid obtained during bronchoscopy is considered to be the gold standard for the sampling of the pulmonary compartment. However, a bronchoscopy is an invasive procedure and an unknown dilution factor is introduced. Direct aspiration of pulmonary oedema fluid has been suggested, but this method is only available in patients with severe pulmonary oedema.[48] Alternatively, exhaled breath condensate (EBC) can be used to non-invasively sample the pulmonary compartment. Serial samples of EBC could be used to monitor ARDS development or response to treatment. However, sample collection is time consuming and introduces an unknown dilution factor as well.[49, 50]

Nevertheless, biomarkers used for the identification of less heterogeneous ARDS phenotypes do not require to be ARDS specific, provided that they adequately stratify patients with ARDS. Plasma biomarkers for inflammation have been combined with clinical and physiological variables using advanced statistical modelling to identify a hyperinflammatory and hypoinflammatory ARDS phenotype.[51] Patients that were in a hyperinflammatory state benefitted more from treatment with higher PEEP or simvastatin than patients in a relatively hypoinflammatory state.[51-54] In contrast, the original randomized controlled trials did not find a treatment benefit. Thus, ARDS treatment could be personalised based on the identification of a biomarker derived phenotype.

In **Part I. Personalised treatment of ARDS based on biomarkers**, we examine the independent predictive value of biomarkers in ARDS, assess non-invasive measurement of pulmonary inflammation, and use a latent class analysis in order to identify heterogeneity of treatment effect in patients with community-acquired pneumonia, the main risk factor for the development of ARDS.

Physiologically derived phenotypes in ARDS

Mechanical ventilation according to the ARDS Network trial is considered to be the standard of care in patients with ARDS.[5] However, this mechanical ventilation strategy is a standardized one-size-fits-all approach. Physiological or respiratory parameters, e.g. oxygenation, driving pressure or transpulmonary pressure, can be used to identify more homogeneous subgroups in patients with ARDS or to adjust mechanical ventilation settings following treatment response.

Both the AECC definition of ARDS and the Berlin definition of ARDS included the degree of hypoxemia as a measure for disease severity.[2, 15] A lower PaO_2/FiO_2 ratio is associated with an increase in pulmonary oedema and non-aerated lung tissue, and

likely introduces a heterogeneous treatment effect.[55, 56] It has been suggested that the application of higher PEEP could improve survival in patients with moderate to severe ARDS, but is not beneficial in patients with mild ARDS.[57] In addition, predictive enrichment based on decreased oxygenation has been used in the clinical trial for prone positioning.[6, 58] Driving pressure and pulmonary dead space fraction have also been proposed to establish phenotypes, as both are independently associated with mortality rate in ARDS.[59, 60]

However, oxygenation, driving pressure, and dead space fraction are dependent on mechanical ventilator settings.[61] A change in PEEP likely alters PaO_2/FiO_2 ratio, driving pressure (or compliance), and pulmonary dead space fraction.[27] Instead of a mechanical ventilator setting dependent variable, we should try to identify intrinsic characteristics of the ARDS lung over a range of mechanical ventilator settings. The intrinsic characteristic of the ARDS lung that is subject of this thesis is recruitability.

Recruitability is the amount of collapsed lung tissue that has the potential for (re)aeration.[62] A lung CT scan at various pressure levels is considered to be the gold standard for the assessment of anatomical lung recruitment.[63, 64] PEEP titration based on CT scan is not used in clinical practice, as it requires patient transport, causes radiation exposure, and does not assess changes in respiratory mechanics over time. Multiple PEEP titration approaches at the bedside have been developed to estimate recruitability.[65, 66] Except for a secondary analysis using transpulmonary pressure based PEEP titration, none has shown to reduce mortality rate in patients with ARDS.[67-69]

Electrical impedance tomography (EIT) can be used to personalise treatment in patients with ARDS based on regional ventilation distribution. EIT is a non-invasive monitoring tool that reconstructs a dynamic cross-sectional image of ventilation based on changes in thoracic impedance.[70] The sum of all pixels within a given region of interest form the EIT plethysmogram, which represents the tidal volume delivered to the lung. There is a strong correlation between EIT plethysmogram and lung aeration measured by CT scan or functional end-expiratory lung volume.[71, 72]

Multiple EIT parameters have been described in order to find optimal ventilation during a PEEP trial.[73-76] In addition, alveolar collapse and overdistention can be quantified by examining pixel compliance during a decremental PEEP trial.[77] The pixel compliance can be calculated as the difference in EIT plethysmogram, as a surrogate for regional lung volume, divided by the driving pressure. The best pixel compliance can be calculated during a PEEP trial. A decrease in compliance at higher PEEP levels is an indication of alveolar overdistention and a decrease in compliance at lower PEEP levels

indicates alveolar collapse. The number of studies that use EIT to titrate PEEP is limited. In **Part II. Personalised treatment of ARDS based on recruitability**, we discussed the indications and limitations of a higher PEEP strategy and used EIT to personalise mechanical ventilation in patients with ARDS.

AIMS AND OUTLINE OF THIS THESIS

Despite decades of research in patients with ARDS, only a few interventions have shown to improve clinical outcomes.[12] Heterogeneity in patients with ARDS, and subsequent heterogeneity of treatment effect are likely to be the cause of multiple negative trials.[3] In patients with ARDS it is crucial to select the patients that are likely to respond to treatment, also known as predictive enrichment.[22] The aim of this thesis is to personalise treatment of patients with ARDS based on biomarkers or recruitability.

In **Part I. Personalised treatment of ARDS based on biomarkers**, we examine systemic biomarkers and biomarkers of pulmonary inflammation in order to identify biologically derived phenotypes for future research. In **Chapter 2**, we performed a systematic review of biomarkers that were independently associated with ARDS development and mortality. The majority of identified biomarkers for ARDS were measured in plasma and were associated with inflammation, epithelial injury or endothelial injury. These biomarkers reflected systemic inflammation, and not necessarily pulmonary inflammation. In **Chapter 3**, the link between mechanical ventilation and local pulmonary inflammation is described in a narrative review according to the purinergic signalling hypothesis. Pulmonary extracellular adenosine-triphosphate (ATP) is a key molecule in the development of lung injury, and – if assessed in fluid derived from the lungs – may be used to monitor ARDS or ventilation-induced lung injury. In **Chapter 4**, we examined the feasibility of measuring pulmonary extracellular ATP in exhaled breath condensate (EBC) of patients with increased minute ventilation following an exercise test. In **Chapter 5,** we performed a pilot study to examine whether biomarkers were readily detectable in swivel-derived EBC. Swivel-derived EBC provides the opportunity to collect EBC samples simple and fast in large prospective cohorts. In **Chapter 6**, we focussed on patients with community-acquired pneumonia, the primary risk factor for ARDS. In a secondary analysis of two randomized controlled trials, we used a latent class analysis based on both clinical parameters and biomarkers to identify subgroups in patients with community-acquired pneumonia that were more likely to respond to adjuvant treatment with corticosteroids.

In **Part II. Personalised treatment of ARDS based on recruitability**, advanced respiratory monitoring was used to personalise mechanical ventilation in patients with ARDS. In **Chapter 7**, we describe the rationale for the use of higher PEEP preceded by a recruitment manoeuvre according to the Open Lung Concept. In **Chapter 8**, we performed a small retrospective study and briefly describe how a recruitment manoeuvre and the use of high airway pressures could improve oxygenation and prevent the use of veno-venous extracorporeal membrane oxygenation in patients with severe ARDS. In February 2020, the first patient with coronavirus disease (COVID-19) was admitted to the ICU in the Netherlands, and a wave of patients with COVID-19 related ARDS followed. In **Chapter 9**, we used electrical impedance tomography (EIT) to titrate PEEP in patients with COVID-19 related ARDS based on both minimal alveolar overdistention and collapse. In **Chapter 10**, EIT was used to personalise PEEP in patients with COVID-19 related ARDS. We compared EIT guided PEEP with the PEEP set by the one-size-fits-all PEEP-FiO_2 table, and assessed the baseline characteristics of patients that required significantly lower or higher PEEP according to EIT guided PEEP titration.

REFERENCES

1. Thompson BT, Chambers RC, Liu KD: **Acute Respiratory Distress Syndrome**. *The New England journal of medicine* 2017, **377**(19):1904-1905.
2. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS: **Acute respiratory distress syndrome: the Berlin Definition**. *Jama* 2012, **307**(23):2526-2533.
3. Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, Gattinoni L, van Haren F, Larsson A, McAuley DF *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**(8):788-800.
4. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE: **Acute respiratory distress in adults**. *Lancet (London, England)* 1967, **2**(7511):319-323.
5. Brower R, Matthay M, Morris A, Schoenfeld D, Thompson B, Wheeler A, Network. TARDS: **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**(18):1301-1308.
6. Guerin C, Reignier J, Richard JC, Beuret P, Gacouin A, Boulain T, Mercier E, Badet M, Mercat A, Baudin O *et al*: **Prone positioning in severe acute respiratory distress syndrome**. *The New England journal of medicine* 2013, **368**(23):2159-2168.
7. National Heart L, Blood Institute Acute Respiratory Distress Syndrome Clinical Trials N, Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, deBoisblanc B, Connors AF, Jr., Hite RD *et al*: **Comparison of two fluid-management strategies in acute lung injury**. *The New England journal of medicine* 2006, **354**(24):2564-2575.
8. Papazian L, Forel JM, Gacouin A, Penot-Ragon C, Perrin G, Loundou A, Jaber S, Arnal JM, Perez D, Seghboyan JM *et al*: **Neuromuscular blockers in early acute respiratory distress syndrome**. *The New England journal of medicine* 2010, **363**(12):1107-1116.
9. Brower RG, Lanken PN, MacIntyre N, Matthay MA, Morris A, Ancukiewicz M, Schoenfeld D, Thompson BT, National Heart L, Blood Institute ACTN: **Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome**. *The New England journal of medicine* 2004, **351**(4):327-336.
10. Meade MO, Cook DJ, Guyatt GH, Slutsky AS, Arabi YM, Cooper DJ, Davies AR, Hand LE, Zhou Q, Thabane L *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**. *Jama* 2008, **299**(6):637-645.
11. Mercat A, Richard JC, Vielle B, Jaber S, Osman D, Diehl JL, Lefrant JY, Prat G, Richecoeur J, Nieszkowska A *et al*: **Positive end-expiratory pressure setting in adults with acute lung injury and acute respiratory distress syndrome: a randomized controlled trial**. *Jama* 2008, **299**(6):646-655.
12. Matthay MA, McAuley DF, Ware LB: **Clinical trials in acute respiratory distress syndrome: challenges and opportunities**. *The Lancet Respiratory medicine* 2017, **5**(6):524-534.
13. Writing Group for the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial I, Cavalcanti AB, Suzumura EA, Laranjeira LN, Paisani DM, Damiani LP, Guimaraes HP, Romano ER, Regenga MM, Taniguchi LNT *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: A Randomized Clinical Trial**. *Jama* 2017, **318**(14):1335-1345.
14. Kent DM, Steyerberg E, van Klaveren D: **Personalized evidence based medicine: predictive approaches to heterogeneous treatment effects**. *BMJ* 2018, **363**:k4245.

15. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R: **The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination**. *American journal of respiratory and critical care medicine* 1994, **149**(3 Pt 1):818-824.
16. Tomashefski JF, Jr.: **Pulmonary pathology of acute respiratory distress syndrome**. *ClinChest Med* 2000, **21**(3):435-466.
17. Penuelas O, Aramburu JA, Frutos-Vivar F, Esteban A: **Pathology of acute lung injury and acute respiratory distress syndrome: a clinical-pathological correlation**. *Clin Chest Med* 2006, **27**(4):571-578; abstract vii-viii.
18. Esteban A, Fernandez-Segoviano P, Frutos-Vivar F, Aramburu JA, Najera L, Ferguson ND, Alia I, Gordo F, Rios F: **Comparison of clinical criteria for the acute respiratory distress syndrome with autopsy findings**. *Annals of internal medicine* 2004, **141**(6):440-445.
19. Sarmiento X, Guardiola JJ, Almirall J, Mesalles E, Mate JL, Soler M, Klamburg J: **Discrepancy between clinical criteria for diagnosing acute respiratory distress syndrome secondary to community acquired pneumonia with autopsy findings of diffuse alveolar damage**. *Respiratory medicine* 2011, **105**(8):1170-1175.
20. Ferguson ND, Frutos-Vivar F, Esteban A, Fernandez-Segoviano P, Aramburu JA, Najera L, Stewart TE: **Acute respiratory distress syndrome: underrecognition by clinicians and diagnostic accuracy of three clinical definitions**. *Critical care medicine* 2005, **33**(10):2228-2234.
21. Thille AW, Esteban A, Fernandez-Segoviano P, Rodriguez JM, Aramburu JA, Penuelas O, Cortes-Puch I, Cardinal-Fernandez P, Lorente JA, Frutos-Vivar F: **Comparison of the Berlin definition for acute respiratory distress syndrome with autopsy**. *American journal of respiratory and critical care medicine* 2013, **187**(7):761-767.
22. 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**(2):147-155.
23. Ware LB, Matthay MA, Mebazaa A: **Designing an ARDS trial for 2020 and beyond: focus on enrichment strategies**. *Intensive care medicine* 2020, **46**(12):2153-2156.
24. Sinha P, Calfee CS: **Phenotypes in acute respiratory distress syndrome: moving towards precision medicine**. *Current opinion in critical care* 2019, **25**(1):12-20.
25. Matthay MA, Arabi YM, Siegel ER, Ware LB, Bos LDJ, Sinha P, Beitler JR, Wick KD, Curley MAQ, Constantin JM *et al*: **Phenotypes and personalized medicine in the acute respiratory distress syndrome**. *Intensive care medicine* 2020, **46**(12):2136-2152.
26. Alipanah N, Calfee CS: **Phenotyping in acute respiratory distress syndrome: state of the art and clinical implications**. *Current opinion in critical care* 2022, **28**(1):1-8.
27. Caironi P, Carlesso E, Cressoni M, Chiumello D, Moerer O, Chiurazzi C, Brioni M, Bottino N, Lazzerini M, Bugedo G *et al*: **Lung recruitability is better estimated according to the Berlin definition of acute respiratory distress syndrome at standard 5 cm H2O rather than higher positive end-expiratory pressure: a retrospective cohort study**. *Critical care medicine* 2015, **43**(4):781-790.
28. Sheu CC, Gong MN, Zhai R, Chen F, Bajwa EK, Clardy PF, Gallagher DC, Thompson BT, Christiani DC: **Clinical characteristics and outcomes of sepsis-related vs non-sepsis-related ARDS**. *Chest* 2010, **138**(3):559-567.
29. Calfee CS, Eisner MD, Ware LB, Thompson BT, Parsons PE, Wheeler AP, Korpak A, Matthay MA, Acute Respiratory Distress Syndrome Network NHL, Blood I: **Trauma-associated lung injury dif-**

fers clinically and biologically from acute lung injury due to other clinical disorders. *Critical care medicine* 2007, **35**(10):2243-2250.

30. Schenck EJ, Oromendia C, Torres LK, Berlin DA, Choi AMK, Siempos, II: **Rapidly Improving ARDS in Therapeutic Randomized Controlled Trials**. *Chest* 2019, **155**(3):474-482.
31. Liao KM, Chen CW, Hsiue TR, Lin WC: **Timing of acute respiratory distress syndrome onset is related to patient outcome**. *J Formos Med Assoc* 2009, **108**(9):694-703.
32. Monchi M, Bellenfant F, Cariou A, Joly LM, Thebert D, Laurent I, Dhainaut JF, Brunet F: **Early predictive factors of survival in the acute respiratory distress syndrome. A multivariate analysis**. *American journal of respiratory and critical care medicine* 1998, **158**(4):1076-1081.
33. Calfee CS, Janz DR, Bernard GR, May AK, Kangelaris KN, Matthay MA, Ware LB: **Distinct molecular phenotypes of direct vs indirect ARDS in single-center and multicenter studies**. *Chest* 2015, **147**(6):1539-1548.
34. Gattinoni L, Pelosi P, Suter PM, Pedoto A, Vercesi P, Lissoni A: **Acute respiratory distress syndrome caused by pulmonary and extrapulmonary disease. Different syndromes?** *American journal of respiratory and critical care medicine* 1998, **158**(1):3-11.
35. Goodman LR, Fumagalli R, Tagliabue P, Tagliabue M, Ferrario M, Gattinoni L, Pesenti A: **Adult respiratory distress syndrome due to pulmonary and extrapulmonary causes: CT, clinical, and functional correlations**. *Radiology* 1999, **213**(2):545-552.
36. Thille AW, Richard JC, Maggiore SM, Ranieri VM, Brochard L: **Alveolar recruitment in pulmonary and extrapulmonary acute respiratory distress syndrome: comparison using pressure-volume curve or static compliance**. *Anesthesiology* 2007, **106**(2):212-217.
37. Constantin JM, Jabaudon M, Lefrant JY, Jaber S, Quenot JP, Langeron O, Ferrandiere M, Grelon F, Seguin P, Ichai C *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**(10):870-880.
38. Group RC, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A *et al*: **Dexamethasone in Hospitalized Patients with Covid-19**. *The New England journal of medicine* 2021, **384**(8):693-704.
39. Group WHOREAfC-TW, Sterne JAC, Murthy S, Diaz JV, Slutsky AS, Villar J, Angus DC, Annane D, Azevedo LCP, Berwanger O *et al*: **Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis**. *Jama* 2020, **324**(13):1330-1341.
40. Villar J, Ferrando C, Martinez D, Ambros A, Munoz T, Soler JA, Aguilar G, Alba F, Gonzalez-Higueras E, Conesa LA *et al*: **Dexamethasone treatment for the acute respiratory distress syndrome: a multicentre, randomised controlled trial**. *The Lancet Respiratory medicine* 2020, **8**(3):267-276.
41. Garcia-Laorden MI, Lorente JA, Flores C, Slutsky AS, Villar J: **Biomarkers for the acute respiratory distress syndrome: how to make the diagnosis more precise**. *Annals of translational medicine* 2017, **5**(14):283.
42. Villar J, Slutsky AS: **GOLDEN anniversary of the acute respiratory distress syndrome: still much work to do!** *Current opinion in critical care* 2017, **23**(1):4-9.
43. Frohlich S, Murphy N, Boylan JF: **ARDS: progress unlikely with non-biological definition**. *British journal of anaesthesia* 2013, **111**(5):696-699.
44. Ware LB, Koyama T, Billheimer DD, Wu W, Bernard GR, Thompson BT, Brower RG, Standiford TJ, Martin TR, Matthay MA *et al*: **Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury**. *Chest* 2010, **137**(2):288-296.

45. Terpstra ML, Aman J, van Nieuw Amerongen GP, Groeneveld AB: **Plasma biomarkers for acute respiratory distress syndrome: a systematic review and meta-analysis***. *Critical care medicine* 2014, **42**(3):691-700.
46. van der Zee P, Rietdijk W, Somhorst P, Endeman H, Gommers D: **A systematic review of biomarkers multivariately associated with acute respiratory distress syndrome development and mortality**. *Crit Care* 2020, **24**(1):243.
47. McNeil JB, Shaver CM, Kerchberger VE, Russell DW, Grove BS, Warren MA, Wickersham NE, Ware LB, McDonald WH, Bastarache JA: **Novel Method for Noninvasive Sampling of the Distal Airspace in Acute Respiratory Distress Syndrome**. *American journal of respiratory and critical care medicine* 2018, **197**(8):1027-1035.
48. Bowler RP, Duda B, Chan ED, Enghild JJ, Ware LB, Matthay MA, Duncan MW: **Proteomic analysis of pulmonary edema fluid and plasma in patients with acute lung injury**. *American journal of physiology Lung cellular and molecular physiology* 2004, **286**(6):L1095-1104.
49. Bondesson E, Jansson LT, Bengtsson T, Wollmer P: **Exhaled breath condensate-site and mechanisms of formation**. *J Breath Res* 2009, **3**(1):016005.
50. Carter SR, Davis CS, Kovacs EJ: **Exhaled breath condensate collection in the mechanically ventilated patient**. *Respiratory medicine* 2012, **106**(5):601-613.
51. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA, Network NA: **Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials**. *The Lancet Respiratory medicine* 2014, **2**(8):611-620.
52. Maddali MV, Churpek M, Pham T, Rezoagli E, Zhuo H, Zhao W, He J, Delucchi KL, Wang C, Wickersham N *et al*: **Validation and utility of ARDS subphenotypes identified by machine-learning models using clinical data: an observational, multicohort, retrospective analysis**. *The Lancet Respiratory medicine* 2022.
53. McAuley DF, Laffey JG, O'Kane CM, Perkins GD, Mullan B, Trinder TJ, Johnston P, Hopkins PA, Johnston AJ, McDowell C *et al*: **Simvastatin in the acute respiratory distress syndrome**. *The New England journal of medicine* 2014, **371**(18):1695-1703.
54. Calfee CS, Delucchi KL, Sinha P, Matthay MA, Hackett J, Shankar-Hari M, McDowell C, Laffey JG, O'Kane CM, McAuley DF *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**(9):691-698.
55. Cressoni M, Chiumello D, Algieri I, Brioni M, Chiurazzi C, Colombo A, Colombo A, Crimella F, Guanziroli M, Tomic I *et al*: **Opening pressures and atelectrauma in acute respiratory distress syndrome**. *Intensive care medicine* 2017, **43**(5):603-611.
56. Maiolo G, Collino F, Vasques F, Rapetti F, Tonetti T, Romitti F, Cressoni M, Chiumello D, Moerer O, Herrmann P *et al*: **Reclassifying Acute Respiratory Distress Syndrome**. *American journal of respiratory and critical care medicine* 2018, **197**(12):1586-1595.
57. Briel M, Meade M, Mercat A, Brower RG, Talmor D, Walter SD, Slutsky AS, Pullenayegum E, Zhou Q, Cook D *et al*: **Higher vs lower positive end-expiratory pressure in patients with acute lung injury and acute respiratory distress syndrome: systematic review and meta-analysis**. *Jama* 2010, **303**(9):865-873.
58. Gattinoni L, Carlesso E, Taccone P, Polli F, Guerin C, Mancebo J: **Prone positioning improves survival in severe ARDS: a pathophysiologic review and individual patient meta-analysis**. *Minerva anestesiologica* 2010, **76**(6):448-454.

59. Amato MB, Meade MO, Slutsky AS, Brochard L, Costa EL, Schoenfeld DA, Stewart TE, Briel M, Talmor D, Mercat A *et al*: **Driving pressure and survival in the acute respiratory distress syndrome**. *The New England journal of medicine* 2015, **372**(8):747-755.
60. Nuckton TJ, Alonso JA, Kallet RH, Daniel BM, Pittet JF, Eisner MD, Matthay MA: **Pulmonary dead-space fraction as a risk factor for death in the acute respiratory distress syndrome**. *The New England journal of medicine* 2002, **346**(17):1281-1286.
61. Villar J, Perez-Mendez L, Blanco J, Anon JM, Blanch L, Belda J, Santos-Bouza A, Fernandez RL, Kacmarek RM, Spanish Initiative for Epidemiology S *et al*: **A universal definition of ARDS: the PaO2/FiO2 ratio under a standard ventilatory setting--a prospective, multicenter validation study**. *Intensive care medicine* 2013, **39**(4):583-592.
62. Gattinoni L, Caironi P, Cressoni M, Chiumello D, Ranieri VM, Quintel M, Russo S, Patroniti N, Cornejo R, Bugedo G: **Lung recruitment in patients with the acute respiratory distress syndrome**. *The New England journal of medicine* 2006, **354**(17):1775-1786.
63. Gattinoni L, Collino F, Maiolo G, Rapetti F, Romitti F, Tonetti T, Vasques F, Quintel M: **Positive end-expiratory pressure: how to set it at the individual level**. *Annals of translational medicine* 2017, **5**(14):288.
64. Lu Q, Constantin JM, Nieszkowska A, Elman M, Vieira S, Rouby JJ: **Measurement of alveolar derecruitment in patients with acute lung injury: computerized tomography versus pressure-volume curve**. *Crit Care* 2006, **10**(3):R95.
65. 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**(11):1429-1438.
66. Hess DR: **Recruitment Maneuvers and PEEP Titration**. *Respiratory care* 2015, **60**(11):1688-1704.
67. Talmor D, Sarge T, Malhotra A, O'Donnell CR, Ritz R, Lisbon A, Novack V, Loring SH: **Mechanical ventilation guided by esophageal pressure in acute lung injury**. *The New England journal of medicine* 2008, **359**(20):2095-2104.
68. Beitler JR, Sarge T, Banner-Goodspeed VM, Gong MN, Cook D, Novack V, Loring SH, Talmor D, Group EP-S: **Effect of Titrating Positive End-Expiratory Pressure (PEEP) With an Esophageal Pressure-Guided Strategy vs an Empirical High PEEP-Fio2 Strategy on Death and Days Free From Mechanical Ventilation Among Patients With Acute Respiratory Distress Syndrome: A Randomized Clinical Trial**. *Jama* 2019, **321**(9):846-857.
69. Sarge T, Baedorf-Kassis E, Banner-Goodspeed V, Novack V, Loring SH, Gong MN, Cook D, Talmor D, Beitler JR, Group EP-S: **Effect of Esophageal Pressure-guided Positive End-Expiratory Pressure on Survival from Acute Respiratory Distress Syndrome: A Risk-based and Mechanistic Reanalysis of the EPVent-2 Trial**. *American journal of respiratory and critical care medicine* 2021, **204**(10):1153-1163.
70. Bachmann MC, Morais C, Bugedo G, Bruhn A, Morales A, Borges JB, Costa E, Retamal J: **Electrical impedance tomography in acute respiratory distress syndrome**. *Crit Care* 2018, **22**(1):263.
71. Victorino JA, Borges JB, Okamoto VN, Matos GF, Tucci MR, Caramez MP, Tanaka H, Sipmann FS, Santos DC, Barbas CS *et al*: **Imbalances in regional lung ventilation: a validation study on electrical impedance tomography**. *American journal of respiratory and critical care medicine* 2004, **169**(7):791-800.
72. Hinz J, Hahn G, Neumann P, Sydow M, Mohrenweiser P, Hellige G, Burchardi H: **End-expiratory lung impedance change enables bedside monitoring of end-expiratory lung volume change**. *Intensive care medicine* 2003, **29**(1):37-43.

73. Shono A, Kotani T: **Clinical implication of monitoring regional ventilation using electrical impedance tomography**. *Journal of intensive care* 2019, **7**:4.
74. Kobylianskii J, Murray A, Brace D, Goligher E, Fan E: **Electrical impedance tomography in adult patients undergoing mechanical ventilation: A systematic review**. *Journal of critical care* 2016, **35**:33-50.
75. Blankman P, Hasan D, Erik G, Gommers D: **Detection of 'best' positive end-expiratory pressure derived from electrical impedance tomography parameters during a decremental positive end-expiratory pressure trial**. *Crit Care* 2014, **18**(3):R95.
76. Yoshida T, Piraino T, Lima CAS, Kavanagh BP, Amato MBP, Brochard L: **Regional Ventilation Displayed by Electrical Impedance Tomography as an Incentive to Decrease Positive End-Expiratory Pressure**. *American journal of respiratory and critical care medicine* 2019, **200**(7):933-937.
77. Costa EL, Borges JB, Melo A, Suarez-Sipmann F, Toufen C, Jr., Bohm SH, Amato MB: **Bedside estimation of recruitable alveolar collapse and hyperdistension by electrical impedance tomography**. *Intensive care medicine* 2009, **35**(6):1132-1137.

Part I

Personalised treatment of ARDS based on biomarkers

2

A systematic review of biomarkers multivariately associated with Acute Respiratory Distress Syndrome development and mortality

Philip van der Zee MD[1], Wim Rietdijk PhD[1], Peter Somhorst MSc[1], Henrik Endeman MD PhD[1], Diederik Gommers MD PhD[1]

[1] Department of Adult Intensive Care, Erasmus Medical Center Rotterdam, The Netherlands

Published: Crit Care 2020 Vol. 24 Issue 1

ABSTRACT

Background: Heterogeneity of acute respiratory distress syndrome (ARDS) could be reduced by identification of biomarker based phenotypes. The set of ARDS biomarkers to prospectively define these phenotypes remains to be established.

Objective: To provide an overview of the biomarkers that were multivariately associated with ARDS development or mortality.

Data sources: We performed a systematic search in Embase, MEDLINE, Web of Science, Cochrane CENTRAL, and Google Scholar from inception until 6 March 2020.

Study selection: Studies assessing biomarkers for ARDS development in critically ill patients at-risk for ARDS and mortality due to ARDS adjusted in multivariate analyses were included.

Data extraction and synthesis: We included 35 studies for ARDS development (10,667 patients at-risk for ARDS) and 53 for ARDS mortality (15,344 patients with ARDS). These studies were too heterogeneous to be used in a meta-analysis, as time until outcome and the variables used in the multivariate analyses varied widely between studies. After qualitative inspection, high plasma levels of angiopoeitin-2 and receptor for advanced glycation end products (RAGE) were associated with an increased risk of ARDS development. None of the biomarkers (plasma angiopoeitin-2, C-reactive protein, interleukin-8, RAGE, surfactant protein-D, and Von Willebrand factor) were clearly associated with mortality.

Conclusions: Biomarker data reporting and variables used in multivariate analyses differed greatly between studies. Angiopoeitin-2 and RAGE in plasma were positively associated with increased risk of ARDS development. None of the biomarkers independently predicted mortality. Therefore, we suggested to structurally investigate a combination of biomarkers and clinical parameters in order to find more homogeneous ARDS phenotypes.

PROSPERO identifier: CRD42017078957

Keywords: Acute Respiratory Distress Syndrome, Biomarkers, Diagnosis, Mortality

INTRODUCTION

The acute respiratory distress syndrome (ARDS) is a major problem in the intensive care unit (ICU) with a prevalence of 10% and an in-hospital mortality rate of 40%.[1, 2] ARDS pathophysiology is based on a triad of alveolar-capillary membrane injury, high permeability alveolar oedema, and migration of inflammatory cells.[3] This triad is not routinely measured in clinical practice. Therefore, arterial hypoxemia and bilateral opacities on chest imaging following various clinical insults are used as clinical surrogates in the American European Consensus Conference (AECC) definition and the newer Berlin definition of ARDS.[4, 5]

Histologically ARDS is characterized by diffuse alveolar damage (DAD). The correlation between a clinical and histological diagnosis of ARDS is poor.[6] Only half of clinically diagnosed patients with ARDS have histological signs of DAD at autopsy.[7-10] The number of risk factors for ARDS and consequently the heterogeneous histological substrates found in patients with clinical ARDS have been recognized as a major contributor to the negative randomized controlled trial results among patients with ARDS.[11]

It has been suggested that the addition of biomarkers to the clinical definition of ARDS could reduce ARDS heterogeneity by the identification of subgroups.[12-15] A retrospective latent class analysis of large randomized controlled trials identified two ARDS phenotypes largely based on ARDS biomarkers combined with clinical parameters.[16, 17] These phenotypes responded differently to the randomly assigned intervention arms. Prospective studies are required to validate these ARDS phenotypes and their response to interventions. The set of ARDS biomarkers to prospectively define these phenotypes remains to be established.

Numerous biomarkers and their pathophysiological role in ARDS have been described. [12, 18] In an earlier meta-analysis, biomarkers for ARDS development and mortality were examined in univariate analysis.[19] However, pooling of univariate biomarker data may result in overestimation of the actual effect. For this reason, we conducted a systematic review and included all biomarkers that were multivariately associated with ARDS development or mortality. This study provides a synopsis of ARDS biomarkers that could be used for future research in the identification of ARDS phenotypes.

METHODS

This systematic review was prospectively registered in PROSPERO International Prospective Register of Systematic Reviews (PROSPERO identifier CRD42017078957) and performed according to the Transparent Reporting of Systematic Reviews and Meta-analyses (PRISMA) Statement.[20] After the search strategy, two reviewers (PZ, PS, and/or WG) separately performed study eligibility criteria, data extraction, and quality assessment. Any discrepancies were resolved by consensus, and if necessary, a third reviewer was consulted.

We searched for studies that included biomarkers that were associated with ARDS development in critically ill patients at-risk for ARDS and mortality in the ARDS population in multivariate analyses adjusted for background characteristics. We did not perform a meta-analysis, because the raw data in all studies was either not transformed or log transformed resulting in varying risk ratios and confidence intervals. In addition, the majority of studies used different biomarker concentration cut-offs, resulting in varying concentration increments for risk ratios. Lastly, the number of days until mortality and variables used in multivariate analysis differed between studies. For these reasons we limited this study to a systematic review, as the multivariate odds ratios were not comparable and pooling would result in non-informative estimates.[21]

Search strategy

We performed a systematic search in Embase, MEDLINE, Web of Science, Cochrane CENTRAL, and Google Scholar from inception until 30 July 2018 with assistance from the Erasmus MC librarian. The search was later updated to 6 March 2020. A detailed description of the systematic search string is presented in **Additional file 1**. In addition, the reference lists of included studies and recent systematic reviews were screened to identify additional eligible studies.

Study eligibility criteria

All retrieved studies were screened on the basis of title and abstract. Studies that did not contain adult patients at-risk for ARDS or with ARDS and any biomarker for ARDS were excluded. The following eligibility criteria were used: human research; adult population; studies in which biomarkers were presented as odds ratios (OR) or risk ratios in multivariate analysis with ARDS development or mortality as outcome of interest; peer-reviewed literature only; and English language. Studies comparing ARDS with healthy control subjects, case series (<10 patients included in the study), and studies presenting gene expression fold change were excluded.

Data extraction

A standardized form was used for data-extraction from all eligible studies. Two clinical endpoints were evaluated in this study: development of ARDS in the at-risk population (patients that did develop ARDS versus critically ill patients that did not) and mortality in the ARDS population (survivors versus non-survivors). The following data were extracted: study design and setting, study population, sample size, the definition of ARDS used in the study, outcome, risk ratio with 95% confidence interval in multivariate analyses, and the variables used in the analyses. In addition, the role of the biomarker in ARDS pathophysiology as reported by the studies was extracted and divided into the following categories: increased endothelial permeability, alveolar epithelial injury, oxidative injury, inflammation, pro-fibrotic, myocardial strain, coagulation, and other. Subsequently, the relative frequency distribution of biomarker roles in ARDS pathophysiology was depicted in a bar chart.

Quality assessment

Methodological quality of the included studies was assessed with the Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in systematic reviews and meta-analyses.[22] Items regarding patient selection, comparability and outcome were assessed using a descriptive approach and a risk-of-bias score, varying between 0 (high risk) and 9 (low risk), was assigned to each study.

RESULTS

Literature search and study selection

A total of 8,125 articles were identified by the initial search and 972 by the updated search (**Figure 1**). After removal of duplicates and reviewing titles and abstracts, we selected 438 articles for full-text review. A total of 86 studies was eligible for data extraction: 35 for ARDS development and 53 for ARDS mortality.

Study characteristics and quality assessment

The study characteristics of the 35 studies for ARDS development are presented in **Table 1**. A total of 10,667 critically ill patients was at-risk for ARDS, of whom 2,419 (24.6%) patients developed ARDS. The majority of studies used the Berlin definition of ARDS (21/35), followed by the AECC criteria of ARDS (13/35). The included biomarkers were measured in plasma, cerebrospinal fluid, and bronchoalveolar lavage fluid. In all studies, the first sample was taken within 72 hours following ICU admission.

Table 1 – Study Characteristics for ARDS development

Study	Study design	Study population	ARDS definition	Outcome	Total (n)	ARDS (n)	Age	Gender Male n (%)	Variables in multivariate analysis	Sample moment
Agrawal 2013[34]	Prospective cohort	Critically ill	AECC	ALI	167	19	69±16	8 (42.1%)	APACHE II score, sepsis	Within 24 hours following admission
Ahasic 2012[35]	Case control	Critically ill	AECC	ARDS	531	175	60.7±17.6	102 (58.2%)	Age, gender, APACHE III score, BMI, ARDS risk factor	Within 48 hours following admission
Aisiku 2016[36]	RCT (TBI trial)	Critically ill neurotrauma	Berlin	ARDS	200	52	29.0 (19.5 IQR)	50 (96.2%)	Gender, injury severity scale, Glasgow coma scale	Within 24 hours following injury
Amat 2000[37]	Case control	Critically ill	AECC	ARDS	35	21	54±16	15 (71.4%)	Not specified	At ICU admission
Bai 2017[38]	Prospective cohort	Critically ill neurotrauma	Berlin	ARDS	50	21	48 (39-57 IQR)	10 (46.7%)	Age, gender, BMI, injury score, blood transfusion, mechanical ventilation, Marshall CT score, Glasgow coma scale	At admission
Bai 2017[38]	Prospective cohort	Critically ill trauma	Berlin	ARDS	42	16	44 (35-56 IQR)	10 (62.5%)	Age, gender, BMI, injury score, blood transfusion, mechanical ventilation, Marshall CT score, Glasgow coma scale	At admission
Bai 2018[39]	Prospective cohort	Stroke patients	Berlin	ARDS	384	60	64 (43-72 IQR)	22 (36.7%)	Age, gender, BMI, onset to treatment time, medical history	Within 6 hours following stroke
Chen 2019[40]	Case control	Critically ill sepsis	Berlin	ARDS	115	57	56,3 ±10,1	40 (70,2%)	Age, gender, BMI, smoking history, COPD, cardiomyopathy, APACHE II score, SOFA score	Within 24 hours following ARDS onset or ICU admission
Du 2016[41]	Prospective cohort	Cardiac surgery patients	AECC	ALI	70	18	57.7±11.6	12 (66.7%)	Age, medical history, BMI, systolic blood pressure	Within 1 hour following surgery
Faust 2020[42]	Prospective cohort	Critically ill trauma	Berlin	ARDS	224	41	44 (30-60 IQR)	37 (90.2%)	Injury severity score, blunt mechanism, pre-ICU shock	At ED

Table 1 – Study Characteristics for ARDS development (*continued*)

Study	Study design	Study population	ARDS definition	Outcome	Total (n)	ARDS (n)	Age	Gender Male n (%)	Variables in multivariate analysis	Sample moment
Faust 2020[42]	Prospective cohort	Critically ill sepsis	Berlin	ARDS	120	45	62 (52-67 IQR)	15 (33.3%)	Lung source of sepsis, shock, age	At ED
Fremont 2010[43]	Case control	Critically ill	AECC	ALI/ARDS	192	107	39 (26-53 IQR)	71 (66.4%)	Not specified	Within 72 hours following ICU admission
Gaudet 2018[44]	Prospective cohort	Critically ill patients	Berlin	ARDS	72	11	56 (51-63 IQR)	8 (72.7%)	Not specified	At inclusion
Hendrickson 2018[45]	Retrospective cohort	Severe traumatic brain injury	Berlin	ARDS	182	50	44±20	42 (84.0%)	Age, acute injury scale, Glasgow coma scale, vasopressor use	Within 10 minutes following ED arrival
Huang 2019[46]	Prospective cohort	Critically ill sepsis	Berlin	ARDS	152	41	63.2 ± 11.0	32 (78.0%)	Age, gender, BMI, smoking history, COPD, cardiomyopathy, APACHE II score, SOFA score	Within 24 hours following ICU admission
Huang 2019[47]	Prospective cohort	Critically ill pancreatitis	Berlin	ARDS	1933	143	49 (42-60 IQR)	87 (60,8%)	Age, gender, aetiology of ARDS, APACHE II score	At admission
Jabaudon 2018[48]	Prospective Cohort	Critically ill	Berlin	ARDS	464	59	62±16	46 (78.0%)	SAPS II, sepsis, shock, pneumonia	Within 6 hours following ICU admission
Jensen 2016[49]	RCT (PASS)	Critically ill	Berlin	ARDS	405	31	NR	NR	Age, gender, APACHE II score, sepsis, eGFR	Within 24 hours following admission
Jensen 2016[49]	RCT (PASS)	Critically ill	Berlin	ARDS	353*	31	NR	NR	Age, gender, APACHE II score, sepsis, eGFR	Within 24 hours following admission
Jones 2020[50]	Prospective cohort	Critically ill sepsis	Berlin	ARDS	672	261	60 (51-69 IQR)	154 (59,0%)	Pulmonary source, APACHE III score	At admission
Jones 2020[50]	Prospective cohort	Critically ill sepsis	Berlin	ARDS	843	NR	NR	NR	Pulmonary source, APACHE III score	Within 48 hours following admission

Table 1 – Study Characteristics for ARDS development (*continued*)

Study	Study design	Study population	ARDS definition	Outcome	Total (n)	ARDS (n)	Age	Gender Male n (%)	Variables in multivariate analysis	Sample moment
Komiya 2011[51]	Cross sectional	Acute respiratory failure	AECC	ALI/ARDS	124	53	78 (69-85 IQR)	34 (64.2%)	Age, systolic blood pressure, LVEF, chest x-ray pleural effusion	Within 2 hours following emergency department arrival
Lee 2011[52]	Prospective cohort	Critically ill	AECC	ALI/ARDS	113	50	57.6±19.1	24 (48.0%)	Sepsis, BMI	Within 24 hours following ICU admission
Lin 2017[53]	Retrospective cohort	Critically ill	Berlin	ARDS	212	83	54.3±20.3	53 (63.9%)	CRP, albumin, serum creatinine, APACHE II score	Within 2 hours following ICU admission
Liu 2017[54]	Prospective cohort	Critically ill	AECC	ALI/ARDS	134	19	69±18	10 (52.6%)	APACHE II, sepsis severity	On arrival at ED
Luo 2017[55]	Retrospective cohort	Severe pneumonia	AECC	ALI/ARDS	157	43	56±19	25 (58.1%)	Lung injury score, SOFA score, PaO_2/FiO_2, blood urea	Day 1 following admission
Meyer 2017[56]	Prospective cohort	Critically ill trauma	Berlin	ARDS	198	100	60±14	62 (62.0%)	APACHE III score, age, gender, ethnicity, pulmonary infection	On arrival at ED or ICU
Mikkelsen 2012[57]	Case control	Critically ill	AECC	ALI/ARDS	48	24	38±20	22 (91.7%)	APACHE III score	In ED
Osaka 2011[58]	Prospective cohort	Pneumonia	AECC	ALI/ARDS	27	6	75 (51-92 range)	4 (66.7%)	Not specified	3 to 5 days following admission
Palakshappa 2016[59]	Prospective cohort	Critically ill	Berlin	ARDS	163	73	58 (52-68 IQR)	42 (57.5%)	APACHE III score, diabetes, BMI, pulmonary sepsis	At ICU admission
Reilly 2018[60]	Prospective cohort	Critically ill sepsis	Berlin	ARDS	703	289	60 (51-69 IQR)	170 (58,8%)	Pulmonary source, APACHE III score	Within 24 hours of ICU admission
Shashaty 2019[61]	Prospective cohort	Critically ill sepsis	Berlin	ARDS	120	44	61 (50-68 IQR)	NR	Age, transfusion, pulmonary source, shock	At ED

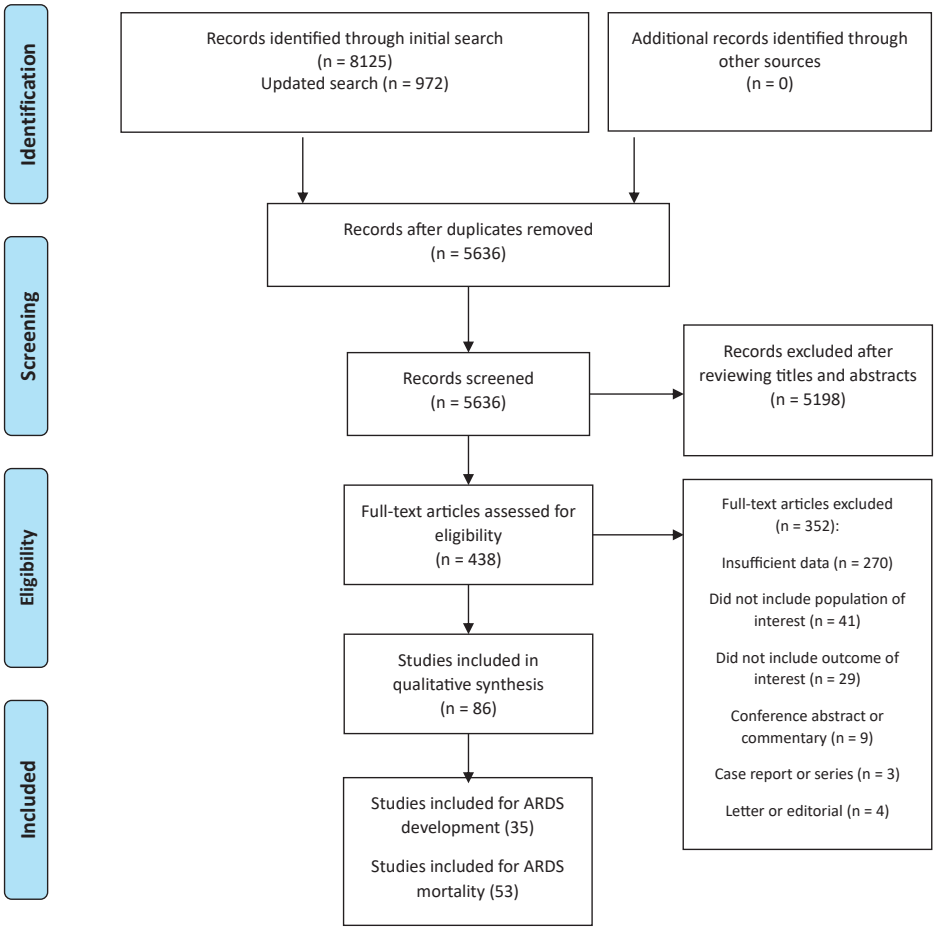
Table 1 – Study Characteristics for ARDS development (*continued*)

Study	Study design	Study population	ARDS definition	Outcome	Total (n)	ARDS (n)	Age	Gender Male n (%)	Variables in multivariate analysis	Sample moment
Shashaty 2019[61]	Prospective cohort	Critically ill trauma	Berlin	ARDS	180	37	41 (25-62 IQR)	NR	Injury severity score, blunt mechanism, transfusion	At presentation
Shaver 2017[62]	Prospective cohort	Critically ill	AECC	ARDS	280	90	54 (44-64 IQR)	54 (60.0%)	Age, APACHE II, sepsis	Day of inclusion
Suzuki 2017[63]	Retrospective cohort	Suspected drug induced lung injury	New bilateral lung infiltration	ALI/ARDS	68	39	72 (65-81IQR)	25 (64.1%)	Gender, age, smoking history, biomarkers	As soon as possible after DLI suspicion
Wang 2019[64]	Prospective cohort	Critically ill sepsis	Berlin	ARDS	109	32	58 ± 10,7	NR	Age, gender, BMI, smoking history, COPD, cardiomyopathy, APACHE II score, SOFA score	Within 24 hours following admission
Ware 2017[65]	Prospective cohort	Critically ill trauma patients	Berlin	ARDS	393	78	42 (26-55)	56 (71.8%)	Not specified	Within 24 hours following inclusion
Xu 2018[66]	Prospective cohort	Critically ill	Berlin	ARDS	158	45	60.0±17.1	35 (77.8%)	APACHE II score, Lung injury prediction score, biomarkers, sepsis	Within 24 hours of ICU admission
Yeh 2017[67]	Prospective cohort	Critically ill	AECC	ALI/ARDS	129	18	65±18	10 (55.6%)	APACHE II score	On arrival at the ED
Ying 2019[68]	Prospective cohort	Critically ill pneumonia	Berlin	ARDS	145	37	61,3 ± 10,4	23 (62,2%)	Age, SOFA score, Lung injury score, heart rate	At admission
				Total[†]	10667	2419				
						24.6%				

*Validating cohort. [†] Some studies included patients from the same cohort.

Abbreviations: AECC American European Consensus Conference definition of ARDS, ALI acute lung injury, APACHE acute physiology and chronic health evaluation, ARDS acute respiratory distress syndrome, BMI body mass index, COPD chronic obstructive pulmonary disease, CRP C-reactive protein, DLI drug-induced lung injury, ED emergency department, eGFR estimated glomerular filtration rate, ICU intensive care unit, LVEF left ventricular ejection fraction, SAPS simplified acute physiology score, SOFA sequential organ failure assessment.

Figure 1. PRISMA Flow diagram for a systematic search



The study characteristics of the 53 studies for ARDS mortality are presented in **Table 2**. A total of 15,344 patients with ARDS were included with an observed mortality rate of 36.0%. The AECC definition of ARDS was used in the majority of included studies (39/53). The included biomarkers were measured in plasma, bronchoalveolar lavage fluid, and urine. All samples were taken within 72 hours following the development of ARDS.

The median quality of the included publications according to the NOS was 7 (range 4-9) for ARDS development and 8 (range 5-9) for ARDS mortality (**Additional file 2**).

Table 2 – Study Characteristics for ARDS mortality

Study	Study design	Setting	ARDS definition	Outcome	Total (n)	Non-survivors (n)	Age	Gender Male n (%)	Variables in multivariate analysis	Sample moment
Adamzik 2013[69]	Prospective cohort	Single centre	AECC	30-day	47	17	44±13	32 (68. 1%)	SAPS II score, gender, Lung injury score, ECMO, CVVHD, BMI, CRP, procalcitonin	Within 24 hours following ICU admission
Ahasic 2012[35]	Prospective cohort	Multicentre	AECC	60-day	175	78	60.7±17.6	102 (58.3%)	Gender, BMI, cirrhosis, Diabetes, need for red cell transfusion, sepsis, septic shock, trauma	Within 48 hours following ICU admission
Amat 2000[37]	Prospective cohort	Two centre	AECC ARDS	1 month after ICU discharge	21	11	54±16	15 (71.4%)	Not specified	Day 0 ICU
Bajwa 2008[70]	Prospective cohort	Single centre	AECC	60-day	177	70	68.3±15.3	99 (55.9%)	APACHE III score	Within 48 hours following ARDS onset
Bajwa 2009[71]	Prospective cohort	Single centre	AECC	60-day	177	70	62.5 (IQR 29.0)	100 (56.5%)	APACHE III score	Within 48 hours following ARDS onset
Bajwa 2013[32]	RCT (FACTT)	Multicentre	AECC	60-day	826	NR	48 (38-59 IQR)	442 (53.5%)	APACHE III score	Day 0 and 3
Calfee 2008[72]	RCT (ARMA)	Multicentre	AECC	180-day	676	NR	51±17	282 (41.7%)	Age, gender, APACHE III score, sepsis, or trauma	Day 0
Calfee 2009[73]	RCT (ARMA)	Multicentre	AECC	Hospital	778	272	51±17	459 (59.0%)	Age, PaO_2/FiO_2, APACHE III score, sepsis or trauma	Day 0
Calfee 2011[74]	RCT (ARMA)	Multicentre	AECC	90-day	547	186	50±16	227 (41.5%)	APACHE III score, tidal volume	Day 0
Calfee 2012[75]	RCT (FACTT)	Multicentre	AECC	90-day	931	261	50±16	498 (53.5%)	Age, APACHE III score, fluid management strategy	Day 0

Table 2 – Study Characteristics for ARDS mortality (*continued*)

Study	Study design	Setting	ARDS definition	Outcome	Total (n)	Non-survivors (n)	Age	Gender Male n (%)	Variables in multivariate analysis	Sample moment
Calfee 2015[76]	Prospective cohort	Single centre	AECC	Hospital	100	31	58±11	52 (52.0%)	APACHE III score	Day 2 following ICU admission
Calfee 2015[76]	RCT (FACTT)	Multicentre	AECC	90-day	853	259	51±15	444 (52.1%)	APACHE III score	Within 48 hours following ARDS onset
Cartin-Ceba 2015[77]	Prospective cohort	Single centre	AECC	In-hospital	100	36	62.5 (51-75 IQR)	54 (54.0%)	Acute physiology score of APACHE III score, DNR status, McCabe score	Within 24 hours following diagnosis
Chen 2009[78]	Prospective cohort	Single centre	*	28-day	59	26	62±19	35 (59.3%)	APACHE II score, biomarkers	Within 24 hours following diagnosis
Clark 1995[79]	Prospective cohort	Single centre	**	mortality	117	48	43.4±15.4	75 (64.1%)	Lung injury score, risk factor for ARDS, lavage protein concentration	Day 3 following disease onset
Clark 2013[80]	RCT (FACTT)	Multicentre	AECC	60-day	400	106	47 (37-57 IQR)	210 (52.5%)	Age, gender, ethnicity, baseline serum creatinine, ARDS risk factor	Day 1 following inclusion
Dolinay 2012[25]	Prospective cohort	Single centre	AECC	In hospital	28	17	54±14.5	13 (46.4%)	APACHE II score	Within 48 hours following ICU admission
Eisner 2003[81]	RCT (ARMA)	Multicentre	AECC	180-day	565	195	51±17	332 (58.8%)	Ventilation strategy, APACHE III score, PaO_2/FiO_2, creatinine, platelet count	Day 0 following inclusion
Forel 2015[82]	Prospective cohort	Multicentre	Berlin <200mmHg	ICU	51	NR (for ICU)	60±13	40 (78.4%)	Lung injury score	Day 3
Forel 2018[83]	Prospective cohort	Single centre	Berlin <200mmHg	60-day	62	21	59 ± 15	47 (75,8%)	Gender, SOFA score, LIS score	Day 3 following onset of ARDS

Table 2 – Study Characteristics for ARDS mortality (*continued*)

Study	Study design	Setting	ARDS definition	Outcome	Total (n)	Non-survivors (n)	Age	Gender Male n (%)	Variables in multivariate analysis	Sample moment
Guervilly 2011[84]	Prospective cohort	Single centre	AECC	28 day	52	21	58±17	39 (75.0%)	Not specified	Within 24 hours following diagnosis
Kim 2019[85]	Retrospective cohort	Single centre	Berlin	In-hospital	97	63	67,2 (64,3-70,1)	63 (64,3%)	APACHE II score, SOFA score, SAPS II score	Within 48 hours following admission
Lee 2019[86]	Retrospective cohort	Single centre	Berlin	In-hospital	237	154	69 (61-74 IQR)	166 (70,0%)	Age, diabetes mellitus, non-pulmonary source, APACHE II score, SOFA	Within 24 hours following intubation
Lesur 2006[87]	Prospective cohort	Multicentre	AECC	28 day	78	29	63±16	48 (61.5%)	Age, $PaCO_2$, APACHE II score	Within 48 hours following onset of ARDS
Li 2019[88]	Retrospective cohort	Single centre	Berlin	28 day	224	70	64 (46-77 IQR)	140 (62.5%)	APACHE II score, age, gender, BMI, smoking status, alcohol abusing status, risk factors, comorbidities	Within 24 hours following ICU admission
Lin 2010[89]	Prospective cohort	Single centre	AECC ARDS	28 day	63	27	75 (57-83 IQR)	38 (60.3%)	Age, lung injury score, SOFA score, APACHE II score, CRP, biomarkers	Within 24 hours following ARDS onset
Lin 2012[90]	Prospective cohort	Single centre	AECC	30 day	87	27	61 (56-70 IQR)	42 (48.3%)	APACHE II, Lung injury score, creatinine, biomarkers	At inclusion
Lin 2013[91]	Prospective cohort	Single centre	AECC	30 days	78	22	63 (54-68 IQR)	45 (57.7%)	Age, APACHE II score, Lung injury score, PaO_2/FiO_2	Within 10 hours following diagnosis
Madtes 1998[92]	Prospective Cohort	Single centre	***	In hospital	74	33	38 (19-68 Range)	50 (67.6%)	Age, PCP III levels, neutrophils, lung injury score	Day 3 following ARDS onset
McClintock 2006[93]	RCT (ARMA)	Multicentre	AECC	Mortality	579	NR	51±17	333 (57.5%)	Ventilator group assignment	Day 0 following inclusion

Table 2 – Study Characteristics for ARDS mortality (*continued*)

Study	Study design	Setting	ARDS definition	Outcome	Total (n)	Non-survivors (n)	Age	Gender Male n (%)	Variables in multivariate analysis	Sample moment
McClintock 2007[94]	RCT (ARMA)	Multicentre	AECC	Mortality	576	NR	52±17	328 (56.9%)	Gender, ventilator group assignment, eGFR, age, APACHE III score, vasopressor use, sepsis	Day 0 following inclusion
McClintock 2008[95]	Prospective cohort	Two centre	AECC	In hospital	50	21	55±16	28 (56.0%)	Age, gender, SAPS II	Within 48 hours following diagnosis
Menk 2018[96]	Retrospective cohort	Single centre	Berlin	ICU	404	182	50 (37-61 IQR)	265 (65.6%)	Age, gender, APACHE II score, SOFA, severe ARDS, peak airway pressure, pulmonary compliance	Within 24hours following admission
Metkus 2017[97]	RCT (ALVEOLI, FACTT)	Multicentre	AECC	60 day	1057	NR	50.4	549 (51.9%)	Age, gender, trial group assignment	Within 24 hours following inclusion
Mrozek 2016[98]	Prospective cohort	Multicentre	AECC	90 day	119	42	57±17	82 (68.9%)	Age, gender, SAPS II score, PaO_2/FiO_2, sepsis	Within 24 hours following inclusion
Ong 2010[99]	Prospective cohort	Two centre	AECC	28-day in-hospital	24	NR	51±21	30 (53.6%)	Age, gender, PaO_2/FiO_2, tidal volume, plateau pressure, APACHE II score	At inclusion
Parsons 2005[100]	RCT (ARMA)	Multicentre	AECC	180 days or discharge	562	196	NR	NR	Ventilation strategy, APACHE III score, PaO_2/FiO_2, creatinine, platelet count, vasopressor use	At inclusion
Parsons 2005[101]	RCT (ARMA)	Multicentre	AECC	In hospital	781	276	51.6±17.3	319 (40.1%)	Ventilation strategy, APACHE III score, PaO_2/FiO_2, creatinine, platelet count, vasopressor use	Day 0

Table 2 – Study Characteristics for ARDS mortality (*continued*)

Study	Study design	Setting	ARDS definition	Outcome	Total (n)	Non-survivors (n)	Age	Gender Male n (%)	Variables in multivariate analysis	Sample moment
Quesnel 2012[102]	Prospective cohort	Single centre	AECC	28 day	92	37	67 (49-74 IQR)	61 (66.3%)	Age, SAPS II score, malignancy, SOFA score, BAL characteristics	NR
Rahmel 2018[103]	Retrospective cohort	Single centre	AECC	30 day	119	37	43.7±13.3	71 (59.7%)	Age, SOFA score	Within 24 hours following admission
Reddy 2019[104]	Prospective cohort	Single centre	Berlin	30-day	39	19	55 (47,5-61,5)	25 (64,1%)	Not specified	Within 24 hours of ARDS diagnosis
Rivara 2012[105]	Prospective cohort	Single centre	AECC	60 day	177	70	71.5 (59-80 IQR)	98 (55.4%)	APACHE III score	Within 48 hours following diagnosis
Rogers 2019[26]	RCT (SAILS)	Multicentre	AECC	60-day	683	NR	56 (43-65)	335 (49.0%)	Age, race, APACHE III score, GFR, randomization, shock	Within 48 hours following ARDS diagnosis
Sapru 2015[33]	RCT (FACTT)	Multicentre	AECC	60 day	449	109	49.8±15.6	242 (53.9%)	Age, gender, APACHE III score, pulmonary sepsis, fluid management strategy	Upon inclusion
Suratt 2009[106]	RCT (ARMA)	Multicentre	AECC	In hospital	645	222	51±17	381 (59.1%)	Ventilation strategy, age, gender	Day 0
Tang 2014[107]	Prospective cohort	Multicentre	Berlin	In hospital	42	20	72.5±10.8	27 (64.3%)	APACHE II score, PaO_2/FiO_2, CRP, WBC, procalcitonin	Within 24 hours following diagnosis
Tsangaris 2009[108]	Prospective cohort	Single centre	AECC	28 day	52	27	66.1±16.9	32 (59.6%)	APACHE II score, age, genotype	Within 48 hours following admission
Tsangaris 2017[109]	Prospective cohort	Single centre	NR	28 day	53	28	64.6±16.8	33 (62.3%)	Lung injury score	Within 48 hours following diagnosis

Table 2 – Study Characteristics for ARDS mortality (*continued*)

Study	Study design	Setting	ARDS definition	Outcome	Total (n)	Non-survivors (n)	Age	Gender Male n (%)	Variables in multivariate analysis	Sample moment
Tsantes 2013[110]	Prospective cohort	Single centre	AECC	28 day	69	34	64.4±17.9	43 (62.3%)	Age, gender, APACHE II score, SOFA score, pulmonary parameters, serum lactate	Within 48 hours following diagnosis
Tseng 2014[30]	Prospective cohort	Single centre	AECC ARDS	ICU	56	16	70.6±9.2	31 (55.4%)	APACHE II score, SOFA score, SAPS II score	Day 1 following ICU admission
Wang 2017[31]	Prospective cohort	Multicentre	Berlin	60 day	167	62	76.5 (19-95 range)	112 (67.1%)	Age, gender, APACHE II score	Day 1 following diagnosis
Wang 2018[111]	Retrospective cohort	Single centre	AECC	Mortality	247	146	62 (48-73 IQR)	162 (65.6%)	Age, cirrhosis, creatinine, PaO_2/FiO_2	Within 24 hours following diagnosis
Ware 2004[112]	RCT (ARMA)	Multicentre	AECC	In hospital	559	193	51±17	332 (59.4%)	Ventilator strategy, APACHE III score, PaO_2/FiO_2, creatinine, platelet count	Day 0 of inclusion
Xu 2017[113]	Retrospective cohort	Single centre	Berlin	28 day	63	27	54 (42-67 IQR)	37 (58.7%)	APACHE II score, PaO_2/FiO_2, procalcitonin	Within 48 following admission
				Total[†]	15344	3914				
						36.0%				

***** Respiratory failure requiring positive pressure ventilation, PF ratio < 200 mmHg, bilateral pulmonary infiltration on chest x-ray, no clinical evidence of left atrial hypertension.

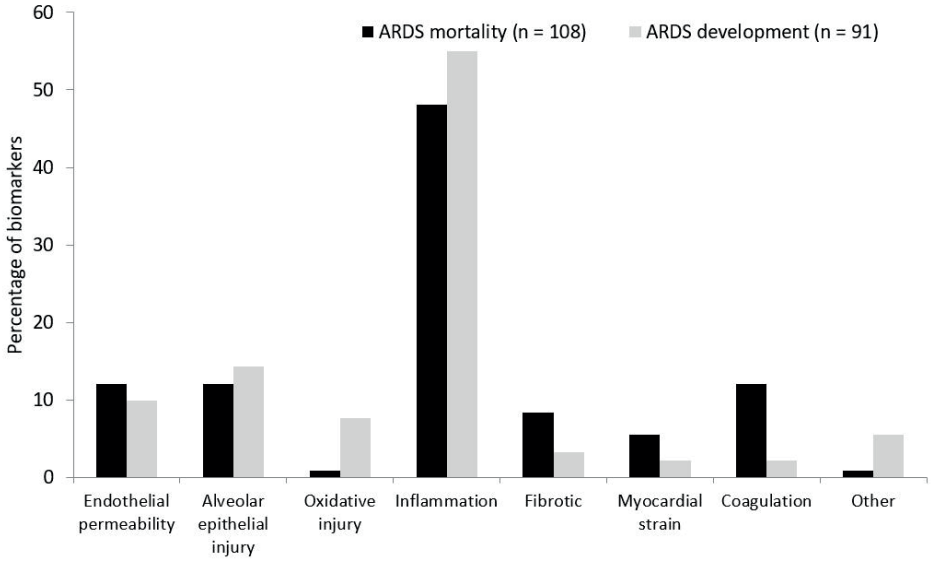
** PF ratio < 150mmHg, PF < 200mmHg with 5 cmH2O PEEP, diffuse parenchymal infiltrates, pulmonary artery wedge pressure < 18 mmHg, no clinical evidence of congestive heart failure.

*** PF ratio < 150mmHg, PF ratio < 200mmHg with 5 cmH2O PEEP, diffuse parenchymal infiltrates, pulmonary artery wedge pressure < 18 mmHg or no clinical evidence of congestive heart failure.

[†] Some studies included patients from the same cohort.

Abbreviations: AECC American European Consensus Conference definition of ARDS, APACHE acute physiology and chronic health evaluation, ARDS acute respiratory distress syndrome, BAL bronchoalveolar lavage, BMI body mass index, CRP C-reactive protein, CVVHD continuous veno-venous haemodialysis, DNR do not resuscitate, ECMO extra corporeal membrane oxygenation, eGFR estimated glomerular filtration rate, FiO_2 fraction of inspired oxygen, ICU intensive care unit, PCP procollagen, No. number, SAPS simplified acute physiology score, SOFA sequential organ failure assessment, WBC white blood cell count.

Figure 2. Biomarker role in ARDS pathophysiology



Biomarkers associated with ARDS development in the at-risk population

A total of 37 biomarkers in plasma, 7 in cerebrospinal fluid, and 1 in bronchoalveolar lavage fluid were assessed in multivariate analyses (**Table 3**). Five studies examined angiopoeitin-2 (Ang-2) and seven studies examined receptor for advanced glycation end products (RAGE). In all studies high plasma levels of Ang-2 and RAGE were significantly associated with an increased risk of ARDS development in the at-risk population. Similar results were seen for surfactant protein-D (SpD) in plasma in all three studies that assessed SpD. In contrast, biomarkers for inflammation as C-reactive protein (CRP), procalcitonin, interleukin-6 and interleukin-8 were not clearly associated with ARDS development. The majority of biomarkers in plasma are surrogates for inflammation in ARDS pathophysiology (**Figure 2**).

Biomarkers associated with mortality in the ARDS population

A total of 49 biomarkers in plasma, 8 in bronchoalveolar lavage fluid, and 3 in urine were included in this study (**Table 4**). Ang-2, CRP, interleukin-8 (IL-8), RAGE, SpD, and Von Willebrand factor (VWF) in plasma were assessed in four or more studies. However, none of these biomarkers was associated with ARDS mortality in all four studies. Similarly to biomarkers in ARDS development, the majority of biomarkers for ARDS mortality in plasma had a pathophysiological role in inflammation (**Figure 2)**. The majority of biomarkers measured in bronchoalveolar lavage fluid had a pro-fibrotic role in ARDS pathophysiology.

Table 3 – Risk ratios for ARDS development in the at-risk population

Biomarkers in plasma	Reference	Biomarker role in ARDS	Sample size	Risk ratio (95% CI)	Cut off	Comment
Adiponectin	Palakshappa 2016[59]	Anti-inflammatory	163	1.12 (1.01-1.25)	per 5mcg/mL	
Angiopoietin-2	Agrawal 2013[34]	Increased endothelial permeability	167	1.8 (1.0-3.4)	per log10	
Angiopoietin-2	Fremont 2010[43]	Increased endothelial permeability	192	2.20 (1.19–4.05)	highest vs lowest quartile	
Angiopoietin-2	Reilly 2018[60]	Increased endothelial permeability	703	1.49 (1.20-1.77)	per log increase	
Angiopoietin-2	Ware 2017[65]	Increased endothelial permeability	393	1.890 (1.322- 2.702)	1st vs 4th quartile	
Angiopoietin-2	Xu 2018[66]	Increased endothelial permeability	158	1.258 (1.137-1.392)		
Advanced oxidant protein products	Du 2016[41]	Oxidative injury	70	1.164 (1.068-1.269)		
Brain natriuretic peptide	Fremont 2010[43]	Myocardial strain	192	0.45 (0.26–0.77)	highest vs lowest quartile	
Brain natriuretic peptide	Komiya 2011[51]	Myocardial strain	124	14.425 (4.382 - 47.483)	> 500 pg/mL	Outcome is CPE
Club cell secretory protein	Jensen 2016[49]	Alveolar epithelial injury	405	2.6 (0.7 - 9.7)	≥ 42.8 ng/mL	Learning cohort
Club cell secretory protein	Jensen 2016[49]	Alveolar epithelial injury	353	0.96 (0.20 - 4.5)	≥ 42.8 ng/mL	Validating cohort
Club cell secretory protein	Lin 2017[53]	Alveolar epithelial injury	212	1.096 (1.085-1.162)		
C reactive protein (CRP)	Bai 2018[39]	Inflammation	384	1.314 (0.620-1.603)		
C reactive protein (CRP)	Chen 2019[40]	Inflammation	115	0.994 (0.978-1.010)		
C reactive protein (CRP)	Huang 2019[46]	Inflammation	152	1.287 (0.295-5.606)	≥ 90.3 mg/L	
C reactive protein (CRP)	Huang 2019[47]	Inflammation	1933	1.008 (1.007-1.010)		
C reactive protein (CRP)	Komiya 2011[51]	Inflammation	124	0.106 (0.035 - 0.323)	> 50 mg/L	Outcome is CPE
C reactive protein (CRP)	Lin 2017[53]	Inflammation	212	1.007 (1.001-1.014)		
C reactive protein (CRP)	Osaka 2011[58]	Inflammation	27	1.029 (0.829-1.293)	per 1mg/dL increase	
C reactive protein (CRP)	Wang 2019[64]	Inflammation	109	1.000 (0.992-1.008)		
C reactive protein (CRP)	Ying 2019[68]	Inflammation	145	1.22 (0.95- 1.68)		
free 2-chlorofatty acid	Meyer 2017[56]	Oxidative injury	198	1.62 (1.25- 2.09)	per log10	

Table 3 – Risk ratios for ARDS development in the at-risk population (*continued*)

Biomarkers in plasma	Reference	Biomarker role in ARDS	Sample size	Risk ratio (95% CI)	Cut off	Comment
total 2-chlorofatty acid	Meyer 2017[56]	Oxidative injury	198	1.82 (1.32- 2.52)	per log10	
free 2-chlorostearic acid	Meyer 2017[56]	Oxidative injury	198	1.82 (1.41- 2.37)	per log10	
total 2-chlorostearic acid	Meyer 2017[56]	Oxidative injury	198	1.78 (1.31- 2.43)	per log10	
Endocan	Gaudet 2018[44]	Leukocyte adhesion inhibition	72	0.001 (0- 0.215)	> 5.36ng/mL	
Endocan	Mikkelsen 2012[57]	Leukocyte adhesion inhibition	48	0.69 (0.49-0.97)	1 unit increase	
Endocan	Ying 2019[68]	Leukocyte adhesion modulation	145	1.57 (1.14-2.25)		
Fibrinogen	Luo 2017[55]	Coagulation	157	1.893 (1.141- 3.142)		
Glutamate	Bai 2017[38]	Non-essential amino acid, neurotransmitter	50	2.229 (1.082 - 2.634)		
Glutamate	Bai 2017[38]	Non-essential amino acid, neurotransmitter	42	0.996 (0.965 - 1.028)		
Glutamate	Bai 2018[39]	Non-essential amino acid	384	3.022 (2.001-4.043)		
Growth arrest specific gene 6	Yeh 2017[67]	Endothelial activation	129	1.6 (1.3- 2.6)		
Insulin like growth factor 1	Ahasic 2012[35]	Pro-fibrotic	531	0.58 (0.42–0.79)	per log10	
IGF Binding Protein 3	Ahasic 2012[35]	Pro-fibrotic	531	0.57 (0.40–0.81)	per log10	
Interleukin-1 beta	Aisiku 2016[36]	Pro-inflammatory	194	0.98 (0.73–1.32)		
Interleukin-1 beta	Chen 2019[40]	Pro-inflammatory	115	1.001 (0.945-1.061)		
Interleukin-1 beta	Huang 2019[46]	Pro-inflammatory	152	0.666 (0.152-2.910)	≥ 11.3 pg/mL	
Interleukin-1 beta	Wang 2019[64]	Pro-inflammatory	109	1.021 (0.982-1.063)		
Interleukin-6	Aisiku 2016[36]	Pro-inflammatory	195	1.24 (1.05–1.49)		
Interleukin-6	Bai 2018[39]	Pro-inflammatory	384	1.194 (0.806-1.364)		
Interleukin-6	Chen 2019[40]	Pro-inflammatory	115	0.998 (0.993-1.003)		
Interleukin-6	Huang 2019[46]	Pro-inflammatory	152	0.512 (0.156-1.678)	≥ 63.7 pg/mL	
Interleukin-6	Yeh 2017[67]	Pro-inflammatory	129	1.4 (0.98-1.7)		

Table 3 – Risk ratios for ARDS development in the at-risk population (*continued*)

Biomarkers in plasma	Reference	Biomarker role in ARDS	Sample size	Risk ratio (95% CI)	Cut off	Comment
Interleukin-8	Agrawal 2013[34]	Pro-inflammatory	167	1.3 (0.97–1.8)	per log10	
Interleukin-8	Aisiku 2016[36]	Pro-inflammatory	194	1.26 (1.04–1.53)		
Interleukin-8	Chen 2019[40]	Pro-inflammatory	115	1.000 (0.996-1.003)		
Interleukin-8	Fremont 2010[43]	Pro-inflammatory	192	1.81 (1.03–3.17)	highest vs lowest quartile	
Interleukin-8	Liu 2017[54]	Pro-inflammatory	134	1.4 (0.98- 1.7)	per log10	
Interleukin-8	Yeh 2017[67]	Pro-inflammatory	129	1.4 (0.92- 1.7)		
Interleukin-10	Aisiku 2016[36]	Anti-inflammatory	195	1.66 (1.22–2.26)		
Interleukin-10	Chen 2019[40]	Anti-inflammatory	115	1.003 (0.998-1.018)		
Interleukin-10	Fremont 2010[43]	Anti-inflammatory	192	2.02 (0.96–4.25)	highest vs lowest quartile	
Interleukin-12p70	Aisiku 2016[36]	Pro-inflammatory	194	1.18 (0.82–1.69)		
Interleukin-17	Chen 2019[40]	Pro-inflammatory	115	1.003 (1.000-1.007)		Not significant
Interleukin-17	Huang 2019[46]	Pro-inflammatory	152	0.644 (0.173-2.405)	≥ 144.55 pg/mL	
Interleukin-17	Wang 2019[64]	Pro-inflammatory	109	1.001 (0.997-1.004)		
Leukotriene B4	Amat 2000[37]	Pro-inflammatory	35	14.3 (2.3 - 88.8)	> 14 pmol/mL	
Microparticles	Shaver 2017[62]	Coagulation	280	0.693 (0.490-0.980)	per 10 microM	
mitochondrial DNA	Faust 2020[42]	Damage associated molecular pattern	224	1.58 (1.14-2.19)		48 hour plasma
mitochondrial DNA	Faust 2020[42]	Damage associated molecular pattern	120	1.52 (1.12-2.06)	per log copies per microliter	48 hour plasma
Myeloperoxidase	Meyer 2017[56]	Pro-inflammatory	198	1.28 (0.89- 1.84)	per log10	
Nitric oxide	Aisiku 2016[36]	Oxidative injury	193	1.60 (0.98–2.90)		
Parkinson disease 7	Liu 2017[54]	Anti-oxidative injury	134	1.8 (1.1- 3.5)	per log10	
Pre B-cell colony enhancing factor	Lee 2011[52]	Pro-inflammatory	113	0.78 (0.43 - 1.41)	per 10 fold increase	
Procalcitonin	Bai 2018[39]	Inflammation	384	1.156 (0.844-1.133)		

Table 3 – Risk ratios for ARDS development in the at-risk population (*continued*)

Biomarkers in plasma	Reference	Biomarker role in ARDS	Sample size	Risk ratio (95% CI)	Cut off	Comment
Procalcitonin	Chen 2019[40]	Inflammation	115	1.020 (0.966-1.077)		
Procalcitonin	Huang 2019[46]	Inflammation	152	2.506 (0.705-8.913)	≥ 13.2 ng/mL	
Procalcitonin	Huang 2019[47]	Inflammation	1933	1.008 (1.000 - 1.016)		Not significant
Procalcitonin	Wang 2019[64]	Inflammation	109	1.019 (0.981-1.058)		
Procollagen III	Fremont 2010[43]	Pro-fibrotic	192	2.90 (1.61–5.23)	highest vs lowest quartile	
Receptor for advanced glycation end products	Fremont 2010[43]	Alveolar epithelial injury	192	3.33 (1.85–5.99)	highest vs lowest quartile	
Receptor for advanced glycation end products	Jabaudon 2018[48]	Alveolar epithelial injury	464	2.25 (1.60- 3.16)	per log10	Baseline
Receptor for advanced glycation end products	Jabaudon 2018[48]	Alveolar epithelial injury	464	4.33 (2.85- 6.56)	per log10	Day 1
Receptor for advanced glycation end products	Jones 2020[50]	Alveolar epithelial injury	672	1.73 (1.35-2.21)		European ancestry
Receptor for advanced glycation end products	Jones 2020[50]	Alveolar epithelial injury	672	2.05 (1.50-2.83)		African ancestry
Receptor for advanced glycation end products	Jones 2020[50]	Alveolar epithelial injury	843	2.56 (2.14-3.06)		European ancestry
Receptor for advanced glycation end products	Ware 2017[65]	Alveolar epithelial injury	393	2.382 (1.638- 3.464)	1st vs 4th quartile	
Receptor interacting protein kinase -3	Shashaty 2019[61]	Increased endothelial permeability	120	1.30 (1.03-1.63)	per 0.5 SD	
Receptor interacting protein kinase -3	Shashaty 2019[61]	Increased endothelial permeability	180	1.83 (1.35-2.48)	per 0.5 SD	
Soluble endothelial selectin	Osaka 2011[58]	Pro-inflammatory	27	1.099 (1.012-1.260)	per 1 ng/mL increase	
Soluble urokinase plasminogen activator receptor	Chen 2019[40]	Pro-inflammatory	115	1.131 (1.002-1.277)		

Table 3 – Risk ratios for ARDS development in the at-risk population (*continued*)

Biomarkers in plasma	Reference	Biomarker role in ARDS	Sample size	Risk ratio (95% CI)	Cut off	Comment
Surfactant protein D	Jensen 2016[49]	Alveolar epithelial injury	405	3.4 (1.0 - 11.4)	≥ 525.6 ng/mL	Learning cohort
Surfactant protein D	Jensen 2016[49]	Alveolar epithelial injury	353	8.4 (2.0 - 35.4)	≥ 525.6 ng/mL	Validating cohort
Surfactant protein D	Suzuki 2017[63]	Alveolar epithelial injury	68	5.31 (1.40-20.15)	per log10	
Tissue inhibitor of matrix metalloproteinase 3	Hendrickson 2018[45]	Decreases endothelial permeability	182	1.4 (1.0-2.0)	1 SD increase	
Tumour necrosis factor alpha	Aisiku 2016[36]	Pro-inflammatory	195	1.03 (0.71–1.51)		
Tumour necrosis factor alpha	Chen 2019[40]	Pro-inflammatory	115	1.002 (0.996-1.009)		
Tumour necrosis factor alpha	Fremont 2010[43]	Pro-inflammatory	192	0.51 (0.27–0.98)	highest vs lowest quartile	
Tumour necrosis factor alpha	Huang 2019[46]	Pro-inflammatory	152	3.999 (0.921-17.375)	≥ 173.0 pg/mL	
Tumour necrosis factor alpha	Wang 2019[64]	Pro-inflammatory	109	1.000 (0.995-1.005)		
Biomarkers in CSF						
Interleukin-1 beta	Aisiku 2016[36]	Pro-inflammatory	174	1.11 (0.80–1.54)		
Interleukin-6	Aisiku 2016[36]	Pro-inflammatory	174	1.06 (0.95–1.19)		
Interleukin-8	Aisiku 2016[36]	Pro-inflammatory	173	1.01 (0.92–1.12)		
Interleukin-10	Aisiku 2016[36]	Anti-inflammatory	174	1.33 (1.00–1.76)		
Interleukin-12p70	Aisiku 2016[36]	Pro-inflammatory	173	1.52 (1.04–2.21)		
Nitric oxide	Aisiku 2016[36]	Oxidative injury	172	1.66 (0.70–3.97)		
Tumour necrosis factor alpha	Aisiku 2016[36]	Pro-inflammatory	174	1.43 (0.97–2.14)		
Biomarkers in BALF						
Soluble trombomodulin	Suzuki 2017[63]	Endothelial injury	68	7.48 (1.60-34.98)		

Abbreviations: CPE cardiopulmonary effusion, CSF cerebrospinal fluid, BALF bronchoalveolar lavage fluid, SD standard deviation.

