ARDS sub-phenotypes - searching for Rorschach amongst the roentgenograms?

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In 1967, Ashbaugh and colleagues first described acute respiratory distress syndrome (ARDS) - an acute illness, characterised by tachypnoea, hypoxaemia and loss of lung compliance occurring after a variety of pulmonary and non-pulmonary insults (including trauma, acute pancreatitis, viral pneumonitis)[1]. This concept is retained as the ARDS illness model within the current consensus definitions, with acute defined as within 7 days of insult, and hypoxaemia categorised using partial pressure of oxygen/fraction of inspired oxygen concentration (PaO_2/FiO_2 ratio) into mild (<40Kpa) , moderate (13.3-26.6Kpa) and severe (<=13.3Kpa) ARDS on a positive end expiratory pressure of >5 cm water[2].

Fifty years on, ARDS remains a clinical challenge. Globally, ARDS remains clinically underrecognized, with an acute hospital mortality of 46% in patients with severe ARDS[3]. Further, after more than 150 randomised controlled trials (RCTs)[4], we do not have a single drug proven to benefit patients with ARDS. Notably, the histopathological *hallmark* of ARDS, diffuse alveolar damage (DAD)[1], is only found in half of the patients, and is difficult to ascertain during acute illness [5]. This clinical challenge led to the hypothesis that the heterogeneity of ARDS will manifest as subpopulations with similar clinical, biological, outcome and/or treatment response characteristics. Further, these subpopulations may be unique to ARDS or shared with other critical illness syndromes.

If we could identify ARDS subpopulations based on clinical and/or biological characteristics, this may highlight molecular mechanisms to target in RCTs, subpopulations with a higher risk of adverse outcomes or greater treatment responses[6]. Calfee and colleagues have led the field of determining such ARDS subpopulations, primarily with data from patients enrolled into RCTs, using latent class analyses (LCA) of clinical and biomarker data. They consistently report a two class model (two ARDS subpopulations or sub-phenotypes) as best fit for the clinical and biomarker data analysed. The *hyperinflammatory* ARDS subpopulation (*Phenotype-2*) is less common, characterised by higher plasma concentrations of cytokines, greater vasopressor use, lower serum bicarbonate concentrations, and a higher prevalence of sepsis, when compared with the more common *hypoinflammatory* ARDS subpopulation (*Phenotype-1*). Importantly, *hyperinflammatory* ARDS has higher mortality and differential treatment response to PEEP, simvastatin, and fluid management.

Further, these phenotypes are stable over the first three days, giving an enrolment window for RCTs and they can be identified with limited biomarker information[6]. A similar two ARDS subpopulation model primarily in patients enrolled into observational cohort study, using only biomarker data and with clustering analysis have also been reported by Bos et al[6]. For such ARDS subpopulations to be useful they must have feasible diagnostic standards to enable categorisation at the bedside, whilst remaining reproducible and biologically informative. We have actively avoided the term 'endotype' as it denotes subpopulations with specific biological mechanisms, whereas these ARDS subpopulations represent a cluster of visible properties and are best referred to as phenotypes.

In this context, let us consider the work by Sinha and colleagues in this issue of Thorax[7]. The authors tested whether the *hyperinflammatory* and *hypoinflammatory* subpopulations reported in RCTs are identifiable in two observational cohorts – a single centre cohort study of Validating Acute Lung Injury markers for Diagnosis (VALID) and a two centre cohort study of Early Assessment of Renal and Lung Injury (EARLI). First they performed latent class analysis (LCA) and showed that a two class model provided best fit for the data from VALID and EARLI cohorts. Second, they report that 3 or 4 marker classifiers (consisting of Interleukin-8, bicarbonate, Protein C and vasopressor-use) developed in RCTs perform adequately in both these cohorts. Consistent with the previous studies, Sinha and colleagues find that the *hyperinflammatory* ARDS contains a high proportion of patients with sepsis (50% vs 18%), require vasopressors (76% and 39%), and higher mortality relative to the *hypoinflammatory* ARDS (pooled mortality 55% and 21% respectively). Interestingly, this mortality difference was not reflected in marked differences in severity of hypoxia. This is important as severity of hypoxia is one of the strongest predictors of outcomes in large observational studies of ARDS[3] and in the predictive validity analyses reported with the consensus definitions[2].