Table 4 – Risk ratios for ARDS mortality in the ARDS population

Biomarkers in plasma	Reference	Biomarker role in ARDS	Sample size	Risk ratio (95% CI)	Cut off	Comment
Activin-A	Kim 2019[85]	Pro-fibrotic	97	2,64 (1,04-6,70)		
Angiopoietin-1 / Angiopoietin-2 ratio	Ong 2010[99]	Modulates endothelial permeability	24	5.52 (1.22-24.9)		
Angiopoietin-2	Calfee 2012[75]	Increased endothelial permeability	931	0.92 (0.73–1.16)	per log10	Infection-related ALI
Angiopoietin-2	Calfee 2012[75]	Increased endothelial permeability	931	1.94 (1.15–3.25)	per log10	Noninfection-related ALI
Angiopoietin-2	Calfee 2015[76]	Increased endothelial permeability	100	2.54 (1.38-4.68)	per log10	Single centre
Angiopoietin-2	Calfee 2015[76]	Increased endothelial permeability	853	1.43 (1.19-1.73)	per log10	Multicentre
Angiotensin 1-9	Reddy 2019[104]	Pro-fibrotic	39	2,24 (1,15-4,39)	Concentration doubled (in Ln)	
Angiotensin 1-10	Reddy 2019[104]	Pro-fibrotic	39	0,36 (0,18-0,72)	Concentration doubled (in Ln)	
Angiotensin converting enzyme	Tsantes 2013[110]	Endothelial permeability, pro-fibrotic	69	1.06 (1.02-1.10)	per 1 unit increase	28 day mortality
Angiotensin converting enzyme	Tsantes 2013[110]	Endothelial permeability, pro-fibrotic	69	1.04 (1.01-1.07)	per 1 unit increase	90 day mortality
NT-pro Brain natriuretic peptide	Bajwa 2008[70]	Myocardial strain	177	2.36 (1.11–4.99)	≥6813 ng/L	
NT- pro Brain natriuretic peptide	Lin 2012[90]	Myocardial strain	87	2.18 (1.54-4.46)	per unit	
Club cell secretory protein	Cartin-Ceba 2015[77]	Alveolar epithelial injury	100	1.09 (0.60-2.02)	per log10	
Club cell secretory protein	Lesur 2006[87]	Alveolar epithelial injury	78	1.37 (1.25-1.83)	Increments of 0.5	
Copeptin	Lin 2012[90]	Osmo-regulatory	87	4.72 (2.48-7.16)	per unit	
C reactive protein (CRP)	Adamzik 2013[69]	Inflammation	47	1.01 (0.9–1.1)	per log10	
C reactive protein (CRP)	Bajwa 2009[71]	Inflammation	177	0.67 (0.52–0.87)	per log10	
C reactive protein (CRP)	Lin 2010[89]	Inflammation	63	2.316 (0.652 - 8.226)		

Table 4 – Risk ratios for ARDS mortality in the ARDS population (*continued*)

Biomarkers in plasma	Reference	Biomarker role in ARDS	Sample size	Risk ratio (95% CI)	Cut off	Comment
C reactive protein (CRP)	Tseng 2014[30]	Inflammation	56	1.265 (0.798-2.005)		Day 3
D-dimer	Tseng 2014[30]	Coagulation	56	1.211 (0.818-1.793)		
Decoy receptor 3	Chen 2009[78]	Immunomodulation	59	4.02 (1.20–13.52)	＞1 ng/ml	Validation cohort
Endocan	Tang 2014[107]	Leukocyte adhesion inhibition	42	1.374 (1.150-1.641)	> 4.96 ng/mL	
Endocan	Tsangaris 2017[109]	Leukocyte adhesion inhibition	53	3.36 (0.74-15.31)	> 13 ng/mL	
Galectin 3	Xu 2017[113]	Pro-fibrotic	63	1.002 (0.978-1.029)	per 1ng/mL	
Granulocyte colony stimulating factor	Suratt 2009[106]	Inflammation	645	1.70 (1.06-2.75)	Quartile 4 vs. Quartile 2	
Growth differentiation factor-15	Clark 2013[80]	Pro-fibrotic	400	2.86 (1.84-4.54)	per log10	
Heparin binding protein	Lin 2013[91]	Inflammation, endothelial permeability	78	1.52 (1.12-2.85)	per log10	
High mobility group protein B1	Tseng 2014[30]	Pro-inflammatory	56	1.002 (1.000-1.004)		Day 1
High mobility group protein B1	Tseng 2014[30]	Pro-inflammatory	56	0.990 (0.968-1.013)		Day 3
Insulin like growth factor	Ahasic 2012[35]	Pro-fibrotic	175	0.70 (0.51–0.95)	per log10	
IGF Binding protein 3	Ahasic 2012[35]	Pro-fibrotic	175	0.69 (0.50–0.94)	per log10	
Intercellular adhesion molecule-1	Calfee 2009[73]	Pro-inflammatory	778	1.22 (0.99–1.49)	per log10	
Intercellular adhesion molecule-1	Calfee 2011[74]	Pro-inflammatory	547	0.74 (0.59–0.95)	per natural log	
Intercellular adhesion molecule-1	McClintock 2008[95]	Pro-inflammatory	50	5.8 (1.1-30.0)	per natural log	
Interleukin-1 beta	Lin 2010[89]	Pro-inflammatory	63	1.355 (0.357-5.140)	per log 10	
Interleukin-6	Calfee 2015[76]	Pro-inflammatory	100	1.81 (1.34-2.45)	per log10	Single centre
Interleukin-6	Calfee 2015[76]	Pro-inflammatory	853	1.24 (1.14-1.35)	per log10	Multicentre
Interleukin-6	Parsons 2005[101]	Pro-inflammatory	781	1.18 (0.93-1.49)	per log10	
Interleukin-8	Amat 2000[37]	Pro-inflammatory	21	0.09 (0.01-1.35)	> 150 pg/mL	
Interleukin-8	Calfee 2011[74]	Pro-inflammatory	547	1.36 (1.15–1.62)	per natural log	

Table 4 – Risk ratios for ARDS mortality in the ARDS population (*continued*)

Biomarkers in plasma	Reference	Biomarker role in ARDS	Sample size	Risk ratio (95% CI)	Cut off	Comment
Interleukin-8	Calfee 2015[76]	Pro-inflammatory	100	1.65 (1.25-2.17)	per log10	Single centre
Interleukin-8	Calfee 2015[76]	Pro-inflammatory	853	1.41 (1.27-1.57)	per log10	Multicentre
Interleukin-8	Cartin-Ceba 2015[77]	Pro-inflammatory	100	1.08 (0.72-1.61)	per log10	
Interleukin-8	Lin 2010[89]	Pro-inflammatory	63	0.935 (0.280-3.114)	per log 10	
Interleukin-8	McClintock 2008[95]	Pro-inflammatory	50	2.0 (1.1-4.0)	per natural log	
Interleukin-8	Parsons 2005[101]	Pro-inflammatory	780	1.73 (1.28-2.34)	per log10	
Interleukin-8	Tseng 2014[30]	Pro-inflammatory	56	1.039 (0.955-1.130)		Day 1
Interleukin-8	Tseng 2014[30]	Pro-inflammatory	56	1.075 (0.940-1.229)		Day 3
Interleukin-10	Parsons 2005[101]	Anti-inflammatory	593	1.23 (0.86-1.76)	per log10	
Interleukin-18	Dolinay 2012[25]	Pro-inflammatory	28	1.60 (1.17-2.20)	per 500pg/mL increase	
Interleukin-18	Rogers 2019[26]	Pro-inflammatory	683	2,2 (1,5-3,1)	≥ 800 pg/mL	
Leukocyte micro particles	Guervilly 2011[84]	Immunomodulation	52	5.26 (1.10 - 24.99)	< 60 elements / µL	
Leukotriene B4	Amat 2000[37]	Pro-inflammatory	21	22.5 (1.1- 460.5)	> 14 pmol/mL	
Neutrophil elastase	Wang 2017[31]	Pro-inflammatory	167	1.76 (p-value 0.002)	1 SD change	Day 1
Neutrophil elastase	Wang 2017[31]	Pro-inflammatory	167	1.58 (p-value 0.06)	1 SD change	Day 3
Neutrophil elastase	Wang 2017[31]	Pro-inflammatory	167	1.70 (p-value 0.001)	1 SD change	Day 7
Neutrophil to lymphocyte ratio	Li 2019[88]	Pro-inflammatory	224	5.815 (1.824 - 18.533)	first - fourth quartile	
Neutrophil to lymphocyte ratio	Wang 2018[111]	Pro-inflammatory	247	1.011 (1.004- 1.017)	per 1% increase	
Neutrophil to lymphocyte ratio	Wang 2018[111]	Pro-inflammatory	247	1.532 (1.095- 2.143)	>14	
Nucleated red blood cells	Menk 2018[96]	Erythrocyte progenitor cell, pro-inflammatory	404	3.21 (1.93- 5.35)	> 220/µL	
Peptidase inhibitor 3	Wang 2017[31]	Anti-inflammatory	167	0.50 (p-value 0.003)	1 SD change	Day 1
Peptidase inhibitor 3	Wang 2017[31]	Anti-inflammatory	167	0.43 (p-value 0.001)	1 SD change	Day 3

Table 4 – Risk ratios for ARDS mortality in the ARDS population (*continued*)

Biomarkers in plasma	Reference	Biomarker role in ARDS	Sample size	Risk ratio (95% CI)	Cut off	Comment
Peptidase inhibitor 3	Wang 2017[31]	Anti-inflammatory	167	0.70 (p-value 0.18)	1 SD change	Day 7
Plasminogen activator inhibitor 1	Cartin-Ceba 2015[77]	Coagulation	100	0.96 (0.62-1.47)	per log10	
Plasminogen activator inhibitor 1 (activity)	Tsangaris 2009[108]	Coagulation	52	1.30 (0.84-1.99)	per 1 unit increase	
Procalcitonin	Adamzik 2013[69]	Inflammation	47	1.01 (0.025–1.2)	per log10	
Procalcitonin	Rahmel 2018[103]	Inflammation	119	0.999 (0.998- 1.001)		
Protein C	McClintock 2008[95]	Coagulation	50	0.5 (0.2-1.0)	per natural log	
Protein C	Tsangaris 2017[109]	Coagulation	53	3.58 (0.73-15.54)	< 41.5 mg/dL	
Receptor for advanced glycation end products	Calfee 2008[72]	Alveolar epithelial injury	676	1.41(1.12–1.78)	per log10	Tidal volume 12 ml/kg
Receptor for advanced glycation end products	Calfee 2008[72]	Alveolar epithelial injury	676	1.03 (0.81–1.31)	per log10	Tidal volume 6 ml/kg
Receptor for advanced glycation end products	Calfee 2015[76]	Alveolar epithelial injury	100	1.98 (1.18-3.33)	per log10	Single centre
Receptor for advanced glycation end products	Calfee 2015[76]	Alveolar epithelial injury	853	1.16 (1.003-1.34)	per log10	Multicentre
Receptor for advanced glycation end products	Cartin-Ceba 2015[77]	Alveolar epithelial injury	100	0.81 (0.50-1.30)	per log10	
Receptor for advanced glycation end products	Mrozek 2016[98]	Alveolar epithelial injury	119	3.1 (1.1-8.9)	-	
Soluble suppression of tumorigenicity-2	Bajwa 2013[32]	Myocardial strain and inflammation	826	1.47 (0.99–2.20)	≥534 ng/mL (day 0)	Day 0
Soluble suppression of tumorigenicity-2	Bajwa 2013[32]	Myocardial strain and inflammation	826	2.94 (2.00–4.33)	≥296 ng/mL (day 3)	Day 3
Soluble triggering receptor expressed on myeloid cells-1	Lin 2010[89]	Pro-inflammatory	63	6.338 (1.607 - 24.998)	per log 10	

Table 4 – Risk ratios for ARDS mortality in the ARDS population (*continued*)

Biomarkers in plasma	Reference	Biomarker role in ARDS	Sample size	Risk ratio (95% CI)	Cut off	Comment
Surfactant protein-A	Eisner 2003[81]	Alveolar epithelial injury	565	0.92 (0.68-1.27)	per 100 ng/ml increment	
Surfactant protein-D	Calfee 2011[74]	Alveolar epithelial injury	547	1.55 (1.27–1.88)	per natural log	
Surfactant protein-D	Calfee 2015[76]	Alveolar epithelial injury	100	1.33 (0.82-2.14)	per log10	Single centre
Surfactant protein-D	Calfee 2015[76]	Alveolar epithelial injury	853	1.09 (0.95-1.24)	per log10	Multicentre
Surfactant protein-D	Eisner 2003[81]	Alveolar epithelial injury	565	1.21 (1.08-1.35)	per 100 ng/ml increment	
Thrombin–antithrombin III complex	Cartin-Ceba 2015[77]	Coagulation	100	1.05 (0.53-2.05)	per log10	
High sensitivity Troponin I	Metkus 2017[97]	Myocardial injury	1057	0.94 (0.64- 1.39)	1st 5th quintile	
Cardiac troponin T	Rivara 2012[105]	Myocardial injury	177	1.44 (1.14-1.81)	per 1ng/mL increase	
Trombomodulin	Sapru 2015[33]	Coagulation	449	2.40 (1.52-3.83)	per log10	Day 0
Trombomodulin	Sapru 2015[33]	Coagulation	449	2.80 (1.69-4.66)	per log10	Day 3
Tumour necrosis factor alpha	Lin 2010[89]	Pro-inflammatory	63	3.691 (0.668-20.998)	per log 10	
Tumour necrosis factor receptor-1	Calfee 2011[74]	Pro-inflammatory	547	1.58 (1.20–2.09)	per natural log	
Tumour necrosis factor receptor-1	Parsons 2005[100]	Pro-inflammatory	562	5.76 (2.63-12.6)	per log10	
Tumour necrosis factor receptor-2	Parsons 2005[100]	Pro-inflammatory	376	2.58 (1.05-6.31)	per log10	
Uric acid	Lee 2019[86]	Antioxidant	237	0,549 (0,293-1,030)	≥ 3,00 mg/dL	
Von Willebrand factor	Calfee 2011[74]	Endothelial activation, coagulation	547	1.57 (1.16– 2.12)	per natural log	
Von Willebrand factor	Calfee 2012[75]	Endothelial activation, coagulation	931	1.51 (1.20–1.90)	per log10	
Von Willebrand factor	Calfee 2015[76]	Endothelial activation, coagulation	853	1.83 (1.46-2.30)	per log10	Multicentre
Von Willebrand factor	Cartin-Ceba 2015[77]	Endothelial activation, coagulation	100	2.93 (0.90-10.7)	per log10	
Von Willebrand factor	Ware 2004[112]	Endothelial activation, coagulation	559	1.6 (1.4 - 2.1)	per SD increment	

Table 4 – Risk ratios for ARDS mortality in the ARDS population (*continued*)

Biomarkers in plasma	Reference	Biomarker role in ARDS	Sample size	Risk ratio (95% CI)	Cut off	Comment
Biomarkers in BALF						
Angiopoietin-2	Tsangaris 2017[109]	Increased endothelial permeability	53	11.18 (1.06-117.48)	> 705 pg/mL	
Fibrocyte percentage	Quesnel 2012[102]	Pro-fibrotic	92	6.15 (2.78-13.64)	>6%	
Plasminogen activator inhibitor 1 (activity)	Tsangaris 2009[108]	Coagulation	52	0.37 (0.06-2.35)	per 1 unit increase	
Procollagen III	Clark 1995[79]	Pro-fibrotic	117	3.6 (1.2-10.7)	≥1.75 U/mL	
Procollagen III	Forel 2015[82]	Pro-fibrotic	51	5.02 (2.06–12.25)	≥9 µg/L	
Transforming growth factor alpha	Madtes 1998[92]	Pro-fibrotic	74	2.3 (0.7- 7.0)	> 1.08 pg/mL	
Transforming growth factor beta 1	Forel 2018[83]	Pro-fibrotic	62	1,003 (0,986-1,019)		
T regulatory cell/CD4+ lymphocyte ratio	Adamzik 2013[69]	Immunomodulation	47	6.5 (1.7–25)	≥7.4 %	
Biomarkers in urine						
Desmosine-to-creatinine ratio	McClintock 2006[93]	Alveolar epithelial injury (elastin breakdown)	579	1.36 (1.02-1.82)	per log10	
Nitric oxide	McClintock 2007[94]	Oxidative injury	576	0.33 (0.20-0.54)	per log10	
Nitric oxide-to-creatinine ratio	McClintock 2007[94]	Oxidative injury	576	0.43 (0.28-0.66)	per log10	

Abbreviations: ALI acute lung injury, BALF bronchoalveolar lavage fluid, SD standard deviation.

DISCUSSION

In the current systematic review, we present a synopsis of biomarkers for ARDS development and mortality tested in multivariate analyses. We did not perform a meta-analysis because of severe data heterogeneity between studies. Upon qualitative inspection, we found that high levels of Ang-2 and RAGE were associated with ARDS development in the at-risk population. None of the biomarkers assessed in four or more studies was associated with an increased mortality rate in all studies. The majority of plasma biomarkers for both ARDS development and mortality are surrogates for inflammation in ARDS pathophysiology.

Previously Terpstra et al.[19] calculated univariate ORs from absolute biomarker concentrations and performed a meta-analysis. They found that 12 biomarkers in plasma were associated with mortality in patients with ARDS. However, a major limitation of their meta-analysis is that these biomarkers were tested in univariate analyses without considering confounders as disease severity scores. Given the high univariate ORs as compared to the multivariate ORs found in this systematic review, the performance of these biomarkers is likely to be overestimated. Jabaudon et al.[23] found in an individual patient data meta-analysis that high concentrations of plasma RAGE were associated with 90-day mortality independent of driving pressure or tidal volume. However, they could not correct for disease severity score as these differed between studies. Unfortunately, we were unable to perform a meta-analysis on multivariate data because of heterogeneity of the included studies, as transformation of raw data, biomarker concentration cut-offs, time until outcome and the variables used in the multivariate analyses varied widely between studies. This could be an incentive to standardize the presentation of ARDS biomarker research in terms of statistics and outcome for future analyses or to make individual patient data accessible.

ARDS biomarkers are presumed to reflect the pathophysiology of ARDS, characterized by alveolar-capillary membrane injury, high permeability alveolar oedema, and migration of inflammatory cells.[3] Previously, Terpstra et al.[19] proposed that biomarkers for ARDS development were correlated with alveolar tissue injury, whereas biomarkers for ARDS mortality correlated more with inflammation. In this systematic review, we found that the majority of biomarkers tested for both ARDS development and mortality were surrogates for inflammation. However, following qualitative inspection, biomarkers for inflammation were not evidently associated with either ARDS development or mortality. In contrast, markers for alveolar epithelial injury (plasma RAGE and SpD) and endothelial permeability (plasma Ang-2), seem to be associated with ARDS development. Therefore, we should consider how we intend to use (a set of) biomarkers in patients with ARDS.

A biomarker for ARDS development should be specific for ARDS, i.e. a biomarker that reflects alveolar injury or alveolar-capillary injury. Half of plasma biomarkers for ARDS development included in this study reflected inflammation. An increase in inflammatory biomarkers is known to correlate with increased disease severity scores.[24-26] In turn, the majority of studies in this review found significantly higher disease severity scores in the critically ill patients that eventually developed ARDS. Thus, plasma biomarkers for inflammation rather represented an estimation of disease severity and its associated increased risk for the development of ARDS. In addition, biomarkers for inflammation in plasma lack the specificity to diagnose ARDS, as they are unlikely to differentiate sepsis with ARDS from sepsis without ARDS. In contrast, locally sampled biomarkers for inflammation, for example in the alveolar space, could potentially diagnose ARDS.[27] Biomarkers used for ARDS mortality or for the identification of less heterogeneous ARDS phenotypes do not require to be ARDS specific, provided that they adequately predict or stratify patients with ARDS.

The heterogeneity of ARDS has been recognized as a major contributor to the negative randomized controlled trial results among patients with ARDS.[11] Therefore, it is necessary to identify homogeneous ARDS phenotypes that are more likely to respond to an intervention. This is known as predictive enrichment.[28] Previously patients with ARDS have been successfully stratified based on clinical parameters, such as ARDS risk factor (pulmonary or extra-pulmonary) or PaO_2/FiO_2 ratio.[29] ARDS biomarkers could be used to stratify patients with ARDS based on biological or pathophysiological phenotype. For example, trials of novel therapies designed to influence vascular permeability may benefit from preferentially enrolling patients with high Ang-2 concentrations. Recently, clinical parameters have been combined with a set of biomarkers in a retrospective latent class analysis. In three trials, two distinct phenotypes were found: hyperinflammatory and hypoinflammatory ARDS.[16, 17] Patients with the hyperinflammatory phenotype had reduced mortality rate with higher positive end-expiratory pressures and with liberal fluid treatment, whereas the trials themselves found no difference between the entire intervention groups. The next step is to validate the identification of ARDS phenotypes based on latent class analysis in prospective studies. An adequate combination of biomarkers and clinical parameters remains to be established. Until now, there is no list of biomarkers that are associated with ARDS development or mortality independently of clinical parameters. This systematic review may guide the selection of ARDS biomarkers used for predictive enrichment.

This systematic review has limitations. First, the intent of this systematic review was to perform a meta-analysis. However, we decided not to perform a meta-analysis, as the biomarker data handling and outcomes varied widely among studies, and pooling

would have resulted in a non-informative estimate.[21] Arguably, this is a positive result, as it refrains us from focusing on the few biomarkers that could be pooled in a meta-analysis and guides us into a direction were multiple biomarkers combined with other parameters are of interest. In a heterogeneous syndrome as ARDS, the one biomarker probably does not exist. Second, the first sampling moment varied between sampling at ICU admission until 72 hours following ICU admission. Initially, ARDS is characterized by an exudative phase followed by a second proliferative phase and late fibrotic phase.[3] The moment of sampling likely influences biomarker concentrations, as both alveolar membrane injury and inflammation increase during the exudative phase. This is also seen in six biomarkers that have been measured at separate days, resulting in a significant change in adjusted OR for four biomarkers (**Table 4**).[30-33] Third, the aim of this systematic review was to assess the independent risk effects of biomarkers measured in various bodily fluid compartments. However, the majority of studies assessed biomarkers in plasma. It remains to be answered whether other bodily fluid compartments, for example from the airways and alveolar space themselves, might outperform ARDS biomarkers in plasma, especially for ARDS development. Fourth, all studies found in this systematic review used a clinical definition of ARDS as standard for ARDS diagnosis. Given the poor correlation between a clinical diagnosis and a histopathological diagnosis of ARDS, these studies are diagnosing a very heterogeneous disease syndrome.[7-10] In order to actually evaluate ARDS development, biomarkers should be compared to a histopathological image of DAD, although acquiring histology poses great challenges by itself. Fifth, as only biomarkers assessed in multivariate analyses were included in this study, new promising biomarkers evaluated in univariate analyses were excluded from this study. Lastly, non-significant biomarkers in multivariate analyses were more likely not to be reported, although some studies report non-significant results nonetheless.

CONCLUSION

In here, we present a list of biomarkers for ARDS mortality and ARDS development tested in multivariate analyses. In multiple studies that assessed Ang-2 and RAGE, high plasma levels were associated with an increased risk of ARDS development. We did not find a biomarker that independently predicted mortality in all studies that assessed the biomarker. Furthermore, biomarker data reporting and variables used in multivariate analyses differed greatly between studies. Taken together, we should look for a combination of biomarkers and clinical parameters in a structured approach in order to find more homogeneous ARDS phenotypes. This systematic review may guide the selection of ARDS biomarkers for ARDS phenotyping.

LIST OF ABBREVIATIONS:

AECC	American European Consensus Conference
Ang-2	angiopoeitin-2
ARDS	acute respiratory distress syndrome
CRP	C-reactive protein
DAD	diffuse alveolar damage
IL-8	interleukin-8
NOS	Newcastle-Ottawa Scale
OR	odds ratio
RAGE	receptor for advanced glycation end products
SpD	surfactant protein-D
VWF	Von Willebrand factor

ACKNOWLEDGEMENTS:

We thank Wan-Jie Gu (abbreviated in the text as WG) for his support in study eligibility evaluation (Nanjing University, China).

We thank Wichor Bramer and Elise Krabbendam (Biomedical Information Specialists Medical Library Erasmus MC) for their support in the literature search.

REFERENCES

1. Maca J, Jor O, Holub M, Sklienka P, Bursa F, Burda M, Janout V, Sevcik P: **Past and Present ARDS Mortality Rates: A Systematic Review**. *Respiratory care* 2017, **62**(1):113-122.
2. Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, Gattinoni L, van Haren F, Larsson A, McAuley DF *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**(8):788-800.
3. Thompson BT, Chambers RC, Liu KD: **Acute Respiratory Distress Syndrome**. *The New England journal of medicine* 2017, **377**(19):1904-1905.
4. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R: **The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination**. *American journal of respiratory and critical care medicine* 1994, **149**(3 Pt 1):818-824.
5. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS: **Acute respiratory distress syndrome: the Berlin Definition**. *Jama* 2012, **307**(23):2526-2533.
6. Tomashefski JF, Jr.: **Pulmonary pathology of acute respiratory distress syndrome**. *ClinChest Med* 2000, **21**(3):435-466.
7. de Hemptinne Q, Remmelink M, Brimioulle S, Salmon I, Vincent JL: **ARDS: a clinicopathological confrontation**. *Chest* 2009, **135**(4):944-949.
8. Kao KC, Hu HC, Chang CH, Hung CY, Chiu LC, Li SH, Lin SW, Chuang LP, Wang CW, Li LF *et al*: **Diffuse alveolar damage associated mortality in selected acute respiratory distress syndrome patients with open lung biopsy**. *Crit Care* 2015, **19**:228.
9. Lorente JA, Cardinal-Fernandez P, Munoz D, Frutos-Vivar F, Thille AW, Jaramillo C, Ballen-Barragan A, Rodriguez JM, Penuelas O, Ortiz G *et al*: **Acute respiratory distress syndrome in patients with and without diffuse alveolar damage: an autopsy study**. *Intensive care medicine* 2015, **41**(11):1921-1930.
10. Thille AW, Esteban A, Fernandez-Segoviano P, Rodriguez JM, Aramburu JA, Penuelas O, Cortes-Puch I, Cardinal-Fernandez P, Lorente JA, Frutos-Vivar F: **Comparison of the Berlin definition for acute respiratory distress syndrome with autopsy**. *American journal of respiratory and critical care medicine* 2013, **187**(7):761-767.
11. Matthay MA, McAuley DF, Ware LB: **Clinical trials in acute respiratory distress syndrome: challenges and opportunities**. *The Lancet Respiratory medicine* 2017, **5**(6):524-534.
12. Garcia-Laorden MI, Lorente JA, Flores C, Slutsky AS, Villar J: **Biomarkers for the acute respiratory distress syndrome: how to make the diagnosis more precise**. *Annals of translational medicine* 2017, **5**(14):283.
13. Villar J, Slutsky AS: **GOLDEN anniversary of the acute respiratory distress syndrome: still much work to do!** *Current opinion in critical care* 2017, **23**(1):4-9.
14. Frohlich S, Murphy N, Boylan JF: **ARDS: progress unlikely with non-biological definition**. *British journal of anaesthesia* 2013, **111**(5):696-699.
15. Ware LB, Koyama T, Billheimer DD, Wu W, Bernard GR, Thompson BT, Brower RG, Standiford TJ, Martin TR, Matthay MA *et al*: **Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury**. *Chest* 2010, **137**(2):288-296.
16. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA, Network NA: **Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials**. *The Lancet Respiratory medicine* 2014, **2**(8):611-620.

17. Famous KR, Delucchi K, Ware LB, Kangelaris KN, Liu KD, Thompson BT, Calfee CS, Network A: **Acute Respiratory Distress Syndrome Subphenotypes Respond Differently to Randomized Fluid Management Strategy**. *American journal of respiratory and critical care medicine* 2017, **195**(3):331-338.
18. Blondonnet R, Constantin JM, Sapin V, Jabaudon M: **A Pathophysiologic Approach to Biomarkers in Acute Respiratory Distress Syndrome**. *Dis Markers* 2016, **2016**:3501373.
19. Terpstra ML, Aman J, van Nieuw Amerongen GP, Groeneveld AB: **Plasma biomarkers for acute respiratory distress syndrome: a systematic review and meta-analysis***. *Critical care medicine* 2014, **42**(3):691-700.
20. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D: **The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration**. *PLoS Med* 2009, **6**(7):e1000100.
21. Egger MS, GD., Altman DG.: **Systematic reviews in health care: meta-analysis in context, Second Edition**: BMJ Publishing Group; 2001.
22. Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P: **The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses**. *Online http://wwwohrica/programs/clinical_epidemiology/oxfordasp (cited 04-07-2018)*.
23. Jabaudon M, Blondonnet R, Pereira B, Cartin-Ceba R, Lichtenstern C, Mauri T, Determann RM, Drabek T, Hubmayr RD, Gajic O *et al*: **Plasma sRAGE is independently associated with increased mortality in ARDS: a meta-analysis of individual patient data**. *Intensive care medicine* 2018, **44**(9):1388-1399.
24. Gardlund B, Sjolin J, Nilsson A, Roll M, Wickerts CJ, Wretlind B: **Plasma levels of cytokines in primary septic shock in humans: correlation with disease severity**. *The Journal of infectious diseases* 1995, **172**(1):296-301.
25. Dolinay T, Kim YS, Howrylak J, Hunninghake GM, An CH, Fredenburgh L, Massaro AF, Rogers A, Gazourian L, Nakahira K *et al*: **Inflammasome-regulated cytokines are critical mediators of acute lung injury**. *American journal of respiratory and critical care medicine* 2012, **185**(11):1225-1234.
26. Rogers AJ, Guan J, Trtchounian A, Hunninghake GM, Kaimal R, Desai M, Kozikowski LA, DeSouza L, Mogan S, Liu KD *et al*: **Association of Elevated Plasma Interleukin-18 Level With Increased Mortality in a Clinical Trial of Statin Treatment for Acute Respiratory Distress Syndrome**. *Critical care medicine* 2019, **47**(8):1089-1096.
27. van der Zee P, van Walree I, Fijen JW, van Houte AJ, van Velzen-Blad H, Rijkers G, Gommers D, Endeman H: **Cytokines and Chemokines Are Detectable in Swivel-Derived Exhaled Breath Condensate (SEBC): A Pilot Study in Mechanically Ventilated Patients**. *Dis Markers* 2020, **2020**:2696317.
28. 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**(2):147-155.
29. Sinha P, Calfee CS: **Phenotypes in acute respiratory distress syndrome: moving towards precision medicine**. *Current opinion in critical care* 2019, **25**(1):12-20.
30. Tseng CC, Fang WF, Leung SY, Chen HC, Chang YC, Wang CC, Chang HC, Lin MC: **Impact of serum biomarkers and clinical factors on intensive care unit mortality and 6-month outcome in relatively healthy patients with severe pneumonia and acute respiratory distress syndrome**. *Dis Markers* 2014, **2014**.

31. Wang T, Zhu Z, Liu Z, Yi L, Yang Z, Bian W, Chen W, Wang S, Li G, Li A *et al*: **Plasma Neutrophil Elastase and Elafin as Prognostic Biomarker for Acute Respiratory Distress Syndrome: A Multicenter Survival and Longitudinal Prospective Observation Study**. *Shock (Augusta, Ga)* 2017, **48**(2):168-174.
32. Bajwa EK, Volk JA, Christiani DC, Harris RS, Matthay MA, Thompson BT, Januzzi JL: **Prognostic and diagnostic value of plasma soluble suppression of tumorigenicity-2 concentrations in acute respiratory distress syndrome**. *Critical care medicine* 2013, **41**(11):2521-2531.
33. Sapru A, Calfee CS, Liu KD, Kangelaris K, Hansen H, Pawlikowska L, Ware LB, Alkhouli MF, Abbot J, Matthay MA: **Plasma soluble thrombomodulin levels are associated with mortality in the acute respiratory distress syndrome**. *Intensive care medicine* 2015, **41**(3):470-478.
34. Agrawal A, Matthay MA, Kangelaris KN, Stein J, Chu JC, Imp BM, Cortez A, Abbott J, Liu KD, Calfee CS: **Plasma angiopoietin-2 predicts the onset of acute lung injury in critically ill patients**. *American journal of respiratory and critical care medicine* 2013, **187**(7):736-742.
35. Ahasic AM, Zhai R, Su L, Zhao Y, Aronis KN, Thompson BT, Mantzoros CS, Christiani DC: **IGF1 and IGFBP3 in acute respiratory distress syndrome**. *Eur J Endocrinol* 2012, **166**(1):121-129.
36. Aisiku IP, Yamal JM, Doshi P, Benoit JS, Gopinath S, Goodman JC, Robertson CS: **Plasma cytokines IL-6, IL-8, and IL-10 are associated with the development of acute respiratory distress syndrome in patients with severe traumatic brain injury**. *Crit Care* 2016, **20**(1).
37. Amat M, Barcons M, Mancebo J, Mateo J, Oliver A, Mayoral JF, Fontcuberta J, Vila L: **Evolution of leukotriene B4, peptide leukotrienes, and interleukin-8 plasma concentrations in patients at risk of acute respiratory distress syndrome and with acute respiratory distress syndrome: Mortality prognostic study**. *Critical care medicine* 2000, **28**(1):57-62.
38. Bai W, Zhu WL, Ning YL, Li P, Zhao Y, Yang N, Chen X, Jiang YL, Yang WQ, Jiang DP *et al*: **Dramatic increases in blood glutamate concentrations are closely related to traumatic brain injury-induced acute lung injury**. *Scientific reports* 2017, **7**(1):5380.
39. Bai W, Li W, Ning YL, Li P, Zhao Y, Yang N, Jiang YL, Liang ZP, Jiang DP, Wang Y *et al*: **Blood glutamate levels are closely related to acute lung injury and prognosis after stroke**. *Front Neurol* 2018, **8**(JAN).
40. Chen D, Wu X, Yang J, Yu L: **Serum plasminogen activator urokinase receptor predicts elevated risk of acute respiratory distress syndrome in patients with sepsis and is positively associated with disease severity, inflammation and mortality**. *Exp Ther Med* 2019, **18**(4):2984-2992.
41. Du SL, Ai J, Zeng XZ, Wan J, Wu X, He JX: **Plasma level of advanced oxidation protein products as a novel biomarker of acute lung injury following cardiac surgery**. *Springerplus* 2016, **5**.
42. Faust HE, Reilly JP, Anderson BJ, Ittner CAG, Forker CM, Zhang P, Weaver BA, Holena DN, Lanken PN, Christie JD *et al*: **Plasma Mitochondrial DNA Levels Are Associated With ARDS in Trauma and Sepsis Patients**. *Chest* 2020, **157**(1):67-76.
43. Fremont RD, Koyama T, Calfee CS, Wu W, Dossett LA, Bossert FR, Mitchell D, Wickersham N, Bernard GR, Matthay MA *et al*: **Acute lung injury in patients with traumatic injuries: Utility of a panel of biomarkers for diagnosis and pathogenesis**. *J Trauma Inj Infect Crit Care* 2010, **68**(5):1121-1127.
44. Gaudet A, Parmentier E, Dubucquoi S, Poissy J, Duburcq T, Lassalle P, De Freitas Caires N, Mathieu D: **Low endocan levels are predictive of Acute Respiratory Distress Syndrome in severe sepsis and septic shock**. *Journal of critical care* 2018, **47**:121-126.
45. Hendrickson CM, Gibb SL, Miyazawa BY, Keating SM, Ross E, Conroy AS, Calfee CS, Pati S, Cohen MJ: **Elevated plasma levels of TIMP-3 are associated with a higher risk of acute respiratory**

distress syndrome and death following severe isolated traumatic brain injury. *Trauma surg acute care open* 2018, **3**(1):e000171.

46. Huang X, Zhao M: **High expression of long non-coding RNA MALAT1 correlates with raised acute respiratory distress syndrome risk, disease severity, and increased mortality in septic patients**. *Int J Clin Exp Pathol* 2019, **12**(5):1877-1887.
47. Huang Y, Xiao J, Cai T, Yang L, Shi F, Wang Y, Li Y, Shi T, Li C, Peng Y *et al*: **Immature granulocytes: A novel biomarker of acute respiratory distress syndrome in patients with acute pancreatitis**. *Journal of critical care* 2019, **50**:303-308.
48. Jabaudon M, Berthelin P, Pranal T, Roszyk L, Godet T, Faure JS, Chabanne R, Eisenmann N, Lautrette A, Belville C *et al*: **Receptor for advanced glycation end-products and ARDS prediction: a multicentre observational study**. *Sci rep* 2018, **8**(1):2603.
49. Jensen JUS, Itenov TS, Thormar KM, Hein L, Mohr TT, Andersen MH, Løken J, Tousi H, Lundgren B, Boesen HC *et al*: **Prediction of non-recovery from ventilator-demanding acute respiratory failure, ARDS and death using lung damage biomarkers: data from a 1200-patient critical care randomized trial**. *Ann Intensive Care* 2016, **6**(1).
50. Jones TK, Feng R, Kerchberger VE, Reilly JP, Anderson BJ, Shashaty MGS, Wang F, Dunn TG, Riley TR, Abbott J *et al*: **Plasma sRAGE acts as a genetically regulated causal intermediate in sepsis-associated acute respiratory distress syndrome**. *American journal of respiratory and critical care medicine* 2020, **201**(1):47-56.
51. Komiya K, Ishii H, Teramoto S, Takahashi O, Eshima N, Yamaguchi O, Ebi N, Murakami J, Yamamoto H, Kadota JI: **Diagnostic utility of C-reactive Protein combined with brain natriuretic peptide in acute pulmonary edema: A cross sectional study**. *Respiratory research* 2011, **12**(1).
52. Lee KA, Gong MN: **Pre-B-cell colony-enhancing factor and its clinical correlates with acute lung injury and sepsis**. *Chest* 2011, **140**(2):382-390.
53. Lin J, Zhang W, Wang L, Tian F: **Diagnostic and prognostic values of Club cell protein 16 (CC16) in critical care patients with acute respiratory distress syndrome**. *J Clin Lab Anal* 2017.
54. Liu XW, Ma T, Cai Q, Wang L, Song HW, Liu Z: **Elevation of Serum PARK7 and IL-8 Levels Is Associated With Acute Lung Injury in Patients With Severe Sepsis/Septic Shock**. *J Intensive Care Med* 2017.
55. Luo J, Yu H, Hu YH, Liu D, Wang YW, Wang MY, Liang BM, Liang ZA: **Early identification of patients at risk for acute respiratory distress syndrome among severe pneumonia: A retrospective cohort study**. *J Thorac Dis* 2017, **9**(10):3979-3995.
56. Meyer NJ, Reilly JP, Feng R, Christie JD, Hazen SL, Albert CJ, Franke JD, Hartman CL, McHowat J, Ford DA: **Myeloperoxidase-derived 2-chlorofatty acids contribute to human sepsis mortality via acute respiratory distress syndrome**. *JCI insight* 2017, **2**(23).
57. Mikkelsen ME, Shah CV, Scherpereel A, Lanken PN, Lassalle P, Bellamy SL, Localio AR, Albelda SM, Meyer NJ, Christie JD: **Lower serum endocan levels are associated with the development of acute lung injury after major trauma**. *J Crit Care* 2012, **27**(5).
58. Osaka D, Shibata Y, Kanouchi K, Nishiwaki M, Kimura T, Kishi H, Abe S, Inoue S, Tokairin Y, Igarashi A *et al*: **Soluble endothelial selectin in acute lung injury complicated by severe pneumonia**. *Int J Med Sci* 2011, **8**(4):302-308.
59. Palakshappa JA, Anderson BJ, Reilly JP, Shashaty MGS, Ueno R, Wu Q, Ittner CAG, Tommasini A, Dunn TG, Charles D *et al*: **Low plasma levels of adiponectin do not explain acute respiratory distress syndrome risk: A prospective cohort study of patients with severe sepsis**. *Crit Care* 2016, **20**(1).

60. Reilly JP, Wang F, Jones TK, Palakshappa JA, Anderson BJ, Shashaty MGS, Dunn TG, Johansson ED, Riley TR, Lim B *et al*: **Plasma angiopoietin-2 as a potential causal marker in sepsis-associated ARDS development: evidence from Mendelian randomization and mediation analysis**. *Intensive care medicine* 2018, **44**(11):1849-1858.
61. Shashaty MGS, Reilly JP, Faust HE, Forker CM, Ittner CAG, Zhang PX, Hotz MJ, Fitzgerald D, Yang W, Anderson BJ *et al*: **Plasma receptor interacting protein kinase-3 levels are associated with acute respiratory distress syndrome in sepsis and trauma: A cohort study**. *Crit Care* 2019, **23**(1).
62. Shaver CM, Woods J, Clune JK, Grove BS, Wickersham NE, McNeil JB, Shemancik G, Ware LB, Bastarache JA: **Circulating microparticle levels are reduced in patients with ARDS**. *Crit Care* 2017, **21**(1).
63. Suzuki A, Taniguchi H, Kondoh Y, Ando M, Watanabe N, Kimura T, Kataoka K, Yokoyama T, Sakamoto K, Hasegawa Y: **Soluble thrombomodulin in bronchoalveolar lavage fluid is an independent predictor of severe drug-induced lung injury**. *Respirology* 2017, **22**(4):744-749.
64. Wang Y, Fu X, Yu B, Ai F: **Long non-coding RNA THRIL predicts increased acute respiratory distress syndrome risk and positively correlates with disease severity, inflammation, and mortality in sepsis patients**. *J Clin Lab Anal* 2019, **33**(6).
65. Ware LB, Zhao Z, Koyama T, Brown RM, Semler MW, Janz DR, May AK, Fremont RD, Matthay MA, Cohen MJ *et al*: **Derivation and validation of a two-biomarker panel for diagnosis of ARDS in patients with severe traumatic injuries**. *Trauma surg acute care open* 2017, **2**(1):e000121.
66. Xu Z, Wu GM, Li Q, Ji FY, Shi Z, Guo H, Yin JB, Zhou J, Gong L, Mei CX *et al*: **Predictive Value of Combined LIPS and ANG-2 Level in Critically Ill Patients with ARDS Risk Factors**. *Mediators of inflammation* 2018, **2018**:1739615.
67. Yeh LC, Huang PW, Hsieh KH, Wang CH, Kao YK, Lin TH, Lee XL: **Elevated plasma levels of Gas6 are associated with acute lung injury in patients with severe sepsis**. *Tohoku J Exp Med* 2017, **243**(3):187-193.
68. Ying J, Zhou D, Gu T, Huang J: **Endocan, a Risk Factor for Developing Acute Respiratory Distress Syndrome among Severe Pneumonia Patients**. *Can Respir J* 2019, **2019**.
69. Adamzik M, Broll J, Steinmann J, Westendorf AM, Rehfeld I, Kreissig C, Peters J: **An increased alveolar CD4 + CD25 + Foxp3 + T-regulatory cell ratio in acute respiratory distress syndrome is associated with increased 30-day mortality**. *Intensive care medicine* 2013, **39**(10):1743-1751.
70. Bajwa EK, Januzzi JL, Gong MN, Thompson BT, Christiani DC: **Prognostic value of plasma N-terminal probrain natriuretic peptide levels in the acute respiratory distress syndrome**. *Critical care medicine* 2008, **36**(8):2322-2327.
71. Bajwa EK, Khan UA, Januzzi JL, Gong MN, Thompson BT, Christiani DC: **Plasma C-reactive protein levels are associated with improved outcome in ARDS**. *Chest* 2009, **136**(2):471-480.
72. Calfee CS, Ware LB, Eisner MD, Parsons PE, Thompson BT, Wickersham N, Matthay MA: **Plasma receptor for advanced glycation end products and clinical outcomes in acute lung injury**. *Thorax* 2008, **63**(12):1083-1089.
73. Calfee CS, Eisner MD, Parsons PE, Thompson BT, Conner Jr ER, Matthay MA, Ware LB: **Soluble intercellular adhesion molecule-1 and clinical outcomes in patients with acute lung injury**. *Intensive care medicine* 2009, **35**(2):248-257.
74. Calfee CS, Ware LB, Glidden DV, Eisner MD, Parsons PE, Thompson BT, Matthay MA: **Use of risk reclassification with multiple biomarkers improves mortality prediction in acute lung injury**. *Critical care medicine* 2011, **39**(4):711-717.

75. Calfee CS, Gallagher D, Abbott J, Thompson BT, Matthay MA: **Plasma angiopoietin-2 in clinical acute lung injury: Prognostic and pathogenetic significance**. *Critical care medicine* 2012, **40**(6):1731-1737.
76. Calfee CS, Janz DR, Bernard GR, May AK, Kangelaris KN, Matthay MA, Ware LB: **Distinct molecular phenotypes of direct vs indirect ARDS in single-center and multicenter studies**. *Chest* 2015, **147**(6):1539-1548.
77. Cartin-Ceba R, Hubmayr RD, Qin R, Peters S, Determann RM, Schultz MJ, Gajic O: **Predictive value of plasma biomarkers for mortality and organ failure development in patients with acute respiratory distress syndrome**. *Journal of critical care* 2015, **30**(1):219.e211-219.e217.
78. Chen CY, Yang KY, Chen MY, Chen HY, Lin MT, Lee YC, Perng RP, Hsieh SL, Yang PC, Chou TY: **Decoy receptor 3 levels in peripheral blood predict outcomes of acute respiratory distress syndrome**. *American journal of respiratory and critical care medicine* 2009, **180**(8):751-760.
79. Clark JG, Milberg JA, Steinberg KP, Hudson LD: **Type III procollagen peptide in the adult respiratory distress syndrome. Association of increased peptide levels in bronchoalveolar lavage fluid with increased risk for death**. *Annals of internal medicine* 1995, **122**(1):17-23.
80. Clark BJ, Bull TM, Benson AB, Stream AR, Macht M, Gaydos J, Meadows C, Burnham EL, Moss M: **Growth differentiation factor-15 and prognosis in acute respiratory distress syndrome: A retrospective cohort study**. *Crit Care* 2013, **17**(3).
81. Eisner MD, Parsons P, Matthay MA, Ware L, Greene K: **Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury**. *Thorax* 2003, **58**(11):983-988.
82. Forel JM, Guervilly C, Hraiech S, Voillet F, Thomas G, Somma C, Secq V, Farnarier C, Payan MJ, Donati SY *et al*: **Type III procollagen is a reliable marker of ARDS-associated lung fibroproliferation**. *Intensive care medicine* 2015, **41**(1):1-11.
83. Forel JM, Guervilly C, Farnarier C, Donati SY, Hraiech S, Persico N, Allardet-Servent J, Coiffard B, Gainnier M, Loundou A *et al*: **Transforming growth factor-β1 in predicting early lung fibroproliferation in patients with acute respiratory distress syndrome**. *PloS one* 2018, **13**(11).
84. Guervilly C, Lacroix R, Forel JM, Roch A, Camoin-Jau L, Papazian L, Dignat-George F: **High levels of circulating leukocyte microparticles are associated with better outcome in acute respiratory distress syndrome**. *Crit Care* 2011, **15**(1).
85. Kim JM, Lee JK, Choi SM, Lee J, Park YS, Lee CH, Yim JJ, Yoo CG, Kim YW, Han SK *et al*: **Diagnostic and prognostic values of serum activin-a levels in patients with acute respiratory distress syndrome**. *BMC pulmonary medicine* 2019, **19**(1).
86. Lee HW, Choi SM, Lee J, Park YS, Lee CH, Yim JJ, Yoo CG, Kim YW, Han SK, Lee SM: **Serum Uric Acid Level as a Prognostic Marker in Patients With Acute Respiratory Distress Syndrome**. *J Intensive Care Med* 2019, **34**(5):404-410.
87. Lesur O, Langevin S, Berthiaume Y, Légaré M, Skrobik Y, Bellemare JF, Lévy B, Fortier Y, Lauzier F, Bravo G *et al*: **Outcome value of Clara cell protein in serum of patients with acute respiratory distress syndrome**. *Intensive care medicine* 2006, **32**(8):1167-1174.
88. Li W, Ai X, Ni Y, Ye Z, Liang Z: **The Association Between the Neutrophil-to-Lymphocyte Ratio and Mortality in Patients With Acute Respiratory Distress Syndrome: A Retrospective Cohort Study**. *Shock (Augusta, Ga)* 2019, **51**(2):161-167.
89. Lin MT, Wei YF, Ku SC, Lin CA, Ho CC, Yu CJ: **Serum soluble triggering receptor expressed on myeloid cells-1 in acute respiratory distress syndrome: A prospective observational cohort study**. *J Formos Med Assoc* 2010, **109**(11):800-809.
90. Lin Q, Fu F, Chen H, Zhu B: **Copeptin in the assessment of acute lung injury and cardiogenic pulmonary edema**. *Respiratory medicine* 2012, **106**(9):1268-1277.

91. Lin Q, Shen J, Shen L, Zhang Z, Fu F: **Increased plasma levels of heparin-binding protein in patients with acute respiratory distress syndrome**. *Crit Care* 2013, **17**(4).
92. Madtes DK, Rubenfeld G, Klima LD, Milberg JA, Steinberg KP, Martin TR, Raghu G, Hudson LD, Clark JG: **Elevated transforming growth factor-α levels in bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome**. *American journal of respiratory and critical care medicine* 1998, **158**(2):424-430.
93. McClintock DE, Starcher B, Eisner MD, Thompson BT, Hayden DL, Church GD, Matthay MA, Wiedemann HP, Arroliga AC, Fisher Jr CJ *et al*: **Higher urine desmosine levels are associated with mortality in patients with acute lung injury**. *American journal of physiology Lung cellular and molecular physiology* 2006, **291**(4):L566-L571.
94. McClintock DE, Ware LB, Eisner MD, Wickersham N, Thompson BT, Matthay MA, Wiedemann HP, Arroliga AC, Fisher Jr CJ, Komara Jr JJ *et al*: **Higher urine nitric oxide is associated with improved outcomes in patients with acute lung injury**. *American journal of respiratory and critical care medicine* 2007, **175**(3):256-262.
95. McClintock D, Zhuo H, Wickersham N, Matthay MA, Ware LB: **Biomarkers of inflammation, coagulation and fibrinolysis predict mortality in acute lung injury**. *Crit Care* 2008, **12**(2):R41.
96. Menk M, Giebelhäuser L, Vorderwülbecke G, Gassner M, Graw JA, Weiss B, Zimmermann M, Wernecke KD, Weber-Carstens S: **Nucleated red blood cells as predictors of mortality in patients with acute respiratory distress syndrome (ARDS): an observational study**. *Ann Intensive Care* 2018, **8**(1).
97. Metkus TS, Guallar E, Sokoll L, Morrow D, Tomaselli G, Brower R, Schulman S, Korley FK: **Prevalence and prognostic association of circulating troponin in the acute respiratory distress syndrome**. *Critical care medicine* 2017, **45**(10):1709-1717.
98. Mrozek S, Jabaudon M, Jaber S, Paugam-Burtz C, Lefrant JY, Rouby JJ, Asehnoune K, Allaouchiche B, Baldesi O, Leone M *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**(5):998-1007.
99. Ong T, McClintock DE, Kallet RH, Ware LB, Matthay MA, Liu KD: **Ratio of angiopoietin-2 to angiopoietin-1 as a predictor of mortality in acute lung injury patients**. *Critical care medicine* 2010, **38**(9):1845-1851.
100. Parsons PE, Matthay MA, Ware LB, Eisner MD: **Elevated plasma levels of soluble TNF receptors are associated with morbidity and mortality in patients with acute lung injury**. *American journal of physiology Lung cellular and molecular physiology* 2005, **288**(3 32-3):L426-L431.
101. Parsons PE, Eisner MD, Thompson BT, Matthay MA, Ancukiewicz M, Bernard GR, Wheeler AP: **Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury**. *Critical care medicine* 2005, **33**(1):1-6.
102. Quesnel C, Piednoir P, Gelly J, Nardelli L, Garnier M, Leçon V, Lasocki S, Bouadma L, Philip I, Elbim C *et al*: **Alveolar fibrocyte percentage is an independent predictor of poor outcome in Patients with acute lung injury**. *Critical care medicine* 2012, **40**(1):21-28.
103. Rahmel T, Rump K, Adamzik M, Peters J, Frey UH: **Increased circulating microRNA-122 is associated with mortality and acute liver injury in the acute respiratory distress syndrome**. *BMC anesthesiology* 2018, **18**(1).
104. Reddy R, Asante I, Liu S, Parikh P, Liebler J, Borok Z, Rodgers K, Baydur A, Louie SG: **Circulating angiotensin peptides levels in Acute Respiratory Distress Syndrome correlate with clinical outcomes: A pilot study**. *PloS one* 2019, **14**(3).

105. Rivara MB, Bajwa EK, Januzzi JL, Gong MN, Thompson BT, Christiani DC: **Prognostic significance of elevated cardiac troponin-T levels in acute respiratory distress syndrome patients**. *PloS one* 2012, **7**(7).
106. Suratt BT, Eisner MD, Calfee CS, Allard JB, Whittaker LA, Engelken DT, Petty JM, Trimarchi T, Gauthier L, Parsons PE: **Plasma granulocyte colony-stimulating factor levels correlate with clinical outcomes in patients with acute lung injury**. *Critical care medicine* 2009, **37**(4):1322-1328.
107. Tang L, Zhao Y, Wang D, Deng W, Li C, Li Q, Huang S, Shu C: **Endocan levels in peripheral blood predict outcomes of acute respiratory distress syndrome**. *Mediators of inflammation* 2014, **2014**.
108. Tsangaris I, Tsantes A, Bonovas S, Lignos M, Kopterides P, Gialeraki A, Rapti E, Orfanos S, Dimopoulou I, Travlou A *et al*: **The impact of the PAI-1 4G/5G polymorphism on the outcome of patients with ALI/ARDS**. *Thromb Res* 2009, **123**(6):832-836.
109. Tsangaris I, Tsantes A, Vrigkou E, Kopterides P, Pelekanou A, Zerva K, Antonakos G, Konstantonis D, Mavrou I, Tsaknis G *et al*: **Angiopoietin-2 Levels as Predictors of Outcome in Mechanically Ventilated Patients with Acute Respiratory Distress Syndrome**. *Dis Markers* 2017, **2017**.
110. Tsantes AE, Kopterides P, Bonovas S, Bagos P, Antonakos G, Nikolopoulos GK, Gialeraki A, Kapsimali V, Kyriakou E, Kokori S *et al*: **Effect of angiotensin converting enzyme gene I/D polymorphism and its expression on clinical outcome in acute respiratory distress syndrome**. *Minerva anestesiologica* 2013, **79**(8):861-870.
111. Wang Y, Ju M, Chen C, Yang D, Hou D, Tang X, Zhu X, Zhang D, Wang L, Ji S *et al*: **Neutrophil-to-lymphocyte ratio as a prognostic marker in acute respiratory distress syndrome patients: A retrospective study**. *J Thorac Dis* 2018, **10**(1):273-282.
112. Ware LB, Eisner MD, Thompson BT, Parsons PE, Matthay MA: **Significance of Von Willebrand factor in septic and nonseptic patients with acute lung injury**. *American journal of respiratory and critical care medicine* 2004, **170**(7):766-772.
113. Xu Z, Li X, Huang Y, Mao P, Wu S, Yang B, Yang Y, Chen K, Liu X, Li Y: **The predictive value of plasma galectin-3 for ards severity and clinical outcome**. *Shock (Augusta, Ga)* 2017, **47**(3):331-336.

ADDITIONAL FILE 1

Supplement Literature search

Embase.com

('respiratory distress syndrome'/exp OR ('respiratory distress'/de AND 'acute disease'/de) OR (((acute OR syndrom* OR adult* OR idiopath*) NEAR/3 respirator* NEAR/3 distress*) OR ards):ab,ti) AND (('biological marker'/de OR marker/de OR 'disease marker'/de OR (marker* OR biomarker*):ab,ti) OR (('diagnostic value'/de OR 'diagnostic accuracy'/de OR 'early diagnosis'/de OR 'prognosis'/de OR 'survival'/exp OR 'mortality'/de OR 'fatality'/de OR 'predictor variable'/de OR 'prediction'/de OR ((diagnos* NEAR/3 (value* OR earl* OR accurac* OR utilit*)) OR prognos* OR surviv* OR mortalit* OR fatal* OR predict* OR recogni*):ab,ti) AND ('blood level'/de OR 'cyclic AMP blood level'/exp OR 'enzyme blood level'/exp OR 'histamine blood level'/exp OR 'protein blood level'/exp OR 'serotonin blood level'/exp OR 'somatostatin blood level'/exp OR blood/de OR 'blood analysis'/de OR 'lung lavage'/de OR 'lavage fluid'/de OR 'bronchoalveolar lavage fluid'/de OR 'expired air'/de OR 'breath analysis'/de OR 'concentration (parameters)'/de OR 'blood sampling'/de OR (((blood OR plasma OR serum OR lavage OR expir* OR air OR exhal* OR breath) NEAR/3 (level* OR concentration* OR profile* OR evaluat* OR mediator* OR sampl* OR analy* OR test*))):ab,ti))) NOT ([animals]/lim NOT [humans]/lim) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Editorial]/lim) AND [english]/lim

Medline Ovid

(Respiratory Distress Syndrome, Adult/ OR (((acute OR syndrom* OR adult* OR idiopath*) ADJ3 respirator* ADJ3 distress*) OR ards).ab,ti.) AND ((biomarker/ OR (marker* OR biomarker*).ab,ti.) OR ((Early Diagnosis/ OR prognosis/ OR survival/ OR mortality/ OR mortality.xs. OR Fatal Outcome/ OR ((diagnos* ADJ3 (value* OR earl* OR accurac* OR utilit*)) OR prognos* OR surviv* OR mortalit* OR fatal* OR predict* OR recogni*).ab,ti.) AND (blood.xs. OR blood/ OR exp Bronchoalveolar Lavage/ OR Breath Tests/ OR Blood Specimen Collection/ OR (((blood OR plasma OR serum OR lavage OR expir* OR air OR exhal* OR breath) ADJ3 (level* OR concentration* OR profile* OR evaluat* OR mediator* OR sampl* OR analy* OR test*))).ab,ti.))) NOT (exp animals/ NOT humans/) NOT (letter OR news OR comment OR editorial OR congresses OR abstracts).pt. AND english.la.

Cochrane CENTRAL

((((acute OR syndrom* OR adult* OR idiopath*) NEAR/3 respirator* NEAR/3 distress*) OR ards):ab,ti) AND (((marker* OR biomarker*):ab,ti) OR ((((diagnos* NEAR/3 (value* OR earl* OR accurac* OR utilit*)) OR prognos* OR surviv* OR mortalit* OR fatal* OR predict*

OR recogni*):ab,ti) AND ((((blood OR plasma OR serum OR lavage OR expir* OR air OR exhal* OR breath) NEAR/3 (level* OR concentration* OR profile* OR evaluat* OR mediator* OR sampl* OR analy* OR test*))):ab,ti)))

Web of science

TS=(((((acute OR syndrom* OR adult* OR idiopath*) NEAR/2 respirator* NEAR/2 distress*) OR ards)) AND (((marker* OR biomarker*)) OR ((((diagnos* NEAR/2 (value* OR earl* OR accurac* OR utilit*)) OR prognos* OR surviv* OR mortalit* OR fatal* OR predict* OR recogni*)) AND ((((blood OR plasma OR serum OR lavage OR expir* OR air OR exhal* OR breath) NEAR/2 (level* OR concentration* OR profile* OR evaluat* OR mediator* OR sampl* OR analy* OR test*)))))) NOT ((animal* OR rat OR rats OR mouse OR mice OR murine OR dog OR dogs OR canine OR cat OR cats OR feline OR rabbit OR cow OR cows OR bovine OR rodent* OR sheep OR ovine OR pig OR swine OR porcine OR veterinar* OR chick* OR zebrafish* OR baboon* OR nonhuman* OR primate* OR cattle* OR goose OR geese OR duck OR macaque* OR avian* OR bird*) NOT (human* OR patient*))) AND DT=(article) AND LA=(english)

Google scholar

“acute|adult respiratory distress”|ards marker|markers|biomarker|biomarkers|”blood |plasma|serum|lavage|air|breath level|concentration|profile|evaluation|analysis|test” “diagnostic value|accuracy|utility”|prognosis|survival|mortality|fatal|prediction

ADDITIONAL FILE 2 QUALITY ASSESSMENT

Table E1 – Newcastle Ottawa Scale for Development of ARDS

No.	Reference	Selection - Representativeness	Selection - Selection non-exposed cohort	Selection - Ascertainment of exposure	Selection - Outcome not present	Comparability - matched for	Outcome - Assessment of outcome	Outcome - Follow-up duration	Outcome - Follow up adequacy	Total Score
1	Agrawal 2013[1]	*	*	*	-	**	*	*	*	8
2	Ahasic 2012[2]	*	-	*	-	**	*	*	*	7
3	Aisiku 2016[3]	-	*	*	-	**	*	*	*	7
4	Amat 2000[4]	*	*	*	-	*	*	*	-	6
5	Bai 2017[5]	-	*	*	-	**	*	*	*	7
5	Bai 2017[5]	-	*	*	-	**	*	*	*	7
6	Bai 2018[6]	*	*	*	-	*	*	*	*	7
7	Chen 2019[7]	*	*	-	-	**	*	-	*	6
8	Du 2016[8]	*	*	*	*	*	*	*	*	8
9	Faust 2020[9]	*	*	*	-	**	*	*	*	8
9	Faust 2020[9]	*	*	*	-	**	*	*	*	8
10	Fremont 2010[10]	*	*	*	-	*	*	*	*	7
11	Gaudet 2018[11]	*	*	*	-	*	*	*	-	6
12	Hendrickson 2018[12]	*	*	*	-	**	*	*	-	7
13	Huang 2019[13]	*	*	*	-	**	*	*	*	8
14	Huang 2019[14]	-	*	*	-	**	*	*	*	7
15	Jabaudon 2018[15]	*	*	*	-	**	*	*	*	8
16	Jensen 2016[16]	*	*	*	-	**	*	*	*	8
17	Jones 2020[17]	*	*	*	-	**	*	*	-	7
17	Jones 2020[17]	*	-	-	-	**	*	*	-	6
18	Komiya 2011[18]	*	-	-	-	*	*	-	*	4
19	Lee 2011[19]	*	*	*	-	*	*	*	-	6
20	Lin 2017[20]	*	*	*	-	**	*	*	*	8
21	Liu 2017[21]	*	*	*	-	**	*	*	-	7
22	Luo 2017[22]	*	*	*	*	**	*	*	*	9
23	Meyer 2017[23]	*	*	*	-	**	*	*	*	8
24	Mikkelsen 2012[24]	*	-	*	-	**	*	*	*	7
25	Osaka 2011[25]	-	*	*	-	*	*	*	*	6
26	Palakshappa 2016[26]	*	*	*	-	**	*	*	*	8
27	Reilly 2018[27]	*	*	*	-	**	*	*	-	7
28	Shashaty 2019[28]	*	-	*	-	**	*	*	-	6
28	Shashaty 2019[28]	*	-	*	-	**	*	*	-	6
29	Shaver 2017[29]	*	*	*	*	**	*	*	*	9

Table E1 – Newcastle Ottawa Scale for Development of ARDS (*continued*)

No.	Reference	Selection - Representativeness	Selection - Selection non-exposed cohort	Selection - Ascertainment of exposure	Selection - Outcome not present	Comparability - matched for	Outcome - Assessment of outcome	Outcome - Follow-up duration	Outcome - Follow up adequacy	Total Score
30	Suzuki 2017[30]	-	*	*	-	*	*	*	*	6
31	Wang 2019[31]	*	*	*	-	**	*	*	-	7
32	Ware 2017[32]	*	*	*	*	*	*	*	-	7
33	Xu 2018[33]	*	*	*	-	**	*	*	*	8
34	Yeh 2017[34]	*	*	*	-	**	*	*	*	8
35	Ying 2019[35]	*	*	*	-	**	*	*	*	8

Selection representativeness: patients without pre-defined subgroups; selection non-exposed cohort: cohort of patients at-risk for ARDS without selection out of a larger cohort; ascertainment of exposure: cohort study (not case-control); outcome not present: PaO_2/FiO_2 ratio presented; comparability: ** variables in multivariate analysis described, * multivariate analysis without description of variables; follow-up duration: at least 7 days, follow-up adequacy: reported no loss to follow-up or missing biomarker values.

Table E2 – Newcastle Ottawa Scale for ARDS Mortality

No.	Reference	Selection - Representativeness	Selection - Selection non-exposed cohort	Selection - Ascertainment of exposure	Selection - Outcome not present	Comparability - matched for	Outcome - Assessment of outcome	Outcome - Follow-up duration	Outcome - Follow up adequacy	Total Score
1	Adamzik 2013[36]	*	*	*	*	**	*	*	*	9
2	Ahasic 2012[2]	*	*	-	*	*	*	*	*	7
3	Amat 2000[4]	*	*	*	*	*	*	*	-	7
4	Bajwa 2008[37]	*	*	-	*	**	*	*	-	8
5	Bajwa 2009[38]	*	*	-	*	**	*	*	-	8
6	Bajwa 2013[39]	*	*	*	*	**	-	*	-	7
7	Calfee 2008[40]	*	*	*	*	**	-	*	-	7
8	Calfee 2009[41]	*	*	*	*	**	*	*	-	8
9	Calfee 2011[42]	*	*	*	*	**	*	*	-	8
10	Calfee 2012[43]	-	*	*	*	**	*	*	-	7
11	Calfee 2015[44] Single centre	*	*	*	*	**	*	-	-	7
11	Calfee 2015[44] Multi centre	*	*	*	*	**	*	*	-	8
12	Cartin-Ceba 2015[45]	*	*	*	*	**	*	-	-	7
13	Chen 2009[46] Validation cohort	*	*	*	*	**	*	*	*	9
14	Clark 1995[47]	*	*	*	*	**	*	-	-	7
15	Clark 2013[48]	*	*	*	*	*	*	*	-	7
16	Dolinay 2012[49]	*	-	-	*	**	*	-	*	6
17	Eisner 2003[50]	*	*	*	*	**	*	*	-	8
18	Forel 2015[51]	*	*	*	*	**	*	-	*	8
19	Forel 2018[52]	*	*	-	*	**	*	*	*	8
20	Guervilly 2011[53]	*	*	*	*	*	*	*	*	8
21	Kim 2019[54]	*	*	*	*	**	*	*	*	9
22	Lee 2019[55]	*	*	-	*	**	*	*	*	8
23	Lesur 2006[56]	*	*	*	*	**	*	*	*	9
24	Li 2018[57]	*	*	*	*	**	*	*	-	8
25	Lin 2010[58]	*	*	-	*	**	*	*	*	8
26	Lin 2012[59]	*	*	*	*	**	*	*	*	9
27	Lin 2013[60]	*	*	*	*	**	*	*	*	9
28	Madtes 1998[61]	*	*	*	*	*	*	-	-	6
29	McClintock 2006[62]	*	*	-	*	*	*	-	-	5
30	McClintock 2007[63]	*	*	*	*	**	*	-	-	7

Table E2 – Newcastle Ottawa Scale for ARDS Mortality *(continued)*

No.	Reference	Selection - Representativeness	Selection - Selection non-exposed cohort	Selection - Ascertainment of exposure	Selection - Outcome not present	Comparability - matched for	Outcome - Assessment of outcome	Outcome - Follow-up duration	Outcome - Follow up adequacy	Total Score
31	McClintock 2008[64]	*	*	*	*	**	*	-	*	8
32	Menk 2018[65]	*	*	*	*	**	*	-	-	7
33	Metkus 2017[66]	*	*	*	*	*	*	*	-	7
34	Mrozek 2016[67]	*	*	*	*	**	*	*	*	9
35	Ong 2010[68]	-	*	*	*	**	*	*	*	8
36	Parsons 2005[69]	*	*	-	*	**	*	*	-	7
37	Parsons 2005[70]	*	*	*	*	**	*	*	-	8
38	Quesnel 2012[71]	*	*	*	*	**	*	*	*	9
39	Rahmel 2018[72]	*	*	*	*	**	*	*	*	9
40	Reddy 2019[73]	*	*	*	*	*	*	*	*	8
41	Rivara 2012[74]	*	*	-	*	**	*	*	*	8
42	Rogers 2019[75]	*	-	*	*	**	*	*	-	7
43	Sapru 2015[76]	*	*	-	*	**	*	*	-	7
44	Suratt 2009[77]	*	*	*	*	*	*	-	-	6
45	Tang 2014[78]	*	*	*	*	**	*	-	*	8
46	Tsangaris 2009[79]	*	*	-	*	**	*	*	*	8
47	Tsangaris 2017[80]	-	*	-	*	**	*	*	*	7
48	Tsantes 2013[81]	*	*	*	*	**	*	*	*	9
49	Tseng 2014[82]	*	*	*	*	**	*	-	*	8
50	Wang 2017[83]	*	*	*	*	**	*	*	*	9
51	Wang 2018[84]	*	*	*	*	*	*	-	*	7
52	Ware 2004[85]	*	*	*	*	**	*	-	-	7
53	Xu 2017[86]	*	*	*	*	**	*	*	*	9

Selection representativeness: patients with ARDS without pre-defined subgroups; selection non-exposed cohort: cohort of patients with ARDS without selection out of a larger cohort; ascertainment of exposure: PaO_2/FiO_2 ratio presented; outcome not present: patient being alive upon inclusion; comparability: ** variables in multivariate analysis described, * multivariate analysis without description of variables; follow-up duration: at least 28 days, follow-up adequacy: reported no loss to follow-up or missing biomarker values.

REFERENCES:

1. Agrawal A, Matthay MA, Kangelaris KN, Stein J, Chu JC, Imp BM, Cortez A, Abbott J, Liu KD, Calfee CS: **Plasma angiopoietin-2 predicts the onset of acute lung injury in critically ill patients**. *American journal of respiratory and critical care medicine* 2013, **187**(7):736-742.
2. Ahasic AM, Zhai R, Su L, Zhao Y, Aronis KN, Thompson BT, Mantzoros CS, Christiani DC: **IGF1 and IGFBP3 in acute respiratory distress syndrome**. *Eur J Endocrinol* 2012, **166**(1):121-129.
3. Aisiku IP, Yamal JM, Doshi P, Benoit JS, Gopinath S, Goodman JC, Robertson CS: **Plasma cytokines IL-6, IL-8, and IL-10 are associated with the development of acute respiratory distress syndrome in patients with severe traumatic brain injury**. *Crit Care* 2016, **20**(1).
4. Amat M, Barcons M, Mancebo J, Mateo J, Oliver A, Mayoral JF, Fontcuberta J, Vila L: **Evolution of leukotriene B4, peptide leukotrienes, and interleukin-8 plasma concentrations in patients at risk of acute respiratory distress syndrome and with acute respiratory distress syndrome: Mortality prognostic study**. *Critical care medicine* 2000, **28**(1):57-62.
5. Bai W, Zhu WL, Ning YL, Li P, Zhao Y, Yang N, Chen X, Jiang YL, Yang WQ, Jiang DP *et al*: **Dramatic increases in blood glutamate concentrations are closely related to traumatic brain injury-induced acute lung injury**. *Scientific reports* 2017, **7**(1):5380.
6. Bai W, Li W, Ning YL, Li P, Zhao Y, Yang N, Jiang YL, Liang ZP, Jiang DP, Wang Y *et al*: **Blood glutamate levels are closely related to acute lung injury and prognosis after stroke**. *Front Neurol* 2018, **8**(JAN).
7. Chen D, Wu X, Yang J, Yu L: **Serum plasminogen activator urokinase receptor predicts elevated risk of acute respiratory distress syndrome in patients with sepsis and is positively associated with disease severity, inflammation and mortality**. *Exp Ther Med* 2019, **18**(4):2984-2992.
8. Du SL, Ai J, Zeng XZ, Wan J, Wu X, He JX: **Plasma level of advanced oxidation protein products as a novel biomarker of acute lung injury following cardiac surgery**. *Springerplus* 2016, **5**.
9. Faust HE, Reilly JP, Anderson BJ, Ittner CAG, Forker CM, Zhang P, Weaver BA, Holena DN, Lanken PN, Christie JD *et al*: **Plasma Mitochondrial DNA Levels Are Associated With ARDS in Trauma and Sepsis Patients**. *Chest* 2020, **157**(1):67-76.
10. Fremont RD, Koyama T, Calfee CS, Wu W, Dossett LA, Bossert FR, Mitchell D, Wickersham N, Bernard GR, Matthay MA *et al*: **Acute lung injury in patients with traumatic injuries: Utility of a panel of biomarkers for diagnosis and pathogenesis**. *J Trauma Inj Infect Crit Care* 2010, **68**(5):1121-1127.
11. Gaudet A, Parmentier E, Dubucquoi S, Poissy J, Duburcq T, Lassalle P, De Freitas Caires N, Mathieu D: **Low endocan levels are predictive of Acute Respiratory Distress Syndrome in severe sepsis and septic shock**. *Journal of critical care* 2018, **47**:121-126.
12. Hendrickson CM, Gibb SL, Miyazawa BY, Keating SM, Ross E, Conroy AS, Calfee CS, Pati S, Cohen MJ: **Elevated plasma levels of TIMP-3 are associated with a higher risk of acute respiratory distress syndrome and death following severe isolated traumatic brain injury**. *Trauma surg acute care open* 2018, **3**(1):e000171.
13. Huang X, Zhao M: **High expression of long non-coding RNA MALAT1 correlates with raised acute respiratory distress syndrome risk, disease severity, and increased mortality in septic patients**. *Int J Clin Exp Pathol* 2019, **12**(5):1877-1887.
14. Huang Y, Xiao J, Cai T, Yang L, Shi F, Wang Y, Li Y, Shi T, Li C, Peng Y *et al*: **Immature granulocytes: A novel biomarker of acute respiratory distress syndrome in patients with acute pancreatitis**. *Journal of critical care* 2019, **50**:303-308.

15. Jabaudon M, Berthelin P, Pranal T, Roszyk L, Godet T, Faure JS, Chabanne R, Eisenmann N, Lautrette A, Belville C *et al*: **Receptor for advanced glycation end-products and ARDS prediction: a multicentre observational study**. *Sci rep* 2018, **8**(1):2603.
16. Jensen JUS, Itenov TS, Thormar KM, Hein L, Mohr TT, Andersen MH, Løken J, Tousi H, Lundgren B, Boesen HC *et al*: **Prediction of non-recovery from ventilator-demanding acute respiratory failure, ARDS and death using lung damage biomarkers: data from a 1200-patient critical care randomized trial**. *Ann Intensive Care* 2016, **6**(1).
17. Jones TK, Feng R, Kerchberger VE, Reilly JP, Anderson BJ, Shashaty MGS, Wang F, Dunn TG, Riley TR, Abbott J *et al*: **Plasma sRAGE acts as a genetically regulated causal intermediate in sepsis-associated acute respiratory distress syndrome**. *American journal of respiratory and critical care medicine* 2020, **201**(1):47-56.
18. Komiya K, Ishii H, Teramoto S, Takahashi O, Eshima N, Yamaguchi O, Ebi N, Murakami J, Yamamoto H, Kadota JI: **Diagnostic utility of C-reactive Protein combined with brain natriuretic peptide in acute pulmonary edema: A cross sectional study**. *Respiratory research* 2011, **12**(1).
19. Lee KA, Gong MN: **Pre-B-cell colony-enhancing factor and its clinical correlates with acute lung injury and sepsis**. *Chest* 2011, **140**(2):382-390.
20. Lin J, Zhang W, Wang L, Tian F: **Diagnostic and prognostic values of Club cell protein 16 (CC16) in critical care patients with acute respiratory distress syndrome**. *J Clin Lab Anal* 2017.
21. Liu XW, Ma T, Cai Q, Wang L, Song HW, Liu Z: **Elevation of Serum PARK7 and IL-8 Levels Is Associated With Acute Lung Injury in Patients With Severe Sepsis/Septic Shock**. *J Intensive Care Med* 2017.
22. Luo J, Yu H, Hu YH, Liu D, Wang YW, Wang MY, Liang BM, Liang ZA: **Early identification of patients at risk for acute respiratory distress syndrome among severe pneumonia: A retrospective cohort study**. *J Thorac Dis* 2017, **9**(10):3979-3995.
23. Meyer NJ, Reilly JP, Feng R, Christie JD, Hazen SL, Albert CJ, Franke JD, Hartman CL, McHowat J, Ford DA: **Myeloperoxidase-derived 2-chlorofatty acids contribute to human sepsis mortality via acute respiratory distress syndrome**. *JCI insight* 2017, **2**(23).
24. Mikkelsen ME, Shah CV, Scherpereel A, Lanken PN, Lassalle P, Bellamy SL, Localio AR, Albelda SM, Meyer NJ, Christie JD: **Lower serum endocan levels are associated with the development of acute lung injury after major trauma**. *J Crit Care* 2012, **27**(5).
25. Osaka D, Shibata Y, Kanouchi K, Nishiwaki M, Kimura T, Kishi H, Abe S, Inoue S, Tokairin Y, Igarashi A *et al*: **Soluble endothelial selectin in acute lung injury complicated by severe pneumonia**. *Int J Med Sci* 2011, **8**(4):302-308.
26. Palakshappa JA, Anderson BJ, Reilly JP, Shashaty MGS, Ueno R, Wu Q, Ittner CAG, Tommasini A, Dunn TG, Charles D *et al*: **Low plasma levels of adiponectin do not explain acute respiratory distress syndrome risk: A prospective cohort study of patients with severe sepsis**. *Crit Care* 2016, **20**(1).
27. Reilly JP, Wang F, Jones TK, Palakshappa JA, Anderson BJ, Shashaty MGS, Dunn TG, Johansson ED, Riley TR, Lim B *et al*: **Plasma angiopoietin-2 as a potential causal marker in sepsis-associated ARDS development: evidence from Mendelian randomization and mediation analysis**. *Intensive care medicine* 2018, **44**(11):1849-1858.
28. Shashaty MGS, Reilly JP, Faust HE, Forker CM, Ittner CAG, Zhang PX, Hotz MJ, Fitzgerald D, Yang W, Anderson BJ *et al*: **Plasma receptor interacting protein kinase-3 levels are associated with acute respiratory distress syndrome in sepsis and trauma: A cohort study**. *Crit Care* 2019, **23**(1).

29. Shaver CM, Woods J, Clune JK, Grove BS, Wickersham NE, McNeil JB, Shemancik G, Ware LB, Bastarache JA: **Circulating microparticle levels are reduced in patients with ARDS**. *Crit Care* 2017, **21**(1).
30. Suzuki A, Taniguchi H, Kondoh Y, Ando M, Watanabe N, Kimura T, Kataoka K, Yokoyama T, Sakamoto K, Hasegawa Y: **Soluble thrombomodulin in bronchoalveolar lavage fluid is an independent predictor of severe drug-induced lung injury**. *Respirology* 2017, **22**(4):744-749.
31. Wang Y, Fu X, Yu B, Ai F: **Long non-coding RNA THRIL predicts increased acute respiratory distress syndrome risk and positively correlates with disease severity, inflammation, and mortality in sepsis patients**. *J Clin Lab Anal* 2019, **33**(6).
32. Ware LB, Zhao Z, Koyama T, Brown RM, Semler MW, Janz DR, May AK, Fremont RD, Matthay MA, Cohen MJ *et al*: **Derivation and validation of a two-biomarker panel for diagnosis of ARDS in patients with severe traumatic injuries**. *Trauma surg acute care open* 2017, **2**(1):e000121.
33. Xu Z, Wu GM, Li Q, Ji FY, Shi Z, Guo H, Yin JB, Zhou J, Gong L, Mei CX *et al*: **Predictive Value of Combined LIPS and ANG-2 Level in Critically Ill Patients with ARDS Risk Factors**. *Mediators of inflammation* 2018, **2018**:1739615.
34. Yeh LC, Huang PW, Hsieh KH, Wang CH, Kao YK, Lin TH, Lee XL: **Elevated plasma levels of Gas6 are associated with acute lung injury in patients with severe sepsis**. *Tohoku J Exp Med* 2017, **243**(3):187-193.
35. Ying J, Zhou D, Gu T, Huang J: **Endocan, a Risk Factor for Developing Acute Respiratory Distress Syndrome among Severe Pneumonia Patients**. *Can Respir J* 2019, **2019**.
36. Adamzik M, Broll J, Steinmann J, Westendorf AM, Rehfeld I, Kreissig C, Peters J: **An increased alveolar CD4 + CD25 + Foxp3 + T-regulatory cell ratio in acute respiratory distress syndrome is associated with increased 30-day mortality**. *Intensive care medicine* 2013, **39**(10):1743-1751.
37. Bajwa EK, Januzzi JL, Gong MN, Thompson BT, Christiani DC: **Prognostic value of plasma N-terminal probrain natriuretic peptide levels in the acute respiratory distress syndrome**. *Critical care medicine* 2008, **36**(8):2322-2327.
38. Bajwa EK, Khan UA, Januzzi JL, Gong MN, Thompson BT, Christiani DC: **Plasma C-reactive protein levels are associated with improved outcome in ARDS**. *Chest* 2009, **136**(2):471-480.
39. Bajwa EK, Volk JA, Christiani DC, Harris RS, Matthay MA, Thompson BT, Januzzi JL: **Prognostic and diagnostic value of plasma soluble suppression of tumorigenicity-2 concentrations in acute respiratory distress syndrome**. *Critical care medicine* 2013, **41**(11):2521-2531.
40. Calfee CS, Ware LB, Eisner MD, Parsons PE, Thompson BT, Wickersham N, Matthay MA: **Plasma receptor for advanced glycation end products and clinical outcomes in acute lung injury**. *Thorax* 2008, **63**(12):1083-1089.
41. Calfee CS, Eisner MD, Parsons PE, Thompson BT, Conner Jr ER, Matthay MA, Ware LB: **Soluble intercellular adhesion molecule-1 and clinical outcomes in patients with acute lung injury**. *Intensive care medicine* 2009, **35**(2):248-257.
42. Calfee CS, Ware LB, Glidden DV, Eisner MD, Parsons PE, Thompson BT, Matthay MA: **Use of risk reclassification with multiple biomarkers improves mortality prediction in acute lung injury**. *Critical care medicine* 2011, **39**(4):711-717.
43. Calfee CS, Gallagher D, Abbott J, Thompson BT, Matthay MA: **Plasma angiopoietin-2 in clinical acute lung injury: Prognostic and pathogenetic significance**. *Critical care medicine* 2012, **40**(6):1731-1737.
44. Calfee CS, Janz DR, Bernard GR, May AK, Kangelaris KN, Matthay MA, Ware LB: **Distinct molecular phenotypes of direct vs indirect ARDS in single-center and multicenter studies**. *Chest* 2015, **147**(6):1539-1548.

45. Cartin-Ceba R, Hubmayr RD, Qin R, Peters S, Determann RM, Schultz MJ, Gajic O: **Predictive value of plasma biomarkers for mortality and organ failure development in patients with acute respiratory distress syndrome**. *Journal of critical care* 2015, **30**(1):219.e211-219.e217.
46. Chen CY, Yang KY, Chen MY, Chen HY, Lin MT, Lee YC, Perng RP, Hsieh SL, Yang PC, Chou TY: **Decoy receptor 3 levels in peripheral blood predict outcomes of acute respiratory distress syndrome**. *American journal of respiratory and critical care medicine* 2009, **180**(8):751-760.
47. Clark JG, Milberg JA, Steinberg KP, Hudson LD: **Type III procollagen peptide in the adult respiratory distress syndrome. Association of increased peptide levels in bronchoalveolar lavage fluid with increased risk for death**. *Annals of internal medicine* 1995, **122**(1):17-23.
48. Clark BJ, Bull TM, Benson AB, Stream AR, Macht M, Gaydos J, Meadows C, Burnham EL, Moss M: **Growth differentiation factor-15 and prognosis in acute respiratory distress syndrome: A retrospective cohort study**. *Crit Care* 2013, **17**(3).
49. Dolinay T, Kim YS, Howrylak J, Hunninghake GM, An CH, Fredenburgh L, Massaro AF, Rogers A, Gazourian L, Nakahira K *et al*: **Inflammasome-regulated cytokines are critical mediators of acute lung injury**. *American journal of respiratory and critical care medicine* 2012, **185**(11):1225-1234.
50. Eisner MD, Parsons P, Matthay MA, Ware L, Greene K: **Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury**. *Thorax* 2003, **58**(11):983-988.
51. Forel JM, Guervilly C, Hraiech S, Voillet F, Thomas G, Somma C, Secq V, Farnarier C, Payan MJ, Donati SY *et al*: **Type III procollagen is a reliable marker of ARDS-associated lung fibroproliferation**. *Intensive care medicine* 2015, **41**(1):1-11.
52. Forel JM, Guervilly C, Farnarier C, Donati SY, Hraiech S, Persico N, Allardet-Servent J, Coiffard B, Gainnier M, Loundou A *et al*: **Transforming growth factor-β1 in predicting early lung fibroproliferation in patients with acute respiratory distress syndrome**. *PloS one* 2018, **13**(11).
53. Guervilly C, Lacroix R, Forel JM, Roch A, Camoin-Jau L, Papazian L, Dignat-George F: **High levels of circulating leukocyte microparticles are associated with better outcome in acute respiratory distress syndrome**. *Crit Care* 2011, **15**(1).
54. Kim JM, Lee JK, Choi SM, Lee J, Park YS, Lee CH, Yim JJ, Yoo CG, Kim YW, Han SK *et al*: **Diagnostic and prognostic values of serum activin-a levels in patients with acute respiratory distress syndrome**. *BMC pulmonary medicine* 2019, **19**(1).
55. Lee HW, Choi SM, Lee J, Park YS, Lee CH, Yim JJ, Yoo CG, Kim YW, Han SK, Lee SM: **Serum Uric Acid Level as a Prognostic Marker in Patients With Acute Respiratory Distress Syndrome**. *J Intensive Care Med* 2019, **34**(5):404-410.
56. Lesur O, Langevin S, Berthiaume Y, Légaré M, Skrobik Y, Bellemare JF, Lévy B, Fortier Y, Lauzier F, Bravo G *et al*: **Outcome value of Clara cell protein in serum of patients with acute respiratory distress syndrome**. *Intensive care medicine* 2006, **32**(8):1167-1174.
57. Li W, Ai X, Ni Y, Ye Z, Liang Z: **The Association Between the Neutrophil-to-Lymphocyte Ratio and Mortality in Patients With Acute Respiratory Distress Syndrome: A Retrospective Cohort Study**. *Shock (Augusta, Ga)* 2019, **51**(2):161-167.
58. Lin MT, Wei YF, Ku SC, Lin CA, Ho CC, Yu CJ: **Serum soluble triggering receptor expressed on myeloid cells-1 in acute respiratory distress syndrome: A prospective observational cohort study**. *J Formos Med Assoc* 2010, **109**(11):800-809.
59. Lin Q, Fu F, Chen H, Zhu B: **Copeptin in the assessment of acute lung injury and cardiogenic pulmonary edema**. *Respiratory medicine* 2012, **106**(9):1268-1277.
60. Lin Q, Shen J, Shen L, Zhang Z, Fu F: **Increased plasma levels of heparin-binding protein in patients with acute respiratory distress syndrome**. *Crit Care* 2013, **17**(4).

61. Madtes DK, Rubenfeld G, Klima LD, Milberg JA, Steinberg KP, Martin TR, Raghu G, Hudson LD, Clark JG: **Elevated transforming growth factor-α levels in bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome**. *American journal of respiratory and critical care medicine* 1998, **158**(2):424-430.
62. McClintock DE, Starcher B, Eisner MD, Thompson BT, Hayden DL, Church GD, Matthay MA, Wiedemann HP, Arroliga AC, Fisher Jr CJ *et al*: **Higher urine desmosine levels are associated with mortality in patients with acute lung injury**. *American journal of physiology Lung cellular and molecular physiology* 2006, **291**(4):L566-L571.
63. McClintock DE, Ware LB, Eisner MD, Wickersham N, Thompson BT, Matthay MA, Wiedemann HP, Arroliga AC, Fisher Jr CJ, Komara Jr JJ *et al*: **Higher urine nitric oxide is associated with improved outcomes in patients with acute lung injury**. *American journal of respiratory and critical care medicine* 2007, **175**(3):256-262.
64. McClintock D, Zhuo H, Wickersham N, Matthay MA, Ware LB: **Biomarkers of inflammation, coagulation and fibrinolysis predict mortality in acute lung injury**. *Crit Care* 2008, **12**(2):R41.
65. Menk M, Giebelhäuser L, Vorderwülbecke G, Gassner M, Graw JA, Weiss B, Zimmermann M, Wernecke KD, Weber-Carstens S: **Nucleated red blood cells as predictors of mortality in patients with acute respiratory distress syndrome (ARDS): an observational study**. *Ann Intensive Care* 2018, **8**(1).
66. Metkus TS, Guallar E, Sokoll L, Morrow D, Tomaselli G, Brower R, Schulman S, Korley FK: **Prevalence and prognostic association of circulating troponin in the acute respiratory distress syndrome**. *Critical care medicine* 2017, **45**(10):1709-1717.
67. Mrozek S, Jabaudon M, Jaber S, Paugam-Burtz C, Lefrant JY, Rouby JJ, Asehnoune K, Allaouchiche B, Baldesi O, Leone M *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**(5):998-1007.
68. Ong T, McClintock DE, Kallet RH, Ware LB, Matthay MA, Liu KD: **Ratio of angiopoietin-2 to angiopoietin-1 as a predictor of mortality in acute lung injury patients**. *Critical care medicine* 2010, **38**(9):1845-1851.
69. Parsons PE, Matthay MA, Ware LB, Eisner MD: **Elevated plasma levels of soluble TNF receptors are associated with morbidity and mortality in patients with acute lung injury**. *American journal of physiology Lung cellular and molecular physiology* 2005, **288**(3 32-3):L426-L431.
70. Parsons PE, Eisner MD, Thompson BT, Matthay MA, Ancukiewicz M, Bernard GR, Wheeler AP: **Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury**. *Critical care medicine* 2005, **33**(1):1-6.
71. Quesnel C, Piednoir P, Gelly J, Nardelli L, Garnier M, Leçon V, Lasocki S, Bouadma L, Philip I, Elbim C *et al*: **Alveolar fibrocyte percentage is an independent predictor of poor outcome in Patients with acute lung injury**. *Critical care medicine* 2012, **40**(1):21-28.
72. Rahmel T, Rump K, Adamzik M, Peters J, Frey UH: **Increased circulating microRNA-122 is associated with mortality and acute liver injury in the acute respiratory distress syndrome**. *BMC anesthesiology* 2018, **18**(1).
73. Reddy R, Asante I, Liu S, Parikh P, Liebler J, Borok Z, Rodgers K, Baydur A, Louie SG: **Circulating angiotensin peptides levels in Acute Respiratory Distress Syndrome correlate with clinical outcomes: A pilot study**. *PloS one* 2019, **14**(3).
74. Rivara MB, Bajwa EK, Januzzi JL, Gong MN, Thompson BT, Christiani DC: **Prognostic significance of elevated cardiac troponin-T levels in acute respiratory distress syndrome patients**. *PloS one* 2012, **7**(7).

75. Rogers AJ, Guan J, Trtchounian A, Hunninghake GM, Kaimal R, Desai M, Kozikowski LA, DeSouza L, Mogan S, Liu KD *et al*: **Association of Elevated Plasma Interleukin-18 Level With Increased Mortality in a Clinical Trial of Statin Treatment for Acute Respiratory Distress Syndrome**. *Critical care medicine* 2019, **47**(8):1089-1096.
76. Sapru A, Calfee CS, Liu KD, Kangelaris K, Hansen H, Pawlikowska L, Ware LB, Alkhouli MF, Abbot J, Matthay MA: **Plasma soluble thrombomodulin levels are associated with mortality in the acute respiratory distress syndrome**. *Intensive care medicine* 2015, **41**(3):470-478.
77. Suratt BT, Eisner MD, Calfee CS, Allard JB, Whittaker LA, Engelken DT, Petty JM, Trimarchi T, Gauthier L, Parsons PE: **Plasma granulocyte colony-stimulating factor levels correlate with clinical outcomes in patients with acute lung injury**. *Critical care medicine* 2009, **37**(4):1322-1328.
78. Tang L, Zhao Y, Wang D, Deng W, Li C, Li Q, Huang S, Shu C: **Endocan levels in peripheral blood predict outcomes of acute respiratory distress syndrome**. *Mediators of inflammation* 2014, **2014**.
79. Tsangaris I, Tsantes A, Bonovas S, Lignos M, Kopterides P, Gialeraki A, Rapti E, Orfanos S, Dimopoulou I, Travlou A *et al*: **The impact of the PAI-1 4G/5G polymorphism on the outcome of patients with ALI/ARDS**. *Thromb Res* 2009, **123**(6):832-836.
80. Tsangaris I, Tsantes A, Vrigkou E, Kopterides P, Pelekanou A, Zerva K, Antonakos G, Konstantonis D, Mavrou I, Tsaknis G *et al*: **Angiopoietin-2 Levels as Predictors of Outcome in Mechanically Ventilated Patients with Acute Respiratory Distress Syndrome**. *Dis Markers* 2017, **2017**.
81. Tsantes AE, Kopterides P, Bonovas S, Bagos P, Antonakos G, Nikolopoulos GK, Gialeraki A, Kapsimali V, Kyriakou E, Kokori S *et al*: **Effect of angiotensin converting enzyme gene I/D polymorphism and its expression on clinical outcome in acute respiratory distress syndrome**. *Minerva anestesiologica* 2013, **79**(8):861-870.
82. Tseng CC, Fang WF, Leung SY, Chen HC, Chang YC, Wang CC, Chang HC, Lin MC: **Impact of serum biomarkers and clinical factors on intensive care unit mortality and 6-month outcome in relatively healthy patients with severe pneumonia and acute respiratory distress syndrome**. *Dis Markers* 2014, **2014**.
83. Wang T, Zhu Z, Liu Z, Yi L, Yang Z, Bian W, Chen W, Wang S, Li G, Li A *et al*: **Plasma Neutrophil Elastase and Elafin as Prognostic Biomarker for Acute Respiratory Distress Syndrome: A Multicenter Survival and Longitudinal Prospective Observation Study**. *Shock (Augusta, Ga)* 2017, **48**(2):168-174.
84. Wang Y, Ju M, Chen C, Yang D, Hou D, Tang X, Zhu X, Zhang D, Wang L, Ji S *et al*: **Neutrophil-to-lymphocyte ratio as a prognostic marker in acute respiratory distress syndrome patients: A retrospective study**. *J Thorac Dis* 2018, **10**(1):273-282.
85. Ware LB, Eisner MD, Thompson BT, Parsons PE, Matthay MA: **Significance of Von Willebrand factor in septic and nonseptic patients with acute lung injury**. *American journal of respiratory and critical care medicine* 2004, **170**(7):766-772.
86. Xu Z, Li X, Huang Y, Mao P, Wu S, Yang B, Yang Y, Chen K, Liu X, Li Y: **The predictive value of plasma galectin-3 for ards severity and clinical outcome**. *Shock (Augusta, Ga)* 2017, **47**(3):331-336

3

Excessive Extracellular ATP desensitizes P2Y2 and P2X4 ATP receptors provoking surfactant impairment ending in Ventilation-Induced Lung Injury

Djo Hasan[1,2], Joshua Satalin[3], Philip van der Zee[4], Michaela Kollisch-Singule[3], Paul Blankman[5], Atsuko Shono[6], Peter Somhorst[4], Corstiaan den Uil[4,7], Han Meeder[1,4], Toru Kotani[8] and Gary F. Nieman[3]

[1] Mobile Intensive Care Unit Zuid-West Nederland, Rotterdam, The Netherlands
[2] Department of Surgery, Erasmus Medical Center Rotterdam, The Netherlands
[3] Department of Surgery, Upstate Medical University, Syracuse, USA
[4] Department of Adult Intensive Care, Erasmus Medical Center Rotterdam, The Netherlands
[5] Department of Anesthesiology, Universitair Medisch Centrum Utrecht, The Netherlands
[6] Department of Anesthesiology, Shimane University, Izumo, Japan
[7] Department of Cardiology, Erasmus Medical Center Rotterdam, The Netherlands
[8] Department of Anesthesiology and Critical Care Medicine, Showa University, Tokyo

Published: Int J Mol Sci 2018 Vol. 19 Issue 4

ABSTRACT

Stretching the alveolar epithelial type I (AT I) cells controls the intercellular signalling for the exocytosis of surfactant by the AT II cells through the extracellular release of adenosine triphosphate (ATP) (purinergic signalling). Extracellular ATP is cleared by extracellular ATPases, maintaining its homeostasis and enabling the lung to adapt the exocytosis of surfactant to the demand. Vigorous deformation of the AT I cells by high mechanical power ventilation causes a massive release of extracellular ATP beyond the clearance capacity of the extracellular ATPases. When extracellular ATP reaches levels >100 µM, the ATP receptors of the AT II cells become desensitized and surfactant impairment is initiated. The resulting alteration in viscoelastic properties and in alveolar opening and collapse time-constants leads to alveolar collapse and the redistribution of inspired air from the alveoli to the alveolar ducts, which become pathologically dilated. The collapsed alveoli connected to these dilated alveolar ducts are subject to a massive strain, exacerbating the ATP release. After reaching concentrations >300 µM extracellular ATP acts as a danger-associated molecular pattern, causing capillary leakage, alveolar space oedema, and further deactivation of surfactant by serum proteins. Decreasing the tidal volume to 6 ml/kg or less at this stage cannot prevent further lung injury.

Keywords: extracellular ATP, purinergic signalling, P2X receptors, P2Y receptors, surfactant dysfunction, ventilation-induced lung injury, innate immunity

1. INTRODUCTION

In 1929, Lohmann discovered and isolated adenosine triphosphate (ATP) from liver and muscles.[1, 2] ATP is widely known as an intracellular molecule that is able to transfer energy and is indispensable in living cells.[3, 4] Much later, extracellular ATP was identified and appeared to have a different function than the intracellularly located molecule. The intercellular signalling function of extracellular ATP has been described by Felberg and Hebb in 1948 in perfused cervical superior ganglion of the cat [5] and by Holton in 1959 in the sensory nerves of the rabbit ear.[6] In 1972 Burnstock wrote an article on the hypothesis of the purinergic co-transmission in neurons.[7] However, it took more than 20 years before the intracellular energy source ATP was recognized as an extracellular signalling molecule.[8] Now ATP is established as an important element of purinergic signalling in almost all tissues and the immune system.[9]

It is well documented that potentially lifesaving mechanical ventilation may ironically damage the lungs and increase mortality risk in patients with acute respiratory distress syndrome (ARDS) by causing ventilation-induced lung injury (VILI) [10-13] and ARDS remains a serious clinical problem with mortality close to 40%.[14] Gattinoni, et al. (2016) found that it was not just the individual components of the mechanical breath (i.e., tidal volume, respiratory rate, driving pressure and positive end expiratory pressure — PEEP) that cause VILI but rather the mechanical power that the combination of these components generate.[11] Using continuous mandatory ventilation (CMV—volume control ventilation) with a tidal volume of 38 mL/kg (corresponding to a strain of 2.5). Cressoni, et al. (2016) reported that, in piglets, lung injury cannot be provoked at a respiratory rate of ≤9 /min corresponding to a mechanical power of <12 J/min.[15] Mechanical power of the ventilator is calculated by a formula. Tidal volume, respiratory system elastance, inspiratory-to-expiratory time ratio, airway resistance, respiratory rate and PEEP are include in the equation of the formula.[11] High power mechanical ventilation is defined as any mechanical breath, which exceeds 12 J/min and corresponds to a mechanical ventilation settings with a tidal volume of >38 mL/kg ideal body weight and a respiratory rate of ≥12 / min.[15] Reportedly, intratracheal administration of 400 µL of 5.16 mM ATP in rats leads to alveolar oedema [16] and intratracheal instillation of 50 µL of 100 mM ATP or 200 mM uridine triphosphate (UTP) in mice leads to diffuse alveolar damage resembling the effect of high power mechanical ventilation.[17] Recently, we reported that injurious mechanical ventilation results in vigorous cyclic mechanical deformations of the alveolar epithelial cells resulting in massive release of extracellular ATP from the alveolar epithelial type I (AT I) cell [10]. The high levels of extracellular ATP activate a pro-inflammatory immune response of the innate immune system through purinergic signalling [18-20] which causes diffuse alveolar damage (DAD) [10, 17], the histopathology characteristic of VILI.[21]

In this report, we centred on an important component of VILI: the impairment of the pulmonary surfactant function.[22, 23] Multiple mechanisms for VILI-induced surfactant impairment in the absence of infection have been reported: (1) increased pulmonary vascular permeability resulting in pulmonary oedema with high serum proteins. These serum proteins cause disaggregation and inactivation of surfactant [24], reducing the proportion of functional large aggregates (LAs) significantly in favour of the non-functional small aggregates (SAs) [22, 23]; (2) High mechanical power ventilation may enhance the transport of alveolar surfactant into the airways.[25, 26] However, these explanations do not account for the mechanism of increased surfactant in the lung lavage in the first two hours during mechanical ventilation with high mechanical power. [22, 27] Additionally, the pathogenesis of VILI suggests a mechanism other than capillary leak as the initiating event: High mechanical power ventilation in rats causes surfactant composition changes with surfactant function impairment within one hour and a fall in lung compliance occurs within two hours [22], whereas capillary leak causing overt alveolar oedema detected by a computed tomography (CT) scan does not develop for 2–14 h [28] and histological evidence of alveolar oedema was not detected at two hours. [29]

Therefore, we searched and studied the literature elaborately to find the explanation for the increased surfactant production and the development of surfactant impairment that precede the capillary leak, lung oedema and the pro-inflammatory response of the innate immune system.

2. EXTRACELLULAR RELEASE OF ATP BY AT I AND AT II CELLS AND CLEARANCE OF EXTRACELLULAR ATP

In contrast to the relatively high (3 to 10 mM) intracellular concentrations of ATP in epithelial cells [30] the concentration of extracellular ATP in the medium around the 6HBE14o$^-$ human bronchial epithelial cells [31] and in resting conditions measured in a cell culture of rat AT II cells [32] is much lower at about 2 nM (**Figure 1A**). Mechanical deformation (tonic or cyclic stretching) during ventilation of the AT I cells activates the mechanosensitive P2X7 ATP receptors (P2X7Rs) causing a controlled extracellular release of ATP molecules (**Figure 1B**).[32, 33] In this case the P2X7Rs function as an ATP release channel [34] rather than an intrinsic cation channel or an ATP receptor initiating intracellular signal transduction (P2Y2R and P2X4R in **Figure 1B**).

Extracellular ATP molecules are converted by ATP-converting ecto-enzymes or by soluble extracellular enzymes to adenosine (**Figure 1A–C**).[10, 18] The hydrolysing enzymes

are: Nucleoside triphosphate diphosphohydrolase 1 (NTPD1 or CD39, converts ATP to ADP and ADP to AMP), nucleotide pyrophosphatase/phosphodiesterase (NPP, converts ATP to AMP) and 5′-nucleotidase (5′-NT or CD73, converts AMP to adenosine). Soluble extracellular adenosine deaminase (ADA) converts a proportion of extracellular adenosine to inosine. The remaining adenosine molecule enters the cells via the equilibrative nucleoside transporters (ENT1 and ENT2) and concentrative nucleoside transporters (CNT1 and CNT2). Intracellular adenosine is converted to inosine, hypoxanthine, and AMP by the enzymes ADA, purine nucleoside phosphorylase (PNP) and adenosine kinase (ADK). This process maintains the homeostasis of extracellular ATP in the alveolar walls (**Figure 1A–C**).[10, 18]

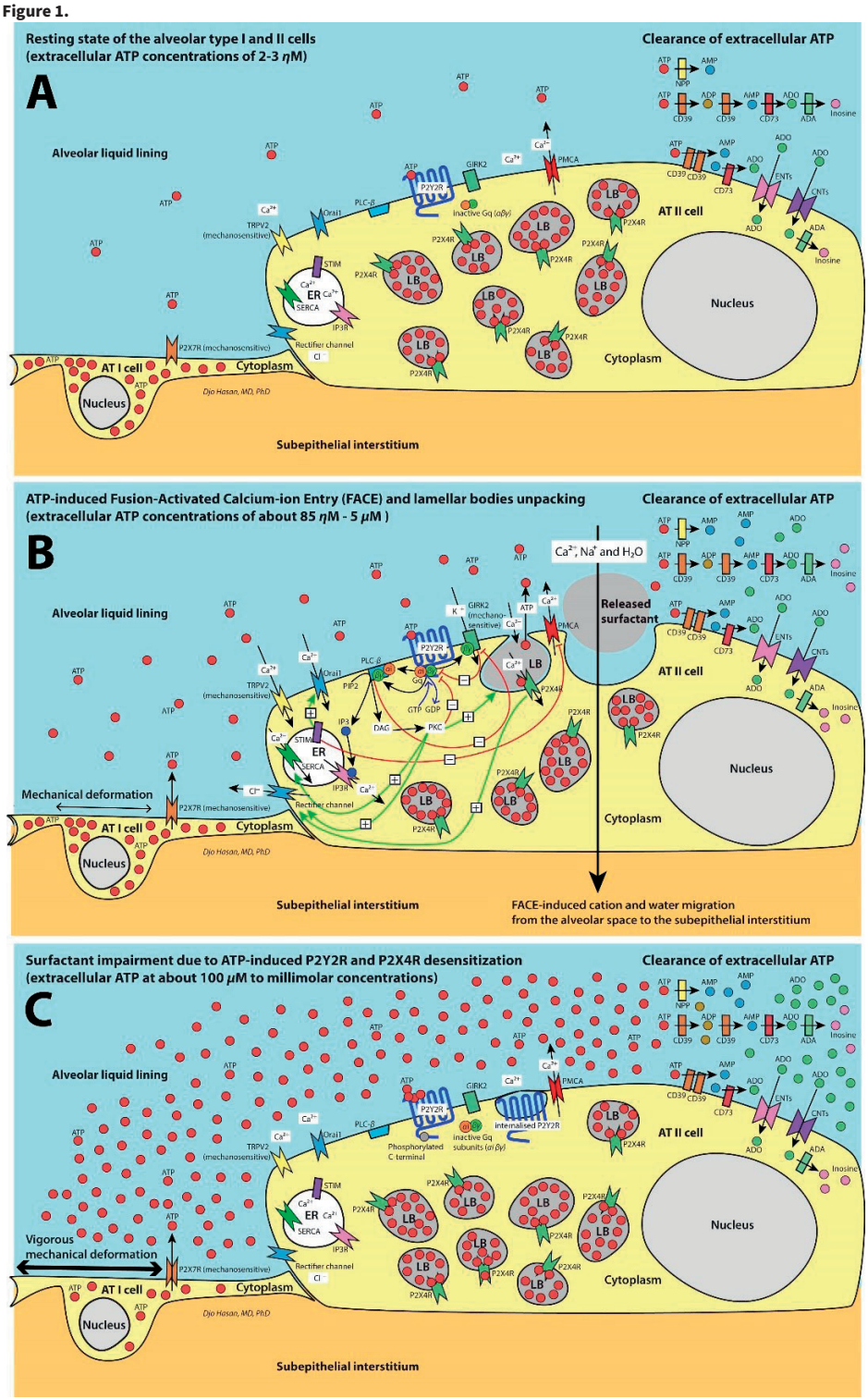
3. PURINERGIC SIGNALLING INCREASES THE AT II CYTOPLASMIC CA^{2+} LEVELS BY THE ENTRY OF EXTRACELLULAR CA^{2+} AND STORE-OPERATED CA^{2+} ENTRY (SOCE)

The extent of the extracellular release of ATP molecules by cyclic stretching of the lung is proportional to the strain (equivalent to the tidal volume), frequency and duration of the ventilation.[32, 33] When the extracellular ATP concentrations reach the half maximum effective concentration (EC_{50}) of 85 to 230 nM in human [35], ATP binds to and activates the P2Y2Rs at the AT II cell membranes (**Figure 1B**).[36] This facilitates the coupling of the Gq/11 molecule (comprising αi and βɣ subunits) to the G protein-coupled receptor (GPCR) structure of the P2Y2Rs. In the basal state, the heteromeric Gq/11 subunits are indissoluble. After coupling to the GPCR, Gq/11 subunits are activated. The activated αi subunit releases a guanosine 5′-diphosphate (GDP) molecule and binds to a guanosine 5′-triphosphate (GTP) molecule followed by the dissociation of the αi and βɣ subunits initiating intracellular signal transduction.[37] The αi and βɣ subunits activate phospholipase C beta (PLC-β) to hydrolyse phosphatidylinositol 4,5-bisphosphate (PIP2) resulting in the formation of the second messengers diacylglycerol (DAG) and inositol triphosphate (IP3).[38] IP3 binds with the IP3 receptors (IP3Rs, a membrane-bound glycoprotein complex functioning as a Ca^{2+} channel sensitive to activation by IP3) causing the release of Ca^{2+} by the endoplasmic reticulum (ER). This process is referred to as store-operated Ca^{2+} entry (SOCE). IP3Rs are important calcium release channels of SOCE.[39] The ryanodine receptor (another important SOCE Ca^{2+} release channel in skeletal muscle, smooth muscle, and cardiac muscle) is not expressed in the lung tissue.[40] SOCE causes Ca^{2+} store depletion that is sensed by the EF-hand and sterile α motif (EF-SAM) regions of Stromal interaction molecule 1 (STIM1, a calcium sensor). This information is transferred to activate the plasma membrane STIM1 Orai1-activating region/CRAC-activating domain (SOAR/CAD) regions through the cytoplasmic C-terminus

1 (CC1) regions of the STIM1 molecules located in the cytoplasm. Then the SOAR/CAD regions activate the calcium release-activated calcium channel protein 1 (Orai1) Ca^{2+} channels at the plasma membrane allowing extracellular Ca^{2+} molecules to enter the cytoplasm. STIM1 and Orai1 belong to the calcium release-activated calcium channel (CRAC) family (**Figure 1B**).[39]

Inward Ca^{2+} current is also generated though the mechanosensitive transient receptor potential cation channel subfamily V member 2 (TRPV2, a non-selective cation channel) during inspiration.[41] In addition, the Gqβɣ subunits of the activated P2Y2Rs simulate (by a direct binding) the K^+ selective inwardly rectifying channel 3 (Kir3) or G protein-coupled inwardly-rectifying K^+ channel 2 (GIRK2) expressed on the AT II cell membrane.[37] Kir3 or GIRK2 is a mechanosensitive channel and can also be activated by mechanical deformation of the AT II cells (**Figure 1B**).[42] Moreover, activation of the P2Y2Rs and the P2X4Rs induces the volume-regulated anion current channel (VRAC). One of the major components of VRAC is the outwardly rectifying Cl^- channel that is sensitive to protein kinase C (PKC) activation.[43, 44] DAG, phosphatidylserine (Ptd-Ser, a component of the AT II cell membrane) and Ca^{2+} are required for the activation of PKC. DAG strikingly increases the affinity of PKC for Ca^{2+}.[45] PKC binds with Ca^{2+} exposing a binding site for Ptd-Ser of the inner part of the cell membrane leading to a redistribution of PKC from the cytosol to the cell membrane.[45] This promotes the trafficking of the lamellar bodies (LBs), docking hemifusion and fusion of the LB membrane with the plasma membrane of the AT II cell (**Figure 2A**).[46] After the development of a fusion pore, further pore expansion is accelerated by an additional elevation of cytoplasm Ca^{2+} levels resulting in the exocytosis of surfactant. It was first thought that the additional elevation in Ca^{2+} levels is achieved by extracellular ATP molecules that reach the P2X4Rs located at the LB membrane through the newly formed fusion pore.[47, 48] But recently, it appeared that the LBs of rat AT II cells contains a high ATP level of about 1.9 mM at a low pH of 5.5.[49] ATP is transported from the cytosol to the LBs through the vesicular nucleotide transporter (VNUT) located on the LB membrane.[50, 51] P2X4Rs are inwardly rectifying cation (Na^+ and Ca^{2+}) channels located at the membrane of the LBs (**Figure 1B**).[49] At pH values lower than 7.4 [49] and at ATP concentrations >100 μM [52] the P2X4Rs are desensitized. Because the fusion pore connects the intravesicular space of the LBs with the extracellular space with a pH value of 7.4 and with low ATP concentrations, the intravesicular pH increases to 7.4 and ATP is released from the LBs to the extracellular space. This causes the intravesicular ATP levels to fall from 1.9 mM to 1–5 μM and within the window of the effective concentrations of the P2X4Rs (as shown in in human embryonic kidney 293—HEK293—cells).[52] This renders the P2X4Rs to become resensitised to ATP stimulation allowing Ca^{2+} ions to enter the cytoplasm (**Figure 1B**).[52, 53]

Figure 1.


A
Resting state of the alveolar type I and II cells
(extracellular ATP concentrations of 2-3 ηM)
Clearance of extracellular ATP
Alveolar liquid lining
AT II cell
AT I cell
Cytoplasm
Nucleus
Subepithelial interstitium
Djo Hosan, MD, PhD
B
ATP-induced Fusion-Activated Calcium-ion Entry (FACE) and lamellar bodies unpacking
(extracellular ATP concentrations of about 85 ηM - 5 μM)
Clearance of extracellular ATP
Ca2+, Na+ and H2O
Released surfactant
Mechanical deformation
FACE-induced cation and water migration
from the alveolar space to the subepithelial interstitium
C
Surfactant impairment due to ATP-induced P2Y2R and P2X4R desensitization
(extracellular ATP at about 100 μM to millimolar concentrations)
Clearance of extracellular ATP
Vigorous mechanical deformation
internalised P2Y2R
Phosphorylated C terminal
inactive Gq subunits (αl βγ)

Schematic presentation of the regulation of surfactant exocytosis. For greater clarity, the high cytosolic adenosine triphosphate (ATP) content is omitted in the figure. (**A**) Resting state of the alveolar epithelial type I (AT I) and AT II cells. (**B**) ATP-induced fusion- activated calcium-ion entry resulting in surfactant exocytosis. (**C**) Excessive extracellular ATP concentrations causing the impairment of surfactant exocytosis. See text for explanation. AT I: Alveolar epithelial type I cell; AT II: Alveolar epithelial type II cell; ER: Endoplasmic reticulum; LB: Lamellar body; P2Y2R and P2X4R: ATP receptors; Gq/11: G protein-coupled receptor molecules comprising αi and βγ subunits; PLC-β: Phospholipase C beta; PIP2: Phosphatidylinositol 4,5-bisphosphate; IP3: Inositol triphosphate; IP3R: Inositol triphosphate receptor, a membrane bound glycoprotein complex functioning as a Ca^{2+} channel sensitive to activation by IP3; STIM1: Stromal interaction molecule 1, a calcium sensor; Orai1: Calcium release-activated calcium channel protein 1, a calcium selective ion channel; TRPV2: Transient receptor potential cation channel subfamily V member 2, a non- selective cation channel; Kir3: K^+ selective inwardly rectifying channel 3 or GIRK2: G protein- coupled inwardly-rectifying K^+ channel 2; DAG: diacylglycerol; PKC: protein kinase C; CD39: Nucleoside triphosphate diphosphohydrolase 1 (NTPD1); NPP: nucleotide pyrophosphatase/phosphodiesterase; CD73: 5′-nucleotidase (5′-NT); ADA: adenosine deaminase; ENTs: Equilibrative nucleoside transporters 1 and 2; CNTs: Concentrative nucleoside transporters 1 and 2; FACE: fusion-activated Ca^{2+} entry; SERCA: sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase channel; PMCA: Plasma membrane Ca^{2+} ATPase channel. Figures extensively adapted from Hasan, et al. (2017) [10] (open access) with permission.

In addition, IP3 can be transported from AT II to other AT II cells, but direct communication between AT II cells can occur only by bridging the AT I cells that separate the AT II cells by means of tunnelling nanotubes (TNTs).[54] TNTs are long membrane projections with a diameter of 50 to 200 nM and a length of up to ~70 µM (the size of several cells).[55] TNTs are capable of transporting signals, organelles, and viruses between AT II cells in the presence of connexin gap junction protein isoform 43 (Cx43).[54] Reportedly, both Cx43 and TNTs are expressed by the AT II cells [56] and intercellular communication through TNTs between alveolar AT II cells that express Cx43 can induce intercellular Ca^{2+} waves by the transmission of IP3 molecules.[54] This process and the activities of outwardly rectifying Cl^- channels and G protein-coupled inwardly-rectifying K^+ channels 2 (GIRK2) reinforce the increase of AT II cytoplasmic Ca^{2+} levels through the paracrine stimulation of the P2Y2Rs by extracellular ATP and the autocrine stimulation of P2X4Rs by vesicular ATP (**Figure 1B**).

The time required for the LB fusion after the activation of the P2Y2Rs ranges from seconds to several minutes.[46] After the initial fusion pore has developed, a perivesicular F-actin coating is formed around the fused LBs (**Figure 2A**). This process is Ca^{2+}-dependent.[57] Despite the accelerated increase in cytoplasm Ca^{2+} levels, surfactant exocytosis is a relatively slow process (lasting several minutes to hours).[46, 58] The amount of the released surfactant by the AT II cells is proportional to the extracellular ATP levels.[32]

4. FUSION OF LYSOSOMES AND LBS WITH THE PLASMA MEMBRANE PLAYS A ROLE IN THE REPAIR OF DAMAGED PLASMA MEMBRANE OF THE AT I AND AT II CELLS, RESPECTIVELY

Belete, et al. (2011) reported that the repair of damaged rat AT I cell monolayers by applying stretch assay or micro puncture assay is facilitated by the subsequent increase in extracellular ATP concentrations. At extracellular concentrations of about 10 μM ATP activates the P2Y2Rs causing the fusion of the membrane of lysosomes with the plasma membrane releasing the lysosomal-associated membrane protein 1 (LAMP-1) and replacing the damaged plasma membrane.[59] The application of apyrase (CD39) that converts ATP to ADP and AMP and after silencing of the expression of P2Y2Rs the plasma membrane repair rate are reduced significantly.[59] We think that similar repair process of the AT II plasma membrane may occur following the fusion of LBs with the plasma membrane.

5. FACE CAUSES A TRANS-EPITHELIAL TRANSPORT OF NA^+, CA^{2+} AND WATER MOLECULES

Besides surfactant exocytosis, FACE causes a trans-epithelial transport of Na^+ and Ca^{2+} molecules from the alveolar space through the P2X4Rs and the cytoplasm of the AT II cells to the sub-epithelial interstitial space. This is followed by a passive water resorption from the alveolar liquid lining to the sub-epithelial interstitial space (**Figure 1B**). Together with the transepithelial transport of Na^+ (through the epithelial Na^+ channel—ENaC) and Cl^- (through the cystic fibrosis transmembrane conductance regulator—CFTR) [68] FACE keeps the alveolar liquid lining as thin as 200 nM with a high density of surfactant phospholipid membranes.[48] The thin alveolar liquid lining promotes the contact between the highly organized multilayer surfactant LAs that are stored in the hypophase beneath the surface active monolayer interfacial film and the surfactant monolayer itself. This facilitates the adsorption of surfactant from the multilayer LAs to the surfactant monolayer film.[48] The surface active monolayer interfacial film forms the basis for an optimal diminution of the surface tension of the air-liquid interface in the alveolar space.[69]

6. SURFACTANT REMODELLING IN THE ALVEOLAR SPACE

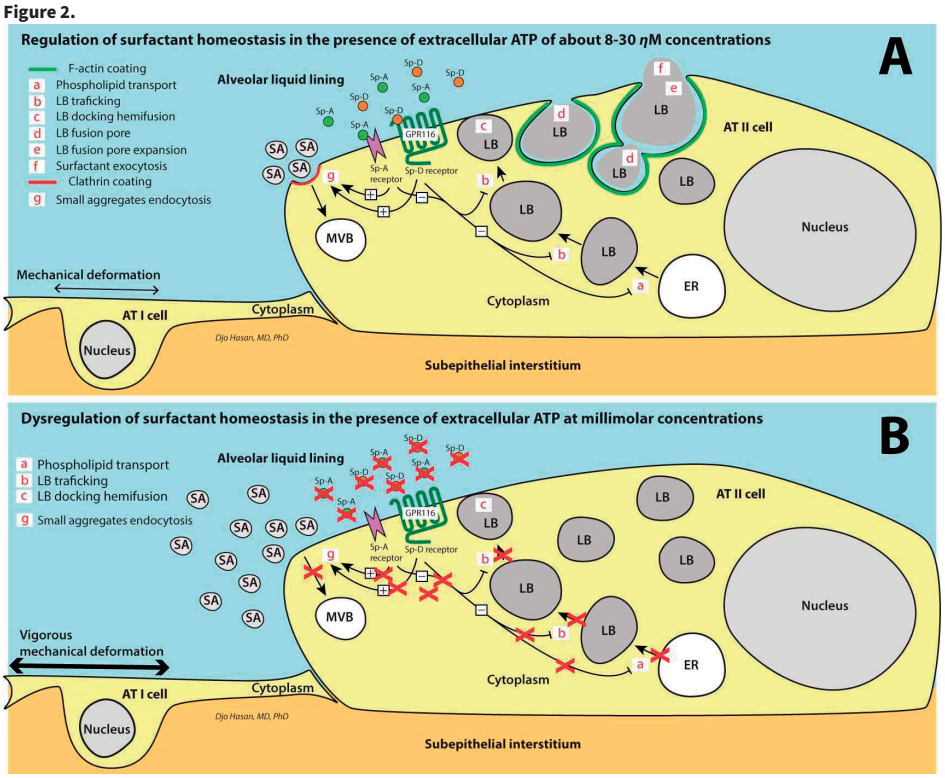
In the alveolar space, surfactant aggregates undergo a remodelling process forming LB-like surfactant compositions and tubular myelin, a lattice-like arrangement of surfactant phospholipid molecules with SP-A and SP-B molecules. These highly organized multilayer surfactant LAs in the extracellular hypophase are thought to be indispensable during the ventilation-induced expansion (inflation) for the adsorption of the phospholipid molecules to the surface active monolayer film at the air-liquid interface (interface film layer) in the alveolar space.[69] SP-B and SP-C are indispensable for the adsorption process [70, 71] and this process is followed by the spreading of the surfactant molecules in the interface film layer.[69] Additionally, the transport of oxygen molecules in a water layer containing SP-B and SP-C-mediated densely packed lipid membranes is significantly faster than through a pure water layer or a water layer with pure phospholipid membranes.[72]

At the beginning of the ventilation-induced compression (deflation) the surfactant molecules in the interface film are not densely packed and still have space to condense causing a rather steep drop in surface tension. Then as deflation progresses the interface monolayer becomes saturated with phospholipid molecules and starts to collapse forming an inward [73, 74] or outward [75] buckling of surfactant bilayers. The outwardly buckled surfactant molecules form bilayer disks that rest above the monolayer.[75] These bilayer disks can either be reincorporated into the monolayer [75] or converted to non-functional SAs and lost into the alveolar space and airways.[26, 69] Similarly, part of the inwardly buckled surfactant bilayers may be reincorporated into the interface monolayer during the adsorption process or may form new multilayer LAs in the hypophase or may be converted to non-functional SAs.[69]

7. SURFACTANT HOMEOSTASIS IN THE ALVEOLAR SPACE

About 10% of the total surfactant molecules is lost and replenished each hour.[69] A small proportion (7–15%) is cleared through the airways [25, 26, 69] presumably after the collapse of the interface monolayer through outward buckling [75] and 20% is cleared by macrophages promoted by GM-CSF (granulocyte-macrophage colony stimulating factor).[69] A very small proportion of surfactant proteins can be detected in the blood [76], but the majority of the “spent” surfactant (about 65%) is taken up by the AT II cells through endocytosis to be recycled.[69] Under basal conditions, SA endocytosis is executed through a clathrin-independent pathway.[60] In contrast, in the presence of secretagogues such as extracellular ATP the uptake of SA is dependent on the clathrin pathway and on both extracellular SP-A and SP-D levels (**Figure 2A**).[60]

Figure 2.



Schematic presentation of the surfactant homeostasis of the alveolar epithelial cells. (**A**) A perivesicular F-actin coating is formed around the fused LBs after the initial LB fusion pore has developed. Several types of fusion pore development are described [57]: (1) 80% of the F-actin- coated fused LBs release surfactant and the LB membrane becomes part of the plasma membrane (kiss-coat-and-release) followed by the disappearance of the F-actin coat; (2) 10% of the F-actin- coated fused LBs discontinued the fusion process and returned inside the cell (kiss-coat-and-run); (3) In the remaining F-actin-coated LBs the fusion process was arrested for a certain time (<20 min) (kiss-coat-and-wait) [57]. The endocytosis of SAs occurs through a clathrin-dependent pathway [60] by the activation of several types of SP-A receptors [60-64] and a SP-D receptor [65]. The SP-D receptor is a GPR116, also known as Ig-Hepta that are highly expressed in the lung [65]. Besides SAs uptake, this process also inhibits the surfactant exocytosis and contributes to the control of extracellular surfactant homeostasis [60, 65]. (**B**) The activation of the pro-inflammatory response of the innate immune system through the activation of the P2X7Rs by extracellular ATP at >300 μM concentrations (ATP molecules at these concentrations act as DAMPs) leads to the recruitment and activation of neutrophils. The recruited and activated neutrophils cause the degradation of SP- D and SP-A leading to a deficiency of SP-D and SP-A [66, 67] preventing the clathrin-dependent recycling of the majority of SAs and aborting the above-mentioned inhibition of the trafficking, semi-fusion and fusion of the LBs with the cell membrane. AT I: Alveolar epithelial type I cell; AT II: Alveolar epithelial type II cell; ER: Endoplasmic reticulum; LB: Lamellar body; MVB: multivesicular body; SP-A: Surfactant protein A; SP-D: Surfactant protein D; SAs: Surfactant small aggregates; LAs: Surfactant large aggregates; GPR116: G protein-coupled receptor 116; DAMPs: danger-associated molecular patterns.

Several SP-A-binding proteins at the cell membrane of the AT II cells are reported: (1) SP-A receptors that bind to A2C and A2R monoclonal anti-idiotype antibodies (SPARs) [61]; (2) surfactant-binding protein BP55 [62]; (3) SP-A receptor with a 50-kD protein core that binds SP-A in a calcium-dependent manner not involving the mannose-binding region of SP-A [63]; (4) a 210-kDa SP-R210 [64]; (5) SP-A receptor that is identified as type II transmembrane protein p63 (CKAP4/p63).[60] Additionally, another receptor that is involved in SA endocytosis is reported: the GPR116 (**Figure 2A**).[65] GPR116 is also known as Ig-Hepta which has Ig-like repeats in the N-terminal extracellular domain and is highly expressed in the lung.[65] GPR116 is thought to be an orphan GPCR carrying an agonistic protein sequence (*Stachel* sequence) that functions as a tethered agonist after the removal or a structural change of the N-terminal from the C-terminal fragment of the *Stachel* sequence.[77] Recently, the activation of the GPR116 by synthetic peptides resembling the C-terminal fragment has been reported.[77] In addition, SP-D may function as a ligand activating the GPR116.[65] Increased SP-D levels in the alveolar liquid lining activate GPR116s. Thus SP-A and SP-D activate the clathrin-dependent 'spent' SA uptake and apparently inhibit the exocytosis of surfactant contributing to the control of extracellular surfactant homeostasis in the alveoli (**Figure 2A**).[60, 65]

8. CLEARANCE OF CA^{2+} IONS FROM THE CYTOPLASM

Clearance of the Ca^{2+} from the AT II cytoplasm occurs by re-entering the endoplasmic reticulum (ER) through sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase channels (SERCAs) that can transfer Ca^{2+} from the cytoplasm to the ER using energy from ATP hydrolysis [78, 79] and/or by leaving the cell through Plasma membrane Ca^{2+} ATPase channels (PMCAs) that are capable of transferring Ca^{2+} from the cytoplasm to the extracellular space (**Figure 1B**).[80] Isoforms of both SERCA and PMCA are expressed in the lung.[78, 80] Under resting conditions, SERCA is bound to phospholamban (SERCA-PLB complex) and the ATPase activity is inhibited. Activation of SERCA-PLB complex occurs after the cytoplasm levels of Ca^{2+} reach micromolar concentration or after phosphorylation by PKC followed by a partial dissociation of PLB from SERCA.[81] In contrast, PMCA is active under resting conditions and is attenuated by activated STIM1 (**Figure 1B**).[82] Restored Ca^{2+} ER levels terminate the stimulation of STIM1 allowing Ca^{2+} ions to be released to the extracellular space. Very high activity of PKC and GIRK2 causes the phosphorylation of the intracellular C-terminal tail of the P2Y2R GPCR molecules causing the ATP receptor to be desensitized (**Figure 1B,C**).[83, 84] GIRK2 can be inhibited by PLCβ through depletion of PIP2 and activation of PKC (**Figure 1B**).[42] These processes control the cytoplasmic Ca^{2+} levels.

9. VENTILATION-INDUCED EXTRACELLULAR ATP: (1) INITIALLY INCREASES THE SURFACTANT RELEASE, (2) HALTS SURFACTANT RELEASE AND PLASMA MEMBRANE REPAIR AT >100 MM CONCENTRATIONS AND (3) TRIGGERS THE PRO-INFLAMMATORY RESPONSE OF THE INNATE IMMUNITY AT >300 MM CONCENTRATIONS

Mechanical ventilation with high mechanical power (>12 J/min) causes vigorous cyclic deformation of the AT I and AT II cells followed by an increased release of extracellular ATP.[10] This proportionally increases the release of surfactant [32] and explains the increase in LA levels in bronchoalveolar lavage fluids (BALFs) in the first hour during ventilation with high mechanical power.[22] Martinez, et al. (2004) confirmed the increase in surfactant exocytosis and increase in respiratory compliance in the first hour of ventilation with high mechanical power in new-born rats.[27] We postulate that after one hour the purinergic receptors of the surfactant release mechanism become desensitized (**Figure 1C**). The mechanism for this desensitization could be explained if extracellular levels of ATP reached ≥100 µM: *In-vitro* exposure of rat glomerular mesangial cells P2Y2Rs to 100 µM ATP during 2 min decreased the sensitivity to stimuli within 1 min. P2Y2Rs reached their maximum desensitization to stimuli within 2 to 4 min. Repetitive stimuli with an interval of 7 minutes led to increasingly weaker responses.[85] Desensitization of the P2Y2Rs occurs through two distinct mechanisms: (1) Phosphorylation of the intracellular C-terminal tail of the P2Y2R GPCR by GIRK2 or by PKC. This prevents the coupling of Gqα and GqβY subunits to the P2Y2R GPCR [84]; (2) Internalization of the P2Y2Rs rendering the receptor inaccessible to ATP binding through an unknown pathway.[86] In HEK293 cells desensitization of the P2X4Rs occurs faster within seconds after a stimulus with 100 µM ATP and being maximally desensitized within 30 to 60 s.[52] The mechanisms of desensitization are: (1) Allosteric change of the P2X4R molecules decreasing the Ca^{2+} pore dimensions [53]; (2) Internalization of the P2X4Rs regulated by Rab5 (a small Ras-like GTPase 5) that promote membrane invagination leading to the endocytosis of P2X4Rs through the clathrin pathway.[87] After desensitization, these receptors become unresponsive to ATP stimuli followed by the absence of cytoplasmic Ca^{2+} response to mechanical deformation of the AT I and AT II cell abolishing the surfactant exocytosis by the AT II cells. Therefore, extracellular ATP concentrations >100 µM desensitize the P2Y2Rs at the plasma membrane of AT II cells and prevent the resensitization of the P2X4Rs in the membrane of LBs leading to the impairment of the surfactant release to the extracellular space. Diminishing surfactant exocytosis involves the disappearance of the FACE-induced trans-epithelial transport of Na^{+}, Ca^{2+} and water molecules from the alveolar space to the interstitium (**Figure 1B,C**). Consequently, the thickness of the alveolar liquid lining increases, reducing the density of surfactant phospholipid membranes in the hypophase. This diminishes

the contact between both the highly organized multilayer surfactant LAs in the hypophase and the surface active monolayer interfacial film. Obviously, this process contributes to the impairment of surfactant function. We postulate that in addition to a halt in the fusion process of LBs with the plasma membrane of AT II cells, the fusion of lysosomes with the plasma membrane of AT I cells is also inhibited by the desensitization of the P2Y2Rs affecting the capacity of the AT II and AT I cells to repair plasma membrane damage.

In addition, increasing extracellular ATP levels results in the up-regulation of the ecto-enzymes and soluble extracellular ATP-converting enzymes CD39 and CD73 leading to a significant increase in extracellular adenosine levels.[88] However, the massive release of ATP in high mechanical power ventilation probably exceeds the capacity of these ATP-converting enzymes to convert ATP molecules. The effective extracellular ATP concentrations to activate the P2X7Rs (in JJ4 macrophage cells and in HEK cells expressing P2X7Rs) starts at about 300 to 1000 μM.[89] In contrast to the P2Y2Rs and P2X4Rs, the P2X7Rs are not subject to desensitization at millimolar or higher extracellular ATP concentrations.[89] P2X7Rs are located at the cell membranes of many immune cells enabling ATP molecules to act as danger associated molecular patterns (DAMPs) activating the pro-inflammatory response of the innate immune system.[10, 18-20] The recruited and activated neutrophils degrade the SP-D and SP-A molecules through a neutrophil serine protease-dependent cleavage and lead to a deficiency of SP-D and SP-A.[66, 67] This prevents the recycling of the majority of SAs (**Figure 2B**).[60, 65] Under nanomolar concentrations of extracellular ATP deficiency of SP-A and SP-D aborts the inhibition of the surfactant exocytosis (**Figure 2A**). However, at >100 μM extracellular ATP concentrations the desensitization of the P2Y2Rs and P2X4Rs prevents surfactant release (**Figure 2B**). The consequences at this stage are that there is a relative increase of non-functional SAs compared to functional LAs and a depletion of LAs in the alveolar space.

10. SURFACTANT DEACTIVATION DEVELOPS SIGNIFICANTLY BEFORE ALVEOLAR SPACE FLOODING CAUSED BY INCREASED CAPILLARY PERMEABILITY

As mentioned above, it is generally assumed that alveolar exudate of serum proteins explains the deactivation of surfactant function [24] and the development of overt lung oedema.[15] However, the group of Lachman (2017) reported that ventilation with high mechanical power for two hours in rats causes a steady increase in the serum C3a levels and a significant increase in lung weight, although the histology of the lung tissue revealed characteristics of diffuse lung injury and a profound interstitial oedema they found very little alveolar oedema.[29] In addition, Cressoni, et al. (2015) found that

alveolar oedema as depicted by serial CT scanning as newly developed densities occurs not earlier than 2.1 to 14.7 h.[28]

We explain this phenomenon by the following consecutive processes: ***First,*** because cytokine levels in mice lung tissue homogenate do not increase earlier than one hour of mechanical ventilation [90] and because the serum complement C3a levels are increased after one hour of high power mechanical ventilation [29], we assume that it takes at more than one hour for the extracellular ATP to reach concentrations >300 µM required to activate the P2X7Rs of the immune cells and initiate the pro-inflammatory response of the innate immune system. ***Second*****,** shortly after the activation of the pro-inflammatory response of the innate immunity, complement components are produced by many cells of the immune system. Induction of the activity of complement C5a and C3a *in-vitro* by moderate concentrations of zymosan (0.01 mg/mL) starts immediately and requires eight hours to reach the maximum level of activation.[91] The small complement fragments C5a and C3a increase vascular permeability in rabbit skin causing capillary leakage of fluid leading to interstitial lung oedema.[92] ***Third,*** the tight junctions between alveolar epithelial cells in the lung (consisting of different types of claudins, zonula occludens-1, occludin, etc.) are an important barrier against exudate formation in the alveolar space [93] and claudin-4 and claudin-18 are expressed in the lung tissue [94]. Wray, et al. (2009) reported that the expression of claudin-4 is increased in the course of three hours of mechanical ventilation with a tidal volume of 20 mL/kg.[95] ***Fourth***, the transcription, activation and extracellular release of IL-1β and the IL-1β -dependent production and activation of matrix metalloproteinase 9 (MMP-9) causing the degradation of the tight junctions proteins zonula occludens-1 and occludin requires additional time.[96] This also applies to the P2X7Rs induced-increase in GSK-3β (glycogen synthase kinase 3β) protein levels that reduce the claudin-18 protein levels.[94] Inhibition of the claudin-4 function results in marked increase in alveolar space oedema.[95]

Therefore, although the interstitial oedema caused by capillary leakage occurs immediately after the initialization of the pro-inflammatory response of the innate immune system the breakdown of the tight junctions requires more time. This breakdown of the tight junctions enables the interstitial fluid to reach the alveolar space leading to alveolar oedema. This explains the observations that interstitial oedema precedes alveolar space flooding by hours [28, 29] and provides the evidence that surfactant function impairment that occurs at 2 hours after the initiation of high power mechanical ventilation in rats [22] is not caused by the disaggregation of surfactant LAs by the extravasated serum containing serum proteins. This rather early surfactant impairment can be explained by the halted FACE-induced trans-epithelial transport of Na^+, Ca^{2+} and water molecules as mentioned above.

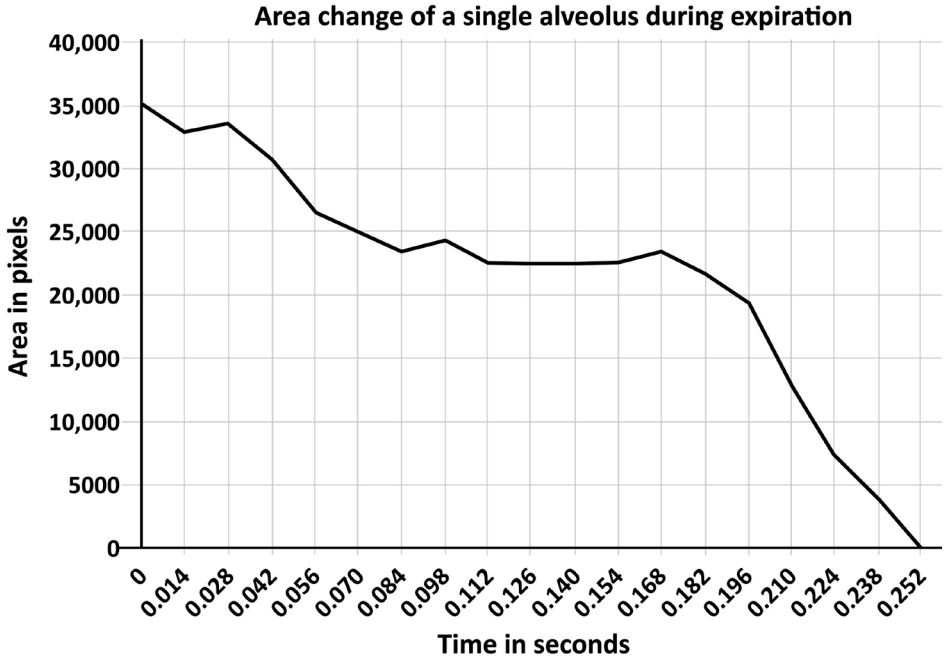
11. SURFACTANT IMPAIRMENT CAUSES CHANGES IN ALVEOLAR MECHANICS EXACERBATING THE RELEASE OF EXTRACELLULAR ATP

We postulate that the magnitude of the extracellular release of ATP molecules by the mechanical deformation of the alveolar epithelial cells during continuous mandatory ventilation with high tidal volumes and low respiratory rate is such that the capacity of the soluble and ecto-enzymes is sufficient to maintain the extracellular ATP within the concentration range of 85 nM to well below 100 μM. Increasing the respiratory rate to reach a mechanical power of >12 J/min boosts the extracellular ATP release beyond the capacity of the extracellular ATPases (CD39, NPP and CD73) to clear and the extracellular ATP levels gradually increase reaching >100 μM and >300 μM concentrations, resulting in surfactant impairment and VILI, respectively.[10, 17] Thus, the healthy lung with intact surfactant function can withstand a strain of 2.5 fairly well [15] as long as the extracellular ATP levels remain below the levels that cause the desensitization of the P2Y2Rs and the PX4Rs.[52, 85]

Using a synchrotron refraction-enhanced computed tomography Sera, et al. (2013) showed that in the healthy murine lung inflation of the lung by increasing the airway pressure from 0 to 8 cm H_2O changes the alveolar duct diameter and not the alveolar space dimensions. At higher airway pressures, the alveolar ducts diameter remains constant and the alveolar space dimensions increase.[97] Therefore, despite the surfactant-induced decrease in surface tension the alveoli require adequate pressure to increase their diameter.

In addition to pressure, time is required to inflate the alveoli.[98] This phenomenon is described as the viscoelastic properties of lung tissue by Suki and Bates.[99] The time and the pressure that are required to inflate the alveoli are proportional to the surface tension. The higher the alveolar surface tension the longer the time and the higher the airway pressure that is required for the alveoli to be inflated.[98, 99] After surfactant deactivation by saline lavage, a pressure of 40 cm H_2O over a 2 second period is required to recruit 80% of collapsed alveoli and 40 s to recruit the remaining alveoli.[98] At lower pressures and shorter time intervals, alveoli are not recruited as tidal volume is redistributed towards the alveolar ducts causing a tremendous enlargement of their size.[100] The dramatic increase in the alveolar duct diameter can be explained by the fact that there are 480 million alveoli [101] and 5600 acinar airways including the alveolar ducts [102] with an alveolar : alveolar duct ratio of 2.9.[100] Redistribution of the tidal volume from the alveoli into the alveolar ducts results in extremely dilated alveolar ducts and pathologic stretching of the alveolar walls of the adjacent alveoli.[100]

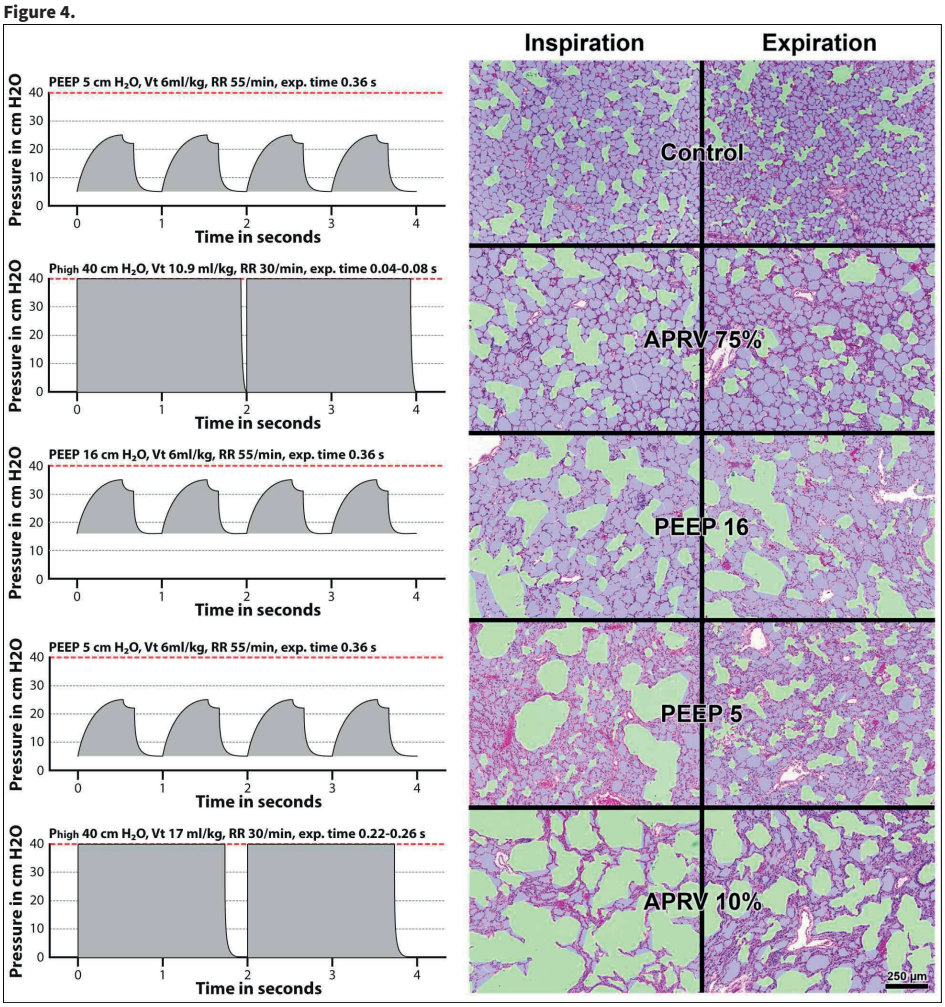
Figure 3.



Graphic presentation of the time course of alveolar collapse during the expiration by releasing an airway pressure of 25 cm H_2O to zero as depicted by *in-vivo* microscopy in rats with surfactant-deactivated lung. The Y-axis represents the alveolar surface areas in pixels and the X-axis is the time. There is a time lag of 0.17 s before alveoli start to collapse after the initiation of the expiratory phase. Furthermore, it takes 0.25 s before the alveoli are fully collapsed. Figure from Satalin, et al. (2016) [103], presented at 'The Open Forum Sessions' during the AARC Congress 2016.

Moreover, alveoli are not only subject to the viscoelastic properties of the lung during inflation, but also during deflation. Satalin, et al. (2016) reported in a surfactant-deactivated lung that there is a lag time of 0.17 s before alveoli begin to collapse after the termination of the inspiratory phase. Furthermore, it takes 0.25 s before the alveoli fully collapse (**Figure 3**).[103] This study demonstrated that the very short expiratory duration using the time-controlled adaptive ventilation (TCAV) protocol (corresponding with the APRV75% group in **Figure 4**) is critical in normalizing air distribution within the alveoli and alveolar duct in a rat Tween-induced ARDS model and is supported the study by Kollisch-Singule, et al. (2014) and illustrated in **Figure 4**.[100] If the expiratory duration is longer than the alveolar collapse-time, these newly recruited alveoli will derecruit with each expiration (**Figures 3 and 4**); if set shorter than the alveolar collapse-time the alveoli will remain inflated during the brief expiration period (**Figures 3 and 4**).[100] Thus, the TCAV protocol stabilizes alveoli by two mechanisms: *pressure* and *time*. Therefore, although ventilation with low mechanical power (<12 J/min) does not cause lung injury in healthy lung [15], ventilation with very low mechanical power corresponding with a tidal volume of 6 ml/kg ideal body weight is injurious for the

Figure 4.



The effect of the ventilator settings on the alveolar mechanics. The left graphics are schematic presentations of the ventilator pressure-time curves belonging to the photomicrographs of the lung presented on the right figure. The lung was fixed at the end-inspiratory pressure (left column of the photomicrographs) and at the end-expiratory pressure (right column of the photomicrographs). The conducting airspaces including the alveolar ducts are colored green, the alveolar spaces are magenta and the alveolar walls are lilac. In APRV75% and APRV10% termination of the expiration is set at an EEF/PEF ratio of 75% and 10%, respectively. In the healthy lung using tissue microscopy after fixing the lung at peak-inspiration and at end-expiration, Kollisch-Singule, et al. (2014) demonstrated that the distribution of tidal volume between the alveoli and the alveolar ducts shows little change during inspiration and expiration ('control') [100]. After surfactant deactivation, there is a redistribution of air at the end of expiration from the alveoli towards the alveolar ducts ('expiration' and 'PEEP 5'). During inspiration, the redistribution towards the alveolar ducts markedly increases causing a tremendous deformation of the alveoli adjacent to these alveolar ducts ('inspiration' and 'PEEP 5'). This results in an increased microstrain (defined as the change in length of the alveolar ducts between inspiration and expiration normalized by their original length). Increasing the PEEP level to 16 cm H_2O decreases the microstrain but not the redistribution of air towards the alveolar ducts ('Inspiration', 'expiration' and 'PEEP 16'). The application of APRV10% with a P_{high} of 40 cm H_2O and expiratory time of 0.22-0.26 s increases the redistribution of air towards the alveolar ducts and the microstrain dramatically ('Inspiration', 'expiration' and 'APRV10%'). By applying APRV75% with a P_{high} of 40 cm H_2O with a shorter expiration time 0f 0.04 to 0.08 s the redistribution of air towards the alveolar ducts and the microstrain much improve but are still not completely restored ('Inspiration', 'expiration' and 'APRV75%') [100]. Thus: in surfactant deactivated lung, a short expiratory time stabilizes the

alveoli and a long expiratory time allows alveolar collapse to occur. By setting the timing of the termination of the expiration relative the PEF, the actual expiration time will change proportional to the time-constant of the alveoli. For instance, in slowly deflating alveoli a longer time is required to reach an EEF/PEF ratio of 75% than in fast deflating alveoli. Consequently, the expiration time in a lung with a high compliance is longer than in a lung with a low compliance. Therefore, this mode is now referred to as the 'time-controlled adaptive ventilation' (TCAV). APRV: airway pressure release ventilation; EEF: end-expiratory flow; PEF: peak-expiratory flow; P_{high}: inspiratory pressure; PEEP: positive end expiratory pressure; Vt: tidal volume; RR: respiratory rate; Exp: Expiratory. Photomicrographs figure from Kollisch-Singule, et al. (2014) [100] with permission.

surfactant-deactivated lung (**Figure 3**).[100, 104] The efficacy of the TCAV protocol was recently demonstrated in experimental pulmonary and extrapulmonary ARDS.[105] The DAD score (reflecting the extent of pulmonary damage) and the expression of biological markers for lung tissue damage (i.e. amphiregulin, vascular cell adhesion molecule 1—VCAM-1, syndecan 1, metalloproteinase 9—MMP9 and decorin) are significantly higher in volume controlled ventilation (VC) with 8ml/kg ideal body weight than in TCAV.[105]

In this perspective, the consequences of mechanical ventilation on the surfactant-deactivated lung are: ***First,*** if the pressure and the duration of the inspiration are inadequate to expand the alveoli tidal volume will be distributed towards the alveolar ducts. This increases the deformation of the alveolar epithelial cells of the adjacent alveoli that are connected to these alveolar ducts tremendously and augments the release of extracellular ATP to a level beyond the capacity of the ATPase enzymes (CD39, NPP and CD 73). ATP will gradually reach >100 μM concentrations causing surfactant impairment and >300 μM concentrations invoking the pro-inflammatory response of the innate immune system injuring the lung tissue. Bellingan, et al. (2014) reported that treatment with interferon-beta-1a (IFN-β-1a) that up-regulates the expression of CD73 reduces the ARDS mortality.[106] ***Second***, even after a successful recruitment manoeuvre (RM) the newly recruited alveoli will collapse and reopen during every breath if PEEP is not set correctly since the expiratory duration with continuous mandatory ventilation is longer than the alveolar collapse-time.

The difficulty of opening the lung with a RM and attempting to stabilize it with PEEP was demonstrated in a recent publication by Cavalcanti, et al. (2017) and the "the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial (ART) Investigators".[107] They reported a study of patients with moderate to severe ARDS. The patients are randomized into a control and an experimental treatment group. The control arm received ventilation with low tidal volume according to the ARDSNet protocol. The experimental strategy arm received the same low tidal volume protocol as the control group with the addition of neuromuscular blockade and RMs with incremental PEEP up to plateau pressure levels of 50 cm H_2O followed by a decremental PEEP trial to identify the PEEP level with the highest respiratory compliance. The mean PEEP level in the control group

was 12.2 cm H_2O and 16.8 cm H_2O in the experimental group. The mean plateau pressure in the experimental group was higher than in the control group but was always below 30 cm H_2O. There is a slight but statistically significant higher mortality in the experimental group compared to the control group (55.3% vs. 49.3%).[107] The RMs in the treatment group may open the lung initially, but soon after the termination of the RMs, the newly recruited alveoli recollapse due to an inadequate PEEP level. Therefore, the investigators opened up the lungs during the RM but the level of PEEP failed to keep the lung open thereafter, providing one explanation for the lack of benefit observed in the experimental group.

12. SUMMARY AND CONCLUSIONS

In the healthy lung, continuous mandatory ventilation with high mechanical power causes an increase in mechanical deformation of the AT I cells followed by an increase in the release of extracellular ATP. This then functions as a signalling molecule for the AT II cells to release surfactant. However, at about 100 µM concentrations, extracellular ATP receptors of the AT II cells become desensitized, surfactant release is halted, and the FACE-induced trans-epithelial transport of Na^+, Ca^{2+}, and water molecules from the alveolar space to the interstitium is diminished, thickening the alveolar liquid lining and impairing the surfactant function. At 300 µM concentrations and above, extracellular ATP initiates the pro-inflammatory response of the innate immune system with immediate increased complement C3 levels causing capillary leakage followed by the disruption of the intercellular junctions of the alveolar epithelial cells, causing overt alveolar space oedema. Surfactant disaggregation by serum proteins further deactivates the surfactant function, leading to a significant alteration in the viscoelastic properties of the lung and the redistribution of the tidal volume towards the alveolar ducts. This boosts the extracellular release of ATP by the alveolar epithelial cells and the pro-inflammatory response of the innate immune system. In addition, the initiated pro-inflammatory response of the innate immunity injuring the lung is followed by a reactive adenosynergic immune paralysis of the immune system and fibrosis.[10] Although extracellular ATP levels can be reduced by a treatment with IFN-β-1a, this may increase adenosine levels.

Future research should be directed into blocking high levels of extracellular ATP combined with improved ventilation strategies. Furthermore, new monitoring systems have to be developed to assess markers of the massively increased purinergic signalling in the lung.

REFERENCES

1. Lohmann K: **Über die Pyrophosphatfraktion im Muskel**. *Naturwissenschaften* 1929, **17**(31):624-625.
2. Langen P, Hucho F: **Karl Lohmann and the discovery of ATP**. *Angew Chem Int Ed Engl* 2008, **47**(10):1824-1827.
3. Williams RJ: **The history and the hypotheses concerning ATP-formation by energised protons**. *FEBS letters* 1978, **85**(1):9-19.
4. Wikstrom MK, Saari HT: **Conformational changes in cytochrome aa3 and ATP synthetase of the mitochondrial membrane and their role in mitochondrial energy transduction**. *Molecular and cellular biochemistry* 1976, **11**(1):17-33.
5. Feldberg W, Hebb C: **The stimulating action of phosphate compounds on the perfused superior cervical ganglion of the cat**. *The Journal of physiology* 1948, **107**(2):210-221.
6. Holton P: **The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves**. *The Journal of physiology* 1959, **145**(3):494-504.
7. Burnstock G: **Purinergic nerves**. *Pharmacological reviews* 1972, **24**(3):509-581.
8. Burnstock G: **Purinergic signalling: Its unpopular beginning, its acceptance and its exciting future**. *Bioessays* 2012, **34**(3):218-225.
9. Burnstock G: **Purinergic signalling: from discovery to current developments**. *ExpPhysiol* 2014, **99**(1):16-34.
10. Hasan D, Blankman P, Nieman GF: **Purinergic signalling links mechanical breath profile and alveolar mechanics with the pro-inflammatory innate immune response causing ventilation-induced lung injury**. *Purinergic signalling* 2017, **13**(3):363-386.
11. Gattinoni L, Tonetti T, Cressoni M, Cadringher P, Herrmann P, Moerer O, Protti A, Gotti M, Chiurazzi C, Carlesso E *et al*: **Ventilator-related causes of lung injury: the mechanical power**. *Intensive care medicine* 2016, **42**(10):1567-1575.
12. Nieman GF, Gatto LA, Habashi NM: **Impact of mechanical ventilation on the pathophysiology of progressive acute lung injury**. *J Appl Physiol (1985)* 2015, **119**(11):1245-1261.
13. Slutsky AS, Ranieri VM: **Ventilator-induced lung injury**. *N Engl J Med* 2013, **369**(22):2126-2136.
14. Rezoagli E, Fumagalli R, Bellani G: **Definition and epidemiology of acute respiratory distress syndrome**. *Annals of translational medicine* 2017, **5**(14):282.
15. Cressoni M, Gotti M, Chiurazzi C, Massari D, Algieri I, Amini M, Cammaroto A, Brioni M, Montaruli C, Nikolla K *et al*: **Mechanical Power and Development of Ventilator-induced Lung Injury**. *Anesthesiology* 2016, **124**(5):1100-1108.
16. Rich PB, Douillet CD, Mahler SA, Husain SA, Boucher RC: **Adenosine triphosphate is released during injurious mechanical ventilation and contributes to lung edema**. *The Journal of trauma* 2003, **55**(2):290-297.
17. Matsuyama H, Amaya F, Hashimoto S, Ueno H, Beppu S, Mizuta M, Shime N, Ishizaka A, Hashimoto S: **Acute lung inflammation and ventilator-induced lung injury caused by ATP via the P2Y receptors: an experimental study**. *RespirRes* 2008, **9**:79.
18. Eltzschig HK, Sitkovsky MV, Robson SC: **Purinergic signaling during inflammation**. *N Engl J Med* 2012, **367**(24):2322-2333.
19. Idzko M, Ferrari D, Eltzschig HK: **Nucleotide signalling during inflammation**. *Nature* 2014, **509**(7500):310-317.
20. Cekic C, Linden J: **Purinergic regulation of the immune system**. *NatRevImmunol* 2016, **16**(3):177-192.

21. Castro CY: **ARDS and diffuse alveolar damage: a pathologist's perspective**. *Semin Thorac Cardiovasc Surg* 2006, **18**(1):13-19.
22. Maruscak AA, Vockeroth DW, Girardi B, Sheikh T, Possmayer F, Lewis JF, Veldhuizen RA: **Alterations to surfactant precede physiological deterioration during high tidal volume ventilation**. *American journal of physiology Lung cellular and molecular physiology* 2008, **294**(5):L974-983.
23. Verbrugge SJ, Bohm SH, Gommers D, Zimmerman LJ, Lachmann B: **Surfactant impairment after mechanical ventilation with large alveolar surface area changes and effects of positive end-expiratory pressure**. *British journal of anaesthesia* 1998, **80**(3):360-364.
24. Martinez Sarrasague M, Cimato A, Rubin de Celis E, Facorro G: **Influence of serum protein and albumin addition on the structure and activity of an exogenous pulmonary surfactant**. *Respiratory physiology & neurobiology* 2011, **175**(3):316-321.
25. Faridy EE: **Effect of ventilation on movement of surfactant in airways**. *Respiration physiology* 1976, **27**(3):323-334.
26. Pettenazzo A, Jobe A, Humme J, Seidner S, Ikegami M: **Clearance of surfactant phosphatidylcholine via the upper airways in rabbits**. *Journal of applied physiology (Bethesda, Md : 1985)* 1988, **65**(5):2151-2155.
27. Martinez F, Lewis J, Copland I, Engelberts D, Kavanagh BP, Post M, Schurch S, Belik J: **Mechanical ventilation effect on surfactant content, function, and lung compliance in the newborn rat**. *Pediatric research* 2004, **56**(1):19-25.
28. Cressoni M, Chiurazzi C, Gotti M, Amini M, Brioni M, Algieri I, Cammaroto A, Rovati C, Massari D, di Castiglione CB *et al*: **Lung inhomogeneities and time course of ventilator-induced mechanical injuries**. *Anesthesiology* 2015, **123**(3):618-627.
29. Petersen B, Busch T, Gaertner J, Haitsma JJ, Krabbendam S, Ebsen M, Lachmann B, Kaisers UX: **Complement activation contributes to ventilator-induced lung injury in rats**. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society* 2016, **67**(6):911-918.
30. Schwiebert EM, Zsembery A: **Extracellular ATP as a signaling molecule for epithelial cells**. *Biochimica et biophysica acta* 2003, **1615**(1-2):7-32.
31. Lazarowski ER, Boucher RC, Harden TK: **Constitutive release of ATP and evidence for major contribution of ecto-nucleotide pyrophosphatase and nucleoside diphosphokinase to extracellular nucleotide concentrations**. *The Journal of biological chemistry* 2000, **275**(40):31061-31068.
32. Patel AS, Reigada D, Mitchell CH, Bates SR, Margulies SS, Koval M: **Paracrine stimulation of surfactant secretion by extracellular ATP in response to mechanical deformation**. *Am J Physiol Lung Cell MolPhysiol* 2005, **289**(3):L489-L496.
33. Furuya K, Tan JJ, Boudreault F, Sokabe M, Berthiaume Y, Grygorczyk R: **Real-time imaging of inflation-induced ATP release in the ex-vivo rat lung**. *American journal of physiology Lung cellular and molecular physiology* 2016:ajplung.00425.02015.
34. Brandao-Burch A, Key ML, Patel JJ, Arnett TR, Orriss IR: **The P2X7 Receptor is an Important Regulator of Extracellular ATP Levels**. *Frontiers in endocrinology* 2012, **3**:41.
35. Brunschweiger A, Muller CE: **P2 receptors activated by uracil nucleotides--an update**. *Current medicinal chemistry* 2006, **13**(3):289-312.
36. Mishra A, Chintagari NR, Guo Y, Weng T, Su L, Liu L: **Purinergic P2X7 receptor regulates lung surfactant secretion in a paracrine manner**. *J Cell Sci* 2011, **124**(Pt 4):657-668.
37. Wettschureck N, Offermanns S: **Mammalian G proteins and their cell type specific functions**. *Physiological reviews* 2005, **85**(4):1159-1204.

38. Berridge MJ: **Inositol trisphosphate and diacylglycerol: two interacting second messengers**. *Annual review of biochemistry* 1987, **56**:159-193.
39. Hogan PG, Rao A: **Store-operated calcium entry: Mechanisms and modulation**. *Biochemical and biophysical research communications* 2015, **460**(1):40-49.
40. Lanner JT, Georgiou DK, Joshi AD, Hamilton SL: **Ryanodine receptors: structure, expression, molecular details, and function in calcium release**. *Cold Spring Harbor perspectives in biology* 2010, **2**(11):a003996.
41. O'Neil RG, Heller S: **The mechanosensitive nature of TRPV channels**. *Pflugers Archiv : European journal of physiology* 2005, **451**(1):193-203.
42. Keselman I, Fribourg M, Felsenfeld DP, Logothetis DE: **Mechanism of PLC-mediated Kir3 current inhibition**. *Channels (Austin, Tex)* 2007, **1**(2):113-123.
43. Clarke LL, Harline MC, Otero MA, Glover GG, Garrad RC, Krugh B, Walker NM, Gonzalez FA, Turner JT, Weisman GA: **Desensitization of P2Y2 receptor-activated transepithelial anion secretion**. *The American journal of physiology* 1999, **276**(4 Pt 1):C777-787.
44. Varela D, Penna A, Simon F, Eguiguren AL, Leiva-Salcedo E, Cerda O, Sala F, Stutzin A: **P2X4 activation modulates volume-sensitive outwardly rectifying chloride channels in rat hepatoma cells**. *The Journal of biological chemistry* 2010, **285**(10):7566-7574.
45. Rasmussen H, Isales CM, Calle R, Throckmorton D, Anderson M, Gasalla-Herraiz J, McCarthy R: **Diacylglycerol production, Ca2+ influx, and protein kinase C activation in sustained cellular responses**. *Endocrine reviews* 1995, **16**(5):649-681.
46. Dietl P, Haller T, Frick M: **Spatio-temporal aspects, pathways and actions of Ca(2+) in surfactant secreting pulmonary alveolar type II pneumocytes**. *Cell Calcium* 2012, **52**(3-4):296-302.
47. Miklavc P, Mair N, Wittekindt OH, Haller T, Dietl P, Felder E, Timmler M, Frick M: **Fusion-activated Ca2+ entry via vesicular P2X4 receptors promotes fusion pore opening and exocytotic content release in pneumocytes**. *Proc Natl Acad Sci USA* 2011, **108**(35):14503-14508.
48. Thompson KE, Korbmacher JP, Hecht E, Hobi N, Wittekindt OH, Dietl P, Kranz C, Frick M: **Fusion-activated cation entry (FACE) via P2X(4) couples surfactant secretion and alveolar fluid transport**. *FASEB J* 2013, **27**(4):1772-1783.
49. Fois G, Winkelmann VE, Bareis L, Staudenmaier L, Hecht E, Ziller C, Ehinger K, Schymeinsky J, Kranz C, Frick M: **ATP is stored in lamellar bodies to activate vesicular P2X4 in an autocrine fashion upon exocytosis**. *The Journal of general physiology* 2018, **150**(2):277-291.
50. Moriyama Y, Hiasa M, Sakamoto S, Omote H, Nomura M: **Vesicular nucleotide transporter (VNUT): appearance of an actress on the stage of purinergic signaling**. *Purinergic signalling* 2017, **13**(3):387-404.
51. Zhong XZ, Cao Q, Sun X, Dong XP: **Activation of lysosomal P2X4 by ATP transported into lysosomes via VNUT/SLC17A9 using V-ATPase generated voltage gradient as the driving force**. *The Journal of physiology* 2016, **594**(15):4253-4266.
52. Stojilkovic SS, Yan Z, Obsil T, Zemkova H: **Structural insights into the function of P2X4: an ATP-gated cation channel of neuroendocrine cells**. *Cellular and molecular neurobiology* 2010, **30**(8):1251-1258.
53. Zemkova H, Khadra A, Rokic MB, Tvrdonova V, Sherman A, Stojilkovic SS: **Allosteric regulation of the P2X4 receptor channel pore dilation**. *Pflugers Archiv : European journal of physiology* 2015, **467**(4):713-726.
54. Lock JT, Parker I, Smith IF: **Communication of Ca(2+) signals via tunneling membrane nanotubes is mediated by transmission of inositol trisphosphate through gap junctions**. *Cell Calcium* 2016, **60**(4):266-272.

55. Rustom A, Saffrich R, Markovic I, Walther P, Gerdes H-H: **Nanotubular Highways for Intercellular Organelle Transport**. *Science* 2004, **303**(5660):1007.
56. Koval M: **Sharing signals: connecting lung epithelial cells with gap junction channels**. *American journal of physiology Lung cellular and molecular physiology* 2002, **283**(5):L875-893.
57. Miklavc P, Wittekindt OH, Felder E, Dietl P: **Ca2+-dependent actin coating of lamellar bodies after exocytotic fusion: a prerequisite for content release or kiss-and-run**. *Annals of the New York Academy of Sciences* 2009, **1152**:43-52.
58. Haller T, Dietl P, Pfaller K, Frick M, Mair N, Paulmichl M, Hess MW, Furst J, Maly K: **Fusion pore expansion is a slow, discontinuous, and Ca2+-dependent process regulating secretion from alveolar type II cells**. *The Journal of cell biology* 2001, **155**(2):279-289.
59. Belete HA, Hubmayr RD, Wang S, Singh RD: **The role of purinergic signaling on deformation induced injury and repair responses of alveolar epithelial cells**. *PloS one* 2011, **6**(11):e27469.
60. Bates SR: **P63 (CKAP4) as an SP-A receptor: implications for surfactant turnover**. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology* 2010, **25**(1):41-54.
61. Strayer DS, Yang S, Jerng HH: **Surfactant protein A-binding proteins. Characterization and structures**. *The Journal of biological chemistry* 1993, **268**(25):18679-18684.
62. Wissel H, Looman AC, Fritzsche I, Rustow B, Stevens PA: **SP-A-binding protein BP55 is involved in surfactant endocytosis by type II pneumocytes**. *The American journal of physiology* 1996, **271**(3 Pt 1):L432-440.
63. Kresch MJ, Christian C, Lu H: **Isolation and partial characterization of a receptor to surfactant protein A expressed by rat type II pneumocytes**. *American journal of respiratory cell and molecular biology* 1998, **19**(2):216-225.
64. Chroneos ZC, Abdolrasulnia R, Whitsett JA, Rice WR, Shepherd VL: **Purification of a cell-surface receptor for surfactant protein A**. *The Journal of biological chemistry* 1996, **271**(27):16375-16383.
65. Fukuzawa T, Ishida J, Kato A, Ichinose T, Ariestanti DM, Takahashi T, Ito K, Abe J, Suzuki T, Wakana S *et al*: **Lung surfactant levels are regulated by Ig-Hepta/GPR116 by monitoring surfactant protein D**. *PloS one* 2013, **8**(7):e69451.
66. Cooley J, McDonald B, Accurso FJ, Crouch EC, Remold-O'Donnell E: **Patterns of neutrophil serine protease-dependent cleavage of surfactant protein D in inflammatory lung disease**. *Journal of leukocyte biology* 2008, **83**(4):946-955.
67. Rubio F, Cooley J, Accurso FJ, Remold-O'Donnell E: **Linkage of neutrophil serine proteases and decreased surfactant protein-A (SP-A) levels in inflammatory lung disease**. *Thorax* 2004, **59**(4):318-323.
68. Ren H, Birch NP, Suresh V: **An Optimised Human Cell Culture Model for Alveolar Epithelial Transport**. *PloS one* 2016, **11**(10):e0165225.
69. Olmeda B, Martinez-Calle M, Perez-Gil J: **Pulmonary surfactant metabolism in the alveolar airspace: Biogenesis, extracellular conversions, recycling**. *Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft* 2017, **209**:78-92.
70. Zuo YY, Veldhuizen RA, Neumann AW, Petersen NO, Possmayer F: **Current perspectives in pulmonary surfactant--inhibition, enhancement and evaluation**. *Biochimica et biophysica acta* 2008, **1778**(10):1947-1977.
71. Parra E, Perez-Gil J: **Composition, structure and mechanical properties define performance of pulmonary surfactant membranes and films**. *Chemistry and physics of lipids* 2015, **185**:153-175.

72. Olmeda B, Villen L, Cruz A, Orellana G, Perez-Gil J: **Pulmonary surfactant layers accelerate O(2) diffusion through the air-water interface**. *Biochimica et biophysica acta* 2010, **1798**(6):1281-1284.
73. Baoukina S, Tieleman DP: **Computer simulations of lung surfactant**. *Biochimica et biophysica acta* 2016, **1858**(10):2431-2440.
74. Sibug-Aga R, Dunn RC: **High-resolution studies of lung surfactant collapse**. *Photochemistry and photobiology* 2004, **80**(3):471-476.
75. Schief WR, Antia M, Discher BM, Hall SB, Vogel V: **Liquid-crystalline collapse of pulmonary surfactant monolayers**. *Biophysical journal* 2003, **84**(6):3792-3806.
76. Mokra D, Kosutova P: **Biomarkers in acute lung injury**. *Respiratory physiology & neurobiology* 2015, **209**:52-58.
77. Demberg LM, Winkler J, Wilde C, Simon KU, Schon J, Rothemund S, Schoneberg T, Promel S, Liebscher I: **Activation of Adhesion G Protein-coupled Receptors: Agonist specificity of *Stachel* sequence-derived peptides**. *The Journal of biological chemistry* 2017, **292**(11):4383-4394.
78. Burk SE, Lytton J, MacLennan DH, Shull GE: **cDNA cloning, functional expression, and mRNA tissue distribution of a third organellar Ca2+ pump**. *The Journal of biological chemistry* 1989, **264**(31):18561-18568.
79. Allen BG, Katz S: **Phosphorylation of cardiac junctional and free sarcoplasmic reticulum by PKC alpha, PKC beta, PKA and the Ca2+/calmodulin-dependent protein kinase**. *Molecular and cellular biochemistry* 1996, **155**(2):91-103.
80. Caride AJ, Filoteo AG, Enyedi A, Verma AK, Penniston JT: **Detection of isoform 4 of the plasma membrane calcium pump in human tissues by using isoform-specific monoclonal antibodies**. *The Biochemical journal* 1996, **316 (Pt 1)**:353-359.
81. Dong X, Thomas DD: **Time-resolved FRET reveals the structural mechanism of SERCA-PLB regulation**. *Biochemical and biophysical research communications* 2014, **449**(2):196-201.
82. Ritchie MF, Samakai E, Soboloff J: **STIM1 is required for attenuation of PMCA-mediated Ca2+ clearance during T-cell activation**. *The EMBO journal* 2012, **31**(5):1123-1133.
83. Garrad RC, Otero MA, Erb L, Theiss PM, Clarke LL, Gonzalez FA, Turner JT, Weisman GA: **Structural basis of agonist-induced desensitization and sequestration of the P2Y2 nucleotide receptor. Consequences of truncation of the C terminus**. *The Journal of biological chemistry* 1998, **273**(45):29437-29444.
84. Flores RV, Hernandez-Perez MG, Aquino E, Garrad RC, Weisman GA, Gonzalez FA: **Agonist-induced phosphorylation and desensitization of the P2Y2 nucleotide receptor**. *Molecular and cellular biochemistry* 2005, **280**(1-2):35-45.
85. Gutierrez AM, Lou X, Erik A, Persson G, Ring A: **Growth hormones reverse desensitization of P2Y(2) receptors in rat mesangial cells**. *Biochemical and biophysical research communications* 2000, **270**(2):594-599.
86. Sromek SM, Harden TK: **Agonist-induced internalization of the P2Y2 receptor**. *Molecular pharmacology* 1998, **54**(3):485-494.
87. Stokes L: **Rab5 regulates internalisation of P2X4 receptors and potentiation by ivermectin**. *Purinergic signalling* 2013, **9**(1):113-121.
88. Eckle T, Fullbier L, Wehrmann M, Khoury J, Mittelbronn M, Ibla J, Rosenberger P, Eltzschig HK: **Identification of ectonucleotidases CD39 and CD73 in innate protection during acute lung injury**. *Journal of immunology (Baltimore, Md : 1950)* 2007, **178**(12):8127-8137.
89. Surprenant A, Rassendren F, Kawashima E, North RA, Buell G: **The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7)**. *Science* 1996, **272**(5262):735-738.

90. Vaneker M, Halbertsma FJ, van Egmond J, Netea MG, Dijkman HB, Snijdelaar DG, Joosten LA, van der Hoeven JG, Scheffer GJ: **Mechanical ventilation in healthy mice induces reversible pulmonary and systemic cytokine elevation with preserved alveolar integrity: an in vivo model using clinical relevant ventilation settings**. *Anesthesiology* 2007, **107**(3):419-426.
91. Morad HO, Belete SC, Read T, Shaw AM: **Time-course analysis of C3a and C5a quantifies the coupling between the upper and terminal Complement pathways in vitro**. *Journal of immunological methods* 2015, **427**:13-18.
92. Williams TJ: **Vascular permeability changes induced by complement-derived peptides**. *Agents and actions* 1983, **13**(5-6):451-455.
93. Wittekindt OH: **Tight junctions in pulmonary epithelia during lung inflammation**. *Pflugers Archiv : European journal of physiology* 2017, **469**(1):135-147.
94. Barth K, Blasche R, Neisser A, Bramke S, Frank JA, Kasper M: **P2X7R-dependent regulation of glycogen synthase kinase 3beta and claudin-18 in alveolar epithelial type I cells of mice lung**. *Histochemistry and cell biology* 2016, **146**(6):757-768.
95. Wray C, Mao Y, Pan J, Chandrasena A, Piasta F, Frank JA: **Claudin-4 augments alveolar epithelial barrier function and is induced in acute lung injury**. *American journal of physiology Lung cellular and molecular physiology* 2009, **297**(2):L219-227.
96. Yang F, Zhao K, Zhang X, Zhang J, Xu B: **ATP Induces Disruption of Tight Junction Proteins via IL-1 Beta-Dependent MMP-9 Activation of Human Blood-Brain Barrier In Vitro**. *Neural plasticity* 2016, **2016**:8928530.
97. Sera T, Yokota H, Tanaka G, Uesugi K, Yagi N, Schroter RC: **Murine pulmonary acinar mechanics during quasi-static inflation using synchrotron refraction-enhanced computed tomography**. *Journal of applied physiology (Bethesda, Md : 1985)* 2013, **115**(2):219-228.
98. Albert SP, DiRocco J, Allen GB, Bates JH, Lafollette R, Kubiak BD, Fischer J, Maroney S, Nieman GF: **The role of time and pressure on alveolar recruitment**. *Journal of applied physiology (Bethesda, Md : 1985)* 2009, **106**(3):757-765.
99. Suki B, Bates JH: **Lung tissue mechanics as an emergent phenomenon**. *Journal of applied physiology (Bethesda, Md : 1985)* 2011, **110**(4):1111-1118.
100. Kollisch-Singule M, Emr B, Smith B, Ruiz C, Roy S, Meng Q, Jain S, Satalin J, Snyder K, Ghosh A *et al*: **Airway pressure release ventilation reduces conducting airway micro-strain in lung injury**. *JAmCollSurg* 2014, **219**(5):968-976.
101. Ochs M, Nyengaard JR, Jung A, Knudsen L, Voigt M, Wahlers T, Richter J, Gundersen HJ: **The number of alveoli in the human lung**. *American journal of respiratory and critical care medicine* 2004, **169**(1):120-124.
102. Barre SF, Haberthur D, Stampanoni M, Schittny JC: **Efficient estimation of the total number of acini in adult rat lung**. *Physiological reports* 2014, **2**(7).
103. Satalin J, Jain SV, Kollisch-Singule MC, Andrews PL, Searles Q, Sweeney T, Gatto LA, Habashi NM, Nieman GF: **Discerning expiratory time vs. pressure in preventing in-vivo alveolar collapse (abstract number 2531914 presented at 'The Open Forum Sessions' AARC Congress 2016, San Antonio, TX.)** *Respiratory care* 2016, **61**(10):OF41.
104. Roy S, Habashi N, Sadowitz B, Andrews P, Ge L, Wang G, Roy P, Ghosh A, Kuhn M, Satalin J *et al*: **Early airway pressure release ventilation prevents ARDS-a novel preventive approach to lung injury**. *Shock (Augusta, Ga)* 2013, **39**(1):28-38.
105. Silva PL, Cruz FF, Samary CDS, Moraes L, de Magalhaes RF, Fernandes MVS, Bose R, Pelegati VB, Carvalho HF, Capelozzi VL *et al*: **Biological Response to Time-Controlled Adaptive Ventilation Depends on Acute Respiratory Distress Syndrome Etiology**. *Critical care medicine* 2018.

106. Bellingan G, Maksimow M, Howell DC, Stotz M, Beale R, Beatty M, Walsh T, Binning A, Davidson A, Kuper M *et al*: **The effect of intravenous interferon-beta-1a (FP-1201) on lung CD73 expression and on acute respiratory distress syndrome mortality: an open-label study**. *The Lancet Respiratory medicine* 2014, **2**(2):98-107.
107. Cavalcanti AB, Suzumura EA, Laranjeira LN, Paisani DM, Damiani LP, Guimaraes HP, Romano ER, Regenga MM, Taniguchi LNT, Teixeira C *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: A Randomized Clinical Trial**. *Jama* 2017, **318**(14):1335-1345.

4

A feasibility study into adenosine triphosphate measurement in exhaled breath condensate: a potential bedside method to monitor alveolar deformation.

Philip van der Zee[1] MD, Peter Somhorst[1] MSc, Jeroen Molinger[1,2] MSc, Djo Hasan[3] MD PhD, Diederik Gommers[1] MD PhD.

[1] Department of Adult Intensive Care, Erasmus Medical Center Rotterdam, The Netherlands
[2] BeLife Human Performance Lab, Rotterdam, The Netherlands
[3] Department of Surgery, Erasmus Medical Center Rotterdam, The Netherlands

Published: Purinergic Signal 2018 Vol. 14 Issue 3

ABSTRACT

Recent research suggested an important role for pulmonary extracellular adenosine triphosphate (ATP) in the development of ventilation induced lung injury. This injury is induced by mechanical deformation of alveolar epithelial cells, which in turn release ATP to the extracellular space. Measuring extracellular ATP in exhaled breath condensate (EBC) may be a non-invasive biomarker for alveolar deformation. Here, we study the feasibility of bedside ATP measurement in EBC. We measured ATP levels in EBC in 10 subjects before and after an exercise test, which increases respiratory parameters and alveolar deformation. EBC lactate concentrations were measured as a dilution marker. We found a significant increase in ATP levels in EBC (before: 73 RLU [IQR 50-209] versus after: 112 RLU [IQR 86-203]; p-value 0.047), and the EBC ATP-to-EBC lactate ratio increased as well (p-value 0.037). We present evidence that bedside measurement of ATP in EBC is feasible and that ATP levels in EBC increase after exercise. Future research should measure ATP levels in EBC during mechanical ventilation as a potential biomarker for alveolar deformation.

Keywords: adenosine triphosphate (ATP), exhaled breath condensate (EBC), exercise test, luciferin-luciferase assay

1. INTRODUCTION

Recently we suggested an important role for pulmonary extracellular adenosine triphosphate (ATP) in the development of ventilation induced lung injury or acute respiratory distress syndrome (ARDS).[1] However, at this moment there is no clinically applicable method to detect extracellular ATP in the lungs.

ATP is omnipresent in cell tissues and the majority of ATP is located in the intracellular space.[2-4] Cells can release ATP molecules after a variety of stimuli (e.g. mechanical deformation, inflammation) and the extracellular ATP concentration increases.[3] In the lungs stretch of the alveolar epithelial type I (AT I) cells result in the extracellular release of ATP.[5-7] Real-time imaging demonstrated that extracellular ATP release occurs simultaneously with mechanical deformation.[8] A nanomolar increase in extracellular ATP stimulates the alveolar epithelial type II cells to release surfactant in the alveolar space.[7, 9-11] Subsequently, extracellular ATP is converted by the CD39 and CD73 enzymes to adenosine and inosine.[3, 4]

The amount of extracellular ATP release correlates with the magnitude of alveolar deformation.[6] Mechanical ventilation can induce severe mechanical deformation and subsequent massive ATP release into the extracellular space. Millimolar concentrations of extracellular ATP act as a danger associated molecular pattern and initiate the pro-inflammatory innate immune response.[3, 12, 13] Prolonged exposure to high levels of extracellular ATP can result in ventilation induced lung injury or ARDS.[1] The measurement of extracellular ATP in the lungs might be a biomarker for alveolar deformation.

ATP in the expired breath can be detected in exhaled breath condensate (EBC).[14-16] EBC is collected by leading exhaled breath air from a subject through a thermo-electric cooling module. The resultant condensate is used for further analyses. EBC collection is a non-invasive method to acquire samples from the respiratory tract and alveoli.[17-19] It is a safe method to assess inflammatory biomarkers in various pulmonary diseases.[20] In addition, EBC contains only few cellular components and low protein levels, indicating virtually no ATP release and low conversion rate.[14] ATP has proven to be stable in EBC for at least 30 minutes.[16] Previous studies used a luciferin-luciferase assay to detect extracellular ATP [14-16], a highly sensitive method to detect ATP.[21] In this study we used a handheld luminometer with a ready to use assay kit. This allowed us to perform ATP measurements in a bedside manner.

We collected EBC from subjects before and after exercise to test whether bedside ATP measurements were feasible. Exercise results in a wide range of physiologic responses, including

a significant increase in respiratory parameters (e.g. tidal volume, respiratory rate, and respiratory minute volume).[22] We hypothesized that the increase in respiratory parameters during exercise resulted in a rise in alveolar deformation and subsequent ATP release into the extracellular space. The aim of this study was to assess the feasibility of bedside ATP measurements and to measure ATP levels in EBC before and after an exercise test.

2. METHODS

2.1 Study Design and Setting

This prospective observational study was performed at BeLife Human Performance Lab, a performance screen and rehabilitation centre. We included subjects between 18 and 75 years old who had a cycle ergometry exercise test at BeLife between October 2017 and January 2018. The exclusion criteria were age <18 years, new onset respiratory symptoms in the past week, and/or a history of unstable respiratory disease (asthma, chronic obstructive pulmonary disease, interstitial lung disease or pulmonary malignancy) requiring changes in therapy in the past three months. The primary outcome of this study was the difference in ATP levels in EBC before and after an exercise test. In addition, ATP levels in EBC were correlated with the following respiratory parameters: respiratory rate, tidal volume, and respiratory minute volume. This study was commissioned by the Department of Adult Intensive Care Medicine of the Erasmus MC Rotterdam, the Netherlands. The study has been performed in accordance with the 1964 Declaration of Helsinki and its later amendments. All subjects gave written informed consent.

2.2 Data Collection

Cycle ergometry exercise test All subjects performed a cycle ergometry exercise test according to the local ramp protocol. The test consisted of a gradual increase in workload until exhaustion. Hemodynamic, metabolic and respiratory parameters, including respiratory rate, tidal volume, and respiratory minute volume, were recorded. Measurement of height, weight, and spirometry (Jaeger Vyntus CPX, Vyaire Medical, USA) were performed before the exercise test. Before and after exercise a capillary blood gas sample was taken. If a capillary blood gas sample after exercise could not be obtained, blood lactate was measured using Lactate Pro2 LT-1730 (Arkray, Japan).

Exhaled breath condensate EBC was collected with the commercially available TurboDECCS System exhaled breath condensator (Disposable Exhaled Condensate Collection Systems, DECCS, Medivac, Italy). A disposable TurboDECCS mouthpiece with saliva filter designed for spontaneously breathing subjects was used. We set condensation temperature at minus 7 °C. EBC was collected twice: once directly before and once five

minutes after the exercise test. Subjects exhaled through the mouthpiece during 15 minutes of tidal breathing. EBC was collected during 15 minutes to collect sufficient sample volume; duration of EBC sampling does not influence adenosine concentrations.[17] In order to minimize sensations of shortness of breath or faintness after the exercise test no nose clip was required.

Luciferin-luciferase assay ATP levels in EBC were measured with luminometry and luciferin-luciferase assay. In this study a 3M ready to use luciferin-luciferase water assay kit (3M Clean-Trace Luminometer LM1, Neuss, Germany) was used. The amount of ATP was expressed in Relative Light Units (RLU). The linearity and sensitivity of this luminometer was confirmed by measurements with different concentrations of sterile pure ATP solutions ranging from 10^{-11} to 10^{-5} M.[23] These ATP concentrations corresponded with 10^{1} to 10^{6} RLU. Two hundred micro litres of EBC was pipetted directly into each assay kit using disposable pipette tips (Filter tip, Greiner Bio-one, Austria). The assay was repeatedly performed every 15 seconds for a duration of two minutes until an equilibrium was reached; i.e. stable RLU values during at least two measurements. In order to decrease intra-assay variability the luciferin-luciferase assay was repeated three times with different assay kits for every EBC sample. Mean ATP level of the three equilibrium values was used in the analyses and intra-assay coefficient of variation (CV) was calculated.

Dilution marker and amylase assay We used EBC lactate as a marker for EBC sample dilution and calculated EBC ATP-to-EBC lactate ratio. In one occasion insufficient sample material was collected and median EBC lactate was used. Lactate in capillary blood gas and EBC was performed on a RapidPoint 500 System (Siemens, Germany, detection limit 180 µmol/L). Subsequently, EBC was stored at minus 80 °C for amylase assay. A colorimetric (405nm) amylase assay was performed to detect possible saliva contamination. Amylase activity was assessed using an Amylase Activity Assay Kit (MAK009, Sigma-Aldrich, USA) and a Varioskan LUX multimode microplate reader (Thermo Fischer Scientific, USA) according to manufacturer protocol.

2.3 Sample Size and Statistical Analysis

We did not calculate a sample size, as the change in ATP levels in EBC before and after exercise is currently unknown. We decided to include 10 subjects in this feasibility study. Baseline characteristics and exercise test data were presented as descriptive statistics. Data was tested for normality. As most data was not normally distributed continuous data were reported as median and interquartile range (IQR). A related-samples Wilcoxon Signed Rank Test was used to assess differences before and after the exercise test. All statistical analyses were performed in IBM SPSS Statistics 21. A p-value <0.05 was considered statistically significant.

3. RESULTS

3.1 Subject Characteristics Before and After the Exercise Test

Twelve subjects were enrolled in this study. One EBC sample obtained before the exercise test contained substantial traces of amylase, while the other samples had an absorbance similar to background signal. We considered this sample to be contaminated with saliva and the subject was excluded from analyses. Another subject was excluded as no EBC was collected despite multiple attempts. The characteristics of the 10 included subjects are presented in **Table 1**. Only two subjects had no medical history, as BeLife is both a performance screen centre and a rehabilitation centre. The results of the exercise tests are shown in **Table 2**. Both hemodynamic and metabolic parameters increased significantly during exercise. Respiratory parameters, including respiratory minute volume, increased significantly as well. This was also reflected in a statistically significant decrease in pCO_2 after the exercise test. In the capillary blood gas there was a significant change in HCO_3^-, base excess, and lactate.

Table 1. Demographic and clinical characteristics of the subjects (n=10).

Characteristic	Median	IQR
Female	n = 8 (80%)	
Age (years)	46	30-53
Height (cm)	170	166-177
Weight (kg)	73.1	61.2-95.2
BMI	26.0	22.1-32.0
BSA (m^2)	1.90	1.74-2.10
Duration of exercise test (min:s)	9:46	7:11 - 11:08
Medical history	Obesity (n = 3) Asthma (n = 1) Surgery (n = 1) Intensive Care admission (n = 1) Essential thrombocytosis (n = 1) M. Crohn (n = 1) No medical history (n = 2)	
Current smoking	n = 1	
Recent respiratory symptoms	n = 2	
Spirometry		
Forced vital capacity (L)	4.00	3.01-4.54
FEV_1 (L)	3.09	2.32-3.89
FEV_1 predicted (%)	100	90-111
FEV_1/VC (%)	80.6	73.7-84.5

BMI Body mass index, BSA Body surface area, FEV_1 Forced expiratory volume in one second, IQR interquartile range, VC Vital capacity.

Table 2. Physiologic variables before and after the exercise test.

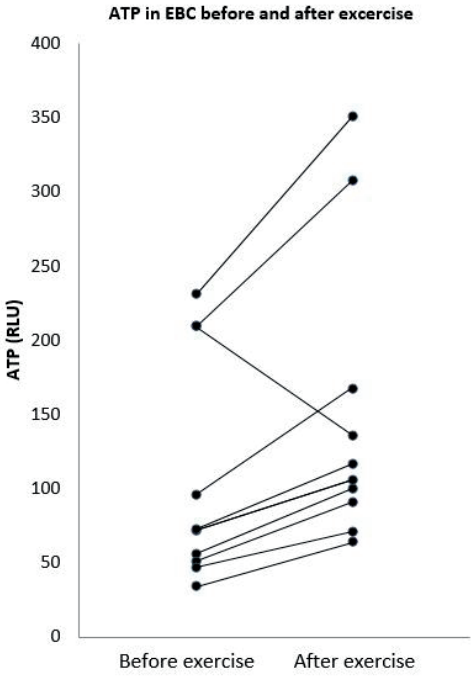
Variables	Unit	Before exercise (Rest)		After exercise (Peak VO_2)		p-value
Hemodynamic parameters						
Heart rate	1/min	87	(74-97)	172	(147-189)	<0.01*
Systolic blood pressure	mmHg	129	(125-158)	185	(167-213)	<0.01*
Diastolic blood pressure	mmHg	77	(68-93)	79	(74-94)	0.959
MAP	mmHg	94	(89-117)	112	(106-131)	<0.01*
Metabolic parameters						
VO_2	mL/min	345	(297-413)	2047	(1599-2436)	<0.01*
Respiratory Exchange Ratio		0.78	(0.72-0.89)	1.10	(1.03-1.27)	0.014*
$PETCO_2$	mmHg	33.75	(27.76-36.25)	33.28	(29.42-38.21)	0.721
$EqCO_2$		33.0	(30.3-35.7)	33.6	(27.7-36.8)	0.959
MET		1.1	(1.0-1.6)	7.7	(6.1-10.9)	<0.01*
Respiratory parameters						
Tidal volume	L	0.744	(0.533-0.883)	2.261	(1.809-2.652)	<0.01*
Respiratory rate	1/min	15.4	(12.7-17.2)	40.1	(31.5-44.1)	<0.01*
Respiratory minute volume	L/min	11.5	(8.7-13.4)	87.1	(64.4-112.3)	<0.01*
Capillary blood gas						
pH		7.408	(7.398-7.442)	7.358	(7.290-7.387)	0.080
pCO_2	mmHg	35.0	(30.2-35.9)	31.2	(27.1-33.9)	0.042*
pO_2	mmHg	75.2	(62.1-85.0)	91.5	(90.9-97.4)	0.068
HCO_3^-	mmol/L	21.5	(21.0-22.1)	15.1	(13.7-19.8)	0.043*
Base excess		-2.1	(-3.2 ; -1.6)	-9.7	(-10.9 ; -4.3)	0.043*
Hematocrit	mmol/L	0.41	(0.35-0.43)	0.43	(0.40-0.46)	0.102
Hemoglobin	mmol/L	8.6	(7.4-9.1)	9.1	(8.3-9.7)	0.066
Oxygen saturation		0.95	(0.92-0.96)	0.96	(0.96-0.98)	0.068
Lactate	mmol/L	1.63	(1.32-1.83)	7.82	(5.63-9.79)	0.018*

Data are presented as median and interquartile range unless stated otherwise. *p-value < 0.05. VO_2 Volume of oxygen consumption, MAP Mean arterial pressure, $PETCO_2$ Partial pressure of exhaled carbon dioxide, MET Metabolic equivalent of a task.

3.2 ATP in Exhaled Breath Condensate

The ATP levels in EBC were detectable in all subjects and increased in nine out of ten subjects (**Figure 1**). ATP levels in EBC increased significantly after exercise (112 RLU, [IQR 86-203]) as compared to before the exercise test (73 RLU, [IQR 50-209]; p-value 0.047) (**Table 3**). Lactate concentrations measured in EBC as a dilution marker did not differ before and after exercise. Comparison of EBC ATP-to-EBC lactate ratio before and after the exercise test resulted in a significant increase (p-value 0.037) as well. The ATP measurements were reproducible with an intra-assay CV of 9.8%. Collected EBC volume was significantly greater after the exercise test, while collection time was similar. No adverse events were observed during this study. We did not find a significant linear correlation between respiratory rate, tidal volume or respiratory minute volume and the amount of ATP detected.

Figure 1. Adenosine triphosphate in exhaled breath condensate before and after exercise



RLU Relative Light Units

Table 3. Adenosine triphosphate in exhaled breath condensate (EBC)

Exhaled breath condensate	Unit	Before exercise (Rest)		After exercise (Peak VO_2)		p-value
EBC ATP	RLU	73	(50-209, Range 34-231)	112	(86-203, Range 64-351)	0.047*
EBC lactate	mmol/L	0.44	(0.41-0.48)	0.45	(0.42-0.49)	0.573
EBC ATP-to-EBC lactate ratio		176	(109-444, Range 78-525)	278	(186-486, Range 131-780)	0.037*
Time of EBC collection	min:s	15:00	(14:48-15:00)	15:00	(14:48-15:00)	0.317
EBC volume	mL	1.3	(0.8-2.0)	1.9	(1.2-2.1)	0.038*

Data are presented as median and interquartile range unless stated otherwise. *p-value < 0.05. RLU Relative Light Units.

4. DISCUSSION

This study showed that the bedside measurement of ATP levels in EBC is feasible. We found a significant increase in ATP levels in EBC after the exercise test as compared to before exercise. Lactate concentrations in EBC, measured as a dilution marker, were similar before and after the exercise test. In addition, we confirmed that EBC collection is simple and safe.

ATP levels in EBC increased in nine out of ten subjects after exercise. Although exercise induces multiple systemic responses, as indicated by a significant increase in physiologic parameters and especially blood lactate concentration, we hypothesized that increased alveolar deformation is the main reason for the observed increase in ATP levels. A systemic origin of increased ATP levels in EBC after exercise is unlikely, as extracellular ATP is rapidly degraded by both soluble and membrane bound ecto-enzymes.[3] In addition, in healthy lungs the tight junctions between adjacent pulmonary epithelium seal the cells and form a barrier between the alveolar air space and the interstitium.[24, 25] Barrier function can diminish following cell damage or inflammation, but it remains intact during brief exercise. This is also reflected by the fact that blood lactate concentration increased significantly, while EBC lactate concentration remained unchanged. As lactate (89 g/mol) is a significantly smaller molecule than ATP (507 g/mol) [26], a rise in lactate concentration in EBC through paracellular transport is more likely to occur. Thus, it is possible that the lung itself is the source of increased ATP levels in EBC after exercise. In one subject ATP levels in EBC did not increase after exercise. This subject was stressed before the exercise test, as indicated by highest heart rate and respiratory parameters at rest. She was in excellent physical condition and recovered fast with a heart rate below baseline at 120 seconds after exercise. Therefore, the difference in physiologic variables before and after the exercise test was smallest in this subject. Other possible explanations for the decrease in ATP levels are contamination of the sample (other than saliva) acquired before the exercise test, or increased instability of ATP after the exercise test due to a change in EBC composition after exercise (e.g. pH).[27, 28]

EBC composition and origin In theory EBC originates from the entire respiratory tract, although the exact origin of EBC remains unclear.[29, 30] The composition of EBC corresponds with the composition of airway lining fluid (ALF) [29], although solute concentrations are significantly lower. EBC is generated in a milieu of air that is nearly saturated with gas-phase water vapour; the majority of EBC consists of evaporated water (up to 99.9%).[17, 31-33] The remainder EBC fluid contains a multitude of volatile and non-volatile compounds. The non-volatile compounds in ALF undergo aerosolization during tidal breathing as small droplets of ALF are released from the airway surfaces.[29, 30, 32] The number of particles detected in exhaled air vary between 0.1 and 4.0 particles per millilitre.[34] Multiple models have been proposed to explain particle aerosolization, including airway turbulence, thermodynamic aerosol formation and the bronchiole fluid film burst (BFFB) model.[30, 35, 36] Airway turbulence, however, is an improbable source of aerosolization in EBC as flow is laminar in the bronchiole at naturally achieved flow rates.[35]

Several studies assessed the influence of exercise on the composition of EBC. The majority of ions and compounds remained unchanged [27], although a significant increase in EBC pH was reported.[27, 28] Both unchanged and increased lactate concentrations in EBC after exercise were observed.[27, 37] The EBC lactate concentrations in this study were in concordance with previously measured concentrations.[38] ATP concentrations in EBC have been measured in patients with COPD, asthma and cystic fibrosis. These studies reported some variability in ATP concentrations.[14-16] However, they did demonstrate a decrease in ATP levels after antibiotic treatment of pulmonary cystic fibrosis exacerbations.[16]

Limitations from this study mainly derived from the low particle concentrations found in EBC and the absence of EBC collection and sample handling standardization. The largest pitfall of analyses of EBC is the unknown amount of fragmented droplet aerosols. According to the BFFB model an increase in respiratory minute volume should lead to an increased number of expired particles.[39] This does not significantly influence EBC sample dilution, as the total amount of exhaled water increases as well.[40] Nevertheless, our subjects had to recover at least five minutes in order to partially restore normal respiratory minute volume. Previous studies reported a wide range in EBC adenosine concentrations and calculated a purine-to-urea ratio to correct for dilution variability.[41-43] Significant amounts of urea and lactate have been observed in EBC.[38] In theory, both can be used as a denominator for the unknown amount of particles that has been aerosolized. Previously urea was used as it is not produced or metabolized in the lungs [44, 45], despite a great within-subject variability in EBC urea concentrations.[17, 46] In our study lactate concentrations were comparable before and after exercise, although lactate can be produced by the respiratory epithelium.[27] As EBC lactate can increase during exercise, an EBC ATP-to-EBC lactate ratio might underestimate the true increase in ATP levels. ATP levels in EBC are near the lower detection limit with the bedside luminometer used in this study. Intra-assay variability was 9.8% despite low ATP levels in EBC; a CV of 10% is considered acceptable.[47] The CV tended to decline as ATP levels in EBC were greater. Increasing the lower detection limit would not only increase test sensitivity, but decrease test variability in the lower ranges as well. According to previously published calibration curves, we estimate that EBC ATP levels in our study were in nanomolar ranges.[23] Although ATP levels measured in EBC are underestimated, as a part of extracellular ATP is rapidly converted to adenosine.[3] Despite supervised EBC collection and saliva filter in the TurboDECCS mouthpiece, one sample tested positive for amylase. According to literature sample contamination rarely occurs and routine amylase assay is not recommended.[17, 33] However, sample contamination is unacceptable when purine concentrations are measured. Therefore, we recommend routine amylase assay in EBC collection of spontaneously breathing subjects. We did not

estimate a sample size to detect a correlation between respiratory parameters and an increase in ATP levels. Moreover, substantial variability between subjects obscured any correlation. Because of the great variability in exhaled aerosol concentrations between subjects longitudinal measurements and intra-individual comparisons are preferable. [48] In addition, the within subject change in ATP levels was assessed, as reference values for inflammatory biomarkers in EBC remain to be established.[18, 29, 49]

5. CONCLUSIONS

In the present study we confirmed that it is feasible to measure ATP levels in EBC in a bedside manner. In addition, ATP levels in EBC increased after exercise, whereas lactate concentrations in EBC remained similar. We hypothesized that ATP levels increased as a result of alveolar deformation. Although EBC collection has some pitfalls and may underestimate alveolar extracellular release of ATP, the non-invasive measurement of ATP levels in EBC holds great potential. Measurement of ATP in EBC may provide a relatively simple and non-invasive method to monitor alveolar deformation. Future studies will focus on the measurement of ATP in EBC during mechanical ventilation.

ACKNOWLEDGMENTS

We thank Wim Rietdijk, PhD, Statistician of the Department of Adult Intensive Care Medicine, Erasmus MC, for his valuable comments on the manuscript.

REFERENCES

1. Hasan D, Blankman P, Nieman GF: **Purinergic signalling links mechanical breath profile and alveolar mechanics with the pro-inflammatory innate immune response causing ventilation-induced lung injury**. *Purinergic signalling* 2017, **13**(3):363-386.
2. Burnstock G: **Physiology and pathophysiology of purinergic neurotransmission**. *Physiological reviews* 2007, **87**(2):659-797.
3. Eltzschig HK, Sitkovsky MV, Robson SC: **Purinergic signaling during inflammation**. *N Engl J Med* 2012, **367**(24):2322-2333.
4. Verkhratsky A, Burnstock G: **Biology of purinergic signalling: its ancient evolutionary roots, its omnipresence and its multiple functional significance**. *Bioessays* 2014, **36**(7):697-705.
5. Patel AS, Reigada D, Mitchell CH, Bates SR, Margulies SS, Koval M: **Paracrine stimulation of surfactant secretion by extracellular ATP in response to mechanical deformation**. *Am J Physiol Lung Cell MolPhysiol* 2005, **289**(3):L489-L496.
6. Belete HA, Hubmayr RD, Wang S, Singh RD: **The role of purinergic signaling on deformation induced injury and repair responses of alveolar epithelial cells**. *PloS one* 2011, **6**(11):e27469.
7. Mishra A, Chintagari NR, Guo Y, Weng T, Su L, Liu L: **Purinergic P2X7 receptor regulates lung surfactant secretion in a paracrine manner**. *J Cell Sci* 2011, **124**(Pt 4):657-668.
8. Furuya K, Tan JJ, Boudreault F, Sokabe M, Berthiaume Y, Grygorczyk R: **Real-time imaging of inflation-induced ATP release in the ex-vivo rat lung**. *American journal of physiology Lung cellular and molecular physiology* 2016:ajplung.00425.02015.
9. Dietl P, Haller T, Frick M: **Spatio-temporal aspects, pathways and actions of Ca(2+) in surfactant secreting pulmonary alveolar type II pneumocytes**. *Cell Calcium* 2012, **52**(3-4):296-302.
10. Miklavc P, Mair N, Wittekindt OH, Haller T, Dietl P, Felder E, Timmler M, Frick M: **Fusion-activated Ca2+ entry via vesicular P2X4 receptors promotes fusion pore opening and exocytotic content release in pneumocytes**. *Proc Natl Acad Sci USA* 2011, **108**(35):14503-14508.
11. Chander A, Sen N, Wu AM, Spitzer AR: **Protein kinase C in ATP regulation of lung surfactant secretion in type II cells**. *The American journal of physiology* 1995, **268**(1 Pt 1):L108-116.
12. Idzko M, Ferrari D, Eltzschig HK: **Nucleotide signalling during inflammation**. *Nature* 2014, **509**(7500):310-317.
13. Burnstock G, Boeynaems JM: **Purinergic signalling and immune cells**. *Purinergic signalling* 2014, **10**(4):529-564.
14. Lazar Z, Huszar E, Kullmann T, Barta I, Antus B, Bikov A, Kollai M, Horvath I: **Adenosine triphosphate in exhaled breath condensate of healthy subjects and patients with chronic obstructive pulmonary disease**. *Inflammation research : official journal of the European Histamine Research Society [et al]* 2008, **57**(8):367-373.
15. Lazar Z, Cervenak L, Orosz M, Galffy G, Komlosi ZI, Bikov A, Losonczy G, Horvath I: **Adenosine triphosphate concentration of exhaled breath condensate in asthma**. *Chest* 2010, **138**(3):536-542.
16. Esther CR, Jr., Alexis NE, Clas ML, Lazarowski ER, Donaldson SH, Ribeiro CM, Moore CG, Davis SD, Boucher RC: **Extracellular purines are biomarkers of neutrophilic airway inflammation**. *The European respiratory journal* 2008, **31**(5):949-956.
17. Horvath I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, Becher G, van Beurden WJ, Corradi M, Dekhuijzen R *et al*: **Exhaled breath condensate: methodological recommendations and unresolved questions**. *The European respiratory journal* 2005, **26**(3):523-548.

18. Carter SR, Davis CS, Kovacs EJ: **Exhaled breath condensate collection in the mechanically ventilated patient**. *Respiratory medicine* 2012, **106**(5):601-613.
19. Hunt J: **Exhaled breath condensate: an overview**. *Immunol Allergy Clin North Am* 2007, **27**(4):587-596; v.
20. Moloney ED, Mumby SE, Gajdocsi R, Cranshaw JH, Kharitonov SA, Quinlan GJ, Griffiths MJ: **Exhaled breath condensate detects markers of pulmonary inflammation after cardiothoracic surgery**. *American journal of respiratory and critical care medicine* 2004, **169**(1):64-69.
21. Douillet CD, Suy S, Zarzaur BL, Robinson WP, 3rd, Milano PM, Boucher RC, Rich PB: **Measurement of free and bound fractions of extracellular ATP in biological solutions using bioluminescence**. *Luminescence* 2005, **20**(6):435-441.
22. Wasserman K, Whipp BJ: **Excercise physiology in health and disease**. *The American review of respiratory disease* 1975, **112**(2):219-249.
23. Omidbakhsh N, Ahmadpour F, Kenny N: **How reliable are ATP bioluminescence meters in assessing decontamination of environmental surfaces in healthcare settings?** *PloS one* 2014, **9**(6):e99951.
24. Wittekindt OH: **Tight junctions in pulmonary epithelia during lung inflammation**. *Pflugers Archiv : European journal of physiology* 2017, **469**(1):135-147.
25. Koval M: **Tight junctions, but not too tight: fine control of lung permeability by claudins**. *American journal of physiology Lung cellular and molecular physiology* 2009, **297**(2):L217-218.
26. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA *et al*: **PubChem Substance and Compound databases**. *Nucleic Acids Res* 2016, **44**(D1):D1202-1213.
27. Greenwald R, Ferdinands JM, Teague WG: **Ionic determinants of exhaled breath condensate pH before and after exercise in adolescent athletes**. *Pediatric pulmonology* 2009, **44**(8):768-777.
28. Riediker M, Danuser B: **Exhaled breath condensate pH is increased after moderate exercise**. *J Aerosol Med* 2007, **20**(1):13-18.
29. Dodig S, Cepelak I: **Exhaled breath condensate--from an analytical point of view**. *Biochem Med (Zagreb)* 2013, **23**(3):281-295.
30. Rosias P: **Methodological aspects of exhaled breath condensate collection and analysis**. *J Breath Res* 2012, **6**(2):027102.
31. Effros RM, Dunning MB, 3rd, Biller J, Shaker R: **The promise and perils of exhaled breath condensates**. *American journal of physiology Lung cellular and molecular physiology* 2004, **287**(6):L1073-1080.
32. Hunt J: **Exhaled breath condensate: an evolving tool for noninvasive evaluation of lung disease**. *The Journal of allergy and clinical immunology* 2002, **110**(1):28-34.
33. Konstantinidi EM, Lappas AS, Tzortzi AS, Behrakis PK: **Exhaled Breath Condensate: Technical and Diagnostic Aspects**. *ScientificWorldJournal* 2015, **2015**:435160.
34. Fairchild CI, Stampfer JF: **Particle concentration in exhaled breath**. *Am Ind Hyg Assoc J* 1987, **48**(11):948-949.
35. Johnson GR, Morawska L: **The mechanism of breath aerosol formation**. *J Aerosol Med Pulm Drug Deliv* 2009, **22**(3):229-237.
36. Malashenko A, Tsuda A, Haber S: **Propagation and breakup of liquid menisci and aerosol generation in small airways**. *J Aerosol Med Pulm Drug Deliv* 2009, **22**(4):341-353.
37. Marek EM, Volke J, Hawener I, Platen P, Muckenhoff K, Marek W: **Measurements of lactate in exhaled breath condensate at rest and after maximal exercise in young and healthy subjects**. *J Breath Res* 2010, **4**(1):017105.

38. Effros RM, Hoagland KW, Bosbous M, Castillo D, Foss B, Dunning M, Gare M, Lin W, Sun F: **Dilution of respiratory solutes in exhaled condensates**. *American journal of respiratory and critical care medicine* 2002, **165**(5):663-669.
39. Almstrand AC, Bake B, Ljungstrom E, Larsson P, Bredberg A, Mirgorodskaya E, Olin AC: **Effect of airway opening on production of exhaled particles**. *Journal of applied physiology (Bethesda, Md : 1985)* 2010, **108**(3):584-588.
40. McCafferty JB, Bradshaw TA, Tate S, Greening AP, Innes JA: **Effects of breathing pattern and inspired air conditions on breath condensate volume, pH, nitrite, and protein concentrations**. *Thorax* 2004, **59**(8):694-698.
41. Esther CR, Jr., Jasin HM, Collins LB, Swenberg JA, Boysen G: **A mass spectrometric method to simultaneously measure a biomarker and dilution marker in exhaled breath condensate**. *Rapid communications in mass spectrometry : RCM* 2008, **22**(5):701-705.
42. Esther CR, Jr., Boysen G, Olsen BM, Collins LB, Ghio AJ, Swenberg JW, Boucher RC: **Mass spectrometric analysis of biomarkers and dilution markers in exhaled breath condensate reveals elevated purines in asthma and cystic fibrosis**. *American journal of physiology Lung cellular and molecular physiology* 2009, **296**(6):L987-993.
43. Esther CR, Jr., Lazaar AL, Bordonali E, Qaqish B, Boucher RC: **Elevated airway purines in COPD**. *Chest* 2011, **140**(4):954-960.
44. Effros RM, Murphy C, Ozker K, Hacker A: **Kinetics of urea exchange in air-filled and fluid-filled rat lungs**. *The American journal of physiology* 1992, **263**(6 Pt 1):L619-626.
45. Zacharasiewicz A, Wilson N, Bush A: **Dilution of respiratory solutes in exhaled condensates**. *American journal of respiratory and critical care medicine* 2003, **167**(5):802; author reply 802.
46. Dwyer TM: **Sampling airway surface liquid: non-volatiles in the exhaled breath condensate**. *Lung* 2004, **182**(4):241-250.
47. Song S, Suh B, Lee SG, Kim JH, Kwon OH: **Comparison of 10% coefficient of variation and 99th percentile cutoff of troponin I in serum and heparinized plasma using Access 2 and DxI 800**. *Clinica chimica acta; international journal of clinical chemistry* 2009, **399**(1-2):117-118.
48. Edwards DA, Man JC, Brand P, Katstra JP, Sommerer K, Stone HA, Nardell E, Scheuch G: **Inhaling to mitigate exhaled bioaerosols**. *Proceedings of the National Academy of Sciences of the United States of America* 2004, **101**(50):17383-17388.
49. Koutsokera A, Loukides S, Gourgoulianis KI, Kostikas K: **Biomarkers in the exhaled breath condensate of healthy adults: mapping the path towards reference values**. *Current medicinal chemistry* 2008, **15**(6):620-630.

5

Cytokines and chemokines are detectable in swivel-derived exhaled breath condensate (SEBC): a pilot study in mechanically ventilated patients

Philip van der Zee[1], Inez van Walree[2], Jan-Willem Fijen[2], Arend-Jan van Houte[3,4], Heleen van Velzen-Blad[3], Ger Rijkers[3,5], Diederik Gommers[1], Henrik Endeman[1,2]

[1] Department of Adult Intensive Care, Erasmus Medical Center Rotterdam, The Netherlands
[2] Department of Adult Intensive Care, Diakonessenhuis Utrecht, The Netherlands
[3] Department of Medical Microbiology and Immunology, St. Antonius Ziekenhuis Nieuwegein, The Netherlands
[4] Department of Medical Microbiology and Clinical Chemistry, Diakonessenhuis Utrecht, The Netherlands
[5] Science Department, University College Roosevelt Middelburg, The Netherlands

Published: Dis Markers 2020 Vol. 2020 Issue 1

ABSTRACT

Introduction: Exhaled breath condensate (EBC) is a non-invasive method to collect samples from the respiratory tract. Usually a thermo-electric cooling module is required to collect sufficient EBC volume for analyses. In here, we assessed the feasibility of cytokine and chemokine detection in EBC collected directly from the ventilator circuit without the use of a cooling module: swivel-derived exhaled breath condensate (SEBC).

Methods: SEBC was prospectively collected from the swivel adapter and stored at -80°C. The objective of this study was to detect cytokines and chemokines in SEBC with a multiplex immunoassay. Secondary outcomes were to assess the correlation between cytokine and chemokine concentrations in SEBC and mechanical ventilation parameters, systemic inflammation parameters, and hemodynamic parameters.

Results: Twenty-nine SEBC samples were obtained from 13 ICU patients. IL-1β, IL-4, IL-8, and IL-17 were detected in more than 90% of SEBC samples and significant correlations between multiple cytokines and chemokines were found. Several significant correlations were found between cytokines and chemokines in SEBC and mechanical ventilation parameters, and serum lactate concentrations.

Conclusion: This pilot study showed that it is feasible to detect cytokines and chemokines in SEBC samples obtained without a cooling module. Despite small sample size, correlations were found between cytokines and chemokines in SEBC and mechanical ventilation parameters, as well as serum lactate concentrations. This simple SEBC collection method provides the opportunity to collect EBC samples in large prospective ICU cohorts.

Keywords: Exhaled breath condensate, inflammation, mechanical ventilation

INTRODUCTION

Pulmonary inflammation is the hallmark of acute respiratory distress syndrome (ARDS) and ventilator-associated pneumonia (VAP).[1, 2] Consecutive measurements of pulmonary inflammation could identify mechanically ventilated patients that develop ARDS or VAP in an early phase of the disease or even patients at risk.

Bronchoscopy with bronchoalveolar lavage is an invasive method that is used to directly detect pulmonary inflammation. However, a bronchoscopy is not routinely performed until clinical or radiological symptoms of ARDS or VAP have developed. Exhaled breath condensate (EBC) is a non-invasive method to sample the airway lining fluid that covers the respiratory tract.[3-5] A variety of inflammatory biomarkers has been detected in EBC.[6, 7] In mechanically ventilated patients, EBC samples are collected by guiding exhaled breath air through a thermo-electric cooling module using additional tubing. Cooling down exhaled breath air is required to collect sufficient sample volume for analyses.[8] The necessity for a cooling module and additional mechanical ventilation tubing prevented the collection of EBC in large prospective cohorts at the intensive care unit (ICU), as it is both complex and time consuming.

Multiplex immunoassays are able to detect cytokines and chemokines in very small sample volumes. A volume of 50 microliters is sufficient to obtain reliable results. In all patients on mechanical ventilation with a heat and moisture exchanger (HME) a small volume of EBC cumulates in the expiratory tubing of the ventilatory circuit: swivel-derived exhaled breath condensate (SEBC).

The hypothesis of this study was that it was feasible to detect cytokines and chemokines in SEBC obtained from mechanically ventilated ICU patients. We collected SEBC material directly from the ventilator circuit and used a multiplex immunoassay to detect cytokines and chemokines.

METHODS

Study design and setting

This prospective observational pilot study was performed in the ICU of Diakonessenhuis, Utrecht, the Netherlands. Adult patients on invasive mechanical ventilation were included in this study. We excluded patients with purulent or haemorrhagic sputum that required active humidification instead of a heat and moisture exchanger (HME). The study was approved by the medical ethical committee (METC) of the Diakonessenhuis

Utrecht. A waiver for informed consent was given due to the non-invasive nature of the study.

Study outcomes

The primary outcome of this feasibility study was to detect cytokines and chemokines in SEBC obtained in standard ICU bedside conditions. Secondary outcomes were to assess the correlation between cytokine and chemokine concentrations in SEBC and mechanical ventilation parameters, systemic inflammation parameters, and hemodynamic parameters.

Data collection

Swivel-derived EBC sample collection SEBC sampling was performed by two researchers (HE and IW) between 8 and 10 a.m. before routine airway care. The tube and the swivel adapter were disconnected and droplets in the swivel adapter were directly aspirated with a 3 mL disposable pipet. The SEBC samples were stored in 2mL containers and immediately cooled in ice. Within 15 minutes following collection the samples were stored at -80°C.

Multiplex immunoassay SEBC samples were analysed with a multiplex immunoassay (Luminex, Austin, TX, R&D Systems Cytokines) according to manufacturer instructions protocol (R&D Systems). The concentrations of a set of cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IFN-ɣ, G-CSF, and TNF-α) and chemokines (MCP-1 and MIP-1β) were measured.

Patient data collection Demographic and clinical characteristics of the patients were retrieved from the patient data management system (PDMS, MetaVision). All patients were ventilated with a Servo-i mechanical ventilator in either pressure control or pressure support mode. Airway pressure levels and fraction of inspired oxygen (FiO_2) were adjusted by the attending physician. The following respiratory variables were recorded at the moment of SEBC sampling: mode of ventilation, tidal volume (Vt), positive end-expiratory pressure (PEEP), peak airway pressure, plateau airway pressure, FiO_2, pulmonary dynamic compliance, and PaO_2/FiO_2 (P/F) ratio. In addition, the mean arterial blood pressure (MAP), heart rate, urinary output in the two hours before sampling, dose of noradrenaline (target MAP >65mmHg), and central temperature were recorded. The following laboratory parameters were assessed at the day of SEBC sampling: serum lactate, haemoglobin, sodium, potassium, CRP, white blood cell count (WBC), platelet count, urea, and creatinine. Arterial blood gas results before the moment of sampling were recorded as well.

Statistical analysis

Descriptive characteristics are shown as median and range. Correlation coefficients were calculated by using Pearson correlation coefficients, provided that at least 10 samples had detectable cytokine or chemokine concentrations. All statistical analyses were performed in IBM SPSS Statistics 21. A difference of $p<0.05$ was considered statistically significant.

RESULTS

Patient characteristics Twenty-nine SEBC samples were obtained from 13 patients (median 2 samples, range 1-4). Patient characteristics are shown in **Table 1**. None of the patients was diagnosed with a VAP or ARDS according to recent definitions.[1, 9] Seven SEBC samples were taken during pressure control ventilation and 22 during pressure support ventilation.

Table 1 Patient characteristics of 13 mechanically ventilated patients

Male (n, %)	7 (54)
Age	69 (37-77)
Reason for admission, n (%)	
- Complications of previous abdominal or vascular surgery	4 (30%)
- Cardiac failure	2 (15%)
- Pneumonia (without severe sepsis or shock)	2 (15%)
- Severe sepsis/septic shock	2 (15%)
- Other	3 (23%)
APACHE II score	25 (14-39)
Peak airway pressure (cmH_2O)	18 (10-36)
Plateau airway pressure (cmH_2O)	12.5 (6-24)
Positive end-expiratory pressure (cmH_2O)	8 (5-18)
Tidal volume (mL/kg predicted body weight)	7.2 (5.1-11.0)
Fraction of inspired oxygen (%)	35 (25-70)
PaO_2/FiO_2 ratio (mmHg)	252 (95-364)
Pulmonary compliance (mL/ cmH_2O)	48 (8-208)

Data are presented as median and range unless stated otherwise.

Feasibility All cytokines and chemokines were detectable in SEBC samples except for IL-2 (**Table 2**). IL-1β, IL-4, IL-8, and IL-17 were detected in more than 90% of SEBC samples, but ranges varied greatly. In addition, we found significant correlations between cytokine and chemokine concentrations (**Table 3**).

Table 2 Concentrations of cytokines and chemokines in SEBC (n = 29)

Cytokine/chemokine	Detection, n (%)	Median (pg/mL)	Range (pg/mL)
IL-1β	26 (93)*	0.12	0.00 - 13.71
IL-2	0	-	-
IL-4	26 (90)	0.26	0.00 - 0.84
IL-5	3 (10)	0.00	0.00 - 0.60
IL-6	15 (52)	0.00	0.00 - 131.80
IL-7	2 (7)	0.00	0.00 - 9.37
IL-8	27 (96)*	2.55	0.00 - 6448.00
IL-10	24 (86)*	0.03	0.00 - 61.14
IL-12	21 (72)	0.04	0.00 - 7.36
IL-13	1 (3)	0.00	0.00 - 1.52
IL-17	26 (90)	1.30	0.00 - 4.75
G-CSF	2 (7)*	0.00	0.00 - 17.90
IFN-ɣ	12 (41)	0.00	0.00 - 96.13
MCP-1	25 (86)	0.86	0.00 - 711.76
MIP-1β	16 (55)	0.22	0.00 - 656.92
TNF-α	12 (41)	0.00	0.00 - 15.51

*one result not available in immunoassay (n = 28). IL interleukin, G-CSF granulocyte colony-stimulating factor, IFN interferon, MCP monocyte chemoattractant protein, MIP macrophage inflammatory protein, TNF tumour necrosis factor.

Table 3 Correlation coefficients between cytokines and chemokines in SEBC

	IL-1β	IL-4	IL-6	IL-8	IL-10	IL-12	IL-17	IFN-ɣ	MCP-1	MIP-1β	TNF -α
IL-1β	-										
IL-4	.021	-									
IL-6	.212	-.063	-								
IL-8	.097	-.156	.963**	-							
IL-10	-.089	-.122	-.045	-.048	-						
IL-12	-.071	-.100	-.027	-.040	.991**	-					
IL-17	.076	.115	-.164	-.212	.257	-.212	-				
IFN-ɣ	-.103	.056	-.062	-.095	.823**	.822**	.063	-			
MCP-1	.060	-.128	.967**	.990**	.045	.058	-.220	-.001	-		
MIP-1β	-.082	-.116	-.006	-.009	.999**	.992**	-.278	.821**	.084	-	
TNF-α	.352	.336	.365	.213	-.02	.024	.282	.411*	.228	.017	-

A positive value indicates a positive correlation, whereas a negative value indicates a negative correlation, * < 0.05 ** < 0.01. Abbreviations: IL interleukin, IFN interferon, MCP monocyte chemoattractant protein, MIP macrophage inflammatory protein, TNF tumour necrosis factor.

Correlations with mechanical ventilation parameters, systemic inflammation, and hemodynamic parameters are shown in the supplementary files. Cytokine and chemokine concentrations in SEBC samples did not differ between patients on pressure control or pressure support ventilation. High Vt (mL/kg PBW) was correlated with IL-10 (r=.391, p<0.05), IL-12 (r=.392, p<0.05), and MIP-1β (r=.397, p<0.05). High P/F ratio was correlated with IL-8 (r=.427, p<0.05) and MCP-1 (r=.381, p<0.05). In addition, we observed significant correlations between high serum lactate and IL-1β (r=.889, p<0.01), IL-6 (r=.817, p<0.01), IL-8 (r=.742, p<0.05), MIP-1β (r=.797, p<0.01), and TNF-α (r=.790, p<0.01).

DISCUSSION

In this pilot study, we showed that it is feasible to detect cytokines and chemokines in SEBC samples obtained directly from the ventilator circuit without the use of a cooling module. The cytokines and chemokines in SEBC correlated significantly with each other. We found correlations between cytokine and chemokine concentrations and mechanical ventilation parameters, as well as high serum lactate. Although the small sample size of this study prevents any definitive conclusions, the measurement of cytokines and chemokines in SEBC has the potential to become a non-invasive bedside method to detect pulmonary inflammation.

In line with previous research, cytokine and chemokine concentrations in SEBC samples were low and concentrations varied widely.[10-15] Both the wide variation and low concentrations are the result of EBC formation in the airways and EBC sample dilution. The exact origin of EBC is uncertain, but McNeil et al. found that there is a correlation between fluids extracted from the HME filter and oedema fluid aspirated directly from the airways.[16] Therefore, it is suggested that EBC originates from the airway lining fluid covering the respiratory tract.[8, 17] Up to 99.9% of EBC consists of evaporated water and only a small proportion consists of both volatile and non-volatile compounds.[3, 18-20] The non-volatile compounds, including cytokines and chemokines, are shed from the airway surfaces as small droplets of airway lining fluid during tidal breathing.[8, 17, 19] The number of droplets detected in exhaled breath air vary greatly resulting in variable sample dilution.[17] Currently there is no consensus on a method to correct for sample dilution. It has been suggested to calculate proportions between substances in order to correct for sample dilution.[21, 22] In our study we found multiple well-known correlations between cytokines and chemokines in SEBC samples. The combination of IL-6 and IL-8 is frequently used in ARDS research[23, 24], whereas IL-13 was only present in combination with high IL-5 concentrations, both are T-helper cell 2 cytokines associated with airway hyperresponsiveness.[25]

Mechanical ventilation with high airway pressures and high tidal volumes is associated with increased mortality in patients with ARDS.[26] Until now, only Gessner et al. found a strong correlation between EBC nitrite levels and tidal volume in an ICU population.[27] In this study, we found significant correlations between high peak airway pressure and G-CSF, and between high tidal volume (mL/kg PBW) and IL-10, IL-12, and MIP-1β. In contrast, Fernandez-Bustamante et al. did not find a difference in EBC cytokines between low tidal volume (6mL/kg) and intermediate tidal volume (10mL/kg) after one hour of mechanical ventilation in healthy perioperative patients.[15] Multiple cytokines were undetectable in their study despite the use of a multiplex immunoassay. The undetectable concentrations could be explained by the small difference in tidal volumes, as a tidal volume of 10mL/kg does not increase mortality rate in ICU patients without ARDS.[28] In addition, in healthy perioperative patients the endothelial barrier function is preserved and might not have been affected by one hour of mechanical ventilation.

Despite the small sample size, we found strong correlations between high serum lactate and IL-1β, IL-6, IL-8, G-CSF, MIP-1β, and TNF-α concentrations in SEBC. Previously we have suggested that ATP levels in EBC samples did not accurately reflect serum lactate in healthy patients.[29] In critically ill patients, the endothelial barrier function is impaired, which could be an explanation for the strong correlations found between serum lactate and cytokines and chemokines in SEBC. Parameters associated with an active infection, such as CRP and white blood cell count, were not significantly correlated with cytokines and chemokines in SEBC samples. Therefore, we hypothesize that the raised concentrations of cytokines and chemokines in SEBC samples is the result of endothelial barrier dysfunction, and not of infection.

This pilot study has several limitations. First, this study was designed as a pilot study with as primary aim to establish the feasibility of detection of cytokines and chemokines in SEBC samples collected without a cooling module. Therefore, sample size was small and the correlations found are likely to overestimate or underestimate the true effect. Second, we did not collect matching plasma samples to measure cytokines and chemokines to correlate with the SEBC samples. Third, no SEBC dilution factors were calculated. We decided to present the raw data as there is no consensus on the calculation of EBC sample dilution.[3, 5] IL-8 could be used as a denominator, as this cytokine is detectable in 96% of measured samples. Lastly, we did not compare SEBC with EBC collected by a cooling module, as there is no gold standard for EBC collection.

Despite these limitations, this pilot study demonstrated that cytokines and chemokines can be detected in SEBC. The only prerequisite is the use of a HME, as active humidification potentially results in sample dilution.[5] This simple SEBC collection method

provides a unique opportunity to collect EBC samples in large prospective ICU cohorts, in order to determine whether cytokines and chemokines are correlated with mechanical ventilation parameters, systemic parameters or even predict the development of ARDS or VAP.

CONCLUSION

This pilot study showed that it is feasible to detect cytokines and chemokines in SEBC samples obtained directly from the ventilator circuit without the use of a cooling module. Although the sample size was small, correlations were found between cytokines and chemokines in SEBC, as well as mechanical ventilation parameters and high serum lactate concentrations. This simple SEBC collection method provides the opportunity to collect EBC samples in large prospective ICU cohorts.

LIST OF ABBREVIATIONS

ARDS	acute respiratory distress syndrome
EBC	exhaled breath condensate
FiO_2	fraction of inspired oxygen
G-CSF	granulocyte colony-stimulating factor
HME	heat and moisture exchanger
ICU	intensive care unit
IFN	interferon
IL	interleukin
MAP	mean arterial blood pressure
MCP	monocyte chemoattractant protein
MIP	macrophage inflammatory protein
METC	medical ethical committee
PEEP	positive end-expiratory pressure
P/F	PaO_2/FiO_2 ratio
SEBC	swivel-derived exhaled breath condensate
TNF	tumour necrosis factor
VAP	ventilator-associated pneumonia
Vt	tidal volume
WBC	white blood cell count

ACKNOWLEDGMENT

We thank Mr. Ben de Jong (Laboratory for Medical Microbiology and Immunology, St. Antonius Hospital) for expert technical assistance.

REFERENCES:

1. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS: **Acute respiratory distress syndrome: the Berlin Definition**. *Jama* 2012, **307**(23):2526-2533.
2. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, Napolitano LM, O'Grady NP, Bartlett JG, Carratala J *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 : an official publication of the Infectious Diseases Society of America* 2016, **63**(5):e61-e111.
3. Horvath I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, Becher G, van Beurden WJ, Corradi M, Dekhuijzen R *et al*: **Exhaled breath condensate: methodological recommendations and unresolved questions**. *The European respiratory journal* 2005, **26**(3):523-548.
4. Hunt J: **Exhaled breath condensate: an overview**. *Immunol Allergy Clin North Am* 2007, **27**(4):587-596; v.
5. Carter SR, Davis CS, Kovacs EJ: **Exhaled breath condensate collection in the mechanically ventilated patient**. *Respiratory medicine* 2012, **106**(5):601-613.
6. Wallace MAG, Pleil JD: **Evolution of clinical and environmental health applications of exhaled breath research: Review of methods and instrumentation for gas-phase, condensate, and aerosols**. *Anal Chim Acta* 2018, **1024**:18-38.
7. Ahmadzai H, Huang S, Hettiarachchi R, Lin JL, Thomas PS, Zhang Q: **Exhaled breath condensate: a comprehensive update**. *Clin Chem Lab Med* 2013, **51**(7):1343-1361.
8. Rosias P: **Methodological aspects of exhaled breath condensate collection and analysis**. *J Breath Res* 2012, **6**(2):027102.
9. Torres A, Niederman MS, Chastre J, Ewig S, Fernandez-Vandellos P, Hanberger H, Kollef M, Li Bassi G, Luna CM, Martin-Loeches I *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 Asociacion Latinoamericana del Torax (ALAT)**. *The European respiratory journal* 2017, **50**(3).
10. Gessner C, Hammerschmidt S, Kuhn H, Seyfarth HJ, Sack U, Engelmann L, Schauer J, Wirtz H: **Exhaled breath condensate acidification in acute lung injury**. *Respiratory medicine* 2003, **97**(11):1188-1194.
11. Gessner C, Scheibe R, Wotzel M, Hammerschmidt S, Kuhn H, Engelmann L, Hoheisel G, Gillissen A, Sack U, Wirtz H: **Exhaled breath condensate cytokine patterns in chronic obstructive pulmonary disease**. *Respiratory medicine* 2005, **99**(10):1229-1240.
12. Sack U, Scheibe R, Wotzel M, Hammerschmidt S, Kuhn H, Emmrich F, Hoheisel G, Wirtz H, Gessner C: **Multiplex analysis of cytokines in exhaled breath condensate**. *Cytometry A* 2006, **69**(3):169-172.
13. Heidemann SM, Sandhu H, Kovacevic N, Phumeetham S, Solomon R: **Detection of tumor necrosis factor-alpha and interleukin-6 in exhaled breath condensate of rats with pneumonia due to staphylococcal enterotoxin B**. *Exp Lung Res* 2011, **37**(9):563-567.
14. Liu HC, Lu MC, Lin YC, Wu TC, Hsu JY, Jan MS, Chen CM: **Differences in IL-8 in serum and exhaled breath condensate from patients with exacerbated COPD or asthma attacks**. *J Formos Med Assoc* 2014, **113**(12):908-914.

15. Fernandez-Bustamante A, Klawitter J, Repine JE, Agazio A, Janocha AJ, Shah C, Moss M, Douglas IS, Tran ZV, Erzurum SC *et al*: **Early effect of tidal volume on lung injury biomarkers in surgical patients with healthy lungs**. *Anesthesiology* 2014, **121**(3):469-481.
16. McNeil JB, Shaver CM, Kerchberger VE, Russell DW, Grove BS, Warren MA, Wickersham NE, Ware LB, McDonald WH, Bastarache JA: **Novel Method for Noninvasive Sampling of the Distal Airspace in Acute Respiratory Distress Syndrome**. *American journal of respiratory and critical care medicine* 2018, **197**(8):1027-1035.
17. Dodig S, Cepelak I: **Exhaled breath condensate--from an analytical point of view**. *Biochem Med (Zagreb)* 2013, **23**(3):281-295.
18. Effros RM, Dunning MB, 3rd, Biller J, Shaker R: **The promise and perils of exhaled breath condensates**. *American journal of physiology Lung cellular and molecular physiology* 2004, **287**(6):L1073-1080.
19. Hunt J: **Exhaled breath condensate: an evolving tool for noninvasive evaluation of lung disease**. *The Journal of allergy and clinical immunology* 2002, **110**(1):28-34.
20. Konstantinidi EM, Lappas AS, Tzortzi AS, Behrakis PK: **Exhaled Breath Condensate: Technical and Diagnostic Aspects**. *ScientificWorldJournal* 2015, **2015**:435160.
21. Effros RM, Hoagland KW, Bosbous M, Castillo D, Foss B, Dunning M, Gare M, Lin W, Sun F: **Dilution of respiratory solutes in exhaled condensates**. *American journal of respiratory and critical care medicine* 2002, **165**(5):663-669.
22. Esther CR, Jr., Jasin HM, Collins LB, Swenberg JA, Boysen G: **A mass spectrometric method to simultaneously measure a biomarker and dilution marker in exhaled breath condensate**. *Rapid communications in mass spectrometry : RCM* 2008, **22**(5):701-705.
23. Calfee CS, Janz DR, Bernard GR, May AK, Kangelaris KN, Matthay MA, Ware LB: **Distinct molecular phenotypes of direct vs indirect ARDS in single-center and multicenter studies**. *Chest* 2015, **147**(6):1539-1548.
24. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA, Network NA: **Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials**. *The Lancet Respiratory medicine* 2014, **2**(8):611-620.
25. Paul WE, Zhu J: **How are T(H)2-type immune responses initiated and amplified?** *Nature reviews Immunology* 2010, **10**(4):225-235.
26. Brower R, Matthay M, Morris A, Schoenfeld D, Thompson B, Wheeler A, Network. TARDS: **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**(18):1301-1308.
27. Gessner C HS, Hartmut K: **Exhaled Breath Condensate Nitrite and Its Relation to Tidal Volume in Acute Lung Injury**. *Chest* 2003, **124**(3):1046-1052.
28. Writing Group for the PI, Simonis FD, Serpa Neto A, Binnekade JM, Braber A, Bruin KCM, Determann RM, Goekoop GJ, Heidt J, Horn J *et al*: **Effect of a Low vs Intermediate Tidal Volume Strategy on Ventilator-Free Days in Intensive Care Unit Patients Without ARDS: A Randomized Clinical Trial**. *Jama* 2018, **320**(18):1872-1880.
29. van der Zee P, Somhorst P, Molinger J, Hasan D, Gommers D: **A feasibility study into adenosine triphosphate measurement in exhaled breath condensate: a potential bedside method to monitor alveolar deformation**. *Purinergic signalling* 2018.

Supplementary files

Table 1 Correlation coefficients between mechanical ventilation parameters and concentrations of cytokines and chemokines in SEBC

	PEEP	Ppeak	Pplat	Compliance	FiO_2 (%)	P/F	Vt/kg
IL-1β	.016	.208	.011	-.107	.195	-.023	-.166
IL-4	-.162	-.051	.234	.068	.019	-.063	-.089
IL-6	.003	.013	-.055	.004	.033	.331	.092
IL-8	-.006	-.129	-.143	.077	-.071	.427*	.172
IL-10	.003	-.190	-.160	.265	.024	.005	.391*
IL-12	.042	-.170	-.101	.298	.081	-.028	.392*
IL-17	-.098	-.069	-.025	.072	.076	-.160	-.129
IFN-ɣ	.092	-.019	.059	.213	.096	-.173	.236
MCP-1	.032	-.105	-.098	.100	-.036	.381*	.195
MIP-1β	-.006	-.197	-.162	.267	.021	.031	.397*
TNF-α	-.033	.288	.207	-.175	.172	-.082	.230

A positive value indicates a positive correlation, whereas a negative value indicates a negative correlation, * < 0.05 ** < 0.01. Abbreviations: IL interleukin, G-CSF granulocyte colony-stimulating factor, IFN interferon, MCP monocyte chemoattractant protein, MIP macrophage inflammatory protein, TNF tumour necrosis factor, PEEP positive end-expiratory pressure, Ppeak peak airway pressure, Pplat plateau airway pressure, FiO_2 fraction of inspired oxygen, P/F PaO_2/FiO_2 ratio, Vt tidal volume.

Table 2 Correlation coefficients between parameters of systemic inflammation and concentrations of cytokines and chemokines in SEBC

	Temperature	CRP	Thrombocytes	Leukocytes	Haemoglobin	Urea	Creatinine
IL-1β	-.099	-.310	-.244	.003	.081	.126	.020
IL-4	-.042	-.194	.276	-.376*	-.131	-.457*	-.445*
IL-6	-.068	.063	-.143	.075	.010	-.077	-.042
IL-8	.054	.092	-.128	.157	.005	-.051	-.076
IL-10	.054	.360	-.062	.039	-.013	.023	.031
IL-12	.035	.337	-.092	-.016	-.038	.027	.065
IL-17	.144	.111	.037	.001	-.393*	.233	.201
IFN-ɣ	.168	.329	.180	-.144	-.138	-.089	-.061
MCP-1	.042	.125	-.129	.116	.033	-.058	-.067
MIP-1β	.060	.351	-.064	.016	-.021	.012	.027
TNF-α	-.069	-.038	.182	-.230	-.255	-.228	-.081

A positive value indicates a positive correlation, whereas a negative value indicates a negative correlation, * < 0.05. Abbreviations: IL interleukin, G-CSF granulocyte colony-stimulating factor, IFN interferon, MCP monocyte chemoattractant protein, MIP macrophage inflammatory protein, TNF tumour necrosis factor, CRP C reactive protein.

Table 3 Correlation coefficients between parameters of circulation and concentrations of cytokines and chemokines in SEBC

Cytokine/chemokine	MAP	UO	Serum lactate	Noradrenaline dose	pH
IL-1β	-.366	-.070	.889**	.316	-.313
IL-4	.293	-.079	.288	.100	.278
IL-6	-.339	.034	.817**	.380	-.079
IL-8	-.305	.113	.742*	-.093	.076
IL-10	-.255	-.112	.321	.521	.022
IL-12	-.259	-.067	.076	.514	-.001
IL-17	.048	.261	.110	-.184	.170
IFN-ɣ	-.268	-.097	.096	.559*	.091
MCP-1	-.300	.112	.233	.626*	.068
MIP-1β	-.263	-.102	.797**	.518	.029
TNF-α	-.427*	-.272	.790**	.389	-.195

A positive value indicates a positive correlation, whereas a negative value indicates a negative correlation, * < 0.05, **p<0.01. Abbreviations: IL interleukin, G-CSF granulocyte colony-stimulating factor, IFN interferon, MCP monocyte chemoattractant protein, MIP macrophage inflammatory protein, TNF tumour necrosis factor, MAP mean arterial pressure, UO urinary output.

6

Community-acquired pneumonia subgroups and differential response to corticosteroids: a secondary analysis of controlled studies

Esther Wittermans[1,11a], Philip van der Zee[2a], Hongchao Qi[3], Ewoudt van de Garde[4,5], Claudine Blum[6], Mirjam Christ-Crain [7], Diederik Gommers[2], Jan Grutters[8,9], Paul Voorn[10], Willem Jan Bos[1,11], Henrik Endeman[2]

[1] Department of Internal Medicine, St. Antonius Hospital Nieuwegein, The Netherlands
[2] Department of Adult Intensive Care, Erasmus Medical Center Rotterdam, The Netherlands
[3] Department of Biostatistics, Erasmus Medical Center Rotterdam, The Netherlands
[4] Department of Clinical Pharmacology, St. Antonius Hospital Nieuwegein, The Netherlands
[5] Division of Pharmacoepidemiology and Clinical Pharmacology, Faculty of Science Utrecht Utrecht, the Netherlands
[6] Department of General Internal & Emergency Medicine, Medical University Clinic Aarau, Switzerland
[7] Department of Endocrinology, University Hospital Basel, Switzerland
[8] Department of Pulmonology, St. Antonius Hospital Nieuwegein, the Netherlands
[9] Division of Heart and Lungs, University Medical Centre Utrecht, the Netherlands
[10] Department of Medical Microbiology and Immunology, St. Antonius Hospital Nieuwegein, the Netherlands
[11] Department of Internal Medicine, University Medical Centre Leiden, the Netherlands
[a] Both authors contributed equally to the manuscript

Published: ERJ Open Res Vol. 8 Issue 1

ABSTRACT

Background Latent class analysis (LCA) has identified subgroups with meaningful treatment implications in acute respiratory distress syndrome. We performed a secondary analysis of three studies to assess whether LCA can identify clinically distinct subgroups in community-acquired pneumonia (CAP) and whether the treatment effect of adjunctive corticosteroids differs between subgroups.

Methods LCA was performed on baseline clinical and biomarker data from the Ovidius trial (n=304) and STEP trial (n=727), both randomised controlled trials investigated adjunctive corticosteroid treatment in CAP, and the observational Triple P cohort (n=201). Analyses were conducted independently in two cohorts (Ovidius-TripleP combined and STEP trial). In both cohorts, differences in clinical outcomes and response to adjunctive corticosteroid treatment were examined between subgroups identified through LCA.

Results A two-class model fitted both cohorts best. Class 2 patients had more signs of systemic inflammation compared to Class 1. In both cohorts, length of stay was longer and in-hospital mortality rate was higher in Class 2. In the Ovidius trial, corticosteroids reduced median length of stay in Class 2 (6.5 vs 9.5 days) but not in Class 1 (p-value for interaction=0.02). In the STEP trial, there was no significant interaction for length of stay. We found no significant interaction between class assignment and adjunctive corticosteroid treatment for secondary outcomes.

Conclusions In two independent cohorts, LCA identified two classes of CAP patients with different clinical characteristics and outcomes. Given the different response to adjunctive corticosteroids in the Ovidius trial, LCA might provide a useful basis to improve patient selection for future trials.

INTRODUCTION

Treatment of community-acquired pneumonia (CAP) is based on early diagnosis and prompt initiation of antibiotic therapy.[1] Despite effective treatment, CAP remains a leading cause of mortality and morbidity worldwide.[2] Adjunctive treatment with corticosteroids might improve clinical outcomes in patients with CAP.[3]

A local immune response is crucial to contain and eliminate the primary infection in CAP.[4] However, an uncontrolled or excessive local immune response could result in systemic inflammation and subsequent multi-organ dysfunction.[5]

Adjunctive treatment with corticosteroids, a potent inhibitor of the immune response, has shown to reduce length of stay (LOS) and time to clinical stability in hospitalised patients with CAP.[3] However, corticosteroids did not lower the mortality rate, and increased the incidence of hospital readmission and hyperglycaemia requiring insulin therapy.[3] Therefore, treatment guidelines do not recommend routine use of corticosteroids in patients with CAP.[1]

In a clinically heterogeneous condition as CAP, it is likely that a subgroup of patients does benefit from corticosteroid treatment.[6] It has been hypothesised that corticosteroid treatment should be given to the subgroup with an excessive systemic inflammation response, whereas patients with a local and controlled immune response should not receive corticosteroid treatment.[7] So far, patients with CAP have been stratified by Pneumonia Severity Index (PSI), initial C-reactive protein concentration, and inflammatory status, but stratification did not result in an unequivocal definition of a subgroup benefiting from corticosteroid therapy and therefore did not result in adjustment of clinical guidelines.[3, 8-10]

In other heterogeneous conditions such as sepsis or acute respiratory distress syndrome, substantial efforts have been made to identify subgroups characterised by different prognoses and responses to treatment.[11] In patients with acute respiratory distress syndrome, a latent class analysis (LCA) was used to identify subgroups with different treatment responses to ventilator and fluid management.[12, 13] The identification of patients that are likely to respond to (corticosteroid) treatment, i.e. predictive enrichment, is a step towards personalised medicine and improved patient selection for future clinical trials.[14]

In this secondary analysis of three controlled studies, we attempted to identify CAP subgroups through LCA of baseline clinical and biomarker data from two randomised

controlled trials and one prospective cohort study. In addition, we examined whether LCA based subgroups were associated with different clinical outcomes and a different response to adjunctive corticosteroids.

MATERIALS AND METHODS

Study population and study design

This is a secondary analysis of demographic, clinical and biomarker data obtained at baseline from patients enrolled in the observational Triple P cohort[15], and two multicentre randomised controlled trials: the Ovidius trial (NCT00471640)[16] and the STEP trial (NCT00973154).[17] All studies included hospitalised adult patients with CAP (see supplementary materials).

In the Ovidius trial, patients with CAP were randomly allocated to receive intravenous dexamethasone 5mg daily or placebo for four days following hospital admission.[16] The STEP trial randomised 727 patients with CAP to either placebo or oral prednisolone 50mg daily for seven days in the per protocol analysis.[17] LOS, the primary endpoint in the Ovidius trial and main secondary endpoint in the STEP trial, was significantly reduced in patients assigned to adjunctive treatment with corticosteroids. Details of the original studies are published elsewhere.[16, 17]

The Ovidius trial and Triple-P study were approved by the Medical Ethics Committee at the St Antonius Hospital. The ethical committees of all participating hospitals and Swissmedic approved the STEP trial.

METHODS

Two separate LCAs were performed for the identification of subgroups: one in a combined cohort of Triple P and the Ovidius trial, and one in the STEP trial. The observational Triple P cohort (n = 201) and the Ovidius trial (n = 304) were combined to obtain a larger sample size. We chose to combine these cohorts as the Triple P cohort preceded the Ovidius trial and reported similar clinical and biomarker data. The Ovidius trial and Triple-P study are two mutually exclusive cohorts. The STEP trial (n = 727) was analysed independently as different clinical and biomarker data were recorded.

After identification of subgroups by LCA, differences in clinical outcomes between these subgroups and the presence of interaction between treatment allocation and LCA de-

fined subgroups were assessed separately in both cohorts (Ovidius-TripleP combined and STEP). For the Ovidius-TripleP cohort, only patients who participated in the Ovidius trial were included in the analysis of the interaction between adjunctive corticosteroids. The primary outcome was LOS and secondary outcomes were ICU-admission, in-hospital mortality, 30-day mortality, and 30-day hospital readmission.

Statistical analysis

Baseline characteristics of the Ovidius-TripleP combined and STEP cohort were presented as count (%) for categorical variables, and mean (standard deviation) or median (interquartile range, IQR) for continuous variables, after testing for normal distribution. Baseline characteristics of both cohorts were compared using an independent samples Students t-test, Mann-Whitney U test, or Chi-squared test, as appropriate.

The DepmixS4 package in R 4.0.0 (R core team, 2020) was used to conduct the LCA. Baseline clinical and biomarker data obtained at hospital admission were used as class-defining variables in the LCA. A full list of class defining variables included in the latent class analysis for each cohort are shown in the supplementary materials. Assignment of patients to classes was performed independently of clinical outcomes. LCA was first conducted in the Ovidius-TripleP cohort, and was repeated independently in the STEP cohort. Missing data were accommodated by estimating model parameters based on the full information maximum likelihood (FIML).[18]

We fitted models with latent classes ranging from two to five classes. To determine the best fitting model, we used the following criteria: 1) clinical interpretability, i.e. whether identified classes corresponded to clinically coherent clusters of clinical and biomarker data; 2) the number of patients assigned to the smallest class, where a model with small class size is statistically less meaningful; and 3) the Bayesian Information Criterion, where a lower number corresponds with improved model fit. For clinical interpretability, all continuous variables in the LCA were rescaled to a z-scale with a mean of zero and standard deviation of 1. Subsequently, clinical interpretability was assessed by two authors independently (PZ, HE). Discrepancies were resolved by consensus, and if necessary, a third author was consulted.

Once the number of classes was determined, patients were assigned to the class with maximum probability of class assignment based on the LCA model. The probability of a patient being assigned to a specific class is a weighted average of the N class-specific probabilities in latent class analysis, so each patient has probabilities assigned to all classes, respectively. For example, a patient with a probability of 90% to be assigned to class 1 and 10% probability to be assigned to class 2 was assigned to class 1. Sub-

sequently, the association between class assignment and baseline characteristics or clinical outcomes was tested using Chi-squared, Mann-Whitney U or independent samples T-test, as appropriate. Finally, for the Ovidius trial and STEP cohort, we tested the interaction between randomly assigned treatment and class on clinical outcomes with Poisson regression model for LOS and Chi-squared test for categorical outcomes. A p-value <0.05 was deemed statistically significant.

RESULTS

Baseline characteristics

Baseline characteristics of both cohorts are presented in **Table 1** and eTable 1. In short, patients in the Ovidius-TripleP cohort were younger, had less comorbidities, and had higher levels of inflammatory biomarkers as compared to patients in the STEP cohort. LOS was longer in the Ovidius-TripleP cohort as compared to the STEP cohort (8.5; 6.0-13.0 days versus 7.0; 4.0-10.0 days, p-value <0.001). Secondary outcomes were similar between both cohorts.

Latent class modelling: identification of number of classes

We fitted latent class models ranging from two to five classes (**Table 2**). First, we examined clinical interpretability by plotting class-defining variables for all models and assessed whether identified classes corresponded to clinically coherent subgroups (**Figure 1** & eFigure 1). In both the Ovidius-TripleP and STEP cohort, a two-class model resulted in two coherent and distinct clinical classes. Addition of a third, fourth or fifth class resulted in further subdivision of patients assigned to Class 2 in the two class model, without adding an additional coherent or distinct clinical class. Subsequently, we explored the number of patients per subgroup in all models (**Table 2**). The addition of a third class to the two-class model resulted in a smaller third class of 58 patients in the Ovidius-TripleP cohort and 72 patients in the STEP cohort. We observed a further decline in the number of patients in the smallest class in a four or five-class model. Lastly, the Bayesian Information Criterion was lowest in the five-class model in both the Ovidius-TripleP cohort and STEP cohort, suggesting a better fit for the five-class model. Even though a data driven approach suggested more than two classes, a three class model did not result in an evident third clinical entity. Thus, clinical interpretability of the two-class models in conjunction with the relatively small number of patients in the three, four or five-class models, led us to proceed with the two-class models for both cohorts. We will refer to the classes as Class 1 and Class 2 in the remainder of the manuscript. For the three class model we show clinical characteristics for each class in the supplementary materials.

Table 1. Baseline characteristics

	Ovidius-TripleP cohort (n = 505)	STEP cohort (n = 727)
Demographic data		
Age (years)	67 (51-78)	73 (60-83)
Male	295 (58.4%)	452 (62.2%)
Caucasian	491 (97.2%)	712 (97.9%)
Duration of symptoms (days)	4 (2-7)	4 (2-7)
Antibiotics at home	130 (25.7%)	164 (22.6%)
Corticosteroids at home	34 (6.7%)	14 (1.9%)
Comorbidities		
Nursing home resident	19 (3.8%)	0 (0.0%)
Cerebrovascular accident	46 (9.1%)	67 (9.2%)
Malignancy	45 (8.9%)	70 (9.6%)
Liver disease	2 (0.4%)	28 (3.9%)
Renal disease	40 (7.9%)	218 (30.0%)
Congestive heart failure	68 (13.5%)	134 (18.4%)
Chronic obstructive pulmonary disease	98 (19.4%)	122 (16.8%)
Diabetes mellitus	77 (15.2%)	139 (19.1%)
Current smoker	81 (16.0%)	188 (25.9%)
Pneumonia severity index score	87 (63-114)	90 (64-113)
Outcome		
Length of stay (days)	8.5 (6.0-13.0)	7.0 (4.0-10.0)
ICU admission	38 (7.5%)	39 (5.4%)
In-hospital mortality	24 (4.8%)	24 (3.3%)
30-day mortality	26 (5.1%)	28 (3.9%)
Readmission	37 (7.3%)	39 (5.4%)

Data are n (%), mean (SD), or median (IQR).

Table 2. Fit statistics for latent class models from two to five class models

Ovidius-TripleP cohort						
Number of Classes	BIC	Number of patients per Class				
		1	2	3	4	5
2	124577.2	411	94			
3	120741.9	153	58	294		
4	120507.3	61	112	296	36	
5	118372.7	33	25	94	108	245

STEP cohort						
Number of Classes	BIC	Number of patients per Class				
		1	2	3	4	5
2	116815.7	574	153			
3	106770.5	99	556	72		
4	71445.1	24	125	466	112	
5	70684.5	132	18	44	434	99

BIC Bayesian information criterion

Figure 1A

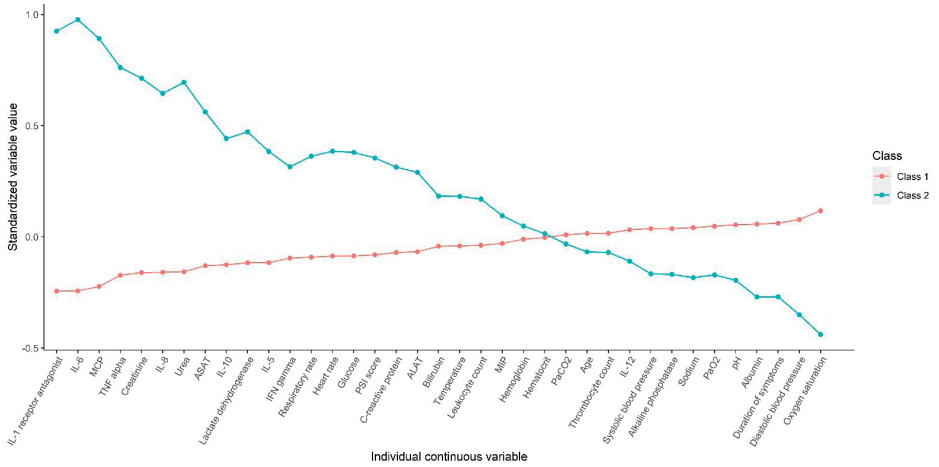


Figure 1B

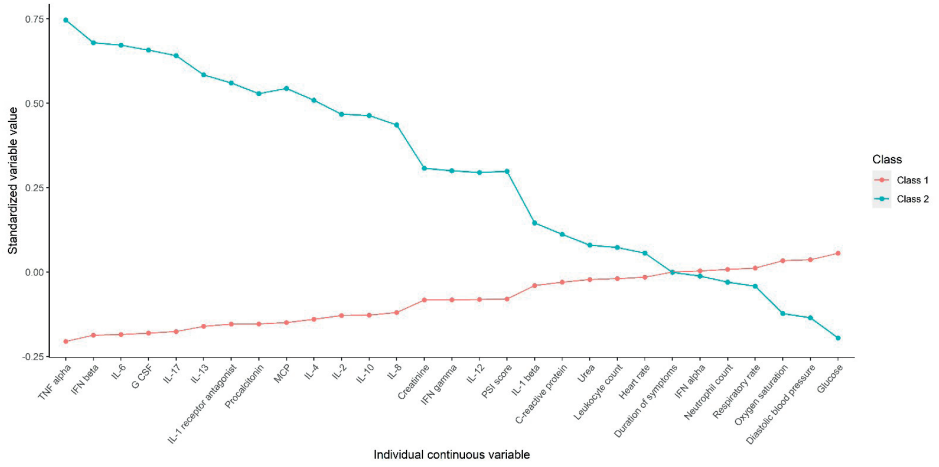


Figure 1

Continuous variables (standardised) by class assignment for the a) Ovidius–TripleP cohort and b) Steroids in Pneumonia (STEP) cohort. Differences between the standardised values of each variable by class (*y*-axis) for the variable shown on the *x*-axis. The variables are sorted by degree of separation between classes: from the maximum positive separation on the left (where the standardised value of class 2 is higher than the standardised value of class 1) to the maximum negative separation on the right (where the standardised value of class 2 to is lower than the standardised value of class 1). The crossover of the lines indicates that the standardised value for this variable was the same for classes 1 and 2 (*i.e.* no difference between class 1 and class 2 for this variable). Therefore, variables near the intersection of both lines are similar in both classes and thus are not class-defining. The method of variable standardisation is described in the methods section. If the standardised value of a certain variable is 1 for a class, it means that the mean value for that variable within that class was one standard deviation higher than the mean value for that variable in the whole cohort. LAT: alanine transaminase; ASAT: aspartate transaminase; G-CSF: granulocyte colony-stimulating factor; IFN: interferon; IL: interleukin; MCP: monocyte chemoattractant protein; MIP:macrophage inflammatory protein; P_{aCO2}: arterial carbon dioxide tension; P_{aO2}: arterial oxygen tension; PSI: pneumonia severity index; TNF: tumour necrosis factor.

Patients were assigned to the class for which the probability of belonging to that class was the highest. Thus al patients in both cohorts were assigned to either class 1 or class 2. In the Ovidius-TripleP cohort, 411 patients were assigned to Class 1 and 94 to Class 2. In the STEP cohort, 574 patients and 153 patients were assigned to Class 1 and Class 2, respectively. Probabilities of class assignment for the two-class model are presented in eFigure 2. The average probability of a patient belonging to the class to which it was assigned was 99.4% for Class 1 and 98.6% Class 2 in the Ovidius-TripleP cohort, and 98.7% for Class 1 and 99.1% for Class 2 in the STEP cohort. This indicated good model fit and robust class assignment.

Class characteristics

Differences between Class 1 and Class 2 in the Ovidius-TripleP cohort are shown in **Figure 1A** and **Table 3**. The most noteworthy and clinically relevant differences were that patients in Class 2 had higher plasma concentration of IL-1 receptor antagonist, IL-6, monocyte chemoattractant protein and tumour necrosis factor alpha compared to Class 1. Furthermore, patients assigned to Class 2 seemed to have more severe illness seeing as they had lower oxygen saturation, lower diastolic blood pressure and had a higher PSI score at admission.

Table 3. Values of variables at baseline stratified by class in the Ovidius-TripleP cohort

Variable	Class 1 (n = 411)	Class 2 (n = 94)	No. missing N (%)
Age (years)	67 [51 - 79]	67 [53 - 76]	0 (0)
Alanine transaminase (U/L)	28 [16 - 44]	28 [19 - 55]	152 (30.1)
Albumin (g/L)	37 [33 - 40]	36 [33 - 38]	339 (67.0)
Alkaline phosphatase (U/L)	90 [70 - 130]	90 [61 - 113]	167 (33.1)
Altered mental status[§]	47 (11.4)	10 (10.6)	11 (2.2)
Aspartate transaminase (U/L)	34 [23 - 51]	38 [25 - 78]*	153 (30.3)
Bilirubin (µmol/L)	12 [9 -16]	16 [12 – 24]*	199 (39.4)
C-reactive protein (mg/L)	196 [94 - 300]	294 [107 – 389]*	9 (1.8)
Cortisol (nmol/L)[§]	226.0 [148.0 – 159.1]	446.8 [322.4 – 691.4]*	23 (4.6)
Corticosteroids at home[§]	30 (7.5)	4 (4.4)	15 (3.0)
Creatinine (µmol/L)	84 [70 – 106]	111 [91 - 157]*	10 (2.0)
Diastolic blood pressure (mmHg)	75 [68 – 83]	70 [60 - 80]*	11 (2.2)
Duration of symptoms (days)	4 [3 – 7]	3 [2 - 5]*	16 (3.2)
Glucose (mmol/L)	7.0 [6.0 - 8.3]	7.5 [6.2 - 9.8]*	39 (7.7)
Heart rate (beats per minute)	95 [82 - 109]	110 [87 - 118]*	9 (1.8)
Haematocrit (L/L)	0.40 [0.36 - 0.43]	0.39 [0.37 - 0.43]	17 (3.4)
Haemoglobin (mmol/L)	8.3 [7.6 - 9.0]	8.3 [7.8 - 9.0]	10 (2.0)
Interferon gamma (pg/mL)	202.1 [16.8 - 288.3]	217.8 [10.0 - 354.9]	213 (42.2)
Interleukin-1 receptor antagonist (pg/mL)	102.8 [18.0- 448.4]	1042.5 [204.2 - 4309.2]*	79 (15.6)

Table 3. Values of variables at baseline stratified by class in the Ovidius-TripleP cohort (*continued*)

Variable	Class 1 (n = 411)	Class 2 (n = 94)	No. missing N (%)
Interleukin-6 (pg/mL)	51.0 [18.0 - 156.3]	749.7 [101.2 - 2209.7]*	63 (12.5)
Interleukin-5 (pg/mL)	0.54 [0.24 - 0.77]	0.46 [0.26 - 0.61]	333 (65.9)
Interleukin-8 (pg/mL)	14.8 [8.1 - 29.3]	59.5 [32.1 - 152.2]*	56 (11.1)
Interleukin-10 (pg/mL)	3.4 [1.4 - 9.0]	15.9 [5.8 - 79.7]*	94 (18.6)
Interleukin-12 (pg/mL)	7.3 [4.1 - 10.5]	8.3 [5.6 - 11.5]	337 (66.7)
Lactate dehydrogenase (U/L)	328 [252 - 480]	435 [313 - 604]*	212 (42.0)
Legionella species[§]	14 (3.4)	6 (6.4)	0 (0)
Leukocyte count (10^9 cells per L)	13.5 [9.5 - 17.7]	14.9 [10.8 - 20.1]	9 (1.8)
Macrophage inflammatory protein (pg/mL)	6.1 [3.7 - 8.5]	6.8 [4.6 - 10.4]	236 (47)
Male[§]	236 (57.4)	59 (62.8)	0 (0)
Monocyte chemoattractant protein (pg/mL)	274.2 [74.7 - 536.6]	918.4 [242.9 - 2463.3]*	46 (9.1)
Oxygen saturation (%)	94 [92 – 97]	94 [88 - 96]*	107 (21.2)
Oxygen therapy[§]	70 (17.0)	30 (31.9)*	312 (61.8)
PaO_2 (kPa)	8.80 [7.80 - 10.38]	8.40 [7.10 - 9.90]*	124 (24.6)
$PaCO_2$ (kPa)	4.40 [4.10 - 4.90]	4.40 [4.00 - 4.85]	124 (24.6)
pH	7.47 [7.44 - 7.50]	7.46 [7.42 - 7.49]	124 (24.6)
Pleural effusion[§]	61 (14.8)	21 (22.3)	9 (1.8)
Pneumonia severity index score	84 [60 - 111]	102 [73 - 126]*	0 (0)
Respiratory rate (breaths per minute)	22 [18 - 30]	25 [20 - 30]*	104 (20.6)
Sodium (mmol/L)	135 [132 - 137]	133 [129 - 137]*	9 (1.8)
S. pneumoniae[§]	85 (20.7)	39 (41.5)*	0 (0)
Systolic blood pressure (mmHg)	131 [120 - 146]	126 [112- 145]	11 (2.2)
Temperature (°C)	38.2 [37.4 - 39.0]	38.5 [37.4 - 39.3]	9 (1.8)
Thrombocyte count (10^9 cells per L)	253 [200 - 317]	237 [177 - 327]	9 (1.8)
Tumour necrosis factor alpha (pg/mL)	5.9 [3.1 - 10.2]	12.4 [6.1 - 29.6]*	224 (44.4)
Urea (mmol/L)	6.4 [4.6 - 9.5]	9.8 [6.3 – 15.2]*	17 (3.4)

Data are shown as Median [IQR] or N (%). * Statistically significant difference between class 1 and class 2. [§]Non class defining variables (variable not included in LCA). Missing data is n (%) for whole cohort.

Differences between Class 1 and Class 2 in the STEP Cohort are shown in **Figure 1B** and **Table 4**. In the STEP-cohort the most noteworthy and clinically relevant differences between classes were higher plasma concentrations of tumour necrosis factor alpha, interferon beta, IL-6, granulocyte colony stimulating factor and IL-17 in class 2 compared to Class 1. Patients in Class 2 also had a higher PSI score compared to Class 1. However there was no difference in oxygen saturation, or diastolic blood pressure.

Table 4. Values of variables at baseline stratified by class in the STEP cohort

	Class 1 (n = 574)	Class 2 (n = 153)	No. missing N (%)
Altered mental status[§]	33 (5.7)	13 (8.5)	0 (0)
C-reactive protein (mg/L)	155 [74 - 247]	171 [93 - 268]	7 (1)
Creatinine (µmol/L)	86 [68 - 109]	98 [72 - 132]*	6 (0.8)
Diastolic blood pressure (mmHg)	70 [60 - 78]	66 [59 - 75]	4 (0.6)
Duration of symptoms (days)	4 [2 - 7]	4 [2 - 7]	17 (2.3)
Glucose (mmol/L)	6.4 [5.5 - 7.7]	6.0 [5.5 - 7.3]	179 (24.6)
Granulocyte colony stimulating factor (pg/mL)	7.0 [7.0 - 8.7]	21.1 [9.3 - 59.3]*	55 (7.6)
Heart rate (beats per minute)	83 [72 - 95]	84 [71 - 101]	4 (0.6)
Interferon alpha (pg/mL)	0.24 [0.24 - 0.33]	0.56 [0.30 - 1.02]*	55 (7.6)
Interferon beta (pg/mL)	22.7 [14.5 – 34.0]	41.3 [22.0 - 74.1]*	55 (7.6)
Interferon gamma (pg/mL)	2.8 [2.8 - 2.8]	2.8 [2.8 - 4.6]*	55 (7.6)
Interleukin-1 beta (pg/mL)	1.0 [1.0 - 1.0]	1.0 [1.0 - 2.8]*	55 (7.6)
Interleukin-1 receptor antagonist (pg/mL)	33.0 [33.0 - 551.5]	1280.1 [33.0 - 6244.1]*	55 (7.6)
Interleukin-2 (pg/mL)	4.4 [4.4 - 4.4]	4.4 [4.4 - 4.4]*	55 (7.6)
Interleukin-4 (pg/mL)	5.5 [5.5 - 5.5]	5.5 [5.5 - 24.4]*	55 (7.6)
Interleukin-6 (pg/mL)	40.6 [14.6 - 102.5]	172.0 [59.7 - 748.4]*	55 (7.6)
Interleukin-8 (pg/mL)	3.9 [1.9 - 9.7]	19.8 [6.6 - 46.1]*	55 (7.6)
Interleukin-10 (pg/mL)	0.9 [0.7 - 1.4]	2.2 [1.3 - 4.8]*	55 (7.6)
Interleukin-12 (pg/mL)	1.1 [1.1 - 1.4]	2.2 [1.3 - 3.7]*	55 (7.6)
Interleukin-13 (pg/mL)	1.3 [1.3 - 1.3]	2.4 [1.3 - 8.8]*	55 (7.6)
Interleukin-17 (pg/mL)	0.57 [0.57 - 0.57]	0.87 [0.57 - 1.86]*	55 (7.6)
Legionella species[§]	11 (1.9)	3 (2.0)	102 (14.0)
Leukocyte count (10^9 cells per L)	11.9 [8.7 - 15.6]	12.2 [9.2 - 15.8]	4 (0.6)
Male[§]	345 (60.1)	107 (69.9)*	0 (0)
Monocyte chemoattractant protein (pg/mL)	39.8 [25.5 - 70.1]	66.6 [37.2 - 242.9]*	55 (7.6)
Neutrophil count (10^9 cells per L)	9.8 [6.9 - 13.2]	10.2 [7.4 - 13.3]	64 (9.7)
Oxygen saturation (%)	95 [92 - 96]	94 [92 - 96]	25 (3.4)
Oxygen therapy[§]	298 (51.9)	79 (51.6)	6 (0.8)
Pleural effusion[§]	65 (11.3)	18 (11.8)	0 (0)
Pneumonia severity index score	88 [63 - 111]	98 [74 - 131]*	0 (0)
Procalcitonin (ng/mL)	0.39 [0.16 - 1.68]	1.14 [0.28 - 10.35]*	133 (18.3)
Respiratory rate (breaths per minute)	20 [18 - 24]	20 [17 - 24]	136 (18.7)
S. pneumoniae[§]	75 (13.1)	31 (20.3)*	104 (14.3)
Tumour necrosis factor alpha (pg/mL)	1.8 [1.8 - 1.9]	2.7 [1.8 - 4.0]*	55 (7.6)
Urea (mmol/L)	6.6 [4.8 – 10.0]	7.9 [5.4 - 13.4]*	37 (5.1)

Data are shown as Median [IQR] or N (%).* Statistically significant difference between class 1 and class 2. [§]Non class defining variables (variable not included in LCA). Missing data is n (%) for whole cohort.

Class prediction with a small number of variables

In order to determine whether classes could be identified based on a reduced number of variables, we tested a three variable model including variables available for both cohorts and differing most between classes (IL-6, TNF-α and oxygen saturation at hospital admission). An Area under the curve (AUC) was calculated to evaluate this reduced model compared to the full model. The AUC was 0.78 and 0.65 respectively for the Ovidius-TripleP cohort and STEP cohort. Contingency tables comparing class membership between reduced and full model are shown in the supplementary material (eTable 2).

Association between class and clinical outcomes

Subsequently, we assessed clinical outcomes in both classes (**Table 5**). In the Ovidius-TripleP cohort, patients in Class 2 had a significantly longer LOS (10.5; 6.5-16.0 days versus 8.0; 6.0-12.0 days, p-value <0.01) and higher rate of ICU admissions. In-hospital mortality and 30-day mortality rates were significantly higher in Class 2. Similar results were observed in the STEP cohort, as patients in Class 2 had longer LOS (7.0; 5.0-12.0 days versus 7.0; 4.0-10.0 days, p-value <0.01), and higher in-hospital mortality rate (**Table 5**).

Table 5. Association between class assignment and clinical outcomes

Ovidius-TripleP cohort			
Clinical outcome	**Class 1 (n = 411)**	**Class 2 (n = 94)**	**p-value**
Length of stay (days)	8.0 (6.0-12.0)	10.5 (6.5-16.0)	<0.01
ICU admission	16 (3.9%)	22 (23.4%)	<0.01
In-hospital mortality	14 (3.4%)	10 (10.6%)	0.01
30-day mortality	15 (3.6%)	11 (11.7%)	<0.01
Readmission	29 (7.1%)	8 (8.5%)	0.79

STEP cohort			
Clinical outcome	**Class 1 (n = 574)**	**Class 2 (n = 153)**	**p-value**
Length of stay (days)	7.0 (4.0-10.0)	7.0 (5.0-12.0)	<0.01
ICU admission	28 (4.9%)	11 (7.2%)	0.35
In-hospital mortality	13 (2.3%)	11 (7.2%)	<0.01
30-day mortality	18 (3.1%)	10 (6.5%)	0.09
Readmission	30 (5.2%)	9 (5.9%)	0.91

Data are N (%) or median (IQR). ICU intensive care unit.

Effect of corticosteroids on outcome stratified by class

Lastly, we used the data from the Ovidius trial and the STEP cohort to determine whether classes responded differently to randomly assigned adjunctive treatment with cortico-

steroids (**Table 6**). In the Ovidius trial, dexamethasone reduced LOS in patients assigned to Class 2 (6.5; 5.5-10.0 days versus 9.5; 5.0-14.5 days), whereas LOS was similar between treatment groups in Class 1 (p-value for interaction 0.02). In the STEP cohort, there was no significant interaction for LOS between class assignment and adjunctive treatment with corticosteroids. In both cohorts, we found no significant interaction for secondary outcomes between class assignment and adjunctive treatment with corticosteroids.

Table 6. Differential response to adjunctive corticosteroid treatment by latent class assignment

Ovidius trial					
	Class 1 (n = 251)		**Class 2 (n = 52)**		
	Corticosteroid (n = 124)	Placebo (n= 128)	Corticosteroid (n = 27)	Placebo (n = 25)	p-value*
Length of stay (days)	6.5 (5.0-8.5)	7.5 (5.5-10.5)	6.5 (5.5-10.0)	9.5 (5.0-14.5)	0.02
ICU admission	4 (3.2)	4 (3.1)	3 (11.1)	6 (24.0)	0.64
In-hospital mortality	7 (5.6)	3 (2.3)	1 (3.7)	5 (20.0)	0.12
30-day mortality	7 (5.6)	4 (3.1)	2 (7.4)	5 (20.0)	0.33
Readmission	6 (4.8)	4 (3.1)	1 (3.7)	3 (12.0)	0.56
STEP cohort					
	Class 1 (n = 574)		**Class 2 (n = 153)**		
	Corticosteroid (n = 285)	Placebo (n= 289)	Corticosteroid (n = 77)	Placebo (n = 76)	p-value*
Length of stay (days)	6.0 (4.0-9.0)	7.0 (5.0-10.0)	7.0 (4.0-11.0)	8.0 (5.0-13.3)	0.46
ICU admission	11 (3.9)	17 (5.9)	6 (7.8)	5 (6.6)	0.61
In-hospital mortality	8 (2.8)	5 (1.7)	5 (6.5)	6 (7.9)	0.71
30-day mortality	11 (3.9)	7 (2.4)	4 (5.2)	6 (7.9)	0.50
Readmission	21 (7.4)	9 (3.1)	5 (6.5)	4 (5.3)	0.69

Data are N (%) or median (IQR). *p-value for interaction between class assignment and corticosteroid treatment. ICU intensive care unit.

DISCUSSION

In this secondary analysis of three controlled studies, LCA identified two distinct classes of CAP patients with different biomarker profiles, clinical characteristics and clinical outcomes. Classes were identified in two independent cohorts, despite multiple significant differences in baseline characteristics between cohorts. In the Ovidius trial, adjunctive treatment with corticosteroids reduced LOS only in patients assigned to Class 2. We found no differential treatment response for length of stay in the STEP cohort or for secondary outcomes in both cohorts.

In both cohorts, Class 2 was characterised by higher concentrations of inflammatory biomarkers, creatinine, and higher PSI scores. Additionally, patients assigned to Class 2 in the Ovidius-TripleP cohort had lower oxygen saturation, lower diastolic blood pressure, and higher incidence of oxygen therapy. In contrast, patients in Class 1 were characterised by lower concentrations of inflammatory plasma biomarkers and lower PSI scores. Furthermore, in the Ovidius-TripleP cohort, cortisol was also higher in Class 2 compared to Class 1, we assume this is explained by the fact patients with more inflammation have an higher activation of the HPA axis and thus higher cortisol levels than patients with lower levels of systemic inflammation because they are more severely ill. Moreover, in both cohorts, LOS was longer, and incidence of ICU admissions and mortality rates were higher in Class 2. Thus, patients in Class 2 had a stronger systemic inflammatory response, whereas patients in Class 1 had fewer signs of systemic inflammation. Patients in Class 2 were more likely to benefit from the anti-inflammatory effects of corticosteroids, whereas the patients assigned to Class 1 were less likely to benefit from the anti-inflammatory effects, at a similar risk of adverse effects.

Corticosteroids reduced length of stay in patients with CAP in the Ovidius trial and in the STEP trial.[16, 17] An individual patient data meta-analysis enrolling data from six randomized controlled trials comparing corticosteroids with placebo in 1506 patients with CAP, including the Ovidius trial and STEP trial, confirmed that adjunctive treatment with corticosteroids reduced LOS.[3] In this meta-analysis, however, the authors could not identify patient subgroups more likely to benefit from corticosteroids based on PSI score (PSI class 1-3 versus PSI class 4-5), initial C-reactive protein concentration (cut-off 188mg/L), initial ICU admission, or systemic inflammatory response syndrome criteria. However, in a clinically heterogeneous condition as CAP, it is unlikely that all patients benefit equally from corticosteroids.[9, 14]

In the Ovidius trial, we found that patients assigned to Class 2 who were treated with corticosteroids showed a significant reduction in LOS, whereas corticosteroids did not reduce LOS in patients assigned to Class 1. These results suggest that the subgroup of CAP patients with signs of a systemic inflammatory response benefit from corticosteroids and patients with a less pronounced systemic inflammatory response do not. However, these results could not be verified in the STEP cohort, even though PSI score was similar between both cohorts. A possible explanation is that LCAs were performed separately in the Ovidius-Triple-P cohort and STEP-cohort and included a different set of class defining variables for each cohort (Figure 1) because available biomarkers differed between both cohorts. Thus the LCA models were not identical in both cohorts. Furthermore, concentrations of inflammatory biomarkers were higher at baseline in the Ovidius cohort compared to the STEP cohort, indicating a more pronounced inflamma-

tory response in the Ovidius cohort that corticosteroids could inhibit. The reduced three variable model - consisting of IL-6, TNF-α, and oxygen saturation – showed that the AUC for class assignment was higher in the Ovidius-TripleP cohort as compared to the STEP cohort. This also suggests that the Ovidius-TripleP cohort relies more on inflammatory response. Adding to the above, in the STEP cohort disease severity, defined by PSI score, was mainly influenced by higher age and more comorbidities, whereas in the Ovidius cohort PSI score was mainly influenced by clinical characteristics and biomarker data indicative of more severe disease. Consequently, clinical variables at baseline did not differ between Class 1 and Class 2 in the STEP cohort, whereas clinical variables at baseline did differ between classes in the Ovidius cohort. Other explanations might be the difference in corticosteroid therapy (dexamethasone versus prednisolone), or the shorter LOS in the STEP cohort (median 8.5; 6.0-13.0 days in Ovidius cohort versus 7.0; 4.0-10.0 days in STEP cohort) making potential differences between classes in the STEP cohort more difficult to detect.

Inflammatory biomarkers contributed more to the determination of classes than clinical data, including C-reactive protein, procalcitonin or leukocyte count. These results suggest that the inflammatory biomarkers were able to identify aspects of CAP pathophysiology that otherwise remained hidden in routinely collected clinical data.

This study has several limitations. First, LCA model selection and interpretation often involves a level of subjectivity.[19] We decided to select a two-class model instead of more classes based on clinical interpretability and the number of patients assigned to the smallest class. Hypothetically, a third class or even a fourth class could have been forced in by generating a smaller cluster of patients with a more extreme set of variables. However, a three or more class model did not result in additional groups with more extreme variables, but in mixed classes without a coherent clinical pattern. Second, we assumed patients in Class 2 to have a systemic inflammatory response and patients in Class 1 to have a more controlled inflammatory response based on distribution of inflammatory biomarkers in plasma. We did not measure the pulmonary response and therefore do not know whether inflammation is indeed contained locally in patients assigned to Class 1. We refrained from using terms as hyperinflammatory or hypoinflammatory, previously proposed in subgroups of patients with ARDS, as all patients are admitted because of CAP, which can hardly be considered a hypoinflammatory condition.[20, 21] Third, this is a secondary analysis which requires prospective validation before definitive conclusions regarding patient subgroup identification and adjunctive corticosteroid treatment can be drawn. Fourth, LOS was calculated from day of hospital admission to day of discharge or day of in-hospital death. Thus LOS was underestimated in patients that died during hospital admission. However, in both cohorts, in-hospital

mortality rate was higher in Class 2 as compared to Class 1. If reported LOS were an underestimation this would mainly be the case in Class 2 and difference in LOS between classes would be even larger than reported. Fifth, the clinical and biomarker data used in this analysis was limited to the data available for both cohorts and to data obtained at time of hospital admission. As the aim of data collection for the original studies was to calculate the PSI score, clinical data used in the LCA resembled the PSI score to some extent and PSI score differed significantly between Class 1 and Class 2 in both cohorts. However, the classes identified by LCA were largely based on biomarker data and thus captured different subgroups of patients than classes based on PSI score only. Lastly, because data was obtained at time of hospital admission, it is unknown whether identified classes remained stable later during the course of CAP.

To our knowledge, this is the first study that identified CAP subgroups through LCA. Because the present study is a proof-of-concept study, our results are not directly applicable for daily clinical practice. Future studies should include validation of our findings in a third independent cohort, after which a clinically useful model with limited number of variables should be developed to ensure applicability. Lastly, validation of these clinical models in predicting response to treatment should be assessed in prospective studies.

In conclusion, we identified two classes of CAP patients with different clinical characteristics, inflammatory profiles and clinical outcomes in two independent cohorts. Furthermore, in the Ovidius trial, adjunctive treatment with corticosteroids reduced LOS only in the patients assigned to Class 2 and not in the patients assigned to Class 1. Given the different response to adjunctive treatment in subgroups in the Ovidius cohort, identification of subgroups might provide a useful basis for improved patient selection in future clinical trials.

FUNDING

St Antonius Research Fund.

REFERENCES:

1. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, Cooley LA, Dean NC, Fine MJ, Flanders SA *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**(7):e45-e67.
2. Troeger C, Blacker BF, Khalil IA, Rao PC, Cao SJ, Zimsen SRM, Albertson S, Stanaway JD, Deshpande A, Farag T *et al*: **Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016**. *Lancet Infectious Diseases* 2018, **18**(11):1191-1210.
3. Briel M, Spoorenberg SMC, Snijders D, Torres A, Fernandez-Serrano S, Meduri GU, Gabarrus A, Blum CA, Confalonieri M, Kasenda B *et al*: **Corticosteroids in Patients Hospitalized With Community-Acquired Pneumonia: Systematic Review and Individual Patient Data Metaanalysis**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2018, **66**(3):346-354.
4. Bermejo-Martin JF, Almansa R, Martin-Fernandez M, Menendez R, Torres A: **Immunological profiling to assess disease severity and prognosis in community-acquired pneumonia**. *Lancet Resp Med* 2017, **5**(12):E35-E36.
5. Rhen T, Cidlowski JA: **Antiinflammatory action of glucocorticoids - New mechanisms for old drugs**. *New Engl J Med* 2005, **353**(16):1711-1723.
6. Chalmers JD: **Corticosteroids for community-acquired pneumonia: a critical view of the evidence**. *European Respiratory Journal* 2016, **48**(4):984-986.
7. Meijvis SCA, van de Garde EMW, Rijkers GT, Bos WJW: **Treatment with anti-inflammatory drugs in community-acquired pneumonia**. *J Intern Med* 2012, **272**(1):25-35.
8. Torres A, Sibila O, Ferrer M, Polverino E, Menendez R, Mensa J, Gabarrus A, Sellares J, Restrepo MI, Anzueto A *et al*: **Effect of corticosteroids on treatment failure among hospitalized patients with severe community-acquired pneumonia and high inflammatory response: a randomized clinical trial**. *Jama* 2015, **313**(7):677-686.
9. Remmelts HH, Meijvis SC, Heijligenberg R, Rijkers GT, Oosterheert JJ, Bos WJ, Endeman H, Grutters JC, Hoepelman AI, Biesma DH: **Biomarkers define the clinical response to dexamethasone in community-acquired pneumonia**. *J Infect* 2012, **65**(1):25-31.
10. Urwyler SA, Blum CA, Coslovsky M, Mueller B, Schuetz P, Christ-Crain M: **Cytokines and Cortisol - predictors of treatment response to corticosteroids in community-acquired pneumonia?** *J Intern Med* 2019, **286**(1):75-87.
11. Scicluna BP, van Vught LA, Zwinderman AH, Wiewel MA, Davenport EE, Burnham KL, Nurnberg P, Schultz MJ, Horn J, Cremer OL *et al*: **Classification of patients with sepsis according to blood genomic endotype: a prospective cohort study**. *Lancet Resp Med* 2017, **5**(10):816-826.
12. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA, Network NA: **Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials**. *The Lancet Respiratory medicine* 2014, **2**(8):611-620.
13. Famous KR, Delucchi K, Ware LB, Kangelaris KN, Liu KD, Thompson BT, Calfee CS, Network A: **Acute Respiratory Distress Syndrome Subphenotypes Respond Differently to Randomized Fluid Management Strategy**. *American journal of respiratory and critical care medicine* 2017, **195**(3):331-338.

14. 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**(2):147-155.
15. Endeman H, Meijvis SC, Rijkers GT, van Velzen-Blad H, van Moorsel CH, Grutters JC, Biesma DH: **Systemic cytokine response in patients with community-acquired pneumonia**. *The European respiratory journal* 2011, **37**(6):1431-1438.
16. Meijvis SC, Hardeman H, Remmelts HH, Heijligenberg R, Rijkers GT, van Velzen-Blad H, Voorn GP, van de Garde EM, Endeman H, Grutters JC *et al*: **Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial**. *Lancet (London, England)* 2011, **377**(9782):2023-2030.
17. Blum CA, Nigro N, Briel M, Schuetz P, Ullmer E, Suter-Widmer I, Winzeler B, Bingisser R, Elsaesser H, Drozdov D *et al*: **Adjunct prednisone therapy for patients with community-acquired pneumonia: a multicentre, double-blind, randomised, placebo-controlled trial**. *Lancet (London, England)* 2015, **385**(9977):1511-1518.
18. Cham H, Reshetnyak E, Rosenfeld B, Breitbart W: **Full Information Maximum Likelihood Estimation for Latent Variable Interactions With Incomplete Indicators**. *Multivariate Behav Res* 2017, **52**(1):12-30.
19. Al Sallakh MA, Rodgers SE, Lyons RA, Sheikh A, Davies GA: **Identifying patients with asthma-chronic obstructive pulmonary disease overlap syndrome using latent class analysis of electronic health record data: a study protocol**. *NPJ Prim Care Respir Med* 2018, **28**(1):22.
20. Sinha P, Calfee CS: **Phenotypes in acute respiratory distress syndrome: moving towards precision medicine**. *Current opinion in critical care* 2019, **25**(1):12-20.
21. Calfee CS, Delucchi KL, Sinha P, Matthay MA, Hackett J, Shankar-Hari M, McDowell C, Laffey JG, O'Kane CM, McAuley DF *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**(9):691-698.

SUPPLEMENTARY MATERIAL

1. Methods
2. Variables included in LCA model for the Ovidius-TripleP cohort and the STEP cohort
3. eFigure 1: Continuous variables by class assignment in a three, four, or five-class model for the Ovidius-TripleP cohort and the STEP cohort.
4. eFigure 2: Probability of class assignment for the Ovidius-TripleP cohort and the STEP cohort.
5. eTable 1 Ovidius-TripleP cohort and STEP cohort baseline characteristics
6. eTable 2 Contingency tables comparing class membership of the reduced model compared to the full model
7. Clinical characteristics for each class in a three-class model in Ovidius-TripleP and STEP cohort

1. METHODS

Definition of CAP

CAP was defined as a new pulmonary infiltrate on chest x-ray, accompanied by at least one of the following criteria: cough, sputum, temperature >38°C (or <35°C), auscultatory findings consistent with pneumonia, C-reactive protein >15 mg/L, leukocyte count >10x10^9 cells/L or <4x10^9 cells/L, or >10% bands in leucocyte differentiation.[1, 2]

Systemic biomarkers

Systemic concentrations of inflammatory biomarkers were measured in plasma samples obtained on the day of hospital admission before administration of any study medication. Samples were stored at -80°C. Analysis was performed using multiplex multi-analyte profiling (Millipore, Billerica, USA), as described previously.[3, 4] Different biomarker panels were used in the Ovidius-TripleP cohort and the STEP cohort (Table 1).

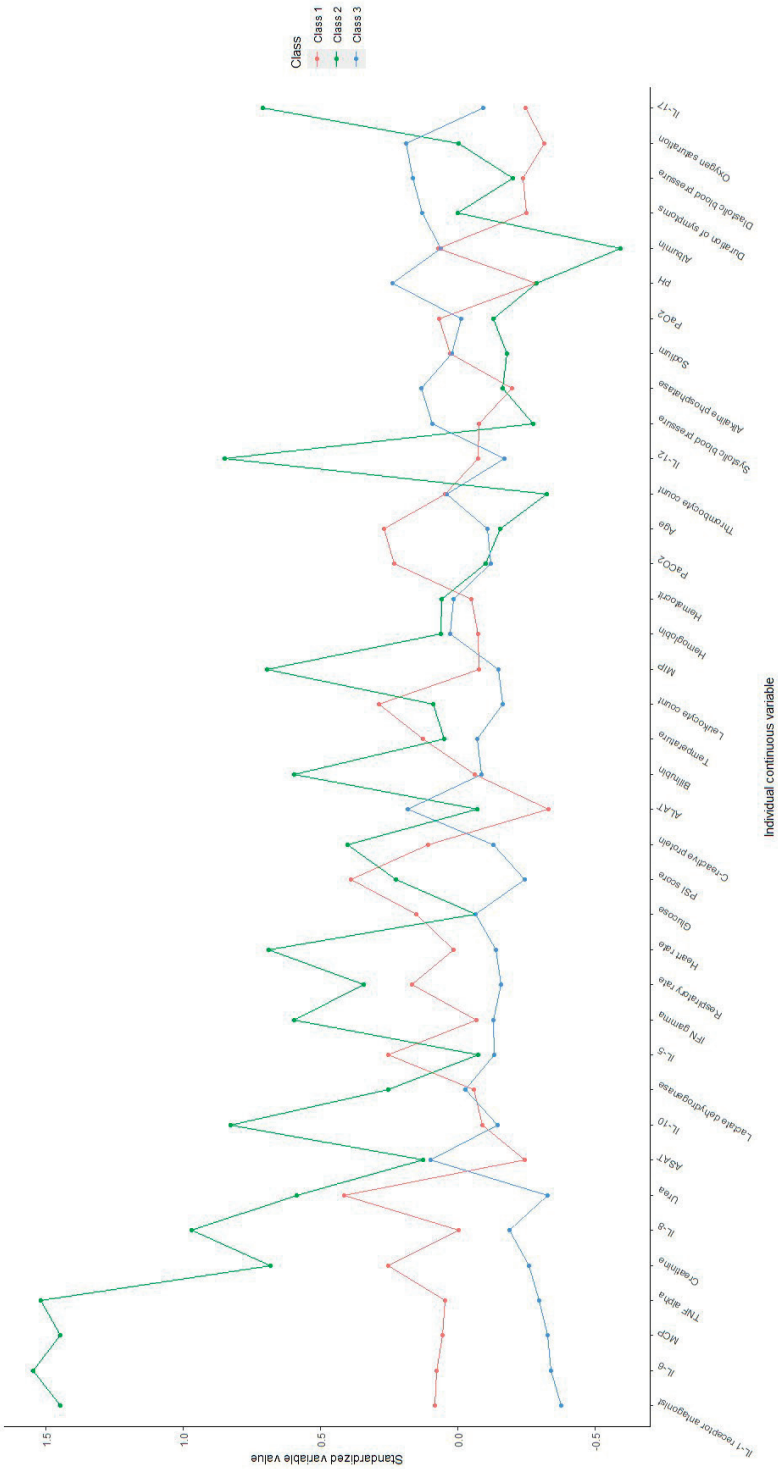
REFERENCES:

1. Fine MJ, Singer DE, Hanusa BH, Lave JR, Kapoor WN: **Validation of a pneumonia prognostic index using the MedisGroups Comparative Hospital Database**. *Am J Med* 1993, **94**(2):153-159.
2. Niederman MS, Mandell LA, Anzueto A, Bass JB, Broughton WA, Campbell GD, Dean N, File T, Fine MJ, Gross PA *et al*: **Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention**. *American journal of respiratory and critical care medicine* 2001, **163**(7):1730-1754.
3. Endeman H, Meijvis SC, Rijkers GT, van Velzen-Blad H, van Moorsel CH, Grutters JC, Biesma DH: **Systemic cytokine response in patients with community-acquired pneumonia**. *The European respiratory journal* 2011, **37**(6):1431-1438.
4. Urwyler SA, Blum CA, Coslovsky M, Mueller B, Schuetz P, Christ-Crain M: **Cytokines and Cortisol - predictors of treatment response to corticosteroids in community-acquired pneumonia?** *J Intern Med* 2019, **286**(1):75-87.

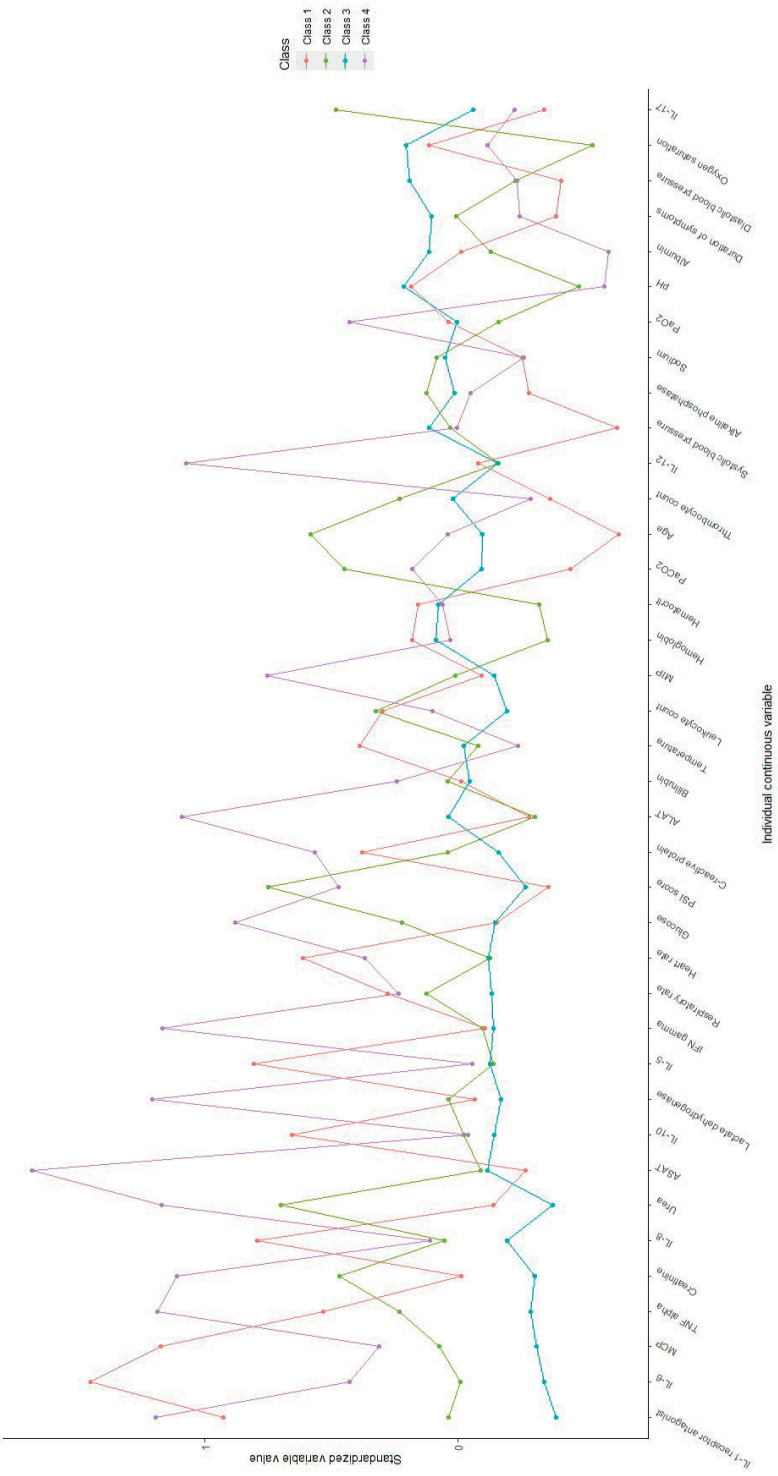
3. eFigure 1

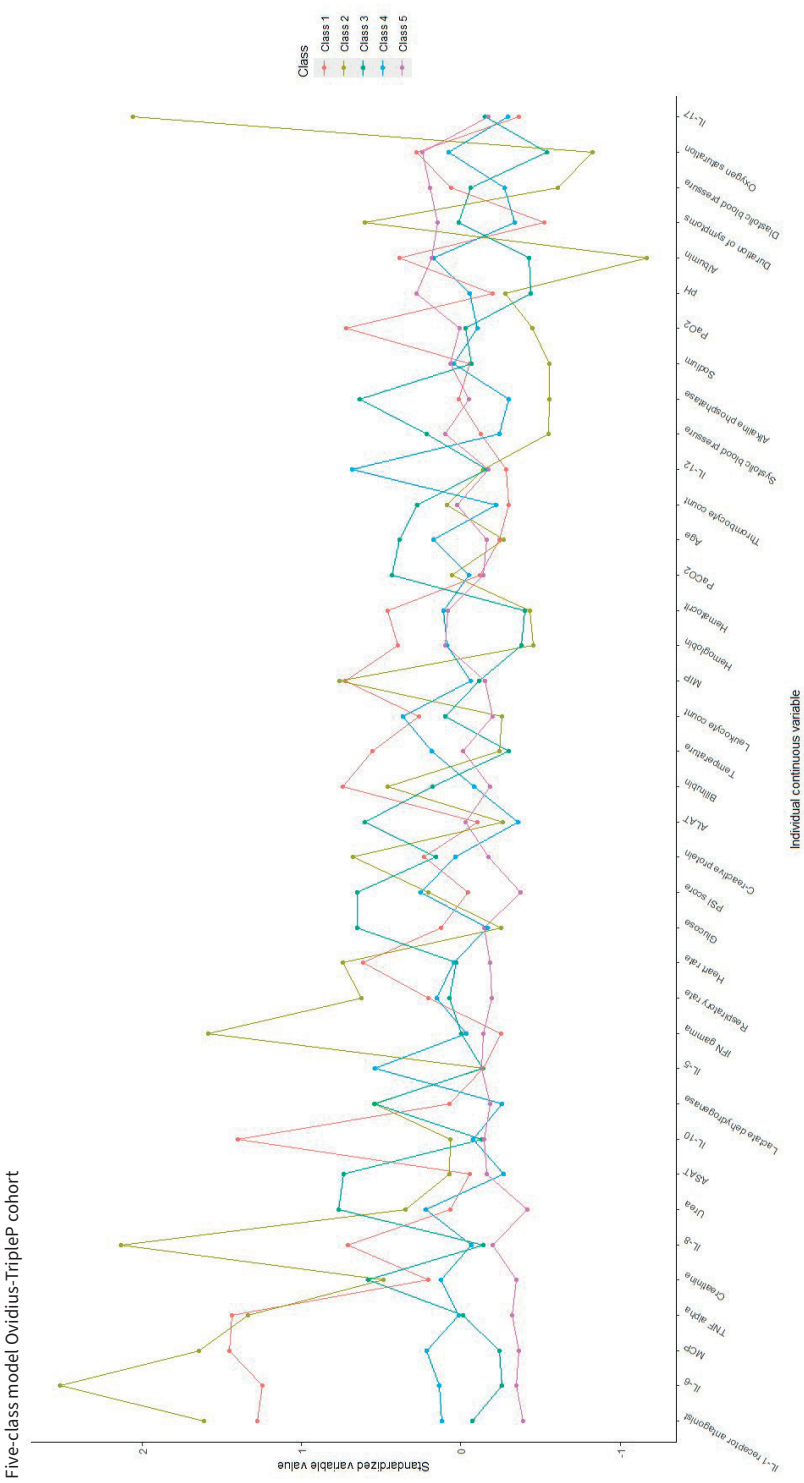
eFigure 1A Continuous variables by class assignment in a three, four, or five-class model in the Ovidius-TripleP cohort. On the Y-axis differences in the standardised values of each variable by subgroup are shown. The individual continuous variables are shown along the x-axis. Variables are sorted by degree of separation between classes.

Three-class model Ovidius-TripleP cohort



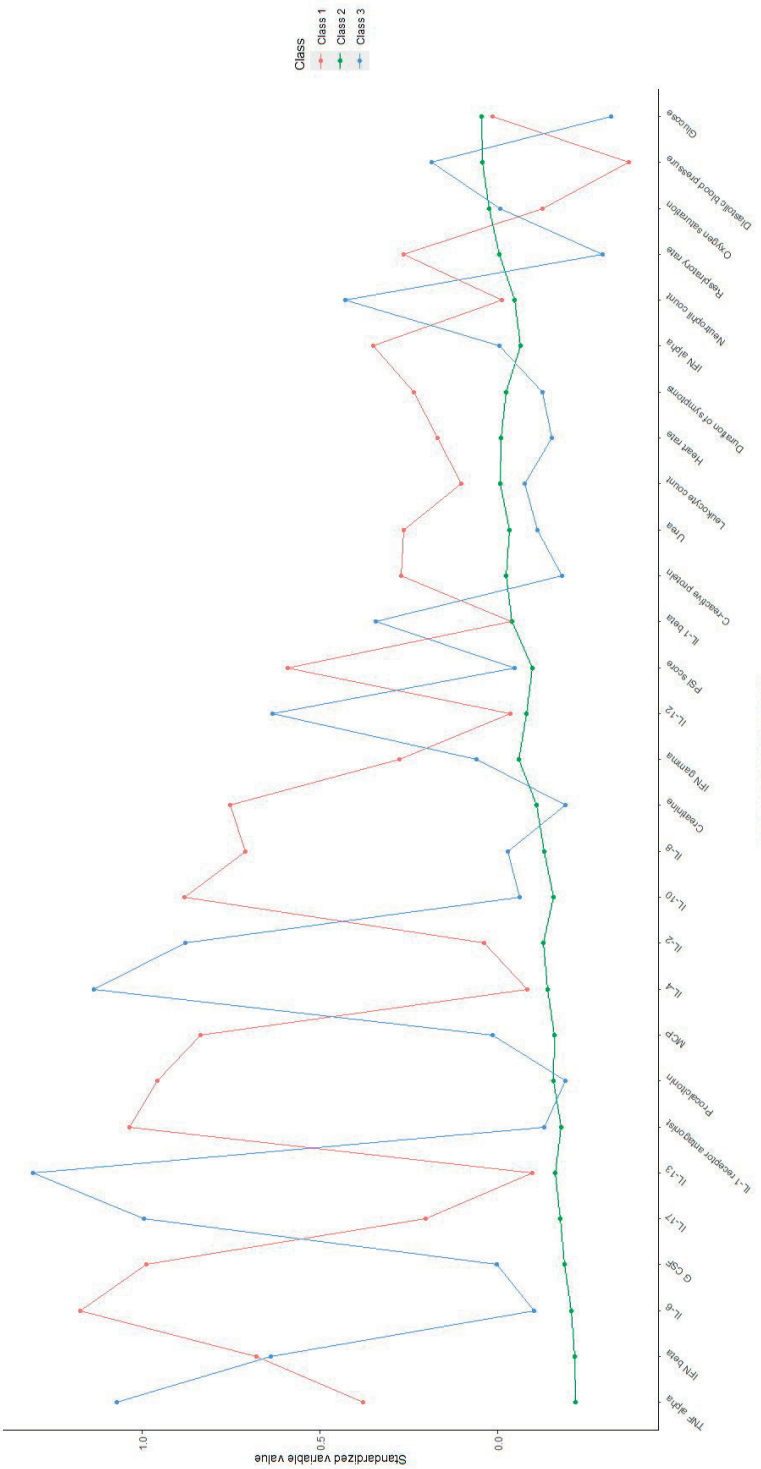
Four-class model Ovidius-TripleP cohort


Class
Class 1
Class 2
Class 3
Class 4
IL-17 receptor antagonist
IL-6
MCP
TNF alpha
Creatinine
IL-8
Urea
ASAT
IL-10
Lactate dehydrogenase
IL-5
IFN gamma
Respiratory rate
Heart rate
Glucose
PSI score
Creactive protein
ALAT
Bilirubin
Temperature
Leukocyte count
MAP
Hemoglobin
Hematocrit
PaCO2
Age
Thrombocyte count
IL-12
Systolic blood pressure
Alkaline phosphatase
Sodium
PaO2
pH
Albumin
Duration of symptoms
Diastolic blood pressure
Oxygen saturation
IL-17
Individual continuous variable
1
0
Standardized variable value

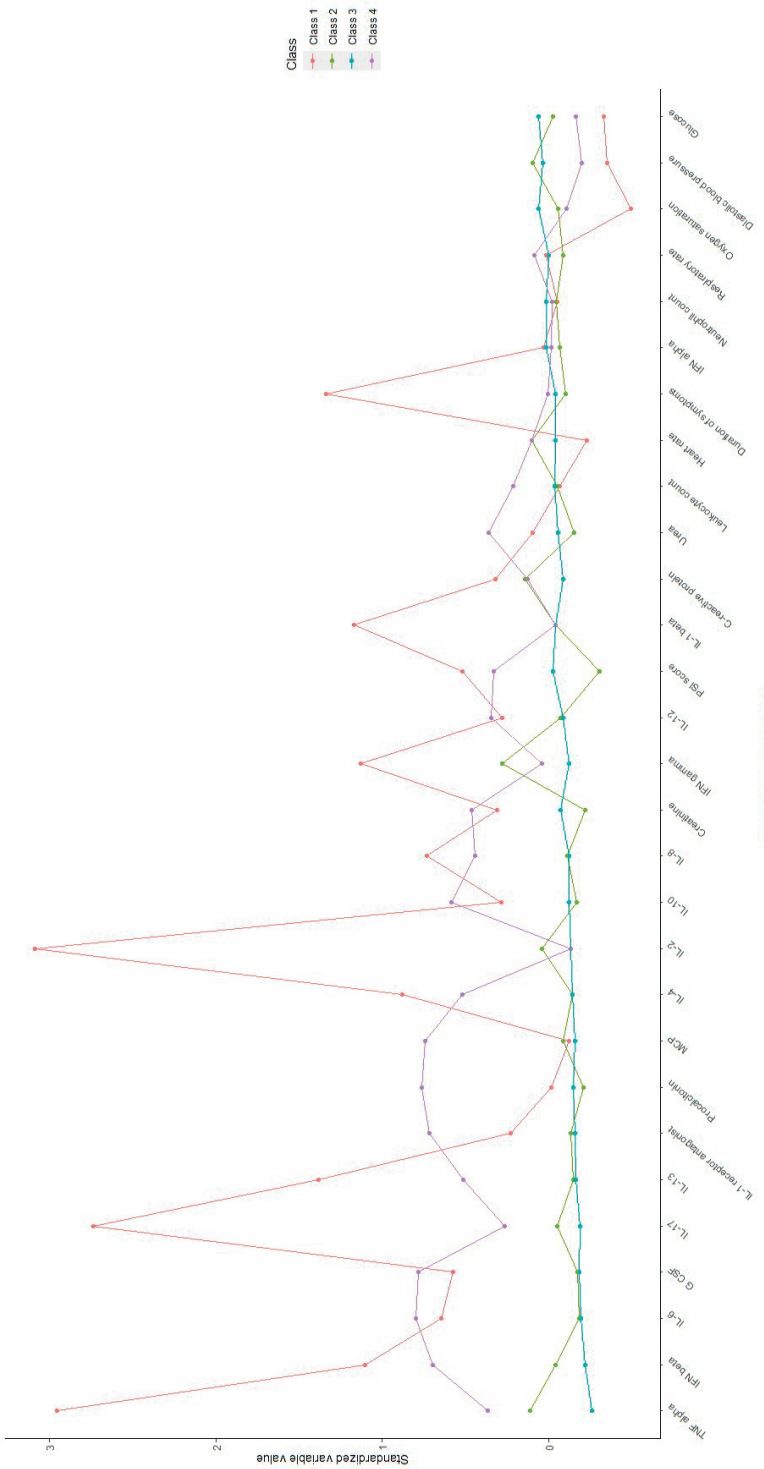


Abbreviations: IL= interleukin; MCP= Monocyte chemoattractant protein; TNF=Tumour necrosis factor; , ASAT= Aspartate transaminase ; IFN= Interferon; PSI= Pneumonia Severity index; ALAT=Alanine transaminase; MIP= Macrophage inflammatory protein.

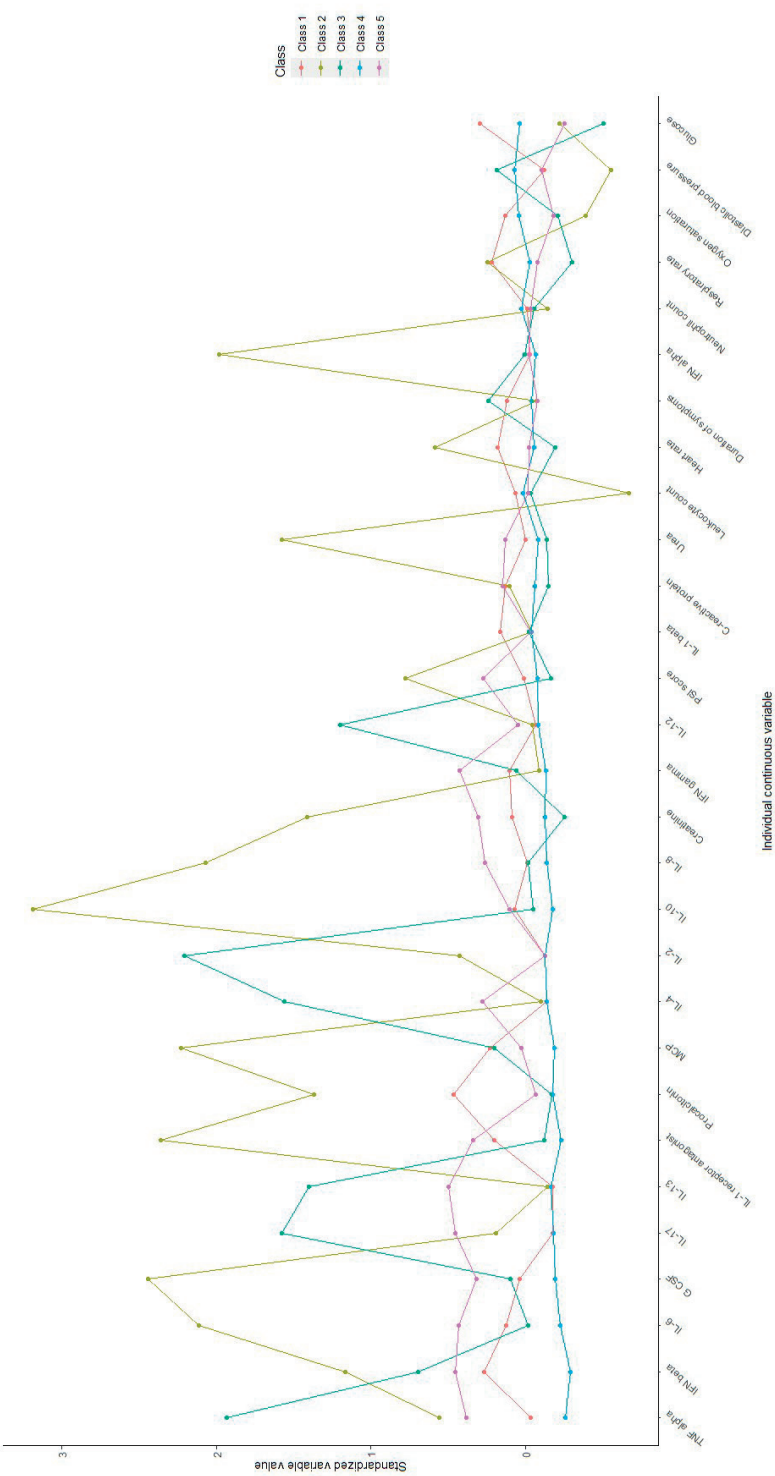
e**Figure 1B Continuous variables by class assignment in a three, four, or five-class model in the STEP cohort**.
On the Y-axis differences in the standardised values of each variable by subgroup are shown. The individual continuous variables are shown along the x-axis. Variables are sorted by degree of separation between classes.
Three-class model STEP cohort



Four-class model STEP cohort



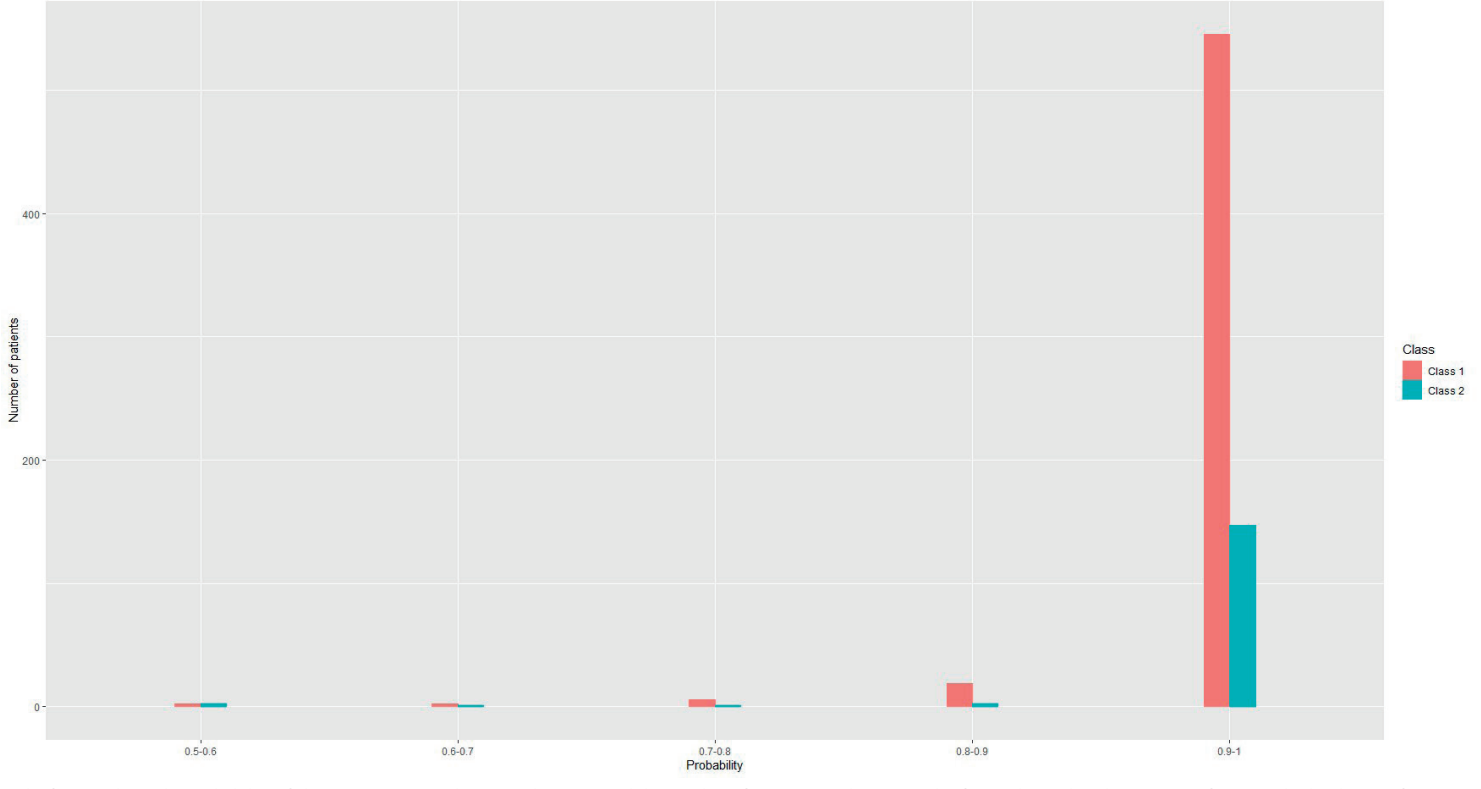
Five-class model STEP cohort



Abbreviations: IL= interleukin; MCP= Monocyte chemoattractant protein; TNF=Tumour necrosis factor; IFN= Interferon; PSI= Pneumonia Severity index; G-CSF= Granulocyte colony-stimulating factor.

4. eFigure 2
eFigure 2A: Probability of class assignment Ovidius-TripleP cohort in a two-class model

eFigure E2B Probability of class assignment STEP cohort in a two-class model



In the figures above the probability of class assignment is shown on the x-axis and the number of patients on the y-axis. This figure shows that the majority of patients had a chance of 90-100% of being assigned to the correct class. For subsequent analyses, patients were assigned to the class with the highest probability of assignment.

5. eTable 1 Baseline characteristics Ovidius-TripleP cohort and STEP cohort

	Ovidius-TripleP cohort (n = 505)	STEP cohort (n = 727)
Demographic data		
Age (years)	67 (51-78)	73 (60-83)
Male	295 (58.4%)	452 (62.2%)
Caucasian	491 (97.2%)	712 (97.9%)
Duration of symptoms (days)	4 (2-7)	4 (2-7)
Antibiotics at home	130 (25.7%)	164 (22.6%)
Corticosteroids at home	34 (6.7%)	14 (1.9%)
Comorbidities		
Nursing home resident	19 (3.8%)	0 (0.0%)
Cerebrovascular accident	46 (9.1%)	67 (9.2%)
Malignancy	45 (8.9%)	70 (9.6%)
Liver disease	2 (0.4%)	28 (3.9%)
Renal disease	40 (7.9%)	218 (30.0%)
Congestive heart failure	68 (13.5%)	134 (18.4%)
Chronic obstructive pulmonary disease	98 (19.4%)	122 (16.8%)
Diabetes mellitus	77 (15.2%)	139 (19.1%)
Current smoker	81 (16.0%)	188 (25.9%)
Clinical data		
Altered mental status*	57 (11.3%)	46 (6.3%)
Pleural effusion	82 (16.2%)	86 (11.8%)
Systolic blood pressure (mmHg)	130 (118-146)	124 (110-140)
Diastolic blood pressure (mmHg)	75 (66-82)	69 (60-78)
Heart rate (beats per minute)	97 (84-111)	83 (72-96)
Respiratory rate (breaths per minute)	24 (20-30)	20 (18-24)
Temperature (°C)	38.2 (37.4-39.0)	37.6 (37.0-38.2)
Oxygen saturation (%)	94 (91-97)	94 (92-96)
Oxygen therapy (yes/no)	100 (19.8%)	377 (51.9%)
Oxygen therapy (L/min)	1 (0-4)	2 (2-4)
Pneumonia severity index score	87 (63-114)	90 (64-113)
Routine laboratory data		
Leukocyte count (10^9 cells per L)	13.8 (9.7-18.4)	12.0 (8.8-15.6)
Neutrophil count (10^9 cells per L)	-	9.9 (6.9-13.3)
Thrombocyte count (10^9 cells per L)	250 (197-318)	-
C-reactive protein (mg/L)	210 (95-317)	160 (79-249)
Procalcitonin (ng/mL)	-	0.46 (0.17-2.50)

5. eTable 1 Baseline characteristics Ovidius-TripleP cohort and STEP cohort (*continued*)

	Ovidius-TripleP cohort (n = 505)	STEP cohort (n = 727)
Haematocrit (L/L)	0.40 (0.37-0.43)	-
Haemoglobin (mmol/L)	8.3 (7.6-9.0)	-
Urea (mmol/L)	6.8 (4.8-10.2)	6.9 (4.9-10.4)
Creatinine (µmol/L)	90 (71-112)	88 (69-113)
Sodium (mmol/L)	134 (131-137)	137 (134-139)
Glucose (mmol/L)	7.1 (6.0-8.6)	7.3 (6.3-8.9)
pH	7.47 (7.44-7.49)	-
PaO_2 (kPa)	8.7 (7.7-10.3)	-
$PaCO_2$ (kPa)	4.4 (4.1-4.9)	-
Alkaline phosphatase (U/L)	90 (68-127)	-
Aspartate transaminase (U/L)	35 (23-52)	-
Alanine transaminase (U/L)	28 (17-45)	-
Lactate dehydrogenase (U/L)	351 (255-518)	-
Bilirubin (µmol/L)	13 (9-17)	-
Albumin (g/L)	37 (33-39)	32 (28-36)
Biomarker data		
Interleukin-1 receptor antagonist (pg/mL)	163.8 (25.1-694.7)	33.0 (33.0-1126.5)
Interleukin-1 beta (pg/mL)	-	1.0 (1.0-1.0)
Interleukin-2 (pg/mL)	-	4.4 (4.4-4.4)
Interleukin-4 (pg/mL)	-	5.5 (5.5-5.5)
Interleukin-5 (pg/mL)	0.5 (0.3-0.7)	-
Interleukin-6 (pg/mL)	72.0 (22.5-248.7)	52.0 (19.0-142.8)
Interleukin-8 (pg/mL)	18.9 (9.1-42.6)	5.0 (2.0-13.0)
Interleukin-10 (pg/mL)	4.5 (1.6-14.2)	1.0 (0.7-1.9)
Interleukin-12 (pg/mL)	7.4 (4.3-10.8)	1.2 (1.1-1.7)
Interleukin-13 (pg/mL)	-	1.3 (1.3-1.3)
Interleukin-17 (pg/mL)	-	0.6 (0.6-0.6)
Tumor necrosis factor alpha (pg/mL)	6.7 (3.6-12.4)	1.7 (1.7-2.3)
Interferon alpha (pg/mL)	-	0.3 (0.3-0.4)
Interferon beta (pg/mL)	-	24.0 (15.0-41.0)
Interferon gamma (pg/mL)	205.9 (12.8-298.6)	2.8 (2.8-2.8)
Monocyte chemoattractant protein (pg/mL)	317.6 (88.5-654.2)	43.0 (27.0-84.8)
Macrophage inflammatory protein (pg/mL)	6.3 (3.9-8.8)	-
Granulocyte colony stimulating factor (pg/mL)	-	7.0 (7.0-13.0)
Causative microorganism		
S. pneumoniae	124 (24.6)	106 (14.6)
H. influenzae	27 (5.3)	-

5. eTable 1 Baseline characteristics Ovidius-TripleP cohort and STEP cohort (*continued*)

	Ovidius-TripleP cohort (n = 505)	**STEP cohort (n = 727)**
Legionella species	20 (4.0)	13 (1.8)
C. burnetii	28 (5.5)	-
Other	96 (19.0)	-
None identified	210 (41.6)	-
Outcome		
Length of stay (days)	8.5 (6.0-13.0)	7.0 (4.0-10.0)
ICU admission	38 (7.5%)	39 (5.4%)
In-hospital mortality	24 (4.8%)	24 (3.3%)
30-day mortality	26 (5.1%)	28 (3.9%)
Readmission	37 (7.3%)	39 (5.4%)

Data are n (%), mean (SD), or median (IQR). * Defined as a state of awareness that differed from the normal awareness of a conscious person, scored by the attending physician.

6. eTable 2 Contingency tables comparing class membership in the reduced model and the full model for Ovidius-TripleP cohort and STEP cohort

Ovidius-TripleP		Full model	
		Class 1	Class 2
Reduced model	Class 1	343	26
	Class 2	68	68

STEP		Full model	
		Class 1	Class 2
Reduced model	Class 1	515	90
	Class 2	59	63

Data are numbers of patients

7. Clinical characteristics for each class for a three-class model in Ovidius-TripleP and STEP cohort
eTable 3 Values of variables at baseline stratified by class in the Ovidius-TripleP cohort

Variable	Class 1 (n=153)	Class 2 (n=58)	Class 3 (n=294)
Temperature (°C)	38.4 [37.4 - 39.1]	38.3 [37.4 - 39.2]	38.1 [37.4 - 39.0]
Leukocyte count (10^9 cells per L)	15.7 [11.1- 20.6]	13.6 [9.2- 18.5]	12.6 [9.4- 16.6]
C-reactive protein (mg/L)	235 [90 - 352]	297 [110- 428]	190 [97 - 271]
Age (years)	72 [60- 81]	66 [41- 76]	63 [50 - 76]
Systolic blood pressure (mmHg)	126 [112- 146]	127 [112 - 143]	134 [120 - 147]
Diastolic blood pressure (mmHg)	70 [62 - 79]	70 [60 - 80]	77 [70 - 85]
Heart rate (beats per minute)	100 [84 - 113]	110 [99 - 126]	94 [82 - 107]
Respiratory rate (breaths per minute)	25 [20 - 30]	25 [20 - 30]	20 [18 - 30]
Haematocrit (L/L)	0.39 [0.36- 0.43]	0.40 [0.37- 0.43]	0.40 [0.37- 0.43]
Urea (mmol/L)	9.0 [6.3 – 13.7]	9.8 [6.4- 15.3]	5.7 [4.3 - 8.4]
Sodium (mmol/L)	134 [131 - 137]	133 [130 - 137]	135 [132 - 137]
Glucose (mmol/L)	7.3 [6.1 - 9.1]	7.4 [6.2- 8.6]	7.0 [6.0 - 8.3]
PaO_2 (kPa)	8.70 [7.50 - 10.80]	8.40 [7.68- 9.50]	8.90 [7.90-10.22]
$PaCO_2$ (kPa)	4.40 [4.10 - 5.10]	4.55 [4.00 - 4.93]	4.40 [4.00 - 4.73]
Creatinine (µmol/L)	99 [81 - 134]	107 [83 - 139]	82 [68 - 100]
Alkaline phosphatase (U/L)	86 [64 - 115]	80 [61 - 110]	96 [71 - 137]
Aspartate transaminase (U/L)	32 [24- 43]	47 [24 - 81]	35 [23 - 60]
Alanine transaminase (U/L)	22 [15 - 33]	28 [20 - 45]	32 [18 - 58]
Lactate dehydrogenase (U/L)	370 [265 - 489]	435 [304 - 547]	326 [248- 502]
Bilirubin (µmol/L)	13 [9 - 16]	18 [14 - 26]	12 [9 - 17]
Albumin (g/L)	37 [33 - 40]	35 [31 - 37]	37 [34 - 39]
Haemoglobin (mmol/L)	8.2 [7.5- 9.0]	8.3 [7.8 - 9.0]	8.4 [7.6 - 9.1]
Thrombocyte count (10^9 cells per L)	261 [197 - 315]	228 [177 - 292]	250 [201 - 324]
Oxygen saturation (%)	93 [90 - 97]	94 [91 - 96]	95 [92 - 97]
Duration of symptoms (days)	3 [2 - 5]	4 [2 - 6]	5 [3 - 7]
Interleukin-1 receptor antagonist (pg/mL)	387.9 [72.9- 1538.6]	1937.5 [628.4- 5823.8]	56.4 [11.4- 242.2]
Interleukin-6 (pg/mL)	220.6 [73.1 - 697.7]	1427.2 [258.1 - 2922.7]	35.6 [15.0 - 81.7]
Interleukin-8 (pg/mL)	37.2 [19.5 - 60.9]	113.6 [42.6 - 267.0]	11.5 [6.6 - 19.1]
Interleukin-10 (pg/mL)	11.1 [3.8- 28.9]	55.6 [10.9- 179.6]	2.2 [1.1- 4.8]
Pneumonia severity index score	106 [76 - 129]	95 [70 - 123]	77 [56 - 102]
Tumour necrosis factor alpha (pg/mL)	9.9 [6.5- 16.2]	32.2 [11.1- 49.0]	5.1 [2.6- 7.7]
Interferon gamma (pg/mL)	239.1 [21.2- 312.5]	195.0 [8.5- 406.7]	182.9 [17.1- 266.9]
Monocyte chemoattractant protein (pg/mL)	462.4 [143.9- 1122.0]	1957.5 [327.3- 3124.5]	226.9 [56.3- 425.0]
Macrophage inflammatory protein (pg/mL)	7.2 [4.9- 9.3]	7.2 [5.2- 12.2]	5.4 [3.4- 7.2]
Interleukin-12 (pg/mL)	9.3 [5.1 - 12.3]	8.5 [5.6 - 11.7]	6.5 [3.8- 10.0]
Interleukin-5 (pg/mL)	0.54 [0.32- 0.81]	0.42 [0.22- 0.60]	0.52 [0.23- 0.67]
pH	7.45 [7.42 - 7.48]	7.45 [7.42 - 7.48]	7.48 [7.45 - 7.50]
Cortisol (nmol/L)	328.6 [225.7 - 540.3]	526.7 [339.3 - 774.7]	195.8 [133.6 - 305.2]
Altered mental status	26 (17.0)	4 (6.9)	27 (9.2)
Pleural effusion	29 (19.0)	15 (25.9)	38 (12.9)
Oxygen therapy	43 (28.1)	18 (31.0)	39 (13.3)
Female	67 (43.8)	23 (39.7)	120 (40.8)

Data are n (%) or mean (SD).

eTable 4 Values of variables at baseline stratified by class in the STEP cohort

Variable	Class 1 (n=99)	Class 2 (n=556)	Class 3 (n=72)
C-reactive protein (mg/L)	190 [72 - 294]	168 [81 - 250]	127 [67 - 210]
Diastolic blood pressure (mmHg)	65 [57 - 72]	70 [60 - 78]	69 [60 - 80]
Heart rate (beats per minute)	88 [72 - 104]	84 [73 - 95]	82 [70 - 95]
Respiratory rate (breaths per minute)	22 [18 - 26]	20 [18 - 24]	20 [16 - 24]
Urea (mmol/L)	9.3 [6.4 - 14.8]	6.6 [4.8 - 9.8]	7.0 [4.5 - 9.9]
Glucose (mmol/L)	6.5 [5.6 - 7.7]	6.5 [5.7 - 7.8]	5.8 [5.2 - 6.5]
Creatinine (µmol/L)	109 [85 - 177]	86 [67 - 108]	84 [70 - 106]
Leukocyte count (10^9 cells per L)	11.5 [7.4 - 17.1]	12.0 [8.7 - 15.9]	12.1 [9.3 - 14.6]
Oxygen saturation (%)	94 [92 - 97]	95 [92 - 96]	94 [92 - 96]
Pneumonia severity index score	106 [78 - 141]	89 [63 - 111]	82 [63 - 105]
Duration of symptoms (days)	4 [2 - 7]	4 [2 - 7]	4 [2 - 6]
Granulocyte colony stimulating factor (pg/mL)	33.0 [13.0 – 114.3]	7.0 [7.0 – 8.0]	14.0 [7.0 – 22.5]
Interferon alpha (pg/mL)	0.67 [0.39 - 1.24]	0.25 [0.25 - 0.30]	0.51 [0.27 - 1.10]
Interferon beta (pg/mL)	58.0 [34.0 - 106.5]	22.0 [14.0 – 33.0]	30.0 [17.0 – 55.0]
Interferon gamma (pg/mL)	2.8 [2.8 - 3.8]	2.8 [2.8 - 2.8]	2.8 [2.8 - 4.2]
Interleukin-1 beta (pg/mL)	1.0 [1.0 - 1.3]	1.0 [1.0 – 1.0]	1.0 [1.0 - 3.5]
Interleukin-1 receptor antagonist (pg/mL)	5375.0 [1466.0 - 11687.3]	33.0 [33.0 – 495.0]	33.0 [33.0 – 733.0]
Interleukin-10 (pg/mL)	3.2 [2.1 - 13.1]	0.9 [0.6 - 1.3]	1.5 [1.0 - 2.7]
Interleukin-12 (pg/mL)	1.8 [1.1 - 2.8]	1.1 [1.1 - 1.4]	2.0 [1.2 - 4.5]
Interleukin-13 (pg/mL)	1.3 [1.3 - 2.5]	1.3 [1.3 - 1.3]	4.0 [1.3 - 13.3]
Interleukin-17 (pg/mL)	0.6 [0.6 - 1.4]	0.6 [0.6 - 0.6]	0.8 [0.6 - 1.7]
Interleukin-2 (pg/mL)	4.4 [4.4 - 4.4]	4.4 [4.4 - 4.4]	4.4 [4.4 - 4.4]
Interleukin-4 (pg/mL)	5.5 [5.5 - 6.9]	5.5 [5.5 - 5.5]	9.0 [5.5 – 32.6]
Interleukin-6 (pg/mL)	540.5 [125.5 - 1422.5]	41.0 [15.0 – 97.0]	73.0 [28.5 - 170.5]
Interleukin-8 (pg/mL)	39.0 [17.8 – 81.0]	4.0 [2.0 – 9.0]	7.0 [4.0 - 16.5]
Monocyte chemoattractant protein (pg/mL)	168.0 [71.3 - 400.3]	39.0 [25.0 – 66.0]	45.0 [27.0 - 74.5]
Tumour necrosis factor alpha (pg/mL)	2.8 [1.7 - 3.9]	1.7 [1.7 - 1.8]	2.5 [1.7 - 3.5]
Procalcitonin (ng/mL)	3.00 [0.60 - 26.36]	0.38 [0.16 - 1.88]	0.39 [0.16 - 1.14]
Neutrophil count (10^9 cells per L)	10.8 [6.6 - 15.4]	9.8 [6.9 - 13.3]	10.1 [7.6 - 12.1]
Altered mental status	8 (8.1)	31 (5.6)	7 (9.7)
Pleural effusion	8 (8.1)	58 (10.4)	17 (23.6)
Oxygen therapy	60 (60.6)	264 (47.5)	53 (73.6)
Female	31 (31.3)	206 (37.1)	38 (52.8)

Data are n (%) or mean (SD).

eTable 5. Association between class assignment and clinical outcomes

Ovidius-TripleP cohort				
Clinical outcome	**Class 1 (n = 153)**	**Class 2 (n = 58)**	**Class 3 (n = 294)**	**p-value**
Length of stay (days)	9.0 (7.0-14.0)	10.3 (6.0-23.8)	8.0 (5.5-11.5)	<0.01
ICU admission	12 (7.8)	14 (24.1)	12 (4.1)	<0.01
In-hospital mortality	11 (2.7)	6 (10.3)	7 (2.4)	<0.01
30-day mortality	13 (8.5)	6 (10.3)	7 (2.4)	<0.01
Readmission	11 (7.2)	4 (6.9)	22 (7.5)	0.98

STEP cohort				
Clinical outcome	**Class 1 (n = 99)**	**Class 2 (n = 556)**	**Class 3 (n = 72)**	**p-value**
Length of stay (days)	8.0 (5.0-13.0)	7.0 (4.0-10.0)	7.0 (5.0-10.3)	<0.01
ICU admission	12 (12.1)	26 (4.7)	1 (1.4)	<0.01
In-hospital mortality	11 (11.1)	11 (2.0)	2 (2.8)	<0.01
30-day mortality	10 (10.1)	16 (2.9)	2 (2.8)	<0.01
Readmission	8 (8.1)	27 (4.9)	4 (5.6)	0.42

Data are N (%) or median (IQR). ICU intensive care unit.

Part II

Personalised treatment of ARDS based on recruitability

7

Recruitment maneuvers and higher PEEP, the so-called Open Lung Concept, in patients with ARDS: rationale, limitations and indications.

Philip van der Zee[1] and Diederik Gommers[1]

[1] Department of Adult Intensive Care, Erasmus Medical Center Rotterdam, The Netherlands

Published: Crit Care 2019 Vol. 23 Issue 1

INTRODUCTION

The acute respiratory distress syndrome (ARDS) is a hypoxemic syndrome primarily treated with supportive mechanical ventilation. Although mechanical ventilation is life-saving, it causes ventilation induced lung injury (VILI). Therefore, the goal of mechanical ventilation is to achieve adequate gas exchange while minimizing lung injury. Multiple mechanical ventilation strategies have been developed to limit VILI. These strategies are based on the pathophysiological concept that alveolar overdistention, shear-stress, and atelectrauma (i.e. the cyclical opening and closing of unstable alveoli) are possible mechanisms that result in VILI. Targets that might aggravate or attenuate VILI, notably tidal volume (V_t) and positive end-expiratory pressure (PEEP), became the subject of extensive research.

The ARDS Network (ARDSNet) trial aimed to reduce overdistention, if necessary at the cost of suboptimal gas exchange.[1] This trial demonstrated that a ventilation strategy with low V_t and limited plateau pressure (P_{plat} ≤30 cmH_2O) reduced mortality rate. Lachmann proposed to reduce atelectrauma and shear-stress with recruitment maneuvers (RM) and subsequent use of higher PEEP: the 'Open Lung Concept' (OLC).[2]

The OLC combined with low V_t seems appealing from a pathophysiological perspective, and it has been very promising in experimental ARDS models.[3, 4] However, clinical evidence is inconsistent. A meta-analysis comparing higher PEEP (13-15 cmH_2O) and low PEEP ventilation strategies reported a reduction in mortality rate, but only in a subgroup analysis including patients with moderate to severe ARDS.[5] Another meta-analysis reported a reduced mortality rate in patients with ARDS treated according to the OLC.[6] Amato and colleagues demonstrated in a multilevel mediation analysis that an increase in PEEP reduced mortality rate in patients with ARDS, but only if this results in a decreased driving pressure.[7]

The recent Alveolar Recruitment for ARDS Trial (ART) renewed the controversies about the efficacy of RMs and application of higher PEEP levels.[8] This trial reported that a RM combined with higher PEEP increased mortality rate in patients with moderate to severe ARDS. It was proposed that the overdistention caused by RMs and higher PEEP might be more harmful than the shear-stress and atelectrauma it prevents.[9] This raises the following question: should we abandon the Open Lung Concept in our patients with ARDS?

In this chapter, we will briefly discuss the pathophysiology of ARDS and VILI, limitations and indications of the OLC, bedside monitoring to guide OLC, and airway pressure release ventilation as an alternative.

The pathophysiology of ARDS and VILI

The pathophysiology of ARDS is based on the trias of alveolar-capillary membrane injury, high-permeability (alveolar) edema, and inflammation.[10] Histologically this is characterized by diffuse alveolar damage.[11] The 'baby lung' model describes the pathophysiological effects of ARDS, mainly edema, on lung mechanics.[12] It is based on observations that atelectasis and edema are preferentially distributed to the dependent lung regions, whereas the independent lung regions are relatively well-aerated. The amount of collapse and edema formation correlates with ARDS severity. Although intrinsic elasticity of the independent lung region is nearly normal, lung function is restricted by the collapsed dependent lung region. Because the ARDS lung is small and not stiff, the term 'baby lung' was proposed.[12]

The pathophysiological trias cannot be routinely measured in clinical practice. Therefore, arterial hypoxemia and bilateral opacities on chest imaging are used as clinical surrogates in the Berlin definition of ARDS.[13] Because the Berlin definition is not based on pathophysiological criteria, it poses several limitations in clinical research. Only half of clinically diagnosed patients with ARDS have diffuse alveolar damage at autopsy.[14] In addition, pulmonary and extrapulmonary insults may induce ARDS, both with a different response to PEEP.[15] As a consequence, ARDS is a heterogeneous syndrome.

The Berlin definition of ARDS specified disease severity according to PaO_2/FiO_2 ratio at a PEEP level of at least 5 cmH_2O. This classification is important, as recruitability is dependent on disease severity. However, PEEP has a major effect on PaO_2/FiO_2 ratio and application of high PEEP could mask ARDS severity. Caironi and colleagues found that 54% of patients with mild ARDS at clinical PEEP (i.e. >5 cmH_2O) were reclassified as either moderate or severe ARDS at 5 cmH_2O PEEP. In addition, the correlation between ARDS severity and lung recruitability significantly improved at 5 cmH_2O.[16] Therefore, a fixed PEEP level should be used to assess disease severity and recruitability.

Injurious mechanical ventilation in experimental models results in diffuse alveolar damage, including interstitial and alveolar edema, hyaline membrane formation, and cell infiltration.[17] Therefore, VILI cannot be distinguished from ARDS and is potentially the most important insult that sustains or aggravates ARDS. As ARDS is characterized by baby lungs, alveolar overdistention of the independent lung is considered to be a major contributor to VILI. Initially it was unclear whether high V_t, high airway pressure, or both resulted in VILI. Dreyfuss and colleagues distinguished V_t from airway pressures in a rat model.[18] Pulmonary edema formation was assessed after 20 minutes of mechanical ventilation according to the following protocols: 1. high pressure (45 cmH_2O) and high V_t (40 ml/kg), 2. high pressure (45 cmH_2O) and lower V_t (19 ml/kg), lower V_t was achieved

by a thoracoabdominal strap avoiding chest wall distention, and 3. negative inspiratory pressure (iron lung) and high V_t (44 ml/kg). They found that edema significantly increased in group 1 and 3 as compared to group 2, indicating that high volume and not high pressure caused lung injury. In addition, in a fourth group they reported that 10 cmH_2O PEEP reduced edema formation.

Protti and colleagues demonstrated the beneficial effect of PEEP in combination with a reduced V_t.[3] In a pig model they divided the end-inspiratory long volume (i.e. strain) in a component generated by PEEP (static strain = PEEP volume/FRC, functional residual capacity) and a component generated by V_t (dynamic strain = V_t/FRC). Four groups were ventilated with a total strain of 2.5 (close to total lung capacity): 1. V_{PEEP} 0% and V_t 100%, 2. V_{PEEP} 25% and V_t 75%, 3. V_{PEEP} 50% and V_t 50%, and 4. V_{PEEP} 75% and V_t 25%. After 54 hours, all pigs in the V_t 100% group died due to massive lung edema, whereas none of the pigs in the V_{PEEP} 75% and V_t 25% group died or developed pulmonary edema. At the end of the experiment, sudden removal of PEEP in the last group did not result in pulmonary edema formation, indicating that the integrity of the alveolar-capillary barrier was preserved and PEEP did not only counteract the extravasation of plasma. PEEP has a protective effect, but V_t should be reduced during the application of high PEEP levels.

The use of higher PEEP levels is accompanied by an increase in P_{plat} above 30 cmH_2O. Since the ARDSNet trial reported that a combination of low V_t and a P_{plat} ≤30 cmH_2O reduced mortality rate, physicians are cautious with the use of high airway pressures. However, P_{plat} is exerted at the entire respiratory system, including the lungs and chest wall. Chest wall elastance varies widely in patients with ARDS and contributes between 20-50% to total respiratory system elastance (E_{RS}).[19] A P_{plat} of 30 cmH_2O exerted at a stiff chest wall (50% of E_{RS}) results in a transpulmonary pressure (P_L) of 15 cmH_2O P_L, whereas a similar P_{plat} exerted at a normal chest wall (20% of E_{RS}) results in a P_L of 24 cmH_2O. Therefore, P_{plat} provides little information about the P_L, i.e. the distending force on the lung.

In conclusion, there is sufficient experimental evidence that high V_t and not high airway pressure is important in the development of VILI. In addition, higher PEEP levels are beneficial if V_t is reduced in order to limit the total strain (overdistention). Thus, a combination of higher PEEP and low V_t should be applied to reduce the development of VILI.

The Open Lung Concept

In 1970 Mead and colleagues developed a mathematical model to estimate intrapulmonary pressures in a heterogeneously ventilated lung.[20] They stated that at the interfaces of open and collapsed lung a transpulmonary pressure of 30 cmH_2O could result

in local pressures of 140 cmH_2O. Based on these estimates Lachmann hypothesized that shear-stress might be the major cause of structural damage and VILI.[2] In order to minimize shear-stress and atelectrauma in heterogeneously ventilated lungs, he proposed 'to open up the lung and keep the lung open'.

Traditionally the OLC consists of a RM to open up the collapsed lung and high PEEP to maintain alveolar stability. According to the LaPlace law ($P = 2\gamma / r$, where P is the pressure within an alveolus, γ is the surface tension of the alveolar wall, and r is the radius of the alveolus) more pressure is required to open a collapsed or deflated alveolus in comparison to an open alveolus. Surfactant impairment in severe ARDS further increases opening pressure as a result of increased surface tension. In addition, opening pressure of collapsed alveoli has to overcome the alveolar retractive force and the compressing force on the alveolus by surrounding lung tissue. The sum of these pressures is estimated to be 45 to 60 cmH_2O in patients with ARDS.[9]

An elegant example of opening the dependent lung, although not by using high airway pressures, is the application of prone positioning. In supine position, the weight of the ventral lungs, heart, and abdominal viscera increases pleural pressure in the dorsal lung regions. The decrease in transpulmonary pressure (airway pressure minus pleural pressure) results in a reduced distending force on the dependent lung. In addition, pulmonary edema in ARDS gradually increases lung mass. Eventually the dependent lung collapses under its own weight and ventilation is redistributed to the baby lung. Application of the prone position changes gravitational forces; the dorsal lung becomes the independent lung region and is re-aerated. Due to conformational shape matching (the anatomic tendency to overdistend ventral lung regions despite gravitational forces) and a greater lung mass on the dorsal side, aeration in prone position is more homogeneously distributed.[21] Perfusion is also distributed more homogeneously in prone position. As a result ventilation-perfusion matching and oxygenation improves.[22] The first large randomized controlled trials could not confirm the theoretical advantages of prone positioning. However, a meta-analysis suggested a reduction in mortality rate in patients with severe ARDS.[23] The beneficial effects of prone positioning were confirmed by the PROSEVA trial.[24] Patients with severe ARDS (PaO_2/FiO_2 ratio <150 mmHg) assigned to the prone group had a significantly lower 28-day mortality rate (16.0%) as compared to the supine group (32.8%). Therefore, opening up the lung by prone position is recommended in severe ARDS.

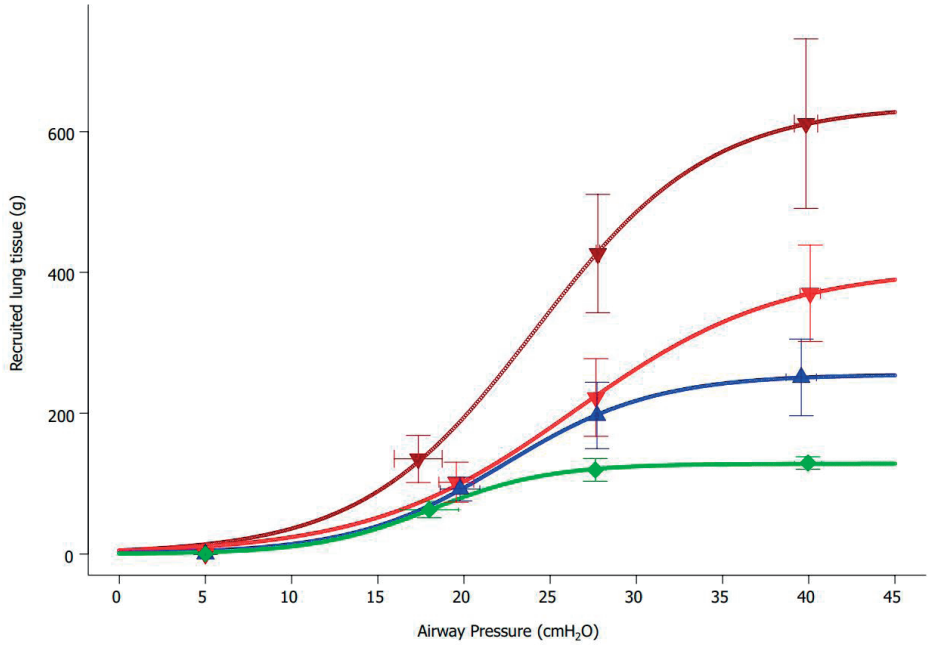
The Open Lung Concept in mild to moderate ARDS

The ATS Clinical Practice Guideline for mechanical ventilation in adult patients with ARDS recommends to limit P_{plat} to 30 cmH_2O, in line with the ARDSNet trial.[25] This

raises the following question: can a lung be fully open at a P_{plat} ≤30 cmH_2O? Cressoni and colleagues investigated whether mechanical ventilation with a P_{plat} of 30 cmH_2O actually recruited the lung.[26] They included 33 patients with mild to severe ARDS. Four CT scans were made: one at 5 cmH_2O PEEP, and three at P_{plat} of 19 ± 0, 28 ± 0, and 40 ± 2 cmH_2O during a <5 second breath holding. Lung recruitment was defined as the amount of lung tissue (grams) which regained inflation as a result of the applied airway pressures (**Figure 1**). They found that the amount of lung recruitment achieved with a P_{plat} increase from 30 to 45 cmH_2O was negligible in patients with mild to moderate ARDS. In contrast, a similar increase in P_{plat} in patients with severe ARDS resulted in a significant amount of lung recruitment. These results confirm that the amount of recruitable tissue increases with ARDS severity.

Multiple clinical studies assessed the effects of RMs in patients with ARDS. A recent meta-analysis included 15 randomized controlled trials (a total of 3,134 patients) that compared the OLC with other mechanical ventilation strategies in patients with ARDS.[6] The authors reported a reduced mortality rate in the patients treated according to the OLC. However, this meta-analysis was performed prior to the ART trial. The multicenter ART included 1,010 patients with moderate to severe ARDS.[8] The objective of this study was to compare RMs with PEEP titrated according to best respiratory system compliance ('High PEEP') with ARDSNet protocol ('Low PEEP'). The initial RM consisted of PEEP increments up to a maximum P_{plat} of 60 cmH_2O. Subsequently, a decremental PEEP trial was performed and the PEEP associated with the best compliance plus 2 cmH_2O was applied. After three cases of resuscitated cardiac arrests the RM was modified to a maximum P_{plat} of 50 cmH_2O. The high PEEP strategy resulted in an increased 28-day mortality rate (55.3% vs 49.3%). There are two major explanations for the increased mortality rate after a RM. A first explanation is the included study population, as 599 of 1,010 patients (59.3%) had moderate ARDS. According to **Figure 1**, an increase of P_{plat} to 60 cmH_2O in moderate ARDS results in a negligible amount of recruited lung tissue at the cost of overdistention. A subgroup analysis supports this hypothesis, as the increase in mortality rate is more pronounced in patients with moderate ARDS, whereas mortality was similar between both groups in patients with severe ARDS. Gattinoni and colleagues estimated the power delivered to the lung during the ART trial. They found that the power delivered to mild ARDS lungs was three times greater than to severe ARDS lungs (1169 Joule versus 390 Joule).[9] Second, the ART trial did not distinguish between responders and non-responders. A mean reduction in driving pressure of only 2 cmH_2O was found, indicating that the RM was inadequate to open up the lung and increase FRC in most patients. In conclusion, this study found an increased mortality rate after the application of a mild recruitment maneuver and subsequent PEEP titration based on best compliance in patients with moderate ARDS.

Figure 1. Lung recruitment as a function of airway pressure.



This figure represents the amount of lung tissue (grams) that is recruited as a function of applied airway pressure. Estimates are based on CT images of patients with ARDS. Green: mild ARDS, blue: moderate ARDS, red: severe ARDS, dark red: severe ARDS with vvECMO. From: Cressoni and colleagues, with permission.[26]

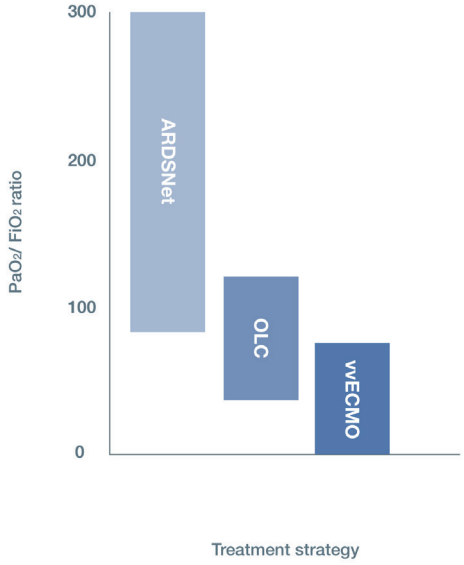
In addition, a trial comparing high-frequency oscillatory ventilation (HFOV) with ARDSNet in patients with moderate to severe ARDS was terminated prematurely, as a trend towards increased mortality in the HFOV group was observed.[27] In this trial HFOV was applied in accordance with the OLC strategy: first a RM was performed by increasing the distending pressure to 40 cmH_2O. Subsequently, mean airway pressure was set at 30 cmH_2O and reduced based on target oxygenation in combination with very low V_t (1-2 ml/kg) and high respiratory frequency. However, as in the ART trial, a subgroup analysis demonstrated that mortality rate was not increased if HFOV was applied in patients with severe ARDS. An individual patient data meta-analysis of four HFOV trials (1,552 patients with ARDS) found that HFOV might even reduce mortality rate in patients with severe ARDS, whereas mortality is increased in patients with mild ARDS.[28] This suggests that a strategy of higher mean airway pressure results in an increased mortality rate in patients with moderate ARDS due to PEEP or distending pressure, whereas in patients with severe ARDS higher mean airway pressure might be beneficial.

The Open Lung Concept in severe ARDS

In patients with severe refractory hypoxemia under ARDSNet protocol there are three possible treatment strategies: 1. maintain ARDSNet protocol and accept hypoxemia, 2.

convert to venovenous extracorporeal membrane oxygenation (vvECMO), or 3. initiate mechanical ventilation according to the OLC, thus accepting airway pressures above 30 cmH_2O (**Figure 2**). The EOLIA trial compared early application of vvECMO with ARDSNet protocol in patients with very severe ARDS.[29] They found that vvECMO did not reduce 60-day mortality rate. In addition, vvECMO is associated with a high complication rate (up to 40%), including intracranial hemorrhages resulting in death.[30]

Figure 2. Indication for the Open Lung Concept in ARDS



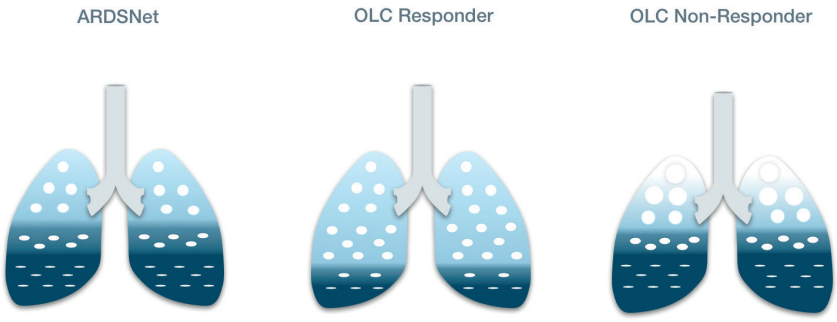
This figure represents the indication for ARDSNet protocol, OLC and vvECMO according to PaO_2/FiO_2 ratio.

In a retrospective analysis of patients treated according to the OLC that met the EOLIA inclusion criteria, we observed a 30-day mortality of 25% as compared to 35-46% in the EOLIA trial.[29] This supports our hypothesis that there is an indication for the OLC in patients with severe ARDS. However, it is essential that RMs and high P_{plat} are guided by strict monitoring.

Inspiratory pressure is limited by P_L instead of $P_{plat.}$ P_L is estimated with an esophageal balloon catheter. An inspiratory P_L of <25 cmH_2O is considered to be lung protective ventilation regardless of P_{plat}.[19] Grasso and colleagues measured P_L in 14 patients with severe ARDS that were referred to their ICU for vvECMO.[19] In half of the patients P_L was above 25 cmH_2O and in these patients vvECMO was initiated. In the other patients P_L was <25 cmH_2O and therefore PEEP was increased from 17 to 22 cmH_2O until P_L was equal to 25 cmH_2O. They accepted airway pressures up to 38 cmH_2O. In these patients oxygenation improved and they did not require vvECMO.

In order to prevent overdistention, it is important to distinguish responders from non-responders to a RM. Responders can be identified by an increase in oxygenation, compliance and/or a significant reduction in driving pressure. The reduction in driving pressure is a direct result of opening up the lung, thereby increasing FRC. In our experience, driving pressure is reduced rapidly after a RM in responders. The extent to which the driving pressure has to decrease in order to be a responder is unclear. The multilevel mediation analysis by Amato and colleagues suggests that a driving pressure of ≤15 cmH_2O reduces mortality rate in patients with ARDS.[7] However, in the ART trial driving pressure was reduced from 13.5 to 11.5 cmH_2O after a RM and still resulted in an increased mortality rate. Although driving pressure decreased initially, an increase was observed afterwards, whereas driving pressure in the control group remained stable. This suggests that not the absolute value of the driving pressure, but maintaining a low stable driving pressure might be more important. In non-responders FRC does not increase after a RM. Thus, PEEP should not be increased as this results in increased overdistention of the baby lung (**Figure 3**).

Figure 3. Responders and non-responders to the Open Lung Concept



Lung aeration at expiration is schematically depicted in ARDSNet protocol (left), in responders (middle) and non-responders (right) to the OLC, i.e. a recruitment maneuver and higher PEEP levels. In responders functional residual capacity increases in response to recruitment , resulting in reduced strain and driving pressure. In non-responders functional residual capacity does not increase following a recruitment maneuver. Subsequent application of higher PEEP levels results in alveolar overdistention. Very light blue: overdistention, light blue: normally aerated lung tissue, and dark blue: collapsed alveoli.

Slow recruitment with Airway Pressure Release Ventilation

Time is an important variable in both alveolar recruitment and stabilization, yet often overlooked. The application of 30 cmH_2O to a lung inflated at 5 cmH_2O for 2 seconds opens up approximately 75% of alveoli.[31] Continuation of 30 cmH_2O for 40 seconds gradually increases the amount of open alveoli to 85%. In the expiratory phase there is a delay of approximately 0.17 seconds before alveolar collapse commences and at 0.25 seconds an alveolus is collapsed.[32] Inspiration time in ARDSNet protocol is too short to

recruit the majority of alveoli and too long to prevent the alveoli from collapsing. Airway pressure release ventilation (APRV) might address both problems. APRV consists of a continuous positive airway pressure (P_{high}) with a brief intermittent release phase (P_{low}) for expiration and CO_2 removal. Patients are allowed to breath spontaneously independent of ventilator cycles. P_{high} slowly recruits the lung and a short P_{low} prevents alveolar collapse. Eventually, the lung is open and stable. However, in experimental models heterogeneity is increased if P_{low} is set too long, giving the alveoli sufficient time to collapse.[33] Zhou and colleagues compared APRV 50% with ARDSNet protocol in patients with moderate to severe ARDS.[34] They found a trend towards a reduced ICU mortality in the APRV group: 19.7% versus 34.3%. The number of ventilator-free days, oxygenation, and respiratory system compliance was in favor of the APRV group. In this study they aimed for a spontaneous minute ventilation of at least 30% of total minute ventilation. The contraction of the diaphragm during spontaneous breathing is more pronounced in the dorsal lung region and assists in opening up even the most dependent lung regions. In conclusion, APRV results in an open lung by slow recruitment, alveolar stabilization and contraction of the diaphragm.

CONCLUSION

The objective of the OLC is to achieve an open and homogeneously ventilated lung. From a pathophysiological perspective the OLC seems beneficial as shear-stress and atelectrauma are reduced. An open and more homogeneously ventilated lung can be achieved by the application of prone position or high airway pressures. In patients with severe ARDS prone position has proven to reduce mortality rate drastically.

Multiple studies using RMs with airway pressures up to 50-60 cmH_2O only improved oxygenation and did not reduce mortality rate. On the contrary, the ART trial found an increased mortality rate when a RM was combined with decremental PEEP titration based on best compliance in patients with moderate to severe ARDS.[8] The application of HFOV and high mean airway pressures in patients with ARDS increased mortality rate as well.[27] However, subgroup analyses of both trials showed that mortality rate increased in patients with moderate ARDS, whereas mortality rate was similar or even reduced in patients with severe ARDS.[28] Apparently, the application of higher PEEP or distending pressures increases mortality in patients with moderate ARDS due to overdistention, despite best PEEP titration. This indicates that high airway pressures should not be used in patients with moderate ARDS.

We propose to apply the OLC in patients with severe ARDS with refractory hypoxemia under ARDSNet protocol, but only if a patient is a responder to recruitment. In patients that do not respond to recruitment, PEEP should be reduced and vvECMO may be considered. As both the OLC and vvECMO require clinical expertise, we recommend to apply this strategy in tertiary referral centers. The exact definition of a responder remains to be elucidated. After a RM driving pressure, oxygenation, and compliance should improve, but to what extent remains unclear.

ACKNOWLEDGMENTS

We thank Nathalie Timmerman for her support in the preparation of figures.

REFERENCES

1. Brower R, Matthay M, Morris A, Schoenfeld D, Thompson B, Wheeler A, Network. TARDS: **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**(18):1301-1308.
2. Lachmann B: **Open up the lung and keep the lung open**. *Intensive care medicine* 1992, **18**(6):319-321.
3. Protti A, Andreis DT, Monti M, Santini A, Sparacino CC, Langer T, Votta E, Gatti S, Lombardi L, Leopardi O *et al*: **Lung stress and strain during mechanical ventilation: any difference between statics and dynamics?** *Critical care medicine* 2013, **41**(4):1046-1055.
4. Jain SV, Kollisch-Singule M, Satalin J, Searles Q, Dombert L, Abdel-Razek O, Yepuri N, Leonard A, Gruessner A, Andrews P *et al*: **The role of high airway pressure and dynamic strain on ventilator-induced lung injury in a heterogeneous acute lung injury model**. *Intensive care medicine experimental* 2017, **5**(1):25.
5. Briel M, Meade M, Mercat A, Brower RG, Talmor D, Walter SD, Slutsky AS, Pullenayegum E, Zhou Q, Cook D *et al*: **Higher vs lower positive end-expiratory pressure in patients with acute lung injury and acute respiratory distress syndrome: systematic review and meta-analysis**. *Jama* 2010, **303**(9):865-873.
6. Lu J, Wang X, Chen M, Cheng L, Chen Q, Jiang H, Sun Z: **An Open Lung Strategy in the Management of Acute Respiratory Distress Syndrome: A Systematic Review and Meta-Analysis**. *Shock (Augusta, Ga)* 2017, **48**(1):43-53.
7. Amato MB, Meade MO, Slutsky AS, Brochard L, Costa EL, Schoenfeld DA, Stewart TE, Briel M, Talmor D, Mercat A *et al*: **Driving pressure and survival in the acute respiratory distress syndrome**. *The New England journal of medicine* 2015, **372**(8):747-755.
8. Writing Group for the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial I, Cavalcanti AB, Suzumura EA, Laranjeira LN, Paisani DM, Damiani LP, Guimaraes HP, Romano ER, Regenga MM, Taniguchi LNT *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: A Randomized Clinical Trial**. *Jama* 2017, **318**(14):1335-1345.
9. Cipulli F, Vasques F, Duscio E, Romitti F, Quintel M, Gattinoni L: **Atelectrauma or volutrauma: the dilemma**. *J Thorac Dis* 2018, **10**(3):1258-1264.
10. Thompson BT, Chambers RC, Liu KD: **Acute Respiratory Distress Syndrome**. *The New England journal of medicine* 2017, **377**(19):1904-1905.
11. Tomashefski JF, Jr.: **Pulmonary pathology of acute respiratory distress syndrome**. *ClinChest Med* 2000, **21**(3):435-466.
12. Gattinoni L, Marini JJ, Pesenti A, Quintel M, Mancebo J, Brochard L: **The "baby lung" became an adult**. *Intensive care medicine* 2016, **42**(5):663-673.
13. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS: **Acute respiratory distress syndrome: the Berlin Definition**. *Jama* 2012, **307**(23):2526-2533.
14. Kao KC, Hu HC, Chang CH, Hung CY, Chiu LC, Li SH, Lin SW, Chuang LP, Wang CW, Li LF *et al*: **Diffuse alveolar damage associated mortality in selected acute respiratory distress syndrome patients with open lung biopsy**. *Crit Care* 2015, **19**:228.
15. Gattinoni L, Pelosi P, Suter PM, Pedoto A, Vercesi P, Lissoni A: **Acute respiratory distress syndrome caused by pulmonary and extrapulmonary disease. Different syndromes?** *American journal of respiratory and critical care medicine* 1998, **158**(1):3-11.

16. Caironi P, Carlesso E, Cressoni M, Chiumello D, Moerer O, Chiurazzi C, Brioni M, Bottino N, Lazzerini M, Bugedo G *et al*: **Lung recruitability is better estimated according to the Berlin definition of acute respiratory distress syndrome at standard 5 cm H2O rather than higher positive end-expiratory pressure: a retrospective cohort study**. *Critical care medicine* 2015, **43**(4):781-790.
17. Slutsky AS, Ranieri VM: **Ventilator-induced lung injury**. *N Engl J Med* 2013, **369**(22):2126-2136.
18. Dreyfuss D, Soler P, Basset G, Saumon G: **High inflation pressure pulmonary edema. Respective effects of high airway pressure, high tidal volume, and positive end-expiratory pressure**. *The American review of respiratory disease* 1988, **137**(5):1159-1164.
19. Grasso S, Terragni P, Birocco A, Urbino R, Del Sorbo L, Filippini C, Mascia L, Pesenti A, Zangrillo A, Gattinoni L *et al*: **ECMO criteria for influenza A (H1N1)-associated ARDS: role of transpulmonary pressure**. *Intensive care medicine* 2012, **38**(3):395-403.
20. Mead J, Takishima T, Leith D: **Stress distribution in lungs: a model of pulmonary elasticity**. *Journal of applied physiology* 1970, **28**(5):596-608.
21. Gattinoni L, Taccone P, Carlesso E, Marini JJ: **Prone position in acute respiratory distress syndrome. Rationale, indications, and limits**. *American journal of respiratory and critical care medicine* 2013, **188**(11):1286-1293.
22. Henderson AC, Sa RC, Theilmann RJ, Buxton RB, Prisk GK, Hopkins SR: **The gravitational distribution of ventilation-perfusion ratio is more uniform in prone than supine posture in the normal human lung**. *Journal of applied physiology (Bethesda, Md : 1985)* 2013, **115**(3):313-324.
23. Sud S, Friedrich JO, Taccone P, Polli F, Adhikari NK, Latini R, Pesenti A, Guerin C, Mancebo J, Curley MA *et al*: **Prone ventilation reduces mortality in patients with acute respiratory failure and severe hypoxemia: systematic review and meta-analysis**. *Intensive care medicine* 2010, **36**(4):585-599.
24. Guerin C, Reignier J, Richard JC, Beuret P, Gacouin A, Boulain T, Mercier E, Badet M, Mercat A, Baudin O *et al*: **Prone positioning in severe acute respiratory distress syndrome**. *The New England journal of medicine* 2013, **368**(23):2159-2168.
25. Fan E, Del Sorbo L, Goligher EC, Hodgson CL, Munshi L, Walkey AJ, Adhikari NKJ, Amato MBP, Branson R, Brower RG *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**(9):1253-1263.
26. Cressoni M, Chiumello D, Algieri I, Brioni M, Chiurazzi C, Colombo A, Colombo A, Crimella F, Guanziroli M, Tomic I *et al*: **Opening pressures and atelectrauma in acute respiratory distress syndrome**. *Intensive care medicine* 2017, **43**(5):603-611.
27. Ferguson ND, Cook DJ, Guyatt GH, Mehta S, Hand L, Austin P, Zhou Q, Matte A, Walter SD, Lamontagne F *et al*: **High-frequency oscillation in early acute respiratory distress syndrome**. *The New England journal of medicine* 2013, **368**(9):795-805.
28. Meade MO, Young D, Hanna S, Zhou Q, Bachman TE, Bollen C, Slutsky AS, Lamb SE, Adhikari NKJ, Mentzelopoulos SD *et al*: **Severity of Hypoxemia and Effect of High-Frequency Oscillatory Ventilation in Acute Respiratory Distress Syndrome**. *American journal of respiratory and critical care medicine* 2017, **196**(6):727-733.
29. Combes A, Hajage D, Capellier G, Demoule A, Lavoue S, Guervilly C, Da Silva D, Zafrani L, Tirot P, Veber B *et al*: **Extracorporeal Membrane Oxygenation for Severe Acute Respiratory Distress Syndrome**. *The New England journal of medicine* 2018, **378**(21):1965-1975.

30. Vaquer S, de Haro C, Peruga P, Oliva JC, Artigas A: **Systematic review and meta-analysis of complications and mortality of veno-venous extracorporeal membrane oxygenation for refractory acute respiratory distress syndrome**. *Ann Intensive Care* 2017, **7**(1):51.
31. Albert SP, DiRocco J, Allen GB, Bates JH, Lafollette R, Kubiak BD, Fischer J, Maroney S, Nieman GF: **The role of time and pressure on alveolar recruitment**. *Journal of applied physiology (Bethesda, Md : 1985)* 2009, **106**(3):757-765.
32. Hasan D, Satalin J, van der Zee P, Kollisch-Singule M, Blankman P, Shono A, Somhorst P, den Uil C, Meeder H, Kotani T *et al*: **Excessive Extracellular ATP Desensitizes P2Y2 and P2X4 ATP Receptors Provoking Surfactant Impairment Ending in Ventilation-Induced Lung Injury**. *International journal of molecular sciences* 2018, **19**(4).
33. Kollisch-Singule M, Emr B, Smith B, Ruiz C, Roy S, Meng Q, Jain S, Satalin J, Snyder K, Ghosh A *et al*: **Airway pressure release ventilation reduces conducting airway micro-strain in lung injury**. *JAmCollSurg* 2014, **219**(5):968-976.
34. Zhou Y, Jin X, Lv Y, Wang P, Yang Y, Liang G, Wang B, Kang Y: **Early application of airway pressure release ventilation may reduce the duration of mechanical ventilation in acute respiratory distress syndrome**. *Intensive care medicine* 2017, **43**(11):1648-1659.

8

vvECMO can be avoided by a transpulmonary pressure guided Open Lung Concept in patients with severe ARDS

Philip van der Zee[1§], Dinis Dos Reis Miranda[1], Han Meeder[1], Henrik Endeman[1], and Diederik Gommers[1]

[1] Department of Adult Intensive Care, Erasmus Medical Center Rotterdam, The Netherlands

Published: Crit Care 2019 Vol. 23 Issue 1 (letter to the editor)

Dear Editor,

The EOLIA trial concluded that vvECMO compared to conventional mechanical ventilation with low tidal volumes and airway pressures ≤30 cmH_2O did not improve survival.[1] Although not statistically significant, the 11% absolute reduction in mortality rate and multiple crossovers to rescue vvECMO were considered to be clinically relevant.[2] However, a conventional mechanical ventilation strategy is likely to be insufficient for patients with severe ARDS, as higher airway pressures are required to maintain lung aeration.[3] Grasso et al. measured the transpulmonary pressure (P_L) in patients with severe ARDS and increased PEEP until P_L was 25 cmH_2O. Fifty percent of patients responded to an increase in airway pressure and did not require vvECMO.[4] We hypothesized that a P_L guided open lung concept (OLC) could improve oxygenation and prevent conversion to vvECMO in patients with severe ARDS.

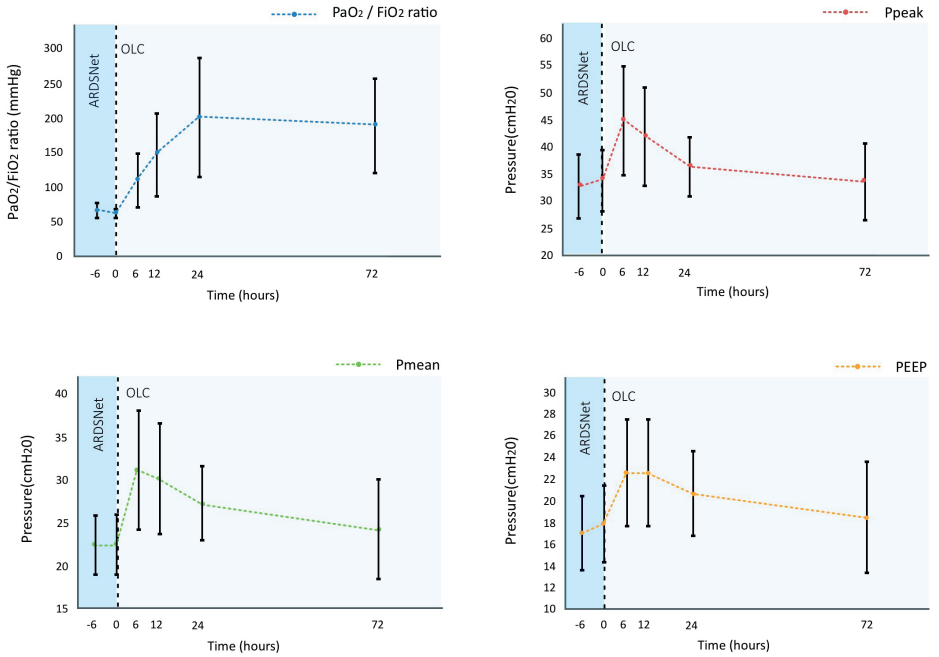
We retrospectively reviewed the records of all patients referred to our ICU between January and May 2018. Eight patients had severe ARDS and had an indication for vvECMO according to the EOLIA trial (demographics are given in the supplementary materials).[1] Before referral protective mechanical ventilation with low tidal volume and a plateau pressure of approximately 30 cmH_2O was applied. PaO_2/FiO_2 ratio was 62±7 mmHg despite the use of neuromuscular blocking agents and prone positioning. After referral, a recruitment manoeuvre was performed and PEEP was increased. P_L was estimated with an oesophageal balloon catheter and we aimed for a P_L ≤25 cmH_2O. In addition, respiratory rate and I:E ratio were increased, thereby generating intrinsic PEEP.

The P_L guided OLC resulted in an increase in PaO_2/FiO_2 ratio to 201±87 mmHg (**Figure 1**) and none of the patients required vvECMO. During the first 6 hours peak airway pressure was increased to 44.9±10.2 cmH_2O, but was reduced to 36.3±5.6 cmH_2O within 24 hours, while PEEP was maintained at 20.6±4.0 cmH_2O. A maximum end-inspiratory P_L of 18±5 cmH_2O was measured. At 72 hours both peak airway pressures and PEEP were reduced to baseline values while oxygenation remained stable.

These data suggest that the OLC improves oxygenation and avoids conversion to vvECMO in patients with severe ARDS. We acknowledge that a recruitment manoeuvre and higher PEEP in patients with moderate to severe ARDS increased mortality in the Alveolar Recruitment Trial.[5] However, the recruitment manoeuvre was standardized and 'recruitability' was not assessed. We hypothesize that a recruitment manoeuvre and higher PEEP is beneficial in patients with large regions of decreased lung aeration. Thus, future research should focus on individual 'recruitability'.[6] Baedorf Kassis et al. introduced a recruitment manoeuvre based on P_L measurements.[7] Other potential

predictors are a decrease in driving pressure, oxygenation response to PEEP-trials, or lung aeration estimated by electrical impedance tomography or ultrasound.

Figure 1. Airway pressures and PaO_2 / FiO_2 ratio after initiation of the OLC



Peak airway pressure, Pmean, PEEP and PaO_2 / FiO_2 ratio as a function of time. The OLC is initiated at T0, i.e. at referral. Mean values and standard deviations are shown. Note that PEEP values are set PEEP levels at the mechanical ventilator. The depicted driving pressure is overestimated as intrinsic PEEP is not shown. FiO_2 fraction of inspired oxygen, PaO_2 partial pressure of arterial oxygen, Ppeak peak airway pressure, Pmean mean airway pressure, PEEP positive end-expiratory pressure.

REFERENCES:

1. Combes A, Hajage D, Capellier G, Demoule A, Lavoue S, Guervilly C, Da Silva D, Zafrani L, Tirot P, Veber B *et al*: **Extracorporeal Membrane Oxygenation for Severe Acute Respiratory Distress Syndrome**. *The New England journal of medicine* 2018, **378**(21):1965-1975.
2. Gattinoni L, Vasques F, Quintel M: **Use of ECMO in ARDS: does the EOLIA trial really help?** *Crit Care* 2018, **22**(1):171.
3. van der Zee P, Gommers D: **Recruitment Maneuvers and Higher PEEP, the So-Called Open Lung Concept, in Patients with ARDS**. *Crit Care* 2019, **23**(1):73.
4. Grasso S, Terragni P, Birocco A, Urbino R, Del Sorbo L, Filippini C, Mascia L, Pesenti A, Zangrillo A, Gattinoni L *et al*: **ECMO criteria for influenza A (H1N1)-associated ARDS: role of transpulmonary pressure**. *Intensive care medicine* 2012, **38**(3):395-403.
5. Writing Group for the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial I, Cavalcanti AB, Suzumura EA, Laranjeira LN, Paisani DM, Damiani LP, Guimaraes HP, Romano ER, Regenga MM, Taniguchi LNT *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: A Randomized Clinical Trial**. *Jama* 2017, **318**(14):1335-1345.
6. Chiumello D, Brochard L, Marini JJ, Slutsky AS, Mancebo J, Ranieri VM, Thompson BT, Papazian L, Schultz MJ, Amato M *et al*: **Respiratory support in patients with acute respiratory distress syndrome: an expert opinion**. *Crit Care* 2017, **21**(1):240.
7. Baedorf Kassis E, Loring SH, Talmor D: **Recruitment maneuvers: using transpulmonary pressure to help Goldilocks**. *Intensive care medicine* 2017, **43**(8):1162-1163.

SUPPLEMENTARY FILES:

Figure 1. Flowchart of patient inclusion
Table 1. Patient demographics
Table 2. Patient parameters
Appendix Mechanical ventilation strategy

Figure 1. Flowchart of patient inclusion

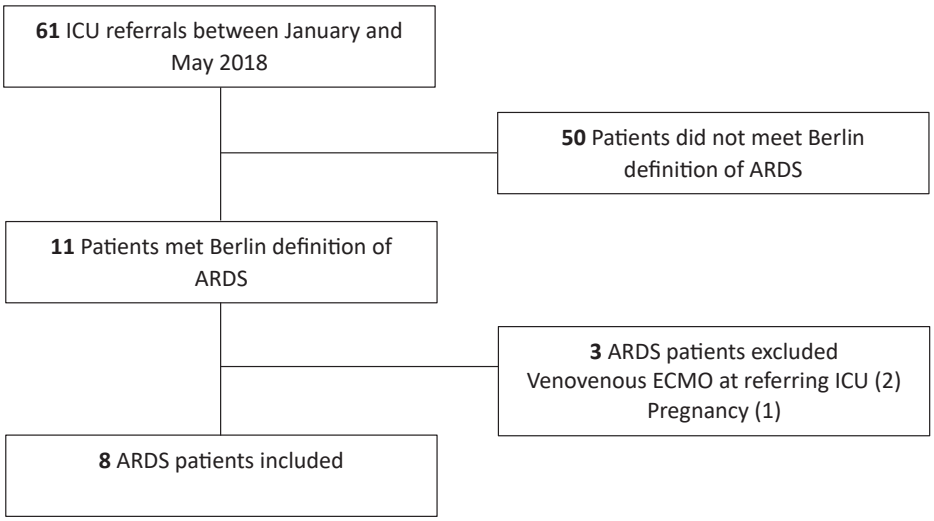


Table 1. Patient demographics

Case	Age	Gender	Admission Diagnosis	PaO_2 / FiO_2 ratio at referral (mmHg)	APACHE IV Score at referral	ICU LoS prior to referral (days)	ICU LoS (days)*	BMI	ICU survival
1	74	M	Influenza, Streptococcus pyogenes	54	108	4	18	24.2	Survivor
2	67	M	Influenza	66	84	1	18	35.8	Survivor
3	27	M	Influenza	73	103	1	33	27.8	Survivor
4	51	M	Invasive aspergillosis	69	98	5	9	20.4	Non-survivor
5	60	F	Influenza	58	128	2	42	36.7	Survivor
6	46	F	Pancreatitis	61	100	2	15	44.1	Survivor
7	64	F	Influenza, Streptococcus pneumoniae	52	147	5	17	29.4	Survivor
8	49	M	Influenza	78	52	2	20	25.5	Survivor

* Total length of stay at the referring ICU and the ICU of the Erasmus MC. ICU intensive care unit, LoS length of stay, BMI body mass index, M male, F female.

Table 2. Patient parameters

	Before referral			After referral					
	0h (T0)	**6h (T6)**	p-value	**12h (T12)**	p-value	**24h (T24)**	p-value	**72h (T72)**	p-value
Mechanical ventilation settings									
RR (1/min)	25.8 ± 3.6	38.4 ± 9.2	0.003	37.4 ± 8.0	0.002	37.8 ± 8.5	0.002	36.3 ± 8.3	0.007
PEEP (cmH_2O)	17.8 ± 3.5	22.6 ± 4.9	0.012	22.6 ± 5.0	0.018	20.6 ± 4.0	0.014	18.4 ± 5.1	0.738
Pmean (cmH_2O)	22.5 ± 3.5	31.1 ± 7.0‡	0.042	30.3 ± 6.5‡	0.105	27.4 ± 4.3	0.242	24.4 ± 5.8	0.734
Ppeak (cmH_2O)	33.9 ± 5.7	44.9 ± 10.2†‡	0.051	41.9 ± 8.9†‡	0.099	36.3 ± 5.6	0.437	33.5 ± 7.1	0.917
I:E ratio	0.79 ± 0.20	1.51 ± 0.45‡	0.006	1.44 ± 0.51	0.025	1.46 ± 0.51	0.018	1.07 ± 0.43	0.133
Vt (mL)	462 ± 69	416 ± 67	0.156	443 ± 103	0.611	428 ± 75	0.369	395 ± 56	0.009
Vt/kg PBW	6.7 ± 0.6	6.1 ± 1.2	0.169	6.5 ± 1.7	0.702	6.3 ± 1.7	0.528	5.8 ± 0.8	0.006
FiO_2 (%)	96 ± 6	79 ± 17†‡	0.034	66 ± 19	0.006	57 ± 14	0.001	54 ± 22	0.003
MV (L/min)	10.9 ± 1.0	16.1 ± 4.5	0.007	16.0 ± 3.6	0.007	15.3 ± 3.1	0.007	14.8 ± 2.9	0.007
Gas exchange									
pH	7.26 ± 0.10	7.30 ± 0.06	0.116	7.32 ± 0.05	0.102	7.34 ± 0.04	0.037	7.36 ± 0.08	0.068
$PaCO_2$ (mmHg)	59.0 ± 16.2	50.1 ± 10.1	0.046	49.4 ± 10.7	0.100	48.3 ± 8.9	0.067	55.1 ± 15.2	0.403
PaO_2 (mmHg)	60.0 ± 7.8	85.6 ± 34.6	0.076	90.1 ± 19.2	0.001	104.6 ± 24.4	0.001	91.2 ± 22.7	0.003
Bicarbonate (mmol/L)	25.2 ± 6.2	24.8 ± 6.1‡	0.404	25.1 ± 4.9‡	0.977	26.0 ± 4.6‡	0.619	30.2 ± 3.8	0.023
Base excess	-1.5 ± 8.7	-2.3 ± 6.0‡	0.468	-1.5 ± 4.4‡	0.982	-0.3 ± 4.3‡	0.601	3.9 ± 3.2	0.089
Oxygen saturation (%)	88 ± 6	94 ± 3†	0.028	97 ± 2	0.003	97 ± 2	0.001	96 ± 4	0.022
PaO_2 / FiO_2 ratio	62 ± 7	110 ± 39*†‡	0.013	149 ± 60	0.005	201 ± 87	0.003	191 ± 68	0.001
Hemodynamic parameters									
Heart rate (1/min)	113 ± 24	107 ± 26	0.348	105 ± 20	0.113	104 ± 20	0.344	108 ± 16	0.609
MAP (mmHg)	75 ± 12	73 ± 11	0.748	77 ± 8	0.749	76 ± 10	0.838	76 ± 13	0.875
Lactate (mmol/L)	2.3 ± 1.0	2.5 ± 0.7*	0.380	1.9 ± 0.5	0.652	2.0 ± 0.8	0.784	2.1 ± 1.4	0.912
Noradrenalin (µg/kg/min)	0.25 ± 0.18	0.43 ± 0.37‡	0.110	0.36 ± 0.36	0.266	0.28 ± 0.23	0.708	0.26 ± 0.44	0.975

Values are mean ± standard deviation. Presented p-values are compared to T0. * p-value < 0.05 as compared to T12; † p-value < 0.05 as compared to T24; ‡ p-value < 0.05 as compared to T72. RR respiratory rate; PEEP positive end-expiratory pressure; Pmean mean airway pressure; Ppeak peak airway pressure; I:E ratio inspiratory : expiratory ratio; Vt tidal volume; PBW predicted body weight; FiO_2 fraction of inspired oxygen; MV minute volume; $PaCO_2$ partial pressure of arterial carbon dioxide; PaO_2 partial pressure of arterial oxygen; MAP mean arterial pressure.

Appendix Mechanical ventilation strategy

Controlled mechanical ventilation with a strive tidal volume of 4-6ml/kg predicted body weight was applied in all patients. A recruitment manoeuvre was performed using stepwise increments in PEEP (usually 5 cmH_2O) while driving pressure was maintained. Peak airway pressure was allowed approximately 20 cmH_2O above the initial peak airway pressure for 2 minutes. The recruitment manoeuvre was discontinued if mean arterial pressure decreased below a predefined cut-off set by the clinician (usually <50 mmHg). Subsequently, peak inspiratory pressures were guided by transpulmonary pressures (P_L) instead of plateau pressures. P_L was estimated with an oesophageal balloon catheter. An inspiratory P_L of <25 cmH_2O was considered to be lung protective ventilation. PEEP was titrated on the basis of a positive end-expiratory P_L and electrical impedance tomography.

9

Electrical impedance tomography for positive end-expiratory pressure titration in COVID-19 related acute respiratory distress syndrome.

9.1

Electrical impedance tomography for positive end-expiratory pressure titration in COVID-19 related ARDS

Philip van der Zee MD[1a], Peter Somhorst MSc[1a], Henrik Endeman MD PhD[1], Diederik Gommers MD PhD[1]

[1] Department of Adult Intensive Care, Erasmus Medical Center Rotterdam, The Netherlands
[a] Both authors contributed equally

Published: Am J Respir Crit Care Med 2020 Vol. 202 Issue 2 (Research letter)

To the editor:

Coronavirus disease 2019 (COVID-19) spreads rapidly and already resulted in severe burden to hospitals and intensive care units (ICU) worldwide. Early reports described progression to acute respiratory distress syndrome (ARDS) in 29% of cases.[1]

It is unknown how to titrate positive end-expiratory pressure (PEEP) in patients with ARDS. Patient survival improved if higher PEEP successfully recruited atelectatic lung tissue.[2] However, excessive PEEP caused alveolar overdistention resulting in reduced patient survival.[3] Therefore, PEEP should be personalised in order to maximize alveolar recruitment and minimize the amount of alveolar overdistention. Electrical impedance tomography (EIT) provides a reliable bedside approach to detect both alveolar overdistention and alveolar collapse.[4]

We describe a case series of COVID-19 patients with moderate to severe ARDS, in whom EIT was applied to personalise PEEP based on lowest relative alveolar overdistention and collapse. Subsequently, we compared this PEEP level with the PEEP that could have been set according to the lower or higher PEEP-FiO_2 table from the ALVEOLI trial.[5] These early experiences may help clinicians to titrate PEEP in COVID-19 patients with ARDS.

METHODS

Study design and inclusion criteria

We conducted this case series between March 1 and March 31 2020 in our tertiary referral ICU (Erasmus MC, Rotterdam, The Netherlands). All consecutive mechanically ventilated patients admitted to the ICU with COVID-19 and moderate to severe ARDS according to the Berlin definition of ARDS were included in this study. COVID-19 was defined as a positive result on polymerase chain reaction of sputum, nasal swab, or pharyngeal swab specimen. The local Medical Ethical Committee approved this study. Informed consent was obtained from all patient's legal representatives.

Study protocol

A PEEP trial was performed daily in all patients according to our local mechanical ventilation protocol. Patients were fully sedated with continuous intravenous infusion of propofol, midazolam and opiates. Persisting spontaneous breathing efforts were prevented with increased sedation or neuromuscular blockade. Arterial blood pressure was measured continuously. Noradrenalin was titrated to maintain a mean arterial blood pressure above 65 mmHg at the start of the PEEP trial.

All patients were ventilated in pressure control mode. FiO_2 was titrated in order to obtain a peripheral oxygen saturation (SpO_2) between 92-95%. The other mechanical ventilation parameters, i.e. PEEP, driving pressure, respiratory rate, and inspiratory/expiratory ratio remained unchanged. Plateau airway pressure and total PEEP were measured during a zero flow state with an inspiratory and expiratory hold procedure, respectively. Absolute transpulmonary pressures were measured with an oesophageal balloon catheter (CooperSurgical, USA or NutriVent, Sidam, Italy). Position and balloon inflation status were tested with chest compression during an expiratory hold manoeuvre.

We monitored bedside ventilation distribution with EIT (Pulmovista 500, Dräger, Germany or Enlight 1800, Timpel, Brazil). An EIT belt was placed around the patient's thorax in the transversal plane corresponding to the 4^{th} to 5^{th} intercostal parasternal space. The belt was placed daily (Pulmovista) or once in three days (Enlight) according to manufacturer's instructions. EIT data were visualized on screen during the entire study protocol without repositioning the EIT belt.

Subsequently, we performed a decremental PEEP trial. PEEP was increased stepwise until PEEP was 10 cmH_2O above baseline PEEP with a minimum PEEP of 24 cmH_2O ($PEEP_{high}$), corresponding to the maximum PEEP advised by the PEEP-FiO_2 table. The PEEP trial was limited to a lower PEEP level in case of hypotension (mean arterial blood pressure <60 mmHg) or desaturation (SpO_2 <88%). $PEEP_{high}$ was maintained for at least one minute. From $PEEP_{high}$, PEEP was reduced in 2 cmH_2O steps of 30 seconds until EIT showed evident collapse. PEEP was reduced an additional 2 cmH_2O in order to confirm a further increase in collapse. The EIT devices provided percentages of relative alveolar overdistention and collapse at every PEEP step. Lastly, total PEEP was set ($PEEP_{set}$) at the PEEP level above the intersection of the curves representing relative alveolar overdistention and collapse (**Figure 1**).[6]

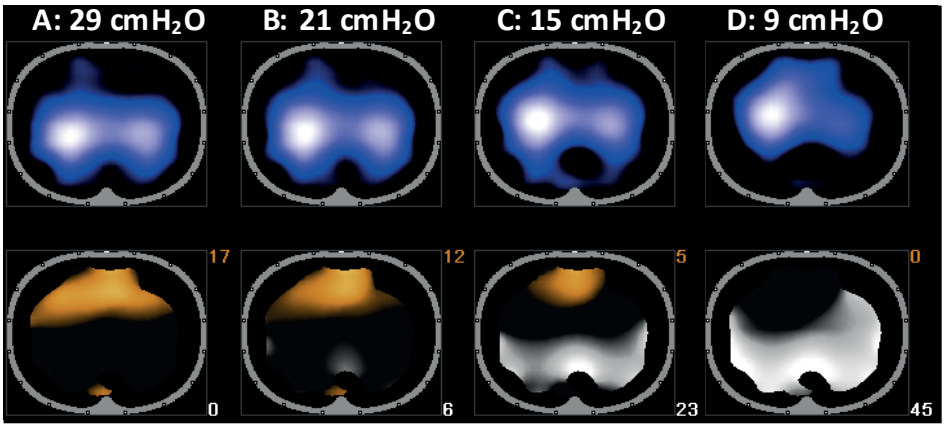
Baseline characteristics and laboratory analyses were retrieved from the patient information system. Diffuse or focal ARDS was established with chest x-ray or lung CT scan similar to the LIVE study.[7]

Statistical analysis

Data were presented as median and inter-quartile range (IQR). Only $PEEP_{set}$ as determined by the first PEEP trial of each patient was used for analyses. The absolute distance in cmH_2O between $PEEP_{set}$ and closest PEEP level that could have been set based on the lower PEEP-FiO_2 table or higher PEEP-FiO_2 table from the ALVEOLI trial was calculated.[5] The Wilcoxon signed-rank test was used to test the difference between $PEEP_{set}$ and the absolute distance to either PEEP-FiO_2 table, and to test the difference in $PEEP_{set}$ between

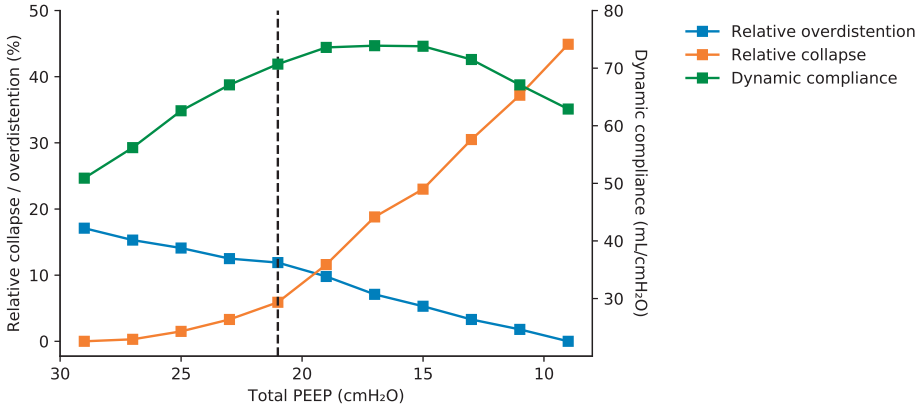
Figure 1 $PEEP_{set}$ based on electrical impedance tomography

Figure 1a. Ventilation distribution at four levels of PEEP.



The top row shows the ventilation distribution in blue, whereas the bottom row shows relative alveolar overdistention in orange and relative alveolar collapse in white. The percentages of relative alveolar overdistention and collapse are presented as well. At a total PEEP of 29 cmH_2O the dorsal areas of the lung are mainly ventilated, while the ventral parts are not ventilated due to overdistention. At a total PEEP of 9 cmH_2O the ventral parts are mainly ventilated (with more ventilation in the right lung than the left lung) and the dorsal parts are not ventilated due to alveolar collapse. At a total PEEP between 15-21 cmH_2O ventilation is mainly distributed to the centre.

Figure 1b. Relative alveolar overdistention, collapse and dynamic compliance.



Relative alveolar overdistention and collapse, and the dynamic compliance of the respiratory system are shown during a decremental PEEP trial. At 29 cmH_2O PEEP there is relative alveolar overdistention but no relative collapse, whereas at 9 cmH_2O PEEP there is relative alveolar collapse but no relative overdistention. Total PEEP was set at the PEEP level above the intersection of the curves representing relative alveolar overdistention and collapse, in this case 21 cmH_2O.[6] Images: Pulmovista 500, Dräger, Germany.

the first and last PEEP trial (up to day 7). Correlations were assessed using Spearman's rank correlation coefficient (ρ).

RESULTS

Study population

We included 15 patients with COVID-19 related ARDS (**Table 1**). Patients had a body mass index (BMI) of 30 kg/m^2 (IQR 27-34 cmH_2O). All patients had high concentrations of C-reactive protein (CRP) and required vasopressors during the first week following ICU admission. In addition, 14 (93%) patients had or progressed to diffuse ARDS on chest x-ray or lung CT scan.

Table 1. Patient characteristics

Gender (M/F)	Age (year)	BMI (kg/m^2)	APACHE IV Score	PaO_2/FiO_2 ratio (mmHg)*	Baseline PEEP (cmH_2O)†	Duration of MV (days)‡	Prone positioning§	DP (cmH_2O) **	P_L (cmH_2O)‖		Compliance (mL/cmH_2O)			CRP†† (mg/L)	ARDS morphology
									Exp.	Insp.	Lung	CW	RS		
F	49	42	79	68	18	8	Yes	12	2	13	104	53	35	530	Diffuse
M	56	33	113	171	20	8	Yes	8	0	8	90	165	58	349	Diffuse
M	65	27	94	54	16	2	Yes	10	2	19	89	103	47	681	Diffuse
M	16	22	74	158	15	1	No	n.a.‡‡	6	19	52	92	33	157	Focal to diffuse
M	72	26	99	163	16	1	No	8	4	12	114	175	69	673	Diffuse
F	59	28	73	116	18	1	Yes	10	5	14	54	189	42	563	Diffuse
F	73	18	125	105	16	0	No	8	2	10	82	134	51	401	Focal to diffuse
F	54	31	94	132	16	2	Yes	13	3	16	43	180	35	526	Diffuse
M	53	31	67	186	16	1	Yes	7	9	14	101	148	60	401	Diffuse
F	62	30	98	134	12	1	No	10	n.a.§§	n.a.§§	n.a.§§	n.a.§§	61	350	Focal to diffuse
M	66	36	124	118	18	1	No	4	4	13	77	88	41	638	Focal
M	68	34	94	134	18	2	Yes	6	-1	14	124	77	47	280	Diffuse
M	56	34	101	148	18	2	Yes	7	n.a.§§	n.a.§§	n.a.§§	n.a.§§	69	331	Diffuse
M	61	29	124	140	18	1	Yes	7	9	14	94	95	47	336	Diffuse
M	65	27	112	100	16	3	Yes	7	5	9	102	146	60	386	Diffuse

* Lowest within 24 hours following ICU admission in our centre.

† Baseline PEEP level at moment of PaO_2/FiO_2 ratio measurement. Baseline PEEP was set at the discretion of the attending clinician.

‡ Number of days on mechanical ventilation at the day of the first PEEP trial.

§ Received at least one session of prone positioning.

** Highest measured value (in cmH_2O) in the first seven days of admission, driving pressure was calculated as the difference between plateau pressure and total PEEP.

‖ Lowest measured end-expiratory value and highest measured end-inspiratory value (in cmH_2O) in the first seven days of admission, absolute transpulmonary pressure was calculated as the difference between airway pressure and oesophageal pressure. Note: the expiratory and inspiratory values are not necessarily measured at the same time and do not reflect transpulmonary driving pressure.

†† Highest measured concentration in the first three days of admission.

‡‡ Unavailable due to loss of data.

§§ Not available due to unsuccessful attempt to place oesophageal balloon catheter.

Abbreviations: ARDS acute respiratory distress syndrome, APACHE acute physiology and chronic health evaluation, BMI body mass index, CW chest wall, CRP C-reactive protein, DP Driving pressure, Exp expiratory, F female, FiO_2 fraction of inspired oxygen, ICU intensive care unit, Insp inspiratory, n.a. not available, M male, MV mechanical ventilation, PEEP positive end-expiratory pressure, P_L transpulmonary pressure, RS respiratory system.

$PEEP_{set}$ in COVID-19 related ARDS

We conducted a total of 63 PEEP trials of which 52 were performed in supine position. Median amount of PEEP trials per patient was 3 (IQR 2-4.5). $PEEP_{set}$ based on EIT was 21 cmH_2O (IQR 16-22 cmH_2O). Driving pressure was below 13 cmH_2O in all patients (**Table 1**). In one PEEP trial (1.6%) we did not reach a $PEEP_{high}$ of 10 cmH_2O above baseline PEEP because of hemodynamic instability (mean arterial blood pressure <60 mmHg). No pneumothoraxes were observed. At 28-days, four patients died (26.7%), three patients were weaning from mechanical ventilation (20.0%), and 8 patients were discharged from the ICU (53.3%).

$PEEP_{set}$ was 2 cmH_2O (IQR 0-5 cmH_2O) above PEEP set by the higher PEEP-FiO_2 table and 10 cmH_2O (IQR 7-14 cmH_2O) above PEEP set by the lower PEEP-FiO_2 table (p-value for the absolute difference 0.01) (**Figure 2A**). There was no correlation between $PEEP_{set}$ and FiO_2 (ρ = 0.11, p-value 0.69). However, we did find a significant correlation between $PEEP_{set}$ and BMI (ρ = 0.76, p-value 0.001) (**Figure 2B**). $PEEP_{set}$ did not change significantly over time (**Figure 2C**).

Figure 2

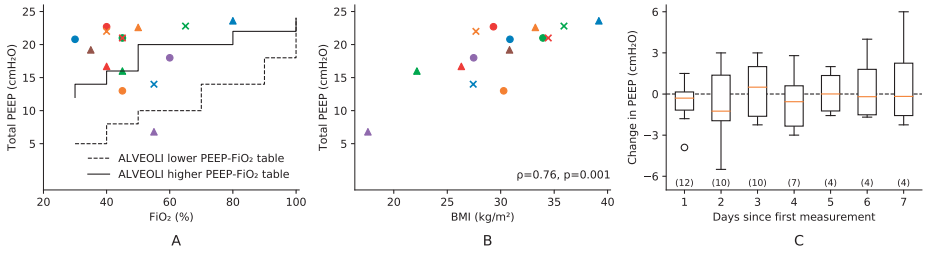


Figure 2a. $PEEP_{set}$ versus higher and lower PEEP-FiO_2 table
The solid and dashed lines represent the PEEP-FiO_2 combination to be used according to the lower and higher PEEP-FiO_2 tables from the ALVEOLI trial. Each marker represents $PEEP_{set}$ at the level of lowest relative alveolar overdistention and collapse as measured with electrical impedance tomography. Only the first PEEP trial of each patient is presented. The crosses indicate subjects that died within 28-days following ICU admission. There was no correlation between $PEEP_{set}$ and FiO_2 (ρ = 0.11, p-value 0.69).

Figure 2b. $PEEP_{set}$ versus body mass index
The correlation between BMI and $PEEP_{set}$ after the first PEEP trial for each patient. Spearman's rank correlation coefficient ρ = 0.76 with p-value 0.001. Similar markers in figure 2a and 2b represent the same patient.

Figure 2c. Change in PEEP as compared to the first PEEP trial
The change in $PEEP_{set}$ as compared to the first PEEP trial, represented by the median (orange line), interquartile range (box) and minimum/maximum values (whiskers). $PEEP_{set}$ did not change significantly over time. The number between parentheses represents the amount of patients measured at that day.

DISCUSSION

In 15 patients with COVID-19 related ARDS, personalised PEEP at the level of lowest relative alveolar overdistention and collapse as measured with EIT resulted in high PEEP. These PEEP levels did not result in high driving pressure or transpulmonary pressure. In addition, PEEP trials did not result in relevant hemodynamic instability or pneumothorax. $PEEP_{set}$ corresponded better to the higher PEEP-FiO_2 table than the lower PEEP-FiO_2 table and was positively correlated with BMI.

In COVID-19 related ARDS, both a low lung recruitability (L-type) and a high lung recruitability phenotype (H-type) have been described based on lung compliance and amount of non-aerated lung tissue on lung CT scan.[8] Especially in patients with the L-type, low PEEP was advised, as higher PEEP would only result in alveolar overdistention without the benefit of alveolar recruitment. In 12 patients with COVID-19 related ARDS, Pan et al.[9] used the recruitment-to-inflation ratio and found that lung recruitability was low as well. However, in our first 15 patients with COVID-19 related ARDS, personalised PEEP at the level of lowest relative alveolar overdistention and collapse as measured with EIT resulted in high PEEP. Perhaps we included only patients with the H-type, but it is more likely that both phenotypes are the extremes of a recruitability continuum. The recruitability continuum represents the amount of non-aerated lung tissue as a result of oedema. Gattinoni et al.[8] already described that one patient with COVID-19 related ARDS could progress from the L-type to the H-type as the amount of non-aerated lung tissue increased. If these results can be generalized, most patients with COVID-19 will become recruitable to some extent. The potential changes in recruitability over time make a personalised PEEP titration approach very interesting, although we did not observe a significant change in $PEEP_{set}$ over time.

In addition, a secondary analysis of the ALVEOLI trial found that higher PEEP improved survival in patients with a hyperinflammatory ARDS phenotype.[10] The hyperinflammatory phenotype could be predicted accurately using interleukin-6, tumour necrosis factor receptor and use of vasopressors. Given the very high CRP concentrations and the use of vasopressors in all our patients, we assumed that the majority of patients in our study were in a hyperinflammatory state.

The LIVE trial predicted PEEP response based on lung morphology, and found that patients with focal ARDS benefited from lower PEEP and patients with diffuse ARDS from higher PEEP.[7] In our study, the majority of patients had or progressed to diffuse ARDS based on chest x-ray or lung CT scan. As a consequence, these COVID-19 patients were likely to respond to higher PEEP.

We realize that availability of EIT is limited in ICUs worldwide. In clinical practice, the PEEP-FiO_2 table is often used, as it is a simple approach to titrate PEEP. This study showed that $PEEP_{set}$ at the level of lowest relative alveolar overdistention and collapse as measured with EIT corresponded better to the higher PEEP-FiO_2 table in 15 patients with COVID-19 related ARDS. However, the patients in our study had a high BMI, resulting in lower transpulmonary pressure and increased PEEP requirement. Higher PEEP should be used with caution in patients with focal ARDS or low BMI. Moreover, response to higher PEEP should always be monitored in terms of driving pressure[2] or oxygenation.[11]

ACKNOWLEDGMENTS

We thank all ICU personnel that enabled us to perform this study.

REFERENCES:

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X *et al*: **Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China**. *Lancet (London, England)* 2020, **395**(10223):497-506.
2. Amato MB, Meade MO, Slutsky AS, Brochard L, Costa EL, Schoenfeld DA, Stewart TE, Briel M, Talmor D, Mercat A *et al*: **Driving pressure and survival in the acute respiratory distress syndrome**. *The New England journal of medicine* 2015, **372**(8):747-755.
3. Writing Group for the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial I, Cavalcanti AB, Suzumura EA, Laranjeira LN, Paisani DM, Damiani LP, Guimaraes HP, Romano ER, Regenga MM, Taniguchi LNT *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: A Randomized Clinical Trial**. *Jama* 2017, **318**(14):1335-1345.
4. Frerichs I, Amato MB, van Kaam AH, Tingay DG, Zhao Z, Grychtol B, Bodenstein M, Gagnon H, Bohm SH, Teschner E *et al*: **Chest electrical impedance tomography examination, data analysis, terminology, clinical use and recommendations: consensus statement of the TRanslational EIT developmeNt stuDy group**. *Thorax* 2017, **72**(1):83-93.
5. Brower RG, Lanken PN, MacIntyre N, Matthay MA, Morris A, Ancukiewicz M, Schoenfeld D, Thompson BT, National Heart L, Blood Institute ACTN: **Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome**. *The New England journal of medicine* 2004, **351**(4):327-336.
6. Pereira SM, Tucci MR, Morais CCA, Simoes CM, Tonelotto BFF, Pompeo MS, Kay FU, Pelosi P, Vieira JE, Amato MBP: **Individual Positive End-expiratory Pressure Settings Optimize Intraoperative Mechanical Ventilation and Reduce Postoperative Atelectasis**. *Anesthesiology* 2018, **129**(6):1070-1081.
7. Constantin JM, Jabaudon M, Lefrant JY, Jaber S, Quenot JP, Langeron O, Ferrandiere M, Grelon F, Seguin P, Ichai C *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**(10):870-880.
8. Gattinoni L, Chiumello D, Caironi P, Busana M, Romitti F, Brazzi L, Camporota L: **COVID-19 pneumonia: different respiratory treatments for different phenotypes?** *Intensive care medicine* 2020.
9. Pan C, Chen L, Lu C, Zhang W, Xia JA, Sklar MC, Du B, Brochard L, Qiu H: **Lung Recruitability in SARS-CoV-2 Associated Acute Respiratory Distress Syndrome: A Single-center, Observational Study**. *American journal of respiratory and critical care medicine* 2020.
10. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA, Network NA: **Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials**. *The Lancet Respiratory medicine* 2014, **2**(8):611-620.
11. Goligher EC, Kavanagh BP, Rubenfeld GD, Adhikari NK, Pinto R, Fan E, Brochard LJ, Granton JT, Mercat A, Marie Richard JC *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**(1):70-76.

9.2

Response letter

Philip van der Zee MD[1]*, Peter Somhorst MSc[1], Henrik Endeman MD PhD[1], Diederik Gommers MD PhD[1]

[1] Department of Adult Intensive Care, Erasmus Medical Center Rotterdam, The Netherlands

Published: Am J Respir Crit Care Med 2020 Vol. 202 Issue 8 (Response letter)

To the editor:

We thank Van den Berg et al. for the opportunity to further discuss our research letter in which positive end-expiratory pressure (PEEP) was titrated at the level of lowest relative alveolar overdistention and collapse based on electrical impedance tomography (EIT).[1] In their comment, the authors argue that PEEP should not be set at the minimum level of both alveolar overdistention and collapse, as alveolar overdistention is potentially more harmful.

We fully agree that alveolar overdistention is harmful to our patients. The Alveolar Recruitment Trial showed us that systematically performed recruitment maneuvers, known to cause alveolar overdistention, increased mortality rate in patients with acute respiratory distress syndrome (ARDS).[2] However, the amount of alveolar overdistention or collapse prior to the application of high airway pressures was unknown. Determining alveolar overdistention and collapse is crucial, as PEEP titration approaches are based on the assumption that there is an optimal compromise between alveolar recruitment (i.e. limit the amount of collapse) and minimizing alveolar overdistention.

Numerous bedside PEEP titration approaches have been described, but none has shown to improve patient survival in large randomized controlled trials. In addition, correlation between different approaches is poor. The explanation is that most bedside PEEP titration approaches have at least one of the following three limitations: 1. the approach does not quantify alveolar recruitment; 2. the respiratory system is assessed as a whole and local lung inhomogeneities remain undetected; and 3. alveolar overdistention is not quantified.

EIT is a functional imaging tool that continuously assesses regional ventilation and lung volume changes at the bedside. As such, EIT is a bedside PEEP titration approach that quantifies both alveolar recruitment and alveolar overdistention, and is able to detect local lung inhomogeneities. However, the amount of studies that used EIT to titrate PEEP in critically ill patients with ARDS is limited. In addition, there is no consensus on how to interpret EIT data.

Blankman et al.[3] compared several EIT derived PEEP titration approaches in post cardiac surgery patients and proposed the intratidal gas distribution index to identify alveolar overdistention in the non-dependent lung regions and to titrate PEEP. In a case series, Yoshida et al.[4] used a ventral-dorsal ventilation distribution of 50%-50% in order to reach homogeneous ventilation and limit alveolar overdistention. In contrast, Franchineau et al.[5] aimed to limit the amount of relative collapse to 15% while

maintaining the lowest percentage of overdistention in patients with extracorporeal membrane oxygenation. Alternatively, we could have aimed for the greatest amount of ventilated pixels or calculate the global inhomogeneity index. We chose to titrate PEEP at the lowest level of relative alveolar overdistention and collapse, as it is a simple and intuitive approach that has proven to be beneficial in mechanically ventilated patients during surgery.[6] This approach resulted in low driving pressures and low transpulmonary pressures in all our patients.

We share the concerns of Van den Berg et al. that alveolar overdistention is harmful to the lungs. Therefore, we quantified the amount of alveolar overdistention before applying higher PEEP in our patients with COVID-19 related ARDS. The Pleural Pressure Working Group's (PLUG) planned RECRUIT project (https://www.plugwgroup.org/), which aims to compare the results of different bedside methods to titrate PEEP based on EIT, might provide us with some answers on how to titrate PEEP using EIT data. In the meantime, we agree with our colleagues to limit the amount of alveolar overdistention in patients with COVID-19 related ARDS by applying prone positioning and quantifying the amount of alveolar overdistention during a PEEP trial.

REFERENCES:

1. van der Zee P, Somhorst P, Endeman H, Gommers D: **Electrical Impedance Tomography for Positive End-expiratory Pressure Titration in COVID-19 Related ARDS**. *American journal of respiratory and critical care medicine* 2020.
2. Writing Group for the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial I, Cavalcanti AB, Suzumura EA, Laranjeira LN, Paisani DM, Damiani LP, Guimaraes HP, Romano ER, Regenga MM, Taniguchi LNT *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: A Randomized Clinical Trial**. *Jama* 2017, **318**(14):1335-1345.
3. Blankman P, Hasan D, Erik G, Gommers D: **Detection of 'best' positive end-expiratory pressure derived from electrical impedance tomography parameters during a decremental positive end-expiratory pressure trial**. *Crit Care* 2014, **18**(3):R95.
4. Yoshida T, Piraino T, Lima CAS, Kavanagh BP, Amato MBP, Brochard L: **Regional Ventilation Displayed by Electrical Impedance Tomography as an Incentive to Decrease Positive End-Expiratory Pressure**. *American journal of respiratory and critical care medicine* 2019, **200**(7):933-937.
5. Franchineau G, Brechot N, Lebreton G, Hekimian G, Nieszkowska A, Trouillet JL, Leprince P, Chastre J, Luyt CE, Combes A *et al*: **Bedside Contribution of Electrical Impedance Tomography to Setting Positive End-Expiratory Pressure for Extracorporeal Membrane Oxygenation-treated Patients with Severe Acute Respiratory Distress Syndrome**. *American journal of respiratory and critical care medicine* 2017, **196**(4):447-457.
6. Pereira SM, Tucci MR, Morais CCA, Simoes CM, Tonelotto BFF, Pompeo MS, Kay FU, Pelosi P, Vieira JE, Amato MBP: **Individual Positive End-expiratory Pressure Settings Optimize Intraoperative Mechanical Ventilation and Reduce Postoperative Atelectasis**. *Anesthesiology* 2018, **129**(6):1070-1081.

10

PEEP-FiO_2 table versus EIT to titrate PEEP in mechanically ventilated patients with COVID-19 related ARDS

Peter Somhorst MSc[*,1], Philip van der Zee MD[*,1], Henrik Endeman MD PhD[1], Diederik Gommers MD PhD[1]

[*] These authors contributed equally to this work.
[1] Department of Adult Intensive Care, Erasmus Medical Center Rotterdam, The Netherlands

Published: Crit Care 2022 Vol. 26 Issue 1

ABSTRACT

Rationale: It is unknown how to titrate positive end-expiratory pressure (PEEP) in patients with COVID-19 related acute respiratory distress syndrome (ARDS). Guidelines recommend the one-size fits all PEEP-FiO_2 table. In this retrospective cohort study, an electrical impedance tomography (EIT) guided PEEP trial was used to titrate PEEP.

Objectives: To compare baseline PEEP according to the high PEEP-FiO_2 table and personalised PEEP following an EIT guided PEEP trial.

Methods: We performed an EIT guided decremental PEEP trial in patients with moderate to severe COVID-19 related ARDS upon intensive care unit admission. PEEP was set at the lowest PEEP above the intersection of curves representing relative alveolar overdistention and collapse. Baseline PEEP was compared with PEEP set according to EIT. We identified patients in whom the EIT guided PEEP trial resulted in a decrease or increase in PEEP of ≥2 cmH_2O.

Measurements and Main Results: We performed a PEEP trial in 75 patients. In 23 (31%) patients PEEP was decreased ≥2 cmH_2O and in 24 (32%) patients PEEP was increased ≥2 cmH_2O. Patients in whom PEEP was decreased had improved respiratory mechanics and more overdistention in the non-dependent lung region at higher PEEP levels. These patients also had a lower BMI, longer time between onset of symptoms and intubation, and higher incidence of pulmonary embolism. Oxygenation improved in patients in whom PEEP was increased.

Conclusions: An EIT guided PEEP trial resulted in a relevant change in PEEP in 63% of patients. These results support the hypothesis that PEEP should be personalised in patients with ARDS.

Keywords: COVID-19, Acute respiratory distress syndrome, mechanical ventilation, positive end-expiratory pressure, electrical impedance tomography

INTRODUCTION

It is unknown how to titrate PEEP in patients with COVID-19 related ARDS. Previous randomized controlled trials in patients with ARDS found that a higher PEEP versus a lower PEEP strategy did not reduce mortality rate.[1-3] However, higher PEEP did reduce mortality rate in patients with severe ARDS and tended to increase mortality rate in the subgroup with mild ARDS.[4] Apparently, there are patient subgroups that benefit from higher PEEP and subgroups that do not benefit from higher PEEP.

Initially, COVID-19 related ARDS was thought to be typical ARDS according to the Berlin definition of ARDS.[5] Later, a phenotype consisting of preserved respiratory system compliance and low lung recruitability was described. The application of lower PEEP was advised.[6, 7] Subsequent studies found that respiratory mechanics of patients with COVID-19 related ARDS and typical ARDS, including respiratory system compliance, were similar between groups. Significant heterogeneity was observed in COVID-19 related ARDS similar to non-COVID ARDS.[8, 9] The high PEEP-FiO_2 table, currently recommended for the treatment of COVID-19 related ARDS, does not take into account patient heterogeneity.[10]

From a pulmonary perspective, PEEP titration is finding a compromise between minimal alveolar overdistention and collapse. The recommended PEEP for lung protective ventilation strategies ranges between 5 cmH_2O and 24 cmH_2O.[11] A strategy consisting of recruitment manoeuvres and titrated PEEP resulted in increased long term mortality in patients with ARDS.[12] Several studies failed to find a benefit of a one-size-fits-all PEEP strategy, and more research into tailoring of PEEP to the individual patient is recommended.[13] Therefore, we think it is crucial to quantify the amount of alveolar overdistention and collapse at the bedside among parameters reflecting respiratory mechanics. EIT can be used for detecting and quantify regional alveolar overdistention and collapse and allows for personalised PEEP titration.[14, 15] In a recent randomized controlled trial in ARDS patients He et al.[16] showed titration using EIT resulted in a decoupling between PEEP and FiO_2, but no difference in long term outcome compared to a PEEP/FiO_2 table. Hsu et al.[17] compared PEEP titrated using EIT to PEEP set based on pressure-volume-loops. They found the EIT lead to lower PEEP and a higher survival rate.

We retrospectively describe a cohort of patients with COVID-19 related ARDS in whom an EIT guided PEEP trial was used to personalise PEEP. The aim of this study was to compare PEEP set by EIT and baseline PEEP according to the high PEEP-FiO_2 table.[1]

METHODS

Study design and inclusion criteria

This is a retrospective analysis of a cohort study conducted between March 1 and June 1 2020 in the general intensive care unit (ICU) of the Erasmus MC, Rotterdam, The Netherlands. The first 15 patients in this study have been described previously.[18] All patients that met the following criteria were included: 1. age ≥16 years; 2. established COVID-19 following a SARS-CoV-2 positive polymerase chain reaction; 3. moderate to severe ARDS according to the Berlin definition of ARDS[5]; and 4. intubated and on controlled mechanical ventilation. The Erasmus MC is a tertiary referral hospital, and some patients were intubated and mechanically ventilated elsewhere before transfer to our ICU. A PEEP trial guided by EIT was performed following admission to the ICU according to our local COVID-19 mechanical ventilation protocol. The PEEP trial was performed once in every patient and was not routinely repeated. We did not perform a PEEP trial if patients had a contra-indication for EIT belt placement (e.g. pacemaker, spinal cord injury), inadequate EIT signal (e.g. thoracic bandages, undrained pneumothorax), or hemodynamic instability (MAP < 60mmHg despite optimization of fluid status and/or use of vasopressors). The Medical Ethical Committee of the Erasmus MC approved this study. According to Dutch legislation no informed consent was required for the retrospective use of anonymized patient data.

Study protocol

All patients were ventilated in pressure control mode. Baseline PEEP was set by the attending clinician. The protocol prescribed using the high PEEP-FiO_2 table, but the clinician had the freedom to choose the PEEP and FiO_2 combination within the limits of the table.[1] Patients were fully sedated (Richmond Agitation-Sedation Scale -5) with continuous intravenous infusion of propofol, midazolam and/or opiates. Persisting spontaneous inspiratory efforts were prevented with increased sedation or neuromuscular blockade (rocuronium). Mean arterial blood pressure (MAP) was measured continuously and noradrenalin was administered to maintain MAP above 65 mmHg prior to the PEEP trial. The fraction of inspired oxygen (FiO_2) was titrated to obtain a peripheral oxygen saturation (SpO_2) between 92-95%.

The PEEP trial was guided by one of two EIT devices, based on availability: Pulmovista 500, Dräger, Germany or Enlight 1800, Timpel, Brazil. An EIT belt containing surface electrodes was placed in the transversal plane at the 4^{th} to 5^{th} intercostal parasternal space according to manufacturer's instructions. Regional ventilation data was visualized on screen during the entire PEEP trial without repositioning the EIT belt. The PEEP titration tool of the EIT devices was used to guide PEEP titration.

A decremental PEEP trial was performed starting at the baseline PEEP according to the high PEEP-FiO_2 table ($PEEP_{base}$). We increased the airway pressure until PEEP was 10 cmH_2O above $PEEP_{base}$ with a minimum of 24 cmH_2O, corresponding to the maximum PEEP advised by the high PEEP-FiO_2 table.[1] The imposed driving pressure (i.e. the difference between PEEP and peak pressure) remained unchanged during the trial. In case of hypotension (MAP <60mmHg) or desaturation (SpO_2 <88%), PEEP was limited to the highest airway pressure without hypotension or desaturation. We aimed to maintain PEEP for at least one minute in order to establish a constant electrical impedance signal. PEEP was reduced in steps of 2 cmH_2O every 30 seconds until continuous EIT monitoring showed evident collapse as compared to maximum PEEP. To confirm a further increase in collapse, PEEP was lowered an additional 2 cmH_2O. Subsequently, we performed a small recruitment manoeuvre at the highest PEEP used during the PEEP trial for 30 seconds. The PEEP titration tools of both EIT devices provided a percentage of relative alveolar overdistention and collapse at every PEEP step. PEEP was set ($PEEP_{set}$) at the lowest PEEP step above the intersection of the curves representing relative alveolar overdistention and collapse, as described previously (see Figure 2 (C)).[18, 19]

Mechanical ventilation, SpO_2 and hemodynamic parameters were recorded at $PEEP_{base}$ and after 30 minutes of $PEEP_{set}$. Plateau airway pressure (Pplat) and total PEEP were measured during an inspiratory and expiratory hold procedure, respectively. We used the last arterial blood gas before and the first arterial blood gas after the decremental PEEP trial for calculation of the PaO_2/FiO_2 ratio at $PEEP_{base}$ and $PEEP_{set}$, respectively. Patient characteristics were extracted from the patient information system.

The primary goal of this study was to compare $PEEP_{base}$ with $PEEP_{set}$. Secondary goals were to compare respiratory mechanics and oxygenation before and after the PEEP trial. Subsequently, we identified the patients in whom $PEEP_{set}$ according to the EIT guided PEEP trial was decreased by ≥2 cmH_2O ($PEEP_{lower}$) or was increased by ≥2 cmH_2O ($PEEP_{higher}$) as compared to $PEEP_{base}$. Patients with a change in $PEEP_{set}$ of less than 2 cmH_2O as compared to $PEEP_{base}$ were assigned to a third group: $PEEP_{equal}$. The change in percentage of relative alveolar overdistention and collapse were reported between PEEP 24 cmH_2O and PEEP 12 cmH_2O, because both PEEP levels were reached during the PEEP trial in 93% (n = 70) of patients.

Statistical analysis

Data were presented as mean (standard deviation), median [25th-75th percentile] or count (percentage). Data was tested for normality using the Shapiro-Wilk test. The Student independent T-test or Mann-Whitney U test was used for the comparison between two groups. A one-way ANOVA or the Kruskal Wallis test was used for the comparison between

three groups. The Student dependent T-test or Wilcoxon signed-rank test was used to compare changes from baseline within patients. A repeated measures ANOVA or Friedman test was used to compare changes over more than two levels. The Chi-square test was used to compare frequencies. Bonferroni correction was applied to correct for multiple testing. Spearman's rank correlation coefficient (ρ) was used for calculation of correlations between variables. A p-value of ≤0.05 was considered to be statistically significant.

RESULTS

Seventy-five mechanically ventilated patients with COVID-19 related ARDS were included in this retrospective cohort study. Patients had a median age of 64 years [54-71] and a body mass index (BMI) of 30.4 kg/m^2 (5.8). Median APACHE IV score at ICU admission was 70 (27) and median time since intubation was 3 days [1-8].

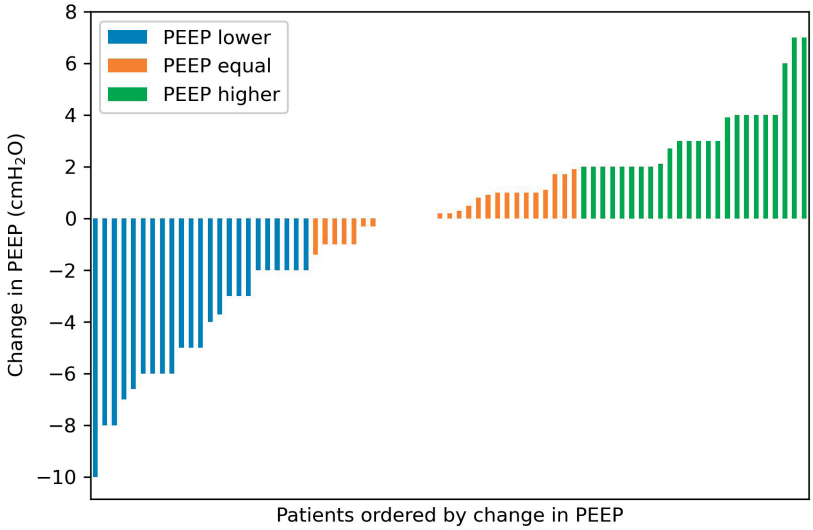
In the entire cohort, we did not observe a difference between the median PEEP level before and after the PEEP trial (**Table 1**). After the PEEP trial, there was a small increase in static compliance and tidal volume. In 31% of patients (n=23), PEEP was decreased by ≥2 cmH_2O and in 32% of patients (n=24) PEEP was increased by ≥2 cmH_2O (**Figure 1**). The remaining 28 patients (37%) were assigned to $PEEP_{equal}$ group. EIT images of a representative patient from the $PEEP_{lower}$ group and the $PEEP_{higher}$ group are shown in **Figure 2**. In 5 patients (7%) a PEEP of 24 cmH_2O could not be applied due to desaturation (n=4) or hypotension (n=1). Desaturation occurred only in the $PEEP_{lower}$ group. One (1%) pneumothorax was observed following central catheter placement.

Table 1: $PEEP_{base}$ versus $PEEP_{set}$.

	$PEEP_{base}$	$PEEP_{set}$	Difference	p-value
Total PEEP (cmH2O)	17.0 [16.0-19.0]	18.0 [14.0-20.0]	0.2 [-2.0-2.0]	1.00
Plateau pressure (cmH2O)	28.0 [25.0-30.8]	28.0 [24.2-30.0]	0.0 [-3.0-2.0]	0.80
Driving pressure (cmH2O)	10.0 [8.0-14.0]	10.0 [7.5-13.0]	-0.5 [-1.0-0.8]	0.083
Tidal volume (mL/kg PBW)	6.5 [5.7-7.0]	6.6 [5.9-7.4]	0.2 [-0.1-0.6]	0.002*
Static compliance (mL/cmH2O)	45 [33-59]	49 [35-64]	4 [-2-8]	0.016*
PaO2 (mmHg)	81 [72-93]	80 [68-96]	0 [-16-13]	1.00
PaO2/FiO2 ratio (mmHg)	162 [110-201]	159 [123-212]	0 [-24-51]	0.92
SpO2 (%)	95 [93-95]	95 [93-96]	0 [-2-2]	1.00
PaCO2 (mmHg)	45 [41-52]	45 [40-53]	-1 [-5-5]	0.71
Systolic Blood Pressure (mmHg)	130 (24)	134 (23)	4 (27)	0.63
Diastolic Blood Pressure (mmHg)	60 [54-65]	61 [54-67]	-1 [-4-3]	1.00
Mean Arterial Pressure (mmHg)	82 [76-91]	83 [77-93]	-1 [-6-5]	1.00
Heart rate (/min)	79 [70-94]	81 [70-92]	1 [-2-4]	0.22

Data are presented as mean (standard deviation) or median [25th and 75th percentile]. * $p < 0.05$

Figure 1 Change in PEEP following the EIT guided PEEP trial.

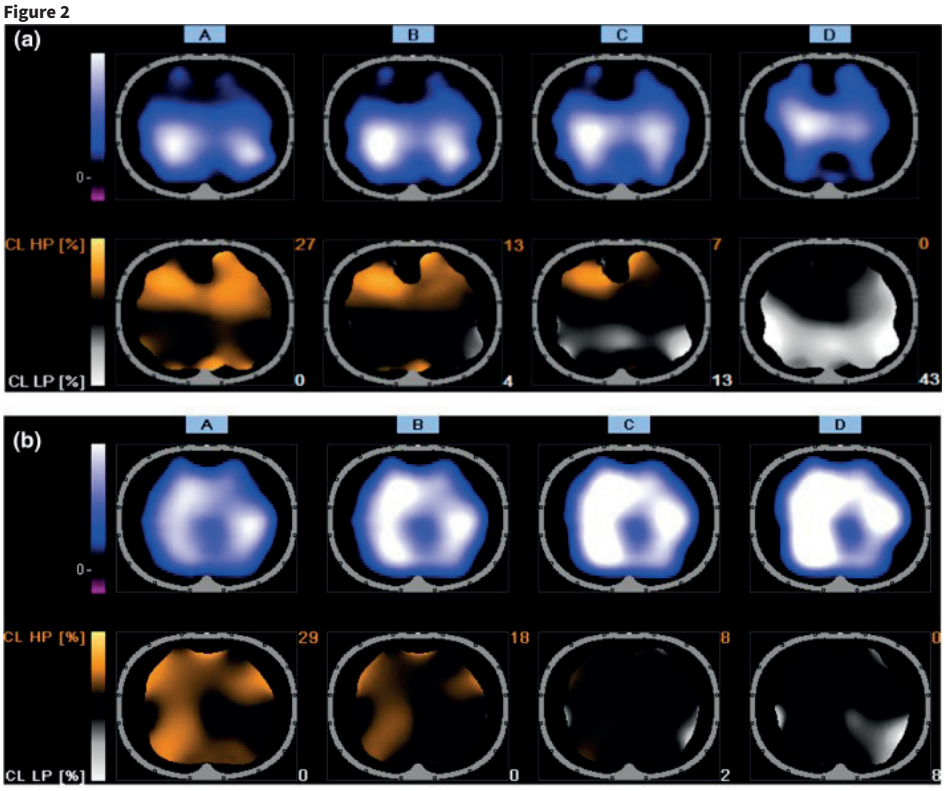


All 75 patients in this cohort are shown on the x-axis. On the y-axis, the change in PEEP ($PEEP_{set}$ - $PEEP_{base}$) is presented. We identified the patients in which $PEEP_{set}$ according to the EIT guided PEEP trial was decreased by ≥2 cmH_2O ($PEEP_{lower}$ in blue) or was increased by ≥2 cmH_2O ($PEEP_{higher}$ in green) as compared to $PEEP_{base}$. Patients with a change in $PEEP_{set}$ of less than 2 cmH_2O as compared to $PEEP_{base}$ were assigned to a third group: $PEEP_{equal}$ (in orange).

Patients in the $PEEP_{lower}$ group had a lower BMI, a higher APACHE IV score, a longer time between onset of symptoms and intubation, a higher d-dimer concentration at ICU admission, and a higher incidence of pulmonary embolism as compared to the other groups (**Table 2**). In the entire cohort, we found a statistically significant correlation between $PEEP_{set}$ and BMI (ρ= 0.59, p-value <0.001), and time between onset of symptoms and intubation (ρ= -0.42, p-value <0.001). We did not observe a correlation between $PEEP_{set}$ and APACHE IV score (ρ= -0.10, p-value 0.41) or d-dimer concentration (ρ= -0.06, p-value 0.66). $PEEP_{set}$ resulted in an increase in tidal volume at the same driving pressure (static compliance was not significantly changed after Bonferroni correction) compared to $PEEP_{base}$ in patients in the $PEEP_{lower}$ group, but not in the other two groups (**Table 3**). In addition, we observed a significant reduction in plateau pressure in the $PEEP_{lower}$ group. In the patients assigned to $PEEP_{higher}$ group, we observed a significant increase in plateau pressure. There was a trend for a higher PaO_2/FiO_2 ratio, but this was not significant after Bonferroni correction.

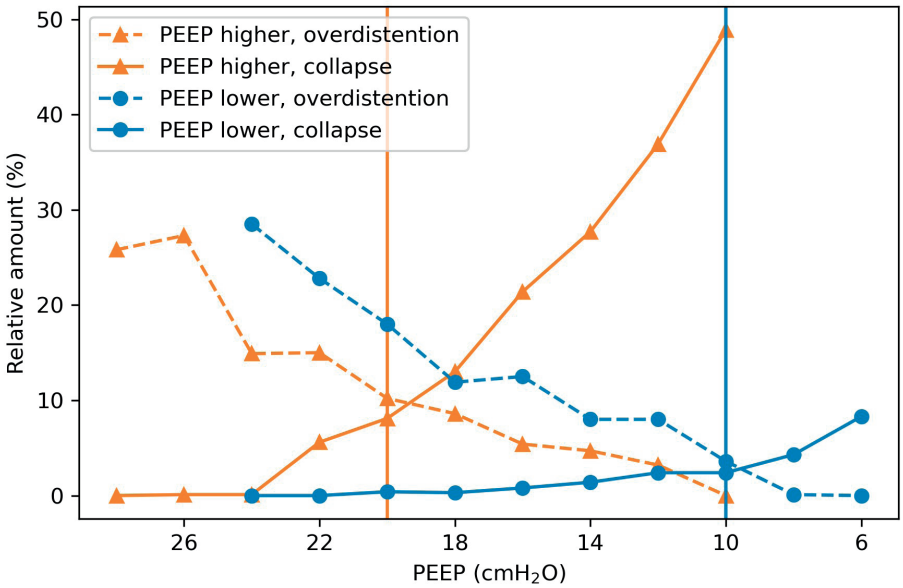
Table 5-8 in de Supplementary Materials show the respiratory mechanics at the baseline PEEP, the highest and lowest PEEP during the decremental PEEP trial and the set PEEP immediately after it was set for the entire cohort (Table 5) and the $PEEP_{lower}$ (Table 6), $PEEP_{equal}$ (Table 7) and and $PEEP_{higher}$ (Table 8) groups.

Figure 2



Overdistention and collapse for two typical patients. A — A patient assigned to the $PEEP_{higher}$ group. The top row displays ventilation distribution at four levels of PEEP (left to right: 28, 20, 16, and 10 cmH_2O). Black indicates no ventilation, various shades of blue indicate ventilation, and white indicates the region where most ventilation is detected. At high PEEP levels ventilation occurs in the dorsal lung regions, whereas the center of ventilation shifts ventrally at lower PEEP levels. The distribution of alveolar overdistention (orange) and alveolar collapse (white) are shown in the bottom row. At high PEEP levels only a small amount of ventilation is detected in the ventral region as a result of overdistention. At low PEEP levels ventilation shifts ventrally as a result of alveolar collapse. High PEEP resulted in a relatively small increase in alveolar overdistention and a large decrease in alveolar collapse. This patient was considered to have high recruitability and total PEEP was set at the lowest PEEP step above the intersection of both curves: 20 cmH_2O.; B — A patient assigned to the $PEEP_{lower}$ group. The top row displays ventilation distribution at four levels of PEEP (left to right: 24, 20, 12, and 6 cmH_2O). A decrease in PEEP resulted in an increase in ventilation (light blue to white) and even at a low PEEP level of 6 cmH_2O ventilation in the dorsal lung regions is more or less preserved. At high PEEP levels, the relative amount of alveolar overdistention is 29%, which indicates severe alveolar overdistention. In contrast, at low PEEP levels the amount of alveolar overdistention is significantly reduced, and only a small amount of alveolar collapse is identified (8%). This patient was considered to have low recruitability and PEEP was set at 10 cmH_2O.

c



C — This plot represents the relative amount of alveolar overdistention and collapse as measured by EIT during a decremental PEEP trial. PEEP was set above at the lowest PEEP step above the intersection of the curves representing relative amount of alveolar overdistention and collapse (vertical lines). The patient in $PEEP_{higher}$ group (orange triangles) had lower amounts of relative overdistention and higher amounts of alveolar collapse at the same PEEP level as compared to the patient assigned to a patient in $PEEP_{lower}$ group (blue dots).

Table 2: Comparison of baseline characteristics between PEEP groups.

	Total (n=75)	$PEEP_{lower}$ (n=23)	$PEEP_{equal}$ (n=28)	$PEEP_{higher}$ (n=24)	p-value
Male gender	59 (79%)	19 (83%)	22 (79%)	18 (75%)	0.82
BMI (kg/m2)	30.4 (5.8)	27.8 (5.6)	31.4 (5.5)	31.6 (5.8)	0.037*
Age (y)	64 [54-71]	66 [60-73]	64 [54-68]	59 [53-70]	0.27
Apache IV score at ICU admission	70 (27)	85 (32)	66 (24)	61 (19)	0.004*
Time since onset symptoms (d)	14 [10-17]	15 [14-26]	14 [8-17]	12 [7-16]	0.061
Time since intubation (d)	3 [1-8]	4 [2-14]	3 [2-6]	2 [1-7]	0.52
Time between onset symptoms and intubation (d)	10 [7-12]	10 [9-13]	7 [6-12]	8 [6-10]	0.046*
Time ventilated in other hospital (d)	1 [0-4]	2 [0-6]	1 [0-3]	1 [0-3]	0.51
28 day mortality	22 (29%)	8 (35%)	7 (25%)	7 (29%)	0.75
D-dimer at admission (mg/L)	1.6 [0.9-3.5]	2.9 [1.5-8.8]	1.2 [0.7-2.8]	1.3 [1.0-2.2]	0.026*
Pulmonary embolism at PEEP trial	13 (17%)	6 (26%)	3 (11%)	4 (17%)	0.35
Proven pulmonary embolism during ICU admission	38 (51%)	17 (74%)	12 (43%)	9 (38%)	0.026*

Data are presented as mean (standard deviation), count (%) or median [25th and 75th percentile]. * $p < 0.05$

Table 3: Comparison of respiratory mechanics between PEEP groups.

		$PEEP_{lower}$ (n=23)	p-value	$PEEP_{equal}$ (n=28)	p-value	$PEEP_{higher}$ (n=24)	p-value
Total PEEP (cmH2O)	$PEEP_{base}$	18.0 [16.4-19.0]		17.0 [15.8-19.6]		17.0 [15.8-18.2]	
	$PEEP_{set}$	14.0 [11.0-16.0]	<0.001*	18.0 [15.8-20.0]	0.48	20.0 [18.0-22.7]	<0.001*
Plateau pressure (cmH2O)	$PEEP_{base}$	29.7 (5.0)		27.9 (3.9)		26.5 (3.3)	
	$PEEP_{set}$	23.9 (4.7)	<0.001*	27.8 (3.8)	1.00	30.1 (4.6)	<0.001*
Driving pressure (cmH2O)	$PEEP_{base}$	11.0 [8.5-14.1]		9.8 [8.0-12.1]		9.0 [8.2-14.0]	
	$PEEP_{set}$	11.0 [7.0-14.0]	0.065	9.5 [7.8-11.9]	0.37	10.0 [9.0-13.0]	1.00
Tidal volume (mL/kg PBW)	$PEEP_{base}$	6.1 (1.2)		6.8 (1.4)		6.5 (0.8)	
	$PEEP_{set}$	6.6 (1.3)	0.002*	7.1 (1.6)	0.060	6.5 (0.8)	1.00
Static compliance (mL/cmH2O)	$PEEP_{base}$	45 (26)		53 (23)		47 (16)	
	$PEEP_{set}$	50 (22)	0.19	55 (19)	1.00	47 (16)	1.00
PaO2 (mmHg)	$PEEP_{base}$	87 (29)		96 (54)		82 (16)	
	$PEEP_{set}$	74 (19)	0.31	89 (23)	1.00	87 (16)	0.65
PaO2/FiO2 ratio (mmHg)	$PEEP_{base}$	141 (59)		188 (102)		162 (66)	
	$PEEP_{set}$	149 (64)	1.00	182 (68)	1.00	185 (72)	0.16
SpO2 (%)	$PEEP_{base}$	94 [93-95]		95 [94-95]		94 [93-95]	
	$PEEP_{set}$	94 [92-96]	1.00	96 [93-97]	1.00	94 [93-96]	0.73
PaCO2 (mmHg)	$PEEP_{base}$	48 [39-57]		44 [39-53]		45 [42-50]	
	$PEEP_{set}$	44 [39-56]	1.00	44 [40-55]	1.00	46 [42-50]	1.00
Systolic Blood Pressure (mmHg)	$PEEP_{base}$	132 (26)		129 (24)		130 (22)	
	$PEEP_{set}$	143 (19)	0.18	128 (24)	1.00	132 (23)	1.00
Diastolic Blood Pressure (mmHg)	$PEEP_{base}$	60 (11)		61 (9)		61 (11)	
	$PEEP_{set}$	63 (13)	0.57	61 (10)	1.00	61 (11)	1.00
Mean Arterial Pressure (mmHg)	$PEEP_{base}$	81 [74-88]		83 [77-93]		82 [76-86]	
	$PEEP_{set}$	89 [80-96]	0.42	81 [76-88]	0.56	83 [76-90]	1.00
Heart rate (/min)	$PEEP_{base}$	83 [70-94]		87 [74-97]		74 [64-80]	
	$PEEP_{set}$	84 [71-94]	1.00	88 [71-95]	1.00	75 [67-84]	0.043*

Data are presented as mean (standard deviation), count (%) or median [25th and 75th percentile]. * $p < 0.05$

The relative percentages of alveolar collapse and overdistention at $PEEP_{set}$ did not differ between groups (**Table 4**). In the $PEEP_{lower}$ group, relative alveolar collapse increased by 8.3% (3.6-15.4%) in the dependent lung region and relative alveolar overdistention decreased by 28.3% (22.2-43.2%) in the non-dependent lung region as a result of a PEEP decrease from 24 to 12 cmH_2O. In contrast, in $PEEP_{higher}$ group this was 24.2% (20.1-29.2%) and 15.1% (4.4-26.2%), respectively.

Table 4: Comparison of alveolar collapse and overdistention between PEEP groups.

	Total (n=75)	$PEEP_{lower}$ (n=23)	$PEEP_{equal}$ (n=28)	$PEEP_{higher}$ (n=24)	p-value
Alveolar collapse					
Collapse at PEEPset	6.1 (3.6)	5.8 (3.9)	6.8 (3.9)	5.5 (2.8)	0.56
Collapse at PEEP 12 cmH2O	17.8 (10.8)	10.2 (7.9)	17.1 (8.8)	24.5 (11.4)	<0.001*
Collapse at PEEP 24 cmH2O	0.0 [0.0-0.6]	0.0 [0.0-0.2]	0.0 [0.0-0.1]	0.4 [0.0-1.1]	0.12
Collapse diff (PEEP 24->12)	17.3 (10.3)	9.8 (7.7)	16.3 (8.0)	24.8 (10.2)	<0.001*
Overdistention					
Overdistention at PEEPset	11.3 [5.4-15.4]	8.8 [4.5-13.1]	10.1 [5.9-15.0]	12.8 [10.2-17.2]	0.20
Overdistention at PEEP 12 cmH2O	5.1 [2.1-9.2]	9.6 [4.9-15.5]	3.3 [1.4-6.1]	5.1 [3.6-7.7]	0.062
Overdistention at PEEP 24 cmH2O	31.3 [26.3-38.4]	37.5 [31.1-56.0]	30.8 [26.3-38.8]	29.6 [20.4-32.6]	0.025*
Hyperdistention diff (PEEP 24->12)	-25.6 [-31.6–17.0]	-28.3 [-43.2–22.2]	-26.5 [-35.0–19.1]	-22.7 [-28.0–14.0]	0.13

Data are presented as mean (standard deviation) or median [25th and 75th percentile]. * $p < 0.05$

DISCUSSION

Based on the EIT guided PEEP trial, PEEP was decreased in 31% of patients and increased in 32% of patients. We found a significant positive correlation between $PEEP_{set}$ and BMI. Patients in $PEEP_{lower}$ group had improved respiratory mechanics after the PEEP trial, and had a lower BMI, longer time between onset of symptoms and intubation, and a higher incidence of pulmonary embolism during ICU admission. In patients in the $PEEP_{lower}$ group, an increase in PEEP resulted in major alveolar overdistention and a small amount of recruitment on EIT. In $PEEP_{higher}$ group, we observed a significant increase in plateau pressure and improved oxygenation after the PEEP trial. In addition, an increase in PEEP resulted in significant alveolar recruitment and small amounts of alveolar overdistention on EIT. Hence, the latter group should be considered as recruitable. The PEEP trial was relatively safe, as 5% of patients had a desaturation and 1% of patients was hypotensive during the PEEP trial.

$PEEP_{set}$ resulted in a trend towards improved respiratory mechanics in the $PEEP_{lower}$ group and improved oxygenation in the $PEEP_{higher}$ group. Both an improved driving pressure and improved oxygenation after a change in PEEP are associated with reduced mortality rate in patients with ARDS.[20] Therefore, we should aim to identify the patients that are likely to respond to PEEP, i.e. recruitability.

Recruitability is the amount of collapsed lung tissue that has the potential for reaeration at higher airway pressures.[21] An increase in PEEP in the patients in $PEEP_{lower}$ group resulted in major alveolar overdistention and a small amount of alveolar recruitment,

whereas the patients in $PEEP_{higher}$ group had significant alveolar recruitment and less alveolar overdistention. In patients with COVID-19 related ARDS, alveolar recruitment does not necessarily result in an increase in static compliance.[22] Thus, patients in $PEEP_{lower}$ group were considered to have low recruitability, patients in $PEEP_{equal}$ group had intermediate recruitability and patients in $PEEP_{higher}$ group had high recruitability.

Until now, we tended to focus on the identification of patients that had high recruitability.[23] However, it might also be beneficial to identify the patients that have low recruitability and are prone to alveolar overdistention. Patients with low recruitability had a lower BMI, a higher incidence of pulmonary embolism, and a longer time between onset of symptoms and intubation. Patients with obesity have lower transpulmonary pressures and lower end-expiratory long volumes as a result of higher pressure from the chest wall.[24] BMI has a positive correlation with recruitability and the use of higher PEEP, as higher PEEP increases transpulmonary pressure and reduces alveolar collapse.[18] In addition, patients in $PEEP_{lower}$ group had a higher incidence of pulmonary embolism during ICU admission. These findings suggest that disturbed pulmonary perfusion, resulting in a ventilation-perfusion mismatch, caused hypoxemia in these patients. Nevertheless, all patients had a reduced static compliance, possibly leading to disturbed minute ventilation or increased dead space fraction as well.[25] Patients in $PEEP_{lower}$ group had a longer time between onset of symptoms and intubation. This could indicate that these patients may have had some form of patient self-inflicted lung injury or pulmonary fibrosis.[26] Unfortunately, we had too few CT scans at the day of PEEP titration to test this hypothesis. The PEEP trial did not reach a maximum PEEP of 24 cmH_2O in 4 (5.3%) patients because of desaturation. These four patients were assigned to the $PEEP_{lower}$ group and had large amounts of alveolar overdistention. Desaturation at high PEEP could be a clear indication of ventilation-perfusion mismatch, likely due to alveolar overdistention.

An observational cohort performed in the Netherlands found a median PEEP titrated by the clinician of 14.0 cmH_2O (11.0-15.0).[27] Two small observational cohorts that used EIT to titrate PEEP found a median PEEP of 12.0 cmH_2O.[28, 29] In our EIT guided population, we found a higher median PEEP of 18.0 cmH_2O (14.0-20.0) as compared to the other studies. Explanations are the relatively high BMI in our cohort and long duration of mechanical ventilation in the cohort of Sella et al.[29] In addition, there is no consensus on how to interpret EIT data obtained during a PEEP trial.[14, 30]

In our study, total PEEP was arbitrarily set at the PEEP level above the intersection of the curves representing relative alveolar overdistention and collapse.[18, 19] We chose this method as it is an intuitive and simple approach that can be performed at the bedside,

but arguably assumes that both alveolar overdistention and collapse are equally harmful.[31] Both Perrier et al.[28] and Sella et al.[29] chose to set PEEP at the intersection of both curves itself, whereas Franchineau et al.[32] chose to limit alveolar collapse at 15%, independent of alveolar overdistention. The last approach favours alveolar collapse over alveolar overdistention and likely resulted in a lower set PEEP as compared to the method used in this study. Future research should focus on the best approach to titrate PEEP based on EIT data and its association with clinical outcomes.

Previous randomized controlled trials in patients with ARDS compared PEEP titrated using EIT to conventional methods. In patients with mild to severe ARDS He et al.[16] showed EIT resulted in a similar PEEP compared to the PEEP/FiO_2 table, but was decoupled from FiO_2. In patients with moderate to severe ARDS Hsu et al.[17] showed PEEP and mortality rate were lower using EIT compared to pressure-volume loops, but mortality rate was high overall (44% in de EIT group and 69% in the control group, vs. 21%-27% in the study by He et al.[16] and 29% in the current study). In our study, PEEP was not changed on average for the entire cohort after titration using EIT, but was changed with ≥2 cmH2O in the majority of patients.

This study has several limitations. First, this retrospective analysis was not prespecified in the study protocol and results should be considered hypothesis-generating. The main purpose of this EIT guided PEEP trial protocol was to improve clinical practice. As a consequence, mechanical ventilation parameters were only recorded at $PEEP_{base}$ and $PEEP_{set}$, limiting a more accurate retrospective analysis of the PEEP trials and EIT data at every PEEP step. A major limitation of this study is the lack of randomization and of the sequence of interventions. All patients received PEEP set by the clinician using the PEEP-FiO_2 table first, and then the EIT guided PEEP trial. A part of the improvements in oxygenation and respiratory mechanics may be due to the PEEP trial itself, instead of the titration of $PEEP_{set}$. This is noticeable in the changes in respiratory mechanics for the $PEEP_{equal}$ group. Second, only patients with COVID-19 related ARDS were included in this study. Although respiratory mechanics in non-COVID-19 related ARDS and typical ARDS seem to be similar, it is uncertain whether results can be generalized to the non-COVID-19 related ARDS population.[8, 9] Third, maximum and minimum PEEP reached in all trials varied. The estimation of the amount of collapse and overdistention is based on the maximum compliance for each EIT pixel. It is probable or even likely maximum compliance is not reached for all pixels, e.g. due to residual collapse in the dependent lung at the highest PEEP level. Therefore, approximately 0% alveolar collapse at PEEP 24 cmH_2O does not necessarily mean that application of higher or prolonged airway pressures cannot result in additional alveolar recruitment. Fourth, we performed a PEEP trial with small steps of 2 cmH_2O and a short step duration of 30 s. Some other

studies report larger steps and longer duration for similar PEEP trials.[15, 28, 32] There is a trade-off between step size, step duration and the time it takes to complete the protocol. After a change in PEEP respiratory mechanics can change in multiple ways with different time frames. By rapidly changing PEEP, we did not allow for slow effects like slow derecruitment, morphological changes to the abdomen and diaphragm, changing hemodynamics and changes in pO_2 and pCO_2. In addition, as a result of the large numbers of patients with COVID-19, we chose a time-efficient study protocol. Fifth, hemodynamic monitoring was limited to continuous measurement of blood pressure and heart rate. PEEP titration is more than balancing alveolar overdistention and collapse, as PEEP influences cardiac output as well.[33] Although the PEEP trials had limited effects on blood pressure and heart rate, we cannot exclude a decrease in cardiac output. In addition, we did not assess pulmonary perfusion with EIT. Hence, EIT guided PEEP titration might have resulted in optimal ventilation, but not necessarily in an optimal ventilation-perfusion match. Sixth, ventilation distribution assessed by EIT is measured in only a small cross-sectional slice of the lung. Ventilation distribution changes when the EIT belt is placed more cranially or caudally, further complicating EIT guided PEEP titration.[34] Seventh, we used devices from two manufacturers to perform the EIT measurements. Although the devices apply the same algorithm by Costa et al.[15] to derive the relative collapse and overdistention, results could vary due to differences in belts, reconstruction models and algorithms. Table 9-16 in the supplementary materials show the results presented in Table 1-4 split by EIT device. Considering the limited data it seems possible the Timpel Enlight 1800 gives higher values overdistention at high PEEP compared to the Dräger Pulmovista 500. Due to the small amount of measurements with the Timpel device (n=7), we were not able to properly compare the devices. Overall, considering only the measurements with the Dräger device (n=68) does not change our interpretation or conclusions.

In conclusion, a PEEP trial guided by EIT as compared to PEEP titration based on the PEEP-FiO_2 table resulted in a clinically relevant change in PEEP in 63% of patients with COVID-19 related ARDS. We found a significant positive correlation between set PEEP and BMI. Patients in whom PEEP was decreased had a lower BMI, a longer time between onset of symptoms and intubation, and a higher incidence of pulmonary embolism. Our results support the hypothesis that PEEP should be personalised in patients with COVID-19 related ARDS in order to reduce the total amount of alveolar overdistention and collapse, i.e. too low or too high PEEP.

REFERENCES

1. Brower RG, Lanken PN, MacIntyre N, Matthay MA, Morris A, Ancukiewicz M, Schoenfeld D, Thompson BT, National Heart L, Blood Institute ACTN: **Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome**. *The New England journal of medicine* 2004, **351**(4):327-336.
2. Meade MO, Cook DJ, Guyatt GH, Slutsky AS, Arabi YM, Cooper DJ, Davies AR, Hand LE, Zhou Q, Thabane L *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**. *Jama* 2008, **299**(6):637-645.
3. Mercat A, Richard JC, Vielle B, Jaber S, Osman D, Diehl JL, Lefrant JY, Prat G, Richecoeur J, Nieszkowska A *et al*: **Positive end-expiratory pressure setting in adults with acute lung injury and acute respiratory distress syndrome: a randomized controlled trial**. *Jama* 2008, **299**(6):646-655.
4. Briel M, Meade M, Mercat A, Brower RG, Talmor D, Walter SD, Slutsky AS, Pullenayegum E, Zhou Q, Cook D *et al*: **Higher vs lower positive end-expiratory pressure in patients with acute lung injury and acute respiratory distress syndrome: systematic review and meta-analysis**. *Jama* 2010, **303**(9):865-873.
5. Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS: **Acute respiratory distress syndrome: the Berlin Definition**. *Jama* 2012, **307**(23):2526-2533.
6. Gattinoni L, Chiumello D, Caironi P, Busana M, Romitti F, Brazzi L, Camporota L: **COVID-19 pneumonia: different respiratory treatments for different phenotypes?** *Intensive care medicine* 2020.
7. Marini JJ, Gattinoni L: **Management of COVID-19 Respiratory Distress**. *Jama* 2020.
8. Ziehr DR, Alladina J, Petri CR, Maley JH, Moskowitz A, Medoff BD, Hibbert KA, Thompson BT, Hardin CC: **Respiratory Pathophysiology of Mechanically Ventilated Patients with COVID-19: A Cohort Study**. *American journal of respiratory and critical care medicine* 2020.
9. Haudebourg AF, Perier F, Tuffet S, de Prost N, Razazi K, Mekontso Dessap A, Carteaux G: **Respiratory Mechanics of COVID-19 vs. Non-COVID-19 Associated Acute Respiratory Distress Syndrome**. *American journal of respiratory and critical care medicine* 2020.
10. Alhazzani W, Moller MH, Arabi YM, Loeb M, Gong MN, Fan E, Oczkowski S, Levy MM, Derde L, Dzierba A *et al*: **Surviving Sepsis Campaign: Guidelines on the Management of Critically Ill Adults with Coronavirus Disease 2019 (COVID-19)**. *Critical care medicine* 2020, **48**(6):e440-e469.
11. Beitler JR: **Lung protection in acute respiratory distress syndrome: what should we target?** *Current opinion in critical care* 2020, **26**(1):26-34.
12. Writing Group for the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial I, Cavalcanti AB, Suzumura EA, Laranjeira LN, Paisani DM, Damiani LP, Guimaraes HP, Romano ER, Regenga MM, Taniguchi LNT *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: A Randomized Clinical Trial**. *Jama* 2017, **318**(14):1335-1345.
13. Sahetya SK: **Searching for the optimal positive end-expiratory pressure for lung protective ventilation**. *Current opinion in critical care* 2020, **26**(1):53-58.
14. Frerichs I, Amato MB, van Kaam AH, Tingay DG, Zhao Z, Grychtol B, Bodenstein M, Gagnon H, Bohm SH, Teschner E *et al*: **Chest electrical impedance tomography examination, data analysis, terminology, clinical use and recommendations: consensus statement of the TRanslational EIT developmeNt stuDy group**. *Thorax* 2017, **72**(1):83-93.

15. Costa EL, Borges JB, Melo A, Suarez-Sipmann F, Toufen C, Jr., Bohm SH, Amato MB: **Bedside estimation of recruitable alveolar collapse and hyperdistension by electrical impedance tomography**. *Intensive care medicine* 2009, **35**(6):1132-1137.
16. He H, Chi Y, Yang Y, Yuan S, Long Y, Zhao P, Frerichs I, Fu F, Moller K, Zhao Z: **Early individualized positive end-expiratory pressure guided by electrical impedance tomography in acute respiratory distress syndrome: a randomized controlled clinical trial**. *Crit Care* 2021, **25**(1):230.
17. Hsu HJ, Chang HT, Zhao Z, Wang PH, Zhang JH, Chen YS, Frerichs I, Moller K, Fu F, Hsu HS *et al*: **Positive end-expiratory pressure titration with electrical impedance tomography and pressure-volume curve: a randomized trial in moderate to severe ARDS**. *Physiol Meas* 2021, **42**(1):014002.
18. van der Zee P, Somhorst P, Endeman H, Gommers D: **Electrical Impedance Tomography for Positive End-Expiratory Pressure Titration in COVID-19-related Acute Respiratory Distress Syndrome**. *American journal of respiratory and critical care medicine* 2020, **202**(2):280-284.
19. Pereira SM, Tucci MR, Morais CCA, Simoes CM, Tonelotto BFF, Pompeo MS, Kay FU, Pelosi P, Vieira JE, Amato MBP: **Individual Positive End-expiratory Pressure Settings Optimize Intraoperative Mechanical Ventilation and Reduce Postoperative Atelectasis**. *Anesthesiology* 2018, **129**(6):1070-1081.
20. Amato MB, Meade MO, Slutsky AS, Brochard L, Costa EL, Schoenfeld DA, Stewart TE, Briel M, Talmor D, Mercat A *et al*: **Driving pressure and survival in the acute respiratory distress syndrome**. *The New England journal of medicine* 2015, **372**(8):747-755.
21. Amato MB, Santiago RR: **The Recruitability Paradox**. *American journal of respiratory and critical care medicine* 2016, **193**(11):1192-1195.
22. Fossali T, Pavlovsky B, Ottolina D, Colombo R, Basile MC, Castelli A, Rech R, Borghi B, Ianniello A, Flor N *et al*: **Effects of Prone Position on Lung Recruitment and Ventilation-Perfusion Matching in Patients With COVID-19 Acute Respiratory Distress Syndrome: A Combined CT Scan/Electrical Impedance Tomography Study**. *Critical care medicine* 2022.
23. van der Zee P, Gommers D: **Recruitment Maneuvers and Higher PEEP, the So-Called Open Lung Concept, in Patients with ARDS**. *Crit Care* 2019, **23**(1):73.
24. Hibbert K, Rice M, Malhotra A: **Obesity and ARDS**. *Chest* 2012, **142**(3):785-790.
25. Saha BK, Ghalib S, Chieng H, Pezzano C, Lydon D, Feustel P, Smith TC, Chopra A: **Correlation of Respiratory Physiologic Parameters in Mechanically Ventilated Coronavirus Disease 2019 Patients**. *Crit Care Explor* 2021, **3**(1):e0328.
26. Brochard L, Slutsky A, Pesenti A: **Mechanical Ventilation to Minimize Progression of Lung Injury in Acute Respiratory Failure**. *American journal of respiratory and critical care medicine* 2017, **195**(4):438-442.
27. Botta M, Tsonas AM, Pillay J, Boers LS, Algera AG, Bos LDJ, Dongelmans DA, Hollmann MW, Horn J, Vlaar APJ *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**(2):139-148.
28. Perier F, Tuffet S, Maraffi T, Alcala G, Victor M, Haudebourg AF, Razazi K, De Prost N, Amato M, Carteaux G *et al*: **Electrical impedance tomography to titrate positive end-expiratory pressure in COVID-19 acute respiratory distress syndrome**. *Crit Care* 2020, **24**(1):678.
29. Sella N, Zarantonello F, Andreatta G, Gagliardi V, Boscolo A, Navalesi P: **Positive end-expiratory pressure titration in COVID-19 acute respiratory failure: electrical impedance tomography vs. PEEP/FiO2 tables**. *Crit Care* 2020, **24**(1):540.

30. van der Zee P, Somhorst P, Endeman H, Gommers D: **Reply to: Electrical Impedance Tomography for Positive End-expiratory Pressure Titration in COVID-19 Related ARDS: Comment**. *American journal of respiratory and critical care medicine* 2020.
31. Gattinoni L, Quintel M, Marini JJ: **Volutrauma and atelectrauma: which is worse?** *Crit Care* 2018, **22**(1):264.
32. Franchineau G, Brechot N, Lebreton G, Hekimian G, Nieszkowska A, Trouillet JL, Leprince P, Chastre J, Luyt CE, Combes A *et al*: **Bedside Contribution of Electrical Impedance Tomography to Setting Positive End-Expiratory Pressure for Extracorporeal Membrane Oxygenation-treated Patients with Severe Acute Respiratory Distress Syndrome**. *American journal of respiratory and critical care medicine* 2017, **196**(4):447-457.
33. Fougeres E, Teboul JL, Richard C, Osman D, Chemla D, Monnet X: **Hemodynamic impact of a positive end-expiratory pressure setting in acute respiratory distress syndrome: importance of the volume status**. *Critical care medicine* 2010, **38**(3):802-807.
34. Bikker IG, Preis C, Egal M, Bakker J, Gommers D: **Electrical impedance tomography measured at two thoracic levels can visualize the ventilation distribution changes at the bedside during a decremental positive end-expiratory lung pressure trial**. *Crit Care* 2011, **15**(4):R193.

SUPPLEMENTARY MATERIALS

Table 5: PEEP trials of all patients.

All patients	Baseline	Highest PEEP	Lowest PEEP	Set PEEP	p value
Total PEEP (cmH2O)	17.0 [16.0-19.0]	27.0 [26.0-28.0]	10.2 [7.0-12.0]	18.0 [14.0-20.0]	<0.001*
Plateau pressure (cmH2O)	28.0 [25.0-30.2]	38.0 [36.0-42.0]	19.5 [17.0-22.0]	28.0 [24.8-30.0]	<0.001*
Driving pressure (cmH2O)	10.0 [8.0-14.0]	11.5 [9.0-15.2]	9.0 [6.7-12.2]	10.0 [7.3-13.0]	<0.001*
Tidal volume (mL/kg PBW)	6.5 [5.7-7.0]	5.2 [4.5-5.8]	6.8 [6.0-7.6]	6.6 [5.9-7.3]	<0.001*
Dynamic compliance (mL/cmH2O)	38 [26-48]	27 [16-35]	42 [32-55]	39 [28-50]	<0.001*
FiO2	60 [60-70]	70 [60-85]	60 [60-70]	60 [60-70]	0.26
SpO2 (%)	95 [93-95]	95 [92-96]	94 [92-96]	95 [93-96]	0.37
PetCO2 (mmHg)	35 [30-42]	38 [32-45]	35 [31-42]	35 [31-42]	<0.001*
Systolic Blood Pressure (mmHg)	128 [115-145]	115 [99-136]	136 [124-150]	131 [116-150]	<0.001*
Diastolic Blood Pressure (mmHg)	60 [54-66]	55 [49-61]	61 [55-69]	61 [54-66]	<0.001*
Mean Arterial Pressure (mmHg)	83 [77-91]	75 [69-83]	85 [80-96]	83 [76-92]	<0.001*
Heart rate (/min)	78 [70-93]	82 [68-93]	80 [69-91]	80 [69-92]	0.52

Data are presented as median and 25th and 75th percentile.

Table 6: PEEP trials of the patients in the $PEEP_{lower}$ group.

$PEEP_{lower}$ (n=23)	Baseline	Highest PEEP	Lowest PEEP	Set PEEP	p value
Total PEEP (cmH2O)	18.0 [16.2-19.0]	26.0 [26.0-28.0]	9.6 [6.8-11.0]	14.0 [11.2-16.0]	<0.001*
Plateau pressure (cmH2O)	28.5 [26.2-32.8]	39.0 [36.0-41.8]	19.0 [16.0-21.0]	24.5 [21.2-28.0]	<0.001*
Driving pressure (cmH2O)	11.0 [8.2-14.0]	12.5 [9.0-16.0]	10.0 [7.2-12.8]	11.5 [7.0-14.0]	<0.001*
Tidal volume (mL/kg PBW)	6.2 [5.2-6.8]	5.1 [3.8-5.7]	6.9 [5.7-7.4]	6.6 [5.5-7.4]	<0.001*
Dynamic compliance (mL/cmH2O)	32 [22-47]	21 [14-35]	38 [28-51]	32 [24-45]	<0.001*
FiO2	40 [40-40]	40 [40-40]	40 [40-40]	40 [40-40]	1.00
SpO2 (%)	94 [93-95]	93 [88-95]	95 [93-96]	94 [92-96]	0.029*
PetCO2 (mmHg)	35 [29-44]	34 [31-44]	32 [29-43]	34 [29-39]	0.032*
Systolic Blood Pressure (mmHg)	136 [121-145]	115 [107-140]	145 [135-164]	143 [131-157]	<0.001*
Diastolic Blood Pressure (mmHg)	61 [50-66]	51 [48-61]	62 [56-70]	62 [54-70]	0.012*
Mean Arterial Pressure (mmHg)	85 [80-89]	72 [67-87]	92 [85-99]	89 [80-95]	<0.001*
Heart rate (/min)	83 [69-88]	84 [70-88]	84 [69-88]	81 [71-91]	0.90

Data are presented as median and 25th and 75th percentile.

Table 7: PEEP trials of the patients in the $PEEP_{equal}$ group.

$PEEP_{equal}$ (n=28)	Baseline	Highest PEEP	Lowest PEEP	Set PEEP	p value
Total PEEP (cmH2O)	17.0 [15.5-19.8]	26.7 [25.5-28.0]	11.0 [8.0-13.9]	18.0 [15.5-20.0]	<0.001*
Plateau pressure (cmH2O)	28.0 [25.5-30.5]	38.0 [36.0-41.0]	20.0 [18.0-22.0]	28.0 [26.0-30.0]	<0.001*
Driving pressure (cmH2O)	10.0 [8.1-12.2]	11.0 [9.2-13.5]	9.0 [6.3-11.0]	9.7 [7.5-11.9]	<0.001*
Tidal volume (mL/kg PBW)	6.5 [5.7-7.3]	5.2 [4.9-5.7]	6.6 [6.0-7.6]	7.0 [6.0-7.5]	<0.001*
Dynamic compliance (mL/cmH2O)	43 [33-51]	28 [25-38]	46 [39-57]	46 [37-54]	<0.001*
FiO2	60 [60-60]	72 [66-79]	60 [60-60]	60 [60-60]	0.39
SpO2 (%)	95 [94-95]	95 [94-96]	94 [91-96]	95 [93-96]	0.081
PetCO2 (mmHg)	35 [31-46]	39 [33-47]	35 [32-44]	35 [33-43]	0.031*
Systolic Blood Pressure (mmHg)	126 [110-142]	114 [99-132]	134 [120-141]	128 [112-144]	<0.001*
Diastolic Blood Pressure (mmHg)	61 [54-65]	54 [51-60]	59 [54-65]	59 [55-63]	0.003*
Mean Arterial Pressure (mmHg)	83 [77-93]	75 [68-84]	82 [79-90]	81 [76-88]	<0.001*
Heart rate (/min)	88 [74-99]	89 [72-101]	86 [72-97]	89 [72-96]	0.80

Data are presented as median and 25th and 75th percentile.

Table 8: PEEP trials of the patients in the $PEEP_{higher}$ group.

$PEEP_{higher}$ (n=24)	Baseline	Highest PEEP	Lowest PEEP	Set PEEP	p value
Total PEEP (cmH2O)	17.0 [15.8-18.2]	27.0 [25.8-29.0]	10.0 [7.0-12.5]	20.0 [18.0-22.7]	<0.001*
Plateau pressure (cmH2O)	26.0 [24.5-28.5]	38.0 [36.0-43.0]	18.0 [17.0-22.5]	30.0 [28.0-32.5]	<0.001*
Driving pressure (cmH2O)	9.0 [8.2-14.0]	11.0 [10.0-16.0]	8.7 [7.0-13.0]	10.0 [9.0-13.0]	<0.001*
Tidal volume (mL/kg PBW)	6.5 [6.0-6.9]	5.1 [4.4-6.3]	6.6 [6.1-7.3]	6.5 [6.0-7.1]	<0.001*
Dynamic compliance (mL/cmH2O)	38 [26-44]	27 [16-34]	41 [33-48]	39 [28-45]	<0.001*
FiO2	80 [75-85]	85 [78-92]	85 [78-92]	85 [78-92]	0.39
SpO2 (%)	94 [93-95]	95 [92-96]	94 [92-97]	94 [93-96]	0.84
PetCO2 (mmHg)	35 [30-38]	39 [35-43]	36 [32-39]	37 [33-41]	0.004*
Systolic Blood Pressure (mmHg)	125 [115-145]	109 [99-130]	134 [124-148]	128 [114-147]	<0.001*
Diastolic Blood Pressure (mmHg)	59 [55-66]	55 [52-62]	61 [54-69]	62 [54-66]	0.007*
Mean Arterial Pressure (mmHg)	82 [76-86]	75 [70-82]	85 [80-96]	83 [76-90]	0.002*
Heart rate (/min)	74 [64-80]	74 [63-84]	75 [64-86]	75 [67-84]	0.12

Data are presented as median and 25th and 75th percentile.

Table 9: $PEEP_{base}$ versus $PEEP_{set}$ for patients where EIT-measurements were performed with the Dräger device.

	$PEEP_{base}$	$PEEP_{set}$	Difference	p-value
Total PEEP (cmH2O)	17.0 [16.0-19.0]	18.0 [14.0-20.9]	0.7 [-2.0-2.0]	1.00
Plateau pressure (cmH2O)	28.0 (4.1)	27.4 (5.1)	-0.5 (4.6)	1.00
Driving pressure (cmH2O)	10.0 [8.1-14.0]	10.0 [7.7-13.0]	-1.0 [-1.0-0.7]	0.080
Tidal volume (mL/kg PBW)	6.5 [5.7-7.0]	6.6 [6.0-7.3]	0.2 [-0.1-0.7]	0.002*
Static compliance (mL/cmH2O)	44 [33-60]	49 [34-64]	4 [-2-8]	0.035*
PaO2 (mmHg)	80 [73-92]	80 [68-95]	0 [-16-13]	1.00
PaO2/FiO2 ratio (mmHg)	161 [113-199]	161 [123-211]	0 [-25-55]	0.90
SpO2 (%)	95 [93-95]	94 [93-96]	0 [-2-2]	1.00
PaCO2 (mmHg)	45 [40-53]	45 [40-52]	-1 [-5-4]	0.57
Systolic Blood Pressure (mmHg)	126 [115-145]	131 [114-147]	-2 [-10-12]	1.00
Diastolic Blood Pressure (mmHg)	60 [54-65]	60 [54-66]	-1 [-4-3]	1.00
Mean Arterial Pressure (mmHg)	83 [76-91]	83 [76-91]	-1 [-6-5]	1.00
Heart rate (/min)	83 [72-95]	81 [70-92]	1 [-1-4]	0.11

Data are presented as mean (standard deviation) or median [25th and 75th percentile]. * $p < 0.05$

Table 10: $PEEP_{base}$ versus $PEEP_{set}$ for patients where EIT-measurements were performed with the Timpel device.

	$PEEP_{base}$	$PEEP_{set}$	Difference	p-value
Total PEEP (cmH2O)	18.0 [15.8-19.8]	17.0 [14.0-18.6]	-2.0 [-3.5-0.0]	0.47
Plateau pressure (cmH2O)	28.0 [23.5-30.5]	26.0 [25.0-27.5]	-3.0 [-3.5-0.5]	0.47
Driving pressure (cmH2O)	11.0 [7.2-11.8]	9.0 [7.5-11.9]	0.0 [-0.8-0.5]	1
Tidal volume (mL/kg PBW)	6.2 [5.7-7.3]	6.2 [5.5-7.4]	-0.0 [-0.0-0.1]	1
Static compliance (mL/cmH2O)	51 [37-55]	52 [38-66]	4 [0-5]	0.66
PaO2 (mmHg)	83 [72-93]	71 [66-107]	6 [-13-24]	1
PaO2/FiO2 ratio (mmHg)	164 [114-216]	159 [112-222]	7 [-18-11]	1
SpO2 (%)	96 [94-97]	96 [94-96]	-1 [-1-2]	1
PaCO2 (mmHg)	44 [42-48]	45 [39-53]	2 [-5-7]	0.69
Systolic Blood Pressure (mmHg)	129 [103-156]	152 [146-164]	4 [-5-54]	1
Diastolic Blood Pressure (mmHg)	52 [49-71]	65 [50-70]	3 [-2-14]	1
Mean Arterial Pressure (mmHg)	81 [75-84]	92 [84-100]	3 [-3-29]	1
Heart rate (/min)	75 [65-82]	71 [62-90]	-3 [-4-0]	0.46

Data are presented as mean (standard deviation) or median [25th and 75th percentile]. * $p < 0.05$

Table 11: Comparison of baseline characteristics between PEEP groups for patients where EIT-measurements were performed with the Dräger device.

	Total (n=68)	$PEEP_{lower}$ (n=19)	$PEEP_{equal}$ (n=25)	$PEEP_{higher}$ (n=24)	p-value
Male gender	53 (78%)	15 (79%)	20 (80%)	18 (75%)	0.91
BMI (kg/m2)	30.5 (6.0)	27.3 (5.7)	31.8 (5.6)	31.6 (5.8)	0.020*
Age (y)	64 [56-70]	66 [62-73]	63 [54-68]	59 [53-70]	0.15
Apache IV score at ICU admission	69 (27)	84 (32)	66 (26)	61 (19)	0.013*
Time since onset symptoms (d)	14 [10-18]	15 [14-27]	15 [8-18]	12 [7-16]	0.037*
Time since intubation (d)	3 [2-9]	4 [2-16]	3 [2-7]	2 [1-7]	0.48
Time between onset symptoms and intubation (d)	10 [7-12]	11 [10-13]	7 [6-12]	8 [6-10]	0.056
Time ventilated in other hospital (d)	1 [0-3]	2 [0-8]	1 [0-3]	1 [0-3]	0.71
28 day mortality	21 (31%)	7 (37%)	7 (28%)	7 (29%)	0.80
D-dimer at admission (mg/L)	1.5 [0.9-3.2]	2.9 [1.3-9.3]	1.2 [0.7-2.8]	1.3 [1.0-2.2]	0.090
Pulmonary embolism at PEEP trial	12 (18%)	5 (26%)	3 (12%)	4 (17%)	0.46
Proven pulmonary embolism during ICU admission	32 (47%)	13 (68%)	10 (40%)	9 (38%)	0.088

Data are presented as mean (standard deviation), count (%) or median [25th and 75th percentile]. * $p < 0.05$

Table 12: Comparison of baseline characteristics between PEEP groups for patients where EIT-measurements were performed with the Timpel device.

	Total (n=7)	$PEEP_{lower}$ (n=4)	$PEEP_{equal}$ (n=3)	p-value
Male gender	6 (86%)	4 (100%)	2 (67%)	0.88
BMI (kg/m2)	27.8 [26.9-29.7]	28.1 [27.7-30.8]	26.3 [26.2-28.6]	0.40
Age (y)	65 [53-72]	58 [49-68]	71 [62-72]	0.63
Apache IV score at ICU admission	74 [58-90]	88 [70-110]	59 [58-68]	0.48
Time since onset symptoms (d)	9 [5-12]	13 [12-14]	4 [4-6]	0.077
Time since intubation (d)	2 [1-4]	4 [2-4]	1 [1-2]	0.20
Time between onset symptoms and intubation (d)	7 [4-8]	9 [8-10]	3 [2-5]	0.12
Time ventilated in other hospital (d)	1 [0-3]	3 [2-4]	1 [0-1]	0.28
28 day mortality	1 (14%)	1 (25%)	0 (0%)	1.00
D-dimer at admission (mg/L)	4.2 [3.3-6.4]	4.2 [3.3-6.4]	n.d.a.	
Pulmonary embolism at PEEP trial	1 (14%)	1 (25%)	0 (0%)	1.00
Proven pulmonary embolism during ICU admission	6 (86%)	4 (100%)	2 (67%)	0.88

Data are presented as mean (standard deviation), count (%) or median [25th and 75th percentile]. * $p < 0.05$, n.d.a.: no data available

11

Summary and general discussion

SUMMARY AND GENERAL DISCUSSION

Despite more than 50 years of clinical research in patients with Acute Respiratory Distress Syndrome (ARDS), only few interventions have shown to significantly improve clinical outcomes.[1, 2] The heterogeneity in risk factor for ARDS, inflammatory status, and respiratory mechanics likely resulted in no overall treatment effect in trials including patients with ARDS.[3, 4] Secondary analyses of negative randomized controlled trials found that some subgroups of patients did benefit from a significant treatment effect.[5, 6] Thus, in patients with ARDS it is crucial to select the patient that is likely to respond to a specific treatment.[7] The aim of this thesis was to personalise treatment of patients with ARDS based on biomarkers or recruitability (**Chapter 1. General introduction**). Ultimately, we aim to identify which patient is likely to benefit from a specific treatment, such as corticosteroids or higher airway pressures.

In **Part I. Personalised treatment of ARDS based on biomarkers**, we examined plasma biomarkers and locally sampled biomarkers in order to identify biologically derived ARDS phenotypes for future research.

First, we performed a systematic review to identify biomarkers independently associated with ARDS development and mortality in **Chapter 2**. We included biomarkers that were obtained in plasma, bronchoalveolar lavage fluid, and even cerebrospinal fluid. Unfortunately, we were unable to perform a meta-analysis because of heterogeneous data presentation in the included studies. We found that the majority of biomarkers in plasma tested for both ARDS development and mortality were surrogates for systemic inflammation, epithelial injury or endothelial injury. Following qualitative inspection, biomarkers for systemic inflammation were not independently associated with either ARDS development or mortality. In contrast, biomarkers for alveolar epithelial injury (plasma receptor for advanced glycation end products and surfactant protein D) and endothelial permeability (plasma angiopoeitin-2), seemed to be associated with ARDS development.

Before we assess biomarkers in patients with ARDS, we must consider how we intend to use these biomarkers.[8] A biomarker for ARDS development should be specific for ARDS, i.e. a biomarker that reflects alveolar injury or alveolar-capillary injury. Locally sampled biomarkers for systemic inflammation, for example in the alveolar space, could potentially diagnose ARDS or establish ARDS severity.[9] Biomarkers for the identification of less heterogeneous ARDS phenotypes do not require to be ARDS specific, provided that they adequately stratify patients with ARDS.

In **Chapter 3**, a link between mechanical ventilation, pulmonary inflammation, and surfactant function impairment is described according to the purinergic signalling hypothesis. Extracellular adenosine-triphosphate (ATP) is thought to be a key molecule in the development of lung injury and ARDS.[10] ATP is released by the alveolar epithelial type I cells as a result of cyclic deformation. Above a certain threshold, extracellular ATP molecules act as a danger-associated molecular pattern and activate the innate immune response through purinergic receptors. Extracellular ATP is a non-specific marker for tissue injury and has a short half-life. We hypothesized that ATP assessed in pulmonary fluid could be used to monitor lung injury or ARDS development.

In **Chapter 4**, we examined the feasibility of measuring pulmonary extracellular ATP in exhaled breath condensate (EBC) of healthy volunteers with increased minute ventilation following an exercise test. We found that ATP could be measured in EBC and concentrations increased after the exercise test. However, as a result of multiple physiological changes, we could not attribute the increase in ATP concentration to the increase in tidal volume or respiratory rate alone.

EBC samples during mechanical ventilation are usually obtained by guiding the expiratory tubing through a thermo-electric cooling module.[11] In **Chapter 5**, we collected the EBC that cumulated in the expiratory tubing during mechanical ventilation and named it swivel-derived exhaled breath condensate (SEBC). We found that biomarkers were readily detectable in 29 samples collected in 13 mechanically ventilated ICU patients. In addition, high tidal volume ventilation was positively correlated with interleukin-10, interleukin-12 and macrophage inflammatory protein-1. SEBC is a relatively simple method to sample the pulmonary compartment and provides opportunities for collection of EBC during mechanical ventilation in large prospective cohorts.

In **Chapter 6**, we aimed to identify a subgroup in patients with community-acquired pneumonia (CAP) that benefit from adjuvant treatment with corticosteroids. We performed a latent class analysis (LCA) in patients with CAP included in one prospective cohort and two randomized controlled trials (Ovidius trial and STEP trial).[12-14] We identified two subgroups based on clinical variables and biomarkers in both cohorts: a relatively hypoinflammatory subgroup and a hyperinflammatory subgroup. Patients in the hyperinflammatory subgroup had higher concentrations of inflammatory biomarkers, and a lower blood pressure and oxygen saturation. In addition, patients in the hyperinflammatory subgroup had longer length of stay, higher mortality rate, and higher incidence of ICU admission. Subsequently, we aimed to identify a heterogeneous treatment effect for treatment with corticosteroids. In the Ovidius cohort, we found that the treatment effect of corticosteroids was greater in patients in the hyperinflamma-

tory subgroup. However, we did not find a heterogeneous treatment effect in the STEP cohort. Possible explanations were the different sets of variables used in both studies or the lower inflammatory biomarker concentrations in the STEP cohort. Future research should focus on further identification of the subgroup in patients with CAP that is likely to benefit from treatment with corticosteroids.

In **Part II. Personalised treatment of ARDS based on recruitability**, advanced respiratory monitoring was used to identify different phenotypes and PEEP responses in patients with ARDS. **Chapter 7** was a narrative review in which we described the rationale and indications for the use of higher PEEP and recruitment manoeuvres, i.e. the open lung concept, in patients with ARDS. We suggested to monitor treatment response and continue or discontinue treatment accordingly. In **Chapter 8**, the open lung concept was applied in patients with severe ARDS and refractory hypoxemia under mechanical ventilation according to the ARDS Network protocol. This small retrospective study showed how a recruitment manoeuvre and higher PEEP could improve oxygenation and prevented the use of veno-venous extracorporeal membrane oxygenation. The majority of patients were responders in terms of oxygenation and we reported an ICU survival rate of 88% (n=8). Although we mentioned recruitability in the discussion, we did not actually measure the amount of alveolar overdistention and collapse.

In **Chapter 9** we used electrical impedance tomography (EIT) in order to visualize alveolar overdistention and collapse during PEEP titration in patients with coronavirus disease (COVID-19) related ARDS. PEEP was set at the minimum relative amount of both alveolar collapse and alveolar overdistention following a decremental PEEP trial. In the first 15 patients in our ICU, we found that PEEP set was positively correlated with body mass index. In addition, we showed that PEEP set was relatively stable over time and did not decrease following respiratory improvement. None of the guidelines for mechanical ventilation in (COVID-19 related) ARDS incorporate a PEEP adjustment for BMI.[15]

In **Chapter 10**, we compared the PEEP set according to the PEEP-FiO_2 table at baseline and the PEEP set according to EIT following a decremental PEEP trial in 75 patients with COVID-19 related ARDS. We found that the majority of patients required either ≥2 cmH_2O higher PEEP or ≥2 cmH_2O lower PEEP as compared to the PEEP-FiO_2 table at baseline. Patients that required less PEEP had a lower BMI, a higher incidence of pulmonary embolism, and significantly more alveolar overdistention at higher PEEP levels. These data illustrated that the one-size-fits-all protocols that are used in most trials for mechanical ventilation do not take into account heterogeneity in patients with ARDS, nor do they account for treatment response. These data underscore the need for personalised mechanical ventilation in order to maximize the heterogeneous treatment effect. Although

PEEP requirement can be partially predicted by BMI, the identification of subgroups in patients with ARDS that require either lower or higher PEEP is still based on the actual PEEP response following a PEEP trial.

RECOMMENDATIONS AND FUTURE PERSPECTIVES

The identification of patient subgroups with a heterogeneous treatment effect poses both great opportunities and great challenges for future research.[5, 6, 16-18] The opportunities are an increased signal-to-noise ratio, resulting in a reduced sample size, a larger treatment effect size, and eventually more positive randomized controlled trials.[19] The challenges are the possibility of phenotype misclassification – i.e. assigning a patient to the wrong phenotype possibly inflicting harm –, premature phenotyping based on limited patient data, and limited external validity.[1, 20] However, the biggest challenge is to construct an approach that correctly identifies a patient at the bedside and enables us to personalise and monitor treatment accordingly.

In **Part I. Personalised treatment of ARDS based on biomarkers**, we aimed to identify biologically derived phenotypes for future research. We searched for biomarkers associated with ARDS in plasma and we examined approaches to obtain locally sampled biomarkers from the pulmonary compartment. We found that there is no single biomarker for ARDS yet, and that biomarkers should be combined with clinical variables in order to establish biologically derived phenotypes. Subsequently, we retrospectively performed a latent class analysis in patients with community-acquired pneumonia.

In order to prospectively establish a biologically derived phenotype in the ICU setting, immediate assessment of biomarkers is a prerequisite. In addition, biologically derived phenotypes are often based on complex multi-variable models. Both complicate the rapid inclusion of patients based on biological phenotypes in trials or the application of phenotypes at the bedside. Simpler alternatives are biological derived phenotypes based on readily available laboratory and clinical variables[21], or the use of a parsimonious model instead of the full models.[5, 22] For example, a three variable model – consisting of interleukin-8, bicarbonate, and protein C – could accurately predict the full model phenotype in patients with ARDS with an accuracy of 94%.[22] A point of care assay has been developed for interleukin-6 and soluble tumour necrosis factor receptor.[23] This point of care assay is currently used in the PHIND trial, which aims to prospectively classify ARDS patients in either a hyperinflammatory or hypoinflammatory phenotype.

Similarly, we aim to assess a heterogeneous treatment effect based on clinical variables and biomarkers in individual patient data of more than six randomized controlled trials that examined the effect of corticosteroids in patients with CAP.[12, 14, 24-27] Based on this heterogeneous treatment effect we are constructing a biologically derived phenotype mainly based on readily available clinical variables. Subsequently, this phenotype will be validated in a prospective cohort. Eventually, the aim is to perform a randomized controlled trial in which treatment with corticosteroids is initiated only in the patients with community-acquired pneumonia that are likely to benefit from adjuvant treatment with corticosteroids.

In **Part II. Personalised treatment of ARDS based on recruitability**, we aimed to identify a subgroup of patients with ARDS that respond to treatment with higher airway pressures. Secondary analyses of previous studies showed that higher PEEP was associated with improved outcomes in patients with severe ARDS, more diffuse ARDS, or a hyperinflammatory status.[5, 28, 29] In patients with COVID-19 related ARDS, we used EIT to personalise PEEP and found that a higher BMI correlated with recruitability, whereas patients with a pulmonary embolism required less PEEP.

If the aim is to predict which patient with ARDS could benefit from a higher PEEP strategy, one could combine disease severity, inflammatory status, lung morphology, and BMI. A patient with severe ARDS, a hyperinflammatory status, diffuse ARDS, and high BMI is likely to benefit from a higher PEEP strategy. In contrast, one could recommend a lower PEEP strategy in patients with mild ARDS, a hypoinflammatory status, focal ARDS, and a low BMI. However, the PEEP response in patients with a contradictory profile, e.g. high BMI with severe but focal ARDS, is more difficult to predict. As a result, a patient could be misclassified as being recruitable or not recruitable and potential harm is inflicted using a higher PEEP strategy, as is suggested by the misclassified patients in the LIVE trial.[28]

The identification of a phenotype or subgroup *predicts* the response to a treatment, the actual *response* following treatment is often not evaluated in randomized controlled trials. Response evaluation, and subsequent treatment continuation or discontinuation, likely reduces the adverse effects of patient misclassification. Especially in randomized controlled trials in which the effects of mechanical ventilation settings are assessed, treatment response can be monitored relatively easily. Secondary analyses of trials comparing PEEP strategies, found that improved oxygenation[30], reduced driving pressure[31], or increased compliance[32] following a PEEP increase, resulted in improved patient outcomes. In contrast, there is no association between improved oxygenation following prone positioning and improved patient outcomes.[33] Alternatively, PEEP response could be monitored and adjusted real-time using EIT or transpulmonary pres-

sure measurements.[34-36] A randomized controlled trial in patients with ARDS that compares the ARDS Network protocol with a treatment protocol that adjusts PEEP daily based on EIT or transpulmonary pressure measurements is feasible and could provide new insights in personalised mechanical ventilation in patients with ARDS.

In addition, the question remains whether phenotypes based on biomarkers or recruitability are specific to the ARDS syndrome, or rather identify subgroups in 'all' critically ill patients. Lung morphology is likely to be ARDS specific by definition. However, the hyperinflammatory phenotype was also identified in critically ill patients without ARDS, and was associated with increased mortality rate.[37, 38] Hence, we could consider to treat critically ill patients in a hyperinflammatory state and with a high BMI with higher PEEP and evaluate treatment response. Instead of focussing on a heterogeneous syndrome diagnosis as ARDS, we could identify treatable traits. These treatable traits could be treated while monitoring treatment response; i.e. response based personalised treatment.

REFERENCES:

1. Alipanah N, Calfee CS: **Phenotyping in acute respiratory distress syndrome: state of the art and clinical implications**. *Current opinion in critical care* 2022, **28**(1):1-8.
2. Matthay MA, McAuley DF, Ware LB: **Clinical trials in acute respiratory distress syndrome: challenges and opportunities**. *The Lancet Respiratory medicine* 2017, **5**(6):524-534.
3. Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, Gattinoni L, van Haren F, Larsson A, McAuley DF *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**(8):788-800.
4. Kent DM, Steyerberg E, van Klaveren D: **Personalized evidence based medicine: predictive approaches to heterogeneous treatment effects**. *BMJ* 2018, **363**:k4245.
5. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA, Network NA: **Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials**. *The Lancet Respiratory medicine* 2014, **2**(8):611-620.
6. Calfee CS, Delucchi KL, Sinha P, Matthay MA, Hackett J, Shankar-Hari M, McDowell C, Laffey JG, O'Kane CM, McAuley DF *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**(9):691-698.
7. 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**(2):147-155.
8. Garcia-Laorden MI, Lorente JA, Flores C, Slutsky AS, Villar J: **Biomarkers for the acute respiratory distress syndrome: how to make the diagnosis more precise**. *Annals of translational medicine* 2017, **5**(14):283.
9. van der Zee P, van Walree I, Fijen JW, van Houte AJ, van Velzen-Blad H, Rijkers G, Gommers D, Endeman H: **Cytokines and Chemokines Are Detectable in Swivel-Derived Exhaled Breath Condensate (SEBC): A Pilot Study in Mechanically Ventilated Patients**. *Dis Markers* 2020, **2020**:2696317.
10. Hasan D, Blankman P, Nieman GF: **Purinergic signalling links mechanical breath profile and alveolar mechanics with the pro-inflammatory innate immune response causing ventilation-induced lung injury**. *Purinergic signalling* 2017, **13**(3):363-386.
11. Rosias P: **Methodological aspects of exhaled breath condensate collection and analysis**. *J Breath Res* 2012, **6**(2):027102.
12. Blum CA, Nigro N, Briel M, Schuetz P, Ullmer E, Suter-Widmer I, Winzeler B, Bingisser R, Elsaesser H, Drozdov D *et al*: **Adjunct prednisone therapy for patients with community-acquired pneumonia: a multicentre, double-blind, randomised, placebo-controlled trial**. *Lancet (London, England)* 2015, **385**(9977):1511-1518.
13. Endeman H, Meijvis SC, Rijkers GT, van Velzen-Blad H, van Moorsel CH, Grutters JC, Biesma DH: **Systemic cytokine response in patients with community-acquired pneumonia**. *The European respiratory journal* 2011, **37**(6):1431-1438.
14. Meijvis SC, Hardeman H, Remmelts HH, Heijligenberg R, Rijkers GT, van Velzen-Blad H, Voorn GP, van de Garde EM, Endeman H, Grutters JC *et al*: **Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial**. *Lancet (London, England)* 2011, **377**(9782):2023-2030.
15. Fan E, Del Sorbo L, Goligher EC, Hodgson CL, Munshi L, Walkey AJ, Adhikari NKJ, Amato MBP, Branson R, Brower RG *et al*: **An Official American Thoracic Society/European Society of Inten-**

sive 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**(9):1253-1263.

16. Bos LD, Schouten LR, van Vught LA, Wiewel MA, Ong DSY, Cremer O, Artigas A, Martin-Loeches I, Hoogendijk AJ, van der Poll T *et al*: **Identification and validation of distinct biological phenotypes in patients with acute respiratory distress syndrome by cluster analysis**. *Thorax* 2017, **72**(10):876-883.
17. Delucchi K, Famous KR, Ware LB, Parsons PE, Thompson BT, Calfee CS, Network A: **Stability of ARDS subphenotypes over time in two randomised controlled trials**. *Thorax* 2018, **73**(5):439-445.
18. Famous KR, Delucchi K, Ware LB, Kangelaris KN, Liu KD, Thompson BT, Calfee CS, Network A: **Acute Respiratory Distress Syndrome Subphenotypes Respond Differently to Randomized Fluid Management Strategy**. *American journal of respiratory and critical care medicine* 2017, **195**(3):331-338.
19. Ware LB, Matthay MA, Mebazaa A: **Designing an ARDS trial for 2020 and beyond: focus on enrichment strategies**. *Intensive care medicine* 2020, **46**(12):2153-2156.
20. Bos LDJ, Sinha P, Dickson RP: **The perils of premature phenotyping in COVID-19: a call for caution**. *The European respiratory journal* 2020, **56**(1).
21. 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**(7):996-1004.
22. Sinha P, Delucchi KL, McAuley DF, O'Kane CM, Matthay MA, Calfee CS: **Development and validation of parsimonious algorithms to classify acute respiratory distress syndrome phenotypes: a secondary analysis of randomised controlled trials**. *The Lancet Respiratory medicine* 2020, **8**(3):247-257.
23. Sinha P, Calfee CS, Cherian S, Brealey D, Cutler S, King C, Killick C, Richards O, Cheema Y, Bailey 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**(12):1209-1218.
24. Torres A, Sibila O, Ferrer M, Polverino E, Menendez R, Mensa J, Gabarrus A, Sellares J, Restrepo MI, Anzueto A *et al*: **Effect of corticosteroids on treatment failure among hospitalized patients with severe community-acquired pneumonia and high inflammatory response: a randomized clinical trial**. *Jama* 2015, **313**(7):677-686.
25. Wittermans E, Vestjens SMT, Spoorenberg SMC, Blok WL, Grutters JC, Janssen R, Rijkers GT, Smeenk F, Voorn GP, van de Garde EMW *et al*: **Adjunctive treatment with oral dexamethasone in non-ICU patients hospitalised with community-acquired pneumonia: a randomised clinical trial**. *The European respiratory journal* 2021, **58**(2).
26. Snijders D, Daniels JM, 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**(9):975-982.
27. Confalonieri M, Urbino R, Potena A, Piattella M, Parigi P, Puccio G, Della Porta R, Giorgio C, Blasi F, Umberger R *et al*: **Hydrocortisone infusion for severe community-acquired pneumonia: a preliminary randomized study**. *American journal of respiratory and critical care medicine* 2005, **171**(3):242-248.
28. Constantin JM, Jabaudon M, Lefrant JY, Jaber S, Quenot JP, Langeron O, Ferrandiere M, Grelon F, Seguin P, Ichai C *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**(10):870-880.

29. Briel M, Meade M, Mercat A, Brower RG, Talmor D, Walter SD, Slutsky AS, Pullenayegum E, Zhou Q, Cook D *et al*: **Higher vs lower positive end-expiratory pressure in patients with acute lung injury and acute respiratory distress syndrome: systematic review and meta-analysis**. *Jama* 2010, **303**(9):865-873.
30. Goligher EC, Kavanagh BP, Rubenfeld GD, Adhikari NK, Pinto R, Fan E, Brochard LJ, Granton JT, Mercat A, Marie Richard JC *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**(1):70-76.
31. Amato MB, Meade MO, Slutsky AS, Brochard L, Costa EL, Schoenfeld DA, Stewart TE, Briel M, Talmor D, Mercat A *et al*: **Driving pressure and survival in the acute respiratory distress syndrome**. *The New England journal of medicine* 2015, **372**(8):747-755.
32. Hodgson CL, Cooper DJ, Arabi Y, King V, Bersten A, Bihari S, Brickell K, Davies A, Fahey C, Fraser J *et al*: **Maximal Recruitment Open Lung Ventilation in Acute Respiratory Distress Syndrome (PHARLAP): A Phase II, Multicenter, Randomized, Controlled Trial**. *American journal of respiratory and critical care medicine* 2019.
33. Albert RK, Keniston A, Baboi L, Ayzac L, Guerin C, Proseva I: **Prone position-induced improvement in gas exchange does not predict improved survival in the acute respiratory distress syndrome**. *American journal of respiratory and critical care medicine* 2014, **189**(4):494-496.
34. He H, Chi Y, Yang Y, Yuan S, Long Y, Zhao P, Frerichs I, Fu F, Moller K, Zhao Z: **Early individualized positive end-expiratory pressure guided by electrical impedance tomography in acute respiratory distress syndrome: a randomized controlled clinical trial**. *Crit Care* 2021, **25**(1):230.
35. Hsu HJ, Chang HT, Zhao Z, Wang PH, Zhang JH, Chen YS, Frerichs I, Moller K, Fu F, Hsu HS *et al*: **Positive end-expiratory pressure titration with electrical impedance tomography and pressure-volume curve: a randomized trial in moderate to severe ARDS**. *Physiol Meas* 2021, **42**(1):014002.
36. Sarge T, Baedorf-Kassis E, Banner-Goodspeed V, Novack V, Loring SH, Gong MN, Cook D, Talmor D, Beitler JR, Group EP-S: **Effect of Esophageal Pressure-guided Positive End-Expiratory Pressure on Survival from Acute Respiratory Distress Syndrome: A Risk-based and Mechanistic Reanalysis of the EPVent-2 Trial**. *American journal of respiratory and critical care medicine* 2021, **204**(10):1153-1163.
37. Heijnen NFL, Hagens LA, Smit MR, Cremer OL, Ong DSY, van der Poll T, van Vught LA, Scicluna BP, Schnabel RM, van der Horst ICC *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**(12):1503-1511.
38. Reddy K, Calfee CS, McAuley DF: **Acute Respiratory Distress Syndrome Subphenotypes beyond the Syndrome: A Step toward Treatable Traits?** *American journal of respiratory and critical care medicine* 2021, **203**(12):1449-1451.

12

Nederlandse samenvatting en discussie

Ondanks ruim 50 jaar aan klinisch onderzoek, zijn er maar weinig interventies die de overleving verbeteren van patiënten met Acute Respiratory Distress Syndrome (ARDS).[1, 2] ARDS is een heterogeen ziektebeeld. De heterogeniteit in onder andere risicofactor voor ARDS, ontstekingsstatus en longmechanica waren waarschijnlijk redenen dat er geen behandeleffect werd aangetoond in de gehele studiegroep.[3, 4] Secundaire analyses van negatieve gerandomiseerde onderzoeken hebben echter aangetoond dat er in sommige subgroepen wel een significant behandeleffect is.[5, 6] Het is dus van cruciaal belang dat wij de juiste patiënt met ARDS selecteren voor een specifieke behandeling.[7] Het doel van dit proefschrift was om de behandeling van patiënten met ARDS te personaliseren op basis van biomarkers of rekruteerbaarheid (de mate waarin het longvolume toeneemt wanneer de luchtwegdruk wordt verhoogd). Uiteindelijk streven wij ernaar om de juiste patiënt te identificeren die waarschijnlijk baat zal hebben bij een specifieke behandeling, zoals met corticosteroïden of hogere luchtwegdrukken.

In **Deel I. Personalisering van de behandeling van ARDS op basis van biomarkers**, hebben wij biomarkers in plasma en lokaal (pulmonaal) verkregen biomarkers onderzocht om fenotypes voor ARDS te identificeren gebaseerd op biomarkers.

Allereerst hebben wij in **Hoofdstuk 2** een systematische literatuurstudie uitgevoerd om biomarkers te identificeren die onafhankelijk geassocieerd zijn met de ontwikkeling of mortaliteit van ARDS. Wij includeerden biomarkers die werden verkregen in plasma, bronchoalveolaire lavagevloeistof en zelfs in cerebrospinale vloeistof. Helaas konden wij geen meta-analyse uitvoeren vanwege de heterogene presentatie van data in de geïncludeerde studies. Wij vonden dat de meerderheid van de onderzochte biomarkers in plasma voor ARDS ontwikkeling en mortaliteit een maat waren voor systemische ontsteking, epitheelbeschadiging of endotheelbeschadiging. Biomarkers voor systemische ontsteking waren niet onafhankelijk geassocieerd met de ontwikkeling van ARDS of mortaliteit. Biomarkers voor alveolaire epitheelbeschadiging (plasma receptor for advanced glycation end products en surfactant protein D) en endotheliale permeabiliteit (plasma-angiopoetin-2) leken daarentegen geassocieerd te zijn met de ontwikkeling van ARDS.

Voordat wij biomarkers gebruiken in patiënten met ARDS, moeten wij overwegen wat het doel van de biomarker moet zijn.[8] Een biomarker voor de ontwikkeling van ARDS moet specifiek zijn voor ARDS, dus een biomarker die een maat is voor alveolaire schade of alveolo-capillaire schade. Lokaal verkregen biomarkers voor systemische ontsteking, bijvoorbeeld in de alveolaire ruimte, kunnen ARDS mogelijk wel diagnosticeren of de ernst van ARDS vaststellen.[9] Biomarkers die worden gebruikt voor de identificatie

van minder heterogene ARDS-fenotypes hoeven niet ARDS-specifiek te zijn, mits deze biomarkers patiënten met ARDS adequaat in groepen kunnen onderverdelen.

In **Hoofdstuk 3** wordt de associatie beschreven tussen purine signalering (purinergic signalling) en mechanische ventilatie, longinflammatie en surfactant dysfunctie. Van extracellulair adenosinetrifosfaat (ATP) wordt verondersteld dat het een sleutelrol heeft in de ontwikkeling van longschade en ARDS.[10] ATP wordt vrijgegeven door de type I alveolaire epitheelcel als gevolg van cyclische vervorming. Wanneer een bepaalde drempelwaarde wordt bereikt, werken extracellulaire ATP-moleculen als een signaalstof voor gevaar (danger-associated molecular pattern) en activeren zij de aangeboren immuunrespons via purine receptoren. Extracellulair ATP is een niet-specifieke biomarker voor weefselbeschadiging en heeft een korte halfwaardetijd. Onze hypothese was dat ATP, wanneer gemeten in vocht afkomstig uit de longen, gebruikt zou kunnen worden om de ontwikkeling van longschade of ARDS te volgen.

In **Hoofdstuk 4** onderzochten wij of het mogelijk is om extracellulair ATP te meten in uitgeademde lucht (exhaled breath condensate, EBC) van gezonde vrijwilligers met een verhoogd ademminuutvolume na een inspanningstest. Wij ontdekten dat ATP kon worden gemeten in EBC en dat de concentraties na de inspanningstest toenamen. Als gevolg van meerdere fysiologische veranderingen konden wij de toegenomen ATP-concentratie echter niet alleen toeschrijven aan het toegenomen teugvolume of de ademhalingsfrequentie.

Het verkrijgen van EBC tijdens mechanische ventilatie gebeurt doorgaans door de expiratoire beademingsslang door een thermo-elektrische koelmodule te leiden.[11] In **Hoofdstuk 5** hebben wij de EBC verzameld die zich ophoopte in de expiratoire beademingsslang en swivel en noemde deze swivel-derived exhaled breath condensate (SEBC). Wij vonden onder andere dat verscheidene biomarkers detecteerbaar waren in 29 monsters die waren verzameld in 13 mechanisch beademde IC-patiënten. Daarnaast vonden wij dat beademing met een groot teugvolume positief was gecorreleerd met interleukine-10, interleukine-12 en macrofaag inflammatoir eiwit-1. SEBC is een relatief eenvoudige methode om het longcompartiment te analyseren en biedt mogelijkheden voor het verzamelen van EBC tijdens mechanische ventilatie in grote prospectieve cohorten.

In **Hoofdstuk 6** hebben wij patiënten met een banale longontsteking (community-acquired pneumonia, CAP) geanalyseerd om een subgroep van patiënten te kunnen identificeren die baat heeft bij adjuvante behandeling met corticosteroïden. Wij voerden een secundaire analyse (latent class analysis, LCA) uit onder patiënten met een CAP die

deel uitmaakten van één prospectief cohort en twee gerandomiseerde gecontroleerde onderzoeken (Ovidius trial en STEP trial).[12-14] In beide cohorten identificeerden wij twee subgroepen op basis van klinische variabelen en biomarkers: een relatief hypo-inflammatoire subgroep en een hyper-inflammatoire subgroep. Patiënten in de hyperinflammatoire subgroep hadden hogere concentraties inflammatoire biomarkers en een lagere bloeddruk en zuurstofsaturatie. Bovendien hadden patiënten in de hyperinflammatoire subgroep een langere opnameduur, een hogere mortaliteit en een hogere incidentie van IC-opname. Vervolgens hebben wij geprobeerd een heterogeen behandeleffect vast te stellen voor adjuvante behandeling met corticosteroïden. In het Ovidius-cohort vonden wij dat het behandeleffect van corticosteroïden groter was bij patiënten in de hyperinflammatoire subgroep. Wij vonden echter geen heterogeen behandeleffect in het STEP-cohort. Mogelijke verklaringen waren de verschillende sets aan variabelen die in beide onderzoeken werden gebruikt of de lagere inflammatoire biomarkerconcentraties in het STEP-cohort. Toekomstig onderzoek moet zich richten op verdere identificatie van de subgroep van patiënten met CAP die waarschijnlijk baat zal hebben bij behandeling met corticosteroïden.

In **Deel II. Personalisering van de behandeling van ARDS op basis van rekruteerbaarheid**, hebben wij geavanceerde monitoring gebruikt tijdens mechanische ventilatie om verschillende fenotypes en behandelrespons vast te stellen in patiënten met ARDS.

In **Hoofdstuk 7** werden de rationale en de indicaties voor het gebruik van hogere positieve eind-expiratoire druk (PEEP) en rekruteren, het zogenaamde openlong concept, in patiënten met ARDS beschreven. Wij stelden voor om de respons op de behandeling te monitoren en de behandeling dienovereenkomstig voort te zetten of te staken.

In **Hoofdstuk 8** werd het open long concept toegepast bij patiënten met ernstig ARDS en refractaire hypoxemie onder beademing volgens het ARDS Network protocol. Deze kleine retrospectieve studie toonde aan hoe rekruteren en hogere PEEP de oxygenatie konden verbeteren en het gebruik van veno-veneuze extracorporale membraanoxygenatie konden voorkomen. De meerderheid van de patiënten verbeterde in termen van oxygenatie en wij rapporteerden een IC-overlevingspercentage van 88% (n=8). Hoewel wij in de discussie de rekruteerbaarheid vermeldden, hebben wij de hoeveelheid alveolaire overdistentie en collaps niet gemeten.

In **Hoofdstuk 9** gebruikten wij elektrische impedantie tomografie (EIT) om alveolaire overdistentie en collaps te visualiseren tijdens PEEP-titratie in patiënten met COVID-19 gerelateerde ARDS. De PEEP werd ingesteld op het niveau met de minimale relatieve hoeveelheid van zowel alveolaire collaps als alveolaire overdistentie na een afbouwende

PEEP trial. Bij de eerste 15 patiënten op onze IC stelden wij vast dat de ingestelde PEEP positief was gecorreleerd met de body mass index (BMI). Daarnaast toonden wij aan dat de ingestelde PEEP relatief stabiel was over de tijd en niet afnam na verbetering van het longbeeld. Geen van de richtlijnen voor mechanische ventilatie in (COVID-19-gerelateerde) ARDS bevat een PEEP-aanpassing voor BMI.[15]

In **Hoofdstuk 10** hebben wij de ingestelde PEEP volgens de PEEP-FiO_2 tabel bij baseline en de ingestelde PEEP volgens EIT na de PEEP trial vergeleken in 75 patiënten met COVID-19-gerelateerde ARDS. Wij vonden dat de meerderheid van de patiënten ≥2 cmH_2O hogere PEEP of ≥2 cmH_2O lagere PEEP nodig had in vergelijking met de PEEP-FiO2-tabel bij baseline. Patiënten die minder PEEP nodig hadden, hadden een lagere BMI, een hogere incidentie van longembolieën en significant meer alveolaire overdistentie bij hogere PEEP niveaus. Deze gegevens illustreren dat de one-size-fits-all protocollen die in de meeste onderzoeken voor mechanische ventilatie worden gebruikt, geen rekening houden met de heterogeniteit tussen patiënten met ARDS, noch met de respons op de behandeling. Deze data onderstrepen de noodzaak van gepersonaliseerde mechanische ventilatie om het heterogene behandeleffect optimaal te benutten. Hoewel de hoeveelheid PEEP gedeeltelijk kan worden voorspeld door BMI, is de identificatie van subgroepen bij patiënten met ARDS die een lagere of hogere PEEP nodig hebben, nog steeds gebaseerd op de daadwerkelijke PEEP respons na een PEEP trial.

Aanbevelingen en toekomstperspectieven

De identificatie van subgroepen van patiënten met een heterogeen behandeleffect biedt zowel grote mogelijkheden als grote uitdagingen voor toekomstig onderzoek.[5, 6, 16-18] De mogelijkheden zijn een verhoogde signaal-ruisverhouding, resulterend in een kleinere benodigde groepsgrootte, een groter behandeleffect en uiteindelijk meer positieve gerandomiseerde gecontroleerde onderzoeken.[19] De uitdagingen zijn de mogelijkheid van verkeerde classificatie van het fenotype - d.w.z. het toewijzen van een patiënt aan het verkeerde fenotype met schade als gevolg -, vroegtijdige fenotypering op basis van beperkte patiëntgegevens en beperkte externe validiteit.[1, 20] De grootste uitdaging is echter om een benadering te vinden die een patiënt correct identificeert aan het bed en ons in staat stelt om de behandeling dienovereenkomstig te personaliseren en te vervolgen.

In **Deel I. Personalisering van de behandeling van ARDS op basis van biomarkers**, wilden wij biologisch afgeleide fenotypes identificeren voor toekomstig onderzoek. Wij zochten naar biomarkers die geassocieerd zijn met ARDS in plasma en wij onderzochten methodes om lokale biomarkers uit het longcompartiment te verkrijgen. Wij ontdekten dat er vooralsnog geen specifieke biomarker voor ARDS is en dat biomarkers moeten

worden gecombineerd met klinische variabelen om biologisch afgeleide fenotypes vast te stellen. Vervolgens hebben wij retrospectief een LCA uitgevoerd bij patiënten met een CAP.

Om prospectief een biologisch afgeleid fenotype vast te stellen op de IC, is vrijwel directe analyse van biomarkers een vereiste. Daarnaast zijn biologisch afgeleide fenotypes vaak gebaseerd op complexe multivariabele modellen. Beide maken de snelle inclusie van patiënten met een biologisch fenotype in trials of de toepassing van fenotypes aan het bed een complexe aangelegenheid. Eenvoudigere alternatieven zijn biologisch afgeleide fenotypes op basis van direct beschikbare laboratorium en klinische variabelen[21], of het gebruik van een eenvoudig model in plaats van de volledige complexe modellen. [5, 22] Zo kan een model met drie variabelen – bestaande uit interleukine-8, bicarbonaat en proteïne C - het fenotype van het volledige model nauwkeurig voorspellen in patiënten met ARDS met een nauwkeurigheid van 94%.[22] Er is een point-of-care-assay ontwikkeld voor interleukine-6 en soluble tumornecrosefactor receptor.[23] Deze point-of-care-assay wordt momenteel gebruikt in de PHIND-studie, die tot doel heeft ARDS-patiënten prospectief te classificeren in een hyperinflammatoir of hypo-inflammatoir fenotype.

Net als in de PHIND-studie, streven wij ernaar om het heterogeen behandeleffect te onderzoeken in individuele patiënten data verkregen tijdens zes gerandomiseerde onderzoeken die het effect van corticosteroïden in patiënten met een CAP onderzochten. [12, 14, 24-27] Op basis van het heterogeen behandeleffect construeren wij een biologisch afgeleid fenotype, voornamelijk gebaseerd op direct beschikbare klinische variabelen. Vervolgens zal dit fenotype gevalideerd worden in een prospectief cohort. Uiteindelijk is het doel om een gerandomiseerde gecontroleerde studie uit te voeren waarin behandeling met corticosteroïden alleen wordt gestart in patiënten met een CAP die waarschijnlijk positief zullen reageren op behandeling met corticosteroïden.

In **Deel II. Personalisering van de behandeling van ARDS op basis van rekruteerbaarheid**, wilden wij een subgroep van patiënten met ARDS identificeren die positief zou kunnen reageren op behandeling met hogere luchtwegdrukken. Secundaire analyses van eerdere onderzoeken toonden aan dat hogere PEEP was geassocieerd met verbeterde uitkomsten onder patiënten met ernstig ARDS, meer diffuus ARDS of een hyperinflammatoire status.[5, 28, 29] In patiënten met COVID-19-gerelateerde ARDS maakten wij gebruik van EIT om PEEP te personaliseren en vonden dat een hogere BMI correleerde met rekruteerbaarheid, terwijl patiënten met een longembolie minder PEEP nodig hadden.

Als het doel is om te voorspellen welke patiënt met ARDS baat zou kunnen hebben bij een hogere PEEP-strategie, zou men de ernst van ARDS, de ontstekingsstatus, de longmorfologie en de BMI kunnen combineren. Een patiënt met ernstig ARDS, een hyperinflammatoire status, diffuus ARDS en een hoge BMI heeft waarschijnlijk baat bij een hogere PEEP-strategie. Daarentegen zou men een lagere PEEP-strategie kunnen aanbevelen bij patiënten met milde ARDS, een hypo-inflammatoire status, focale ARDS en een lage BMI. De PEEP-respons bij patiënten met een tegenstrijdig profiel, bijvoorbeeld een hoge BMI met ernstige maar focale ARDS, is moeilijker te voorspellen. Als gevolg hiervan kan een patiënt ten onrechte worden geclassificeerd als rekruteerbaar of niet rekruteerbaar en wordt potentiële schade toegebracht met behulp van een hogere PEEP-strategie, zoals wordt gesuggereerd door de verkeerd geclassificeerde patiënten in de LIVE-studie.[28]

De identificatie van een fenotype of subgroep voorspelt de respons op een behandeling, de daadwerkelijke respons na behandeling wordt vaak niet geëvalueerd in gerandomiseerde gecontroleerde studies. De evaluatie van de respons en het vervolgens voortzetten of onderbreken van de behandeling verminderen waarschijnlijk de nadelige effecten van het toewijzen van een patiënt aan de verkeerde subgroep. Vooral in trials waarin de effecten van mechanische ventilatie worden onderzocht, kan de respons op de behandeling relatief eenvoudig worden beoordeeld. Uit secundaire analyses van trials die PEEP strategieën hebben vergeleken, blijkt dat een verbeterde oxygenatie[30], een lagere driving pressure[31] of een toename aan compliantie[32] na een PEEP verhoging, resulteerde in betere uitkomsten. Daarentegen is er geen associatie tussen verbeterde oxygenatie na buikligging en overleving.[33] Een alternatief is het opvolgen van de PEEP respons middels EIT of transpulmonale drukmetingen.[34-36] Een gerandomiseerde gecontroleerde trial onder patiënten met ARDS die het ARDS Netwerk protocol vergelijkt met een protocol dat PEEP dagelijks aanpast op basis van EIT of transpulmonale drukmetingen is haalbaar en zou nieuwe inzichten kunnen geven in gepersonaliseerde mechanische ventilatie in patiënten met ARDS.

Tot slot resteert de vraag of fenotypes op basis van biomarkers of rekruteerbaarheid specifiek zijn voor het ARDS syndroom, of ook subgroepen kunnen identificeren onder ‘alle’ ernstig zieke patiënten. Longmorfologie is per definitie ARDS specifiek. Een hyperinflammatoir fenotype werd echter ook geïdentificeerd in ernstig zieke patiënten zonder ARDS en was geassocieerd met een verhoogde mortaliteit.[37, 38] Wij zouden kunnen overwegen om ernstig zieke patiënten met een hyperinflammatoire status en een hoog BMI te behandelen met hogere PEEP om vervolgens de behandelrespons te evalueren. In plaats van ons te richten op een heterogene syndroomdiagnose als ARDS, zouden wij op zoek kunnen gaan naar behandelbare kenmerken. Deze behandelbare kenmerken

kunnen worden behandeld, terwijl de respons wordt opgevolgd; zogenaamde op respons gebaseerde gepersonaliseerde behandeling.

REFERENTIES

1. Alipanah N, Calfee CS: **Phenotyping in acute respiratory distress syndrome: state of the art and clinical implications**. *Current opinion in critical care* 2022, **28**(1):1-8.
2. Matthay MA, McAuley DF, Ware LB: **Clinical trials in acute respiratory distress syndrome: challenges and opportunities**. *The Lancet Respiratory medicine* 2017, **5**(6):524-534.
3. Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, Gattinoni L, van Haren F, Larsson A, McAuley DF *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**(8):788-800.
4. Kent DM, Steyerberg E, van Klaveren D: **Personalized evidence based medicine: predictive approaches to heterogeneous treatment effects**. *BMJ* 2018, **363**:k4245.
5. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA, Network NA: **Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials**. *The Lancet Respiratory medicine* 2014, **2**(8):611-620.
6. Calfee CS, Delucchi KL, Sinha P, Matthay MA, Hackett J, Shankar-Hari M, McDowell C, Laffey JG, O'Kane CM, McAuley DF *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**(9):691-698.
7. 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**(2):147-155.
8. Garcia-Laorden MI, Lorente JA, Flores C, Slutsky AS, Villar J: **Biomarkers for the acute respiratory distress syndrome: how to make the diagnosis more precise**. *Annals of translational medicine* 2017, **5**(14):283.
9. van der Zee P, van Walree I, Fijen JW, van Houte AJ, van Velzen-Blad H, Rijkers G, Gommers D, Endeman H: **Cytokines and Chemokines Are Detectable in Swivel-Derived Exhaled Breath Condensate (SEBC): A Pilot Study in Mechanically Ventilated Patients**. *Dis Markers* 2020, **2020**:2696317.
10. Hasan D, Blankman P, Nieman GF: **Purinergic signalling links mechanical breath profile and alveolar mechanics with the pro-inflammatory innate immune response causing ventilation-induced lung injury**. *Purinergic signalling* 2017, **13**(3):363-386.
11. Rosias P: **Methodological aspects of exhaled breath condensate collection and analysis**. *J Breath Res* 2012, **6**(2):027102.
12. Blum CA, Nigro N, Briel M, Schuetz P, Ullmer E, Suter-Widmer I, Winzeler B, Bingisser R, Elsaesser H, Drozdov D *et al*: **Adjunct prednisone therapy for patients with community-acquired pneumonia: a multicentre, double-blind, randomised, placebo-controlled trial**. *Lancet (London, England)* 2015, **385**(9977):1511-1518.
13. Endeman H, Meijvis SC, Rijkers GT, van Velzen-Blad H, van Moorsel CH, Grutters JC, Biesma DH: **Systemic cytokine response in patients with community-acquired pneumonia**. *The European respiratory journal* 2011, **37**(6):1431-1438.
14. Meijvis SC, Hardeman H, Remmelts HH, Heijligenberg R, Rijkers GT, van Velzen-Blad H, Voorn GP, van de Garde EM, Endeman H, Grutters JC *et al*: **Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial**. *Lancet (London, England)* 2011, **377**(9782):2023-2030.
15. Fan E, Del Sorbo L, Goligher EC, Hodgson CL, Munshi L, Walkey AJ, Adhikari NKJ, Amato MBP, Branson R, Brower RG *et al*: **An Official American Thoracic Society/European Society of Inten-**

sive 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**(9):1253-1263.

16. Bos LD, Schouten LR, van Vught LA, Wiewel MA, Ong DSY, Cremer O, Artigas A, Martin-Loeches I, Hoogendijk AJ, van der Poll T *et al*: **Identification and validation of distinct biological phenotypes in patients with acute respiratory distress syndrome by cluster analysis**. *Thorax* 2017, **72**(10):876-883.
17. Delucchi K, Famous KR, Ware LB, Parsons PE, Thompson BT, Calfee CS, Network A: **Stability of ARDS subphenotypes over time in two randomised controlled trials**. *Thorax* 2018, **73**(5):439-445.
18. Famous KR, Delucchi K, Ware LB, Kangelaris KN, Liu KD, Thompson BT, Calfee CS, Network A: **Acute Respiratory Distress Syndrome Subphenotypes Respond Differently to Randomized Fluid Management Strategy**. *American journal of respiratory and critical care medicine* 2017, **195**(3):331-338.
19. Ware LB, Matthay MA, Mebazaa A: **Designing an ARDS trial for 2020 and beyond: focus on enrichment strategies**. *Intensive care medicine* 2020, **46**(12):2153-2156.
20. Bos LDJ, Sinha P, Dickson RP: **The perils of premature phenotyping in COVID-19: a call for caution**. *The European respiratory journal* 2020, **56**(1).
21. 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**(7):996-1004.
22. Sinha P, Delucchi KL, McAuley DF, O'Kane CM, Matthay MA, Calfee CS: **Development and validation of parsimonious algorithms to classify acute respiratory distress syndrome phenotypes: a secondary analysis of randomised controlled trials**. *The Lancet Respiratory medicine* 2020, **8**(3):247-257.
23. Sinha P, Calfee CS, Cherian S, Brealey D, Cutler S, King C, Killick C, Richards O, Cheema Y, Bailey 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**(12):1209-1218.
24. Torres A, Sibila O, Ferrer M, Polverino E, Menendez R, Mensa J, Gabarrus A, Sellares J, Restrepo MI, Anzueto A *et al*: **Effect of corticosteroids on treatment failure among hospitalized patients with severe community-acquired pneumonia and high inflammatory response: a randomized clinical trial**. *Jama* 2015, **313**(7):677-686.
25. Wittermans E, Vestjens SMT, Spoorenberg SMC, Blok WL, Grutters JC, Janssen R, Rijkers GT, Smeenk F, Voorn GP, van de Garde EMW *et al*: **Adjunctive treatment with oral dexamethasone in non-ICU patients hospitalised with community-acquired pneumonia: a randomised clinical trial**. *The European respiratory journal* 2021, **58**(2).
26. Snijders D, Daniels JM, 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**(9):975-982.
27. Confalonieri M, Urbino R, Potena A, Piattella M, Parigi P, Puccio G, Della Porta R, Giorgio C, Blasi F, Umberger R *et al*: **Hydrocortisone infusion for severe community-acquired pneumonia: a preliminary randomized study**. *American journal of respiratory and critical care medicine* 2005, **171**(3):242-248.
28. Constantin JM, Jabaudon M, Lefrant JY, Jaber S, Quenot JP, Langeron O, Ferrandiere M, Grelon F, Seguin P, Ichai C *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**(10):870-880.

29. Briel M, Meade M, Mercat A, Brower RG, Talmor D, Walter SD, Slutsky AS, Pullenayegum E, Zhou Q, Cook D *et al*: **Higher vs lower positive end-expiratory pressure in patients with acute lung injury and acute respiratory distress syndrome: systematic review and meta-analysis**. *Jama* 2010, **303**(9):865-873.
30. Goligher EC, Kavanagh BP, Rubenfeld GD, Adhikari NK, Pinto R, Fan E, Brochard LJ, Granton JT, Mercat A, Marie Richard JC *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**(1):70-76.
31. Amato MB, Meade MO, Slutsky AS, Brochard L, Costa EL, Schoenfeld DA, Stewart TE, Briel M, Talmor D, Mercat A *et al*: **Driving pressure and survival in the acute respiratory distress syndrome**. *The New England journal of medicine* 2015, **372**(8):747-755.
32. Hodgson CL, Cooper DJ, Arabi Y, King V, Bersten A, Bihari S, Brickell K, Davies A, Fahey C, Fraser J *et al*: **Maximal Recruitment Open Lung Ventilation in Acute Respiratory Distress Syndrome (PHARLAP): A Phase II, Multicenter, Randomized, Controlled Trial**. *American journal of respiratory and critical care medicine* 2019.
33. Albert RK, Keniston A, Baboi L, Ayzac L, Guerin C, Proseva I: **Prone position-induced improvement in gas exchange does not predict improved survival in the acute respiratory distress syndrome**. *American journal of respiratory and critical care medicine* 2014, **189**(4):494-496.
34. He H, Chi Y, Yang Y, Yuan S, Long Y, Zhao P, Frerichs I, Fu F, Moller K, Zhao Z: **Early individualized positive end-expiratory pressure guided by electrical impedance tomography in acute respiratory distress syndrome: a randomized controlled clinical trial**. *Crit Care* 2021, **25**(1):230.
35. Hsu HJ, Chang HT, Zhao Z, Wang PH, Zhang JH, Chen YS, Frerichs I, Moller K, Fu F, Hsu HS *et al*: **Positive end-expiratory pressure titration with electrical impedance tomography and pressure-volume curve: a randomized trial in moderate to severe ARDS**. *Physiol Meas* 2021, **42**(1):014002.
36. Sarge T, Baedorf-Kassis E, Banner-Goodspeed V, Novack V, Loring SH, Gong MN, Cook D, Talmor D, Beitler JR, Group EP-S: **Effect of Esophageal Pressure-guided Positive End-Expiratory Pressure on Survival from Acute Respiratory Distress Syndrome: A Risk-based and Mechanistic Reanalysis of the EPVent-2 Trial**. *American journal of respiratory and critical care medicine* 2021, **204**(10):1153-1163.
37. Heijnen NFL, Hagens LA, Smit MR, Cremer OL, Ong DSY, van der Poll T, van Vught LA, Scicluna BP, Schnabel RM, van der Horst ICC *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**(12):1503-1511.
38. Reddy K, Calfee CS, McAuley DF: **Acute Respiratory Distress Syndrome Subphenotypes beyond the Syndrome: A Step toward Treatable Traits?** *American journal of respiratory and critical care medicine* 2021, **203**(12):1449-1451.

A

PhD Portfolio
List of publications
Curriculum vitae
Dankwoord

PHD PORTFOLIO

Name: Philip van der Zee
Department of Adult Intensive Care, Erasmus MC Rotterdam
Research school: COEUR

PhD period: 2017-2023
Promotor: D. Gommers
Copromotor: H. Endeman

	Organiser	Year	ECTS
Courses			
BROK cursus	Erasmus MC	2017	1.0
Open Clinica	Erasmus MC	2017	0.2
FCCS cursus	NVIC	2017	2.0
Basiscursus proefdierkunde	Universiteit Utrecht	2017	3.0
Soortspecifieke cursus knaagdier en konijn	Universiteit Utrecht	2017	3.0
Imaging and Diagnostics	COEUR	2017	0.2
ARDS masterclass	Erasmus MC	2018	1.5
Microcirculation	AMC	2019	0.6
Brussels ARDS masterclass	ESICM	2019	0.6
Advanced respiratory care research meetings	Erasmus MC	2018-2022	2.0
ZonMW Grant aanvraag	Erasmus MC	2020	0.4
Bronchoscopy	LUMC	2021	0.4
Scientific Integrity	Erasmus MC	2023	0.3
Conferences			
ESICM VIENNA	ESICM	2017	2.0
Mechanical ventilation	VUMC	2017	0.3
Mechanical ventilation	Amphia Breda	2017	0.2
ILD and ICU	Erasmus MC	2018	0.2
ISICEM Brussels	ISICEM	2018	0.2
Inspires	AMC	2018	0.6
Topics in IC	NVIC	2018	0.4
Lung transplant Symposium	Erasmus MC	2019	0.2
ISICEM Brussels	ISICEM	2019	1.0
Hemodynamics	Erasmus MC	2020	0.2
ISICEM Brussels Online	ISICEM	2020	1.0
Didactics			
Bachelor students UCR	UCR	2019	2.0
Bachelor/master students EMC	Erasmus MC	2019	3.0
Practical advanced respiratory monitoring	Erasmus MC	2020-2021	3.0
Bachelor students UCR	UCR	2020	2.0
Bachelor/master students EMC	Erasmus MC	2020	3.0

LIST OF PUBLICATIONS

1. **Van der Zee P**, de Boer A: Pheochromocytoma: a review on preoperative treatment with phenoxybenzamine or doxazosin. *Neth J Med* 2014, 72(4):190-201.

2. Hasan D, Satalin J, **Van der Zee P**, Kollisch-Singule M, Blankman P, Shono A, Somhorst P, den Uil C, Meeder H, Kotani T *et al*: Excessive Extracellular ATP Desensitizes P2Y2 and P2X4 ATP Receptors Provoking Surfactant Impairment Ending in Ventilation-Induced Lung Injury. *International journal of molecular sciences* 2018, 19(4).

3. **Van der Zee P**, Somhorst P, Molinger J, Hasan D, Gommers D: A feasibility study into adenosine triphosphate measurement in exhaled breath condensate: a potential bedside method to monitor alveolar deformation. *Purinergic signalling* 2018.

4. **Van der Zee P**, Dos Reis Miranda D, Meeder H, Endeman H, Gommers D: vvECMO can be avoided by a transpulmonary pressure guided open lung concept in patients with severe ARDS. *Crit Care* 2019, 23(1):133.

5. **Van der Zee P**, Gommers D: Recruitment Maneuvers and Higher PEEP, the So-Called Open Lung Concept, in Patients with ARDS. *Crit Care* 2019, 23(1):73.

6. Endeman H, **Van der Zee P**, van Genderen ME, van den Akker JPC, Gommers D: Progressive respiratory failure in COVID-19: a hypothesis. *The Lancet Infectious diseases* 2020.

7. **Van der Zee P**, Rietdijk W, Somhorst P, Endeman H, Gommers D: A systematic review of biomarkers multivariately associated with acute respiratory distress syndrome development and mortality. *Crit Care* 2020, 24(1):243.

8. **Van der Zee P**, Somhorst P, Endeman H, Gommers D: Electrical Impedance Tomography for Positive End-Expiratory Pressure Titration in COVID-19-related Acute Respiratory Distress Syndrome. *American journal of respiratory and critical care medicine* 2020, 202(2):280-284.

9. **Van der Zee P**, Somhorst P, Endeman H, Gommers D: Reply to: Electrical Impedance Tomography for Positive End-expiratory Pressure Titration in COVID-19 Related ARDS: Comment. *American journal of respiratory and critical care medicine* 2020.

10. **Van der Zee P**, van Walree I, Fijen JW, van Houte AJ, van Velzen-Blad H, Rijkers G, Gommers D, Endeman H: Cytokines and Chemokines Are Detectable in Swivel-Derived Exhaled Breath Condensate (SEBC): A Pilot Study in Mechanically Ventilated Patients. *Dis Markers* 2020, 2020:2696317.

11. Favaron E, Ince C, Hilty MP, Ergin B, **Van der Zee P**, Uz Z, Wendel Garcia PD, Hofmaenner DA, Acevedo CT, van Boven WJ *et al*: Capillary Leukocytes, Microaggregates, and the Response to Hypoxemia in the Microcirculation of Coronavirus Disease 2019 Patients. *Critical care medicine* 2021, 49(4):661-670.

12. Wittermans E, **Van der Zee P**, Qi H, van de Garde EMW, Blum CA, Christ-Crain M, Gommers D, Grutters JC, Voorn GP, Bos WJW *et al*: Community-acquired pneumonia subgroups and differential response to corticosteroids: a secondary analysis of controlled studies. *ERJ Open Res* 2022, 8(1).

13. Tromp K, **Van der Zee P**, Rokx C, van Kampen J, Gommers D, Endeman H: Effect of Methylprednisolone on Inflammation and Coagulation in Patients with Severe COVID-19: A Retrospective Cohort Study. *Biomark Insights* 2021, 16:11772719211021647.

14. Somhorst P, **Van der Zee P**, Endeman H, Gommers D: PEEP-FiO(2) table versus EIT to titrate PEEP in mechanically ventilated patients with COVID-19-related ARDS. *Crit Care* 2022, 26(1):272.

15. Somhorst P, **Van der Zee P**, Endeman H, Gommers D: Reply to: Higher PEEP in intubated COVID-19-associated ARDS patients? We are not sure. *Crit Care* 2022, 26(1):388.

16. Wittermans E, **van der Zee P**, Qi H, Grutters JC, Voorn GP, Bos WJW, van de Garde EMW, Endeman H: Latent class analysis-based subgroups and response to corticosteroids in hospitalised community-acquired pneumonia patients: a validation study. *ERJ Open Res* 2023, 9(2).

CURRICULUM VITAE

Philip Alexander van der Zee was born on the 6th of January 1991 in Rotterdam, The Netherlands. After his graduation at the Emmaus College Rotterdam in 2009, he studied Medicine at Utrecht University faculty of Medicine. He graduated cum laude in 2015. Following graduation, he started in 2016 as a resident not in training (ANIOS) at the Department of Pulmonary Medicine of the University Medical Centre Utrecht. In 2017, Philip started working at the Department of Adult Intensive Care of the Erasmus Medical Centre in Rotterdam. Here he worked as both a resident not in training and as a PhD student under the supervision of prof. dr. Diederik Gommers and dr. Rik Endeman. Following four years (2017-2020) at the Intensive Care Unit, he started at the Department of Pulmonary Medicine of the Erasmus Medical Centre Rotterdam. He began his medical specialist training as a pulmonologist in 2021 under the supervision of dr. Leon van den Toorn and prof. dr. Joachim Aerts.

DANKWOORD

Het dankwoord. Zo mogelijk het belangrijkste onderdeel van een proefschrift. Zeer waarschijnlijk het meest gelezen onderdeel van een proefschrift. Dit proefschrift, inmiddels een uit de hand gelopen hobby, was niet tot stand gekomen zonder de hulp en ondersteuning van vele anderen. Hierbij wil ik alle patiënten, familie, vrienden en collega's hartelijk bedanken die mij hebben ondersteund bij het schrijven van dit boekje. Een aantal mensen wil ik in dit dankwoord in het bijzonder bedanken.

Professor Gommers, beste Diederik, van afdelingshoofd en promotor naar bekende Nederlander en COVID-expert in het tijdsbestek van enkele maanden. Dat kunnen er maar weinig zeggen. Ondanks alle media-aandacht ben je altijd je nuchtere en kritische zelf gebleven. Daarnaast wist je, ondanks alle drukte en aandacht, altijd een moment vrij te maken om met mij mee te kijken naar nieuwe EIT-data of om te discussiëren over de insteek van een manuscript. Dat heb ik altijd zeer op prijs gesteld. Jouw adviezen en kritische correcties hebben dit proefschrift naar een hoger niveau getild. Dank dat jij mij de kans hebt gegeven om onderzoek te doen op deze mooie IC!

Dr. Endeman, beste Rik, dankzij je gedrevenheid, hulp en adviezen is dit proefschrift ontaard in een uit de hand gelopen hobby. Ik weet nog goed dat je een klein jaar nadat ik op de IC was begonnen ook naar het Erasmus bent gekomen. Met jouw holistische blik heb je ervoor gezorgd dat dit proefschrift een combinatie is geworden tussen inflammatie en mechanische ventilatie, en dat beide onlosmakelijk met elkaar verbonden zijn. Zoals een echte opleider betaamt, leidt je op door zelf het goede voorbeeld te geven. Je bent benaderbaar, leergierig, betrokken en gedreven. Het schoolvoorbeeld van 'een goede dokter'. Op naar nog heel wat avondjes Korean BBQ en mooie projecten!

Dr. Hasan, beste Djo, jij hebt me overtuigd om onderzoek te komen doen op de IC van het Erasmus MC. De eerste keer dat ik jou ontmoette, was bedoeld als kennismaking. Uiteindelijk ontaardde dat gesprek in een reeks colleges over beademing, 'purinergic signalling', en vele andere nieuwe ontdekkingen. Ik ken niemand die zo nauwgezet, volledig en enthousiast de literatuur induikt om antwoorden te vinden op de vele vragen die in je opkomen. Hoewel je al enige tijd niet meer werkzaam bent op de IC, was je altijd beschikbaar geweest voor vragen of discussie, dank!

Beste leden van de beoordelingscommissie, **prof. dr. J. Aerts, prof dr. N. Juffermans en prof. dr. R. Pickkers**, dank dat u bereid was om dit proefschrift te lezen en te beoordelen. Tevens dank om zitting te nemen in mijn promotiecommissie.

Beste medeauteurs, hartelijk dank voor jullie samenwerking en kritische opmerkingen op mijn manuscripten. Het proefschrift is er vanzelfsprekend beter op geworden!

Peter Somhorst, beste Peter, wat heb ik de afgelopen jaren veel van je geleerd. Door jou heb ik de beginselen van de EIT en transpulmonale drukmetingen onder de knie gekregen. Tijdens het begin van de COVID-19 pandemie hebben wij bij schier eindeloze hoeveelheden patiënten de beademingsinstellingen geanalyseerd en aangepast. Daarnaast was er gelukkig ook af en toe tijd voor een klein hapje of drankje. Ik zal het nooit vergeten: 'Maak er maar 80 van'.

Jeroen Molinger, beste Jeroen, je enthousiasme, gedrevenheid en kennis van zaken werken aanstekelijk. Daarnaast heb ik dankzij jou een prachtige ingang gekregen bij BeLife om daar proefpersonen te kunnen includeren voor mijn eerste manuscript. Inmiddels zit je al heel wat jaartjes in de Verenigde Staten om daar je kennis te verspreiden. Ik ben blij dat we nog altijd goed contact hebben!

Wim Rietdijk, beste Wim, al vanaf het begin heb jij je zijdelings bezig gehouden met de opzet van mijn promotietraject. Hierbij stak je je mening niet bepaald onder stoelen of banken, ongeacht of dit een manuscript of een willekeurig ander onderwerp betrof. Bij menig manuscript hebben we gediscussieerd over de boodschap die erin moest staan. Als ik de boodschap had uitgelegd, hoorde ik doorgaans: 'Waar staat dat dan?'. Daarnaast was er gelukkig ook tijd voor koffie en etentjes; dank daarvoor!

Esther Wittermans, beste Esther, wat begon als een klein project is uiteindelijk ontaard in een megalomaan project waarbij we meerdere studies uit Nieuwegein en Bazel hebben uitgeplozen. Het resultaat mag er zijn! Dank voor de prettige samenwerking.

Hongchao Qi, dear Hongchao, we requested a statistician at the department of biostatistics. We got more than we asked for! Thank you very much for your help regarding statistics and your invaluable comments on our manuscripts.

Patricia, Ditty, Melanie en Alicija, de 'onderzoeksdames' en het kloppend hart van het onderzoek op de IC. Door jullie voelt het binnenlopen op de onderzoekskamer een beetje als thuiskomen. Bij vragen of logistieke uitdagingen zijn jullie altijd beschikbaar om te helpen en een oplossing te vinden. Zonder jullie was het nooit gelukt om dit proefschrift af te ronden. Heel erg bedankt!

De '**onderzoekskamer**'. Allereerst Tim en Jantine, niet alleen hebben jullie gezorgd voor een fantastische tijd, jullie hebben ook nog eens meegeholpen met tientallen EIT en

drukmetingen. Jullie hulp was onmisbaar, dank jullie wel! Loes, Lisa, Hans, Davy, Ernest, Zoran, Mo, Emanuele, Jim, Julien en Thijs, het was fantastisch om met jullie samen te werken. Jullie hebben het onderzoek doen een stuk leuker gemaakt!

Willem, Frank en John, wat hebben we veel geklust tijdens onze experimenten op de 23e en op de CT kamer. Jullie creativiteit om fondsen voor een opstelling te werven en die daadwerkelijk te bouwen kent geen grenzen. Helaas waren we wat minder creatief met het bestellen van eten tijdens al die avonden. Dank voor de leuke tijd en op naar de komende experimenten!

Els Forman en Marja Klüver, jullie hebben me altijd fantastisch geholpen met het vinden van een gaatje in de volle agenda van Diederik. Dank!

De **stafleden en de verpleging van de Intensive Care van het Erasmus MC**, tijdens mijn promotieonderzoek heb ik heel wat uren gewerkt als ANIOS. Wat heb ik veel geleerd van jullie. Daarnaast heb ik eindeloos metingen verricht bij de patiënten met COVID-19: 'Daar zal je de beademingsboys weer hebben!'. Dank voor de leerzame tijd en jullie hulp!

Leon van den Toorn, Joachim Aerts en de longartsen van het Erasmus MC, inmiddels ben ik al ruim 2 jaar in opleiding tot longarts. Vanaf het begin heb ik me meteen op mijn plek gevoeld. Dank voor de vele leermomenten en de ruimte die ik heb gekregen om mijn onderzoek af te ronden naast de opleiding.

Tot slot mijn familie. Lieve papa en mama, dank jullie wel voor de onvoorwaardelijke steun, trots en liefde die jullie mij altijd hebben gegeven en nog steeds geven. Aan alles komt een einde, zo ook aan het: 'Wanneer is je boekje nou eindelijk eens klaar?!' Ik ben benieuwd wat het volgende onderwerp zal worden.

Lieve Daniël en Frederike, team Eindhoven, dank voor alle steun die jullie mij de afgelopen jaren hebben gegeven. Ik vind het geweldig om te horen dat jullie dichterbij komen wonen! Nu dit boekje af is, ben ik op zoek naar een huis waar ik kan oefenen met klussen voordat ik dat thuis ga doen.

Liever Esther, Pelle en kleine Anna, ik hou ervan om jullie met z'n drietjes te zien keuvelen. Het is een zeer welkome afleiding van wereldse zaken als boekjes schrijven en werken. Wie anders dan Esther en Daniël zou ik moeten vragen als paranimfen om naast me te staan op deze bijzondere dag!

Lieve Nathalie, ik weet soms niet zo goed waar ik jou aan heb verdiend. Je liefde, geduld en zorgzaamheid zijn grenzeloos en je staat altijd voor iedereen klaar. Zonder jouw onvermoeibare steun was het me nooit gelukt om dit proefschrift af te ronden, al was het maar omdat je de voorkant van mijn proefschrift hebt ontworpen. Ik hou van je!