There are several considerations when interpreting these data. First, given the well described compartmentalisation of pulmonary inflammation in ARDS[8], whether changes in cytokines and in neutrophil phenotype measured in peripheral blood truly reflect the changes in alveolar space and pulmonary interstitial space (Figure 1). This hypothesis is supported by the observation that heterogeneity of ARDS extends to its histopathological features[5], with presence of DAD being

associated with higher mortality, and greater likelihood of dying of refractory hypoxaemia compared with other histological patterns of injury. Notable, in Sinha and colleagues' analyses, is the dominance of the *hyperinflammatory* phenotype amongst neutropenic patients, which forces us to consider the centrality of neutrophils in the pathogenesis of ARDS with the alternative being that neutrophil ingress is a damage-associated epiphenomenon. Therefore, it would be useful to perform concurrent sampling to compare the immunological changes in peripheral blood to those within lung parenchyma. Second, as variables used to determine ARDS subpopulations and the definitions and cut offs of these variables differ between studies, despite multiple validations, there is uncertainty on optimal discriminant variables. Amongst the variables examined in these studies, one can discern a range of possible patterns, as shown in Figure-2. If we pool these data from studies thus far, we have a sample size of ~4000 patients, to explore the hypothesis that there may be more than two ARDS subpopulations. This may help overcome the argument that the two ARDS subpopulation model in studies thus far reflect the sample size of each study, rather than all possible ARDS subpopulations due to biological differences. Further, Sinha and colleagues identify a number of additional markers, including a notable divergence of two matrix metalloproteinases(MMP), MMP8 which is increased in the *hypoinflammatory* and MMP9 which is increased in the *hyperinflammatory* subpopulations respectively. They also note a similar divergent relationship between the epithelial damage markers receptor for advanced glycation endproducts (RAGE) and surfactant protein D (SP-D). Understanding the biology of these divergent patterns of related markers, if validated, may provide critical mechanistic insight into the phenotypes identified. Third the most common aetiology of ARDS is infection[3] and the *hyperinflammatory* sub-phenotype is dominated by patients with sepsis often requiring vasopressors. This implies that some of these *hyperinflammatory* sub-phenotype may meet the septic shock definition, and overlap with other clinical and immunological critical illness syndromes. The absence of a marked difference in hypoxia between these ARDS sub-phenotypes also supports this reasoning.

In summary, whilst we are certain that at least two ARDS sub-phenotypes can be identified, uncertainties exist on discriminant variables and features of treatment responsive ARDS subpopulations. To truly enable stratified medicine in ARDS at the bedside, we need to understand

the mechanistic underpinnings of ARDS subpopulations, whilst ensuring diagnostic feasibility, and reliability with near patient testing and validity.

Acknowledgments: MS-H is supported by the National Institute for Health Research Clinician Scientist Award (NIHR-CS-2016-16-011). The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the UK National Institute for Health Research or the Department of Health. ACM is supported by an MRC Clinician Scientist Fellowship (MR/V006118/1).

Ethics approval and consent to participate: Not applicable

Availability of data and material: Not applicable

Competing interests: The authors declare that they have no competing interests

Funding: Not applicable

Authors' contributions: ACM, KK and MSH developed the outline. ACM and KK wrote the first draft and generated the figures. ACM, KK and MSH critically revised the manuscript for important intellectual content, and agreed the final submitted version of the manuscript.

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Figure 1: Compartmentalisation of pulmonary inflammation in ARDS, characterised by bilateral radiographic infiltrates. Mφ macrophage, Mo monocyte, N neutrophil, DC dendritic cell, IL-6 interleukin 6, IL-8 interleukin 8, TNF-α tumour necrosis factor alpha, MMPs-matrix metalloproteinases, NET neutrophil extracellular trap. Created with BioRender.com, radiograph from shutterstock.

Figure 2: ARDS sub phenotypes and pathobiology

Heatmap summarising the variables used for LCA modelling in the respective studies represented by values for the hyperinflammatory subphenotype [6]. These variables and their weighting in the model were extracted from relevant papers and supplemental materials and represent variables used in LCA modelling only. Additional variables reported but not included in LCA modelling are not shown.

Both categorical (top section) and continuous data (lower section) are shown. For categorical data the variables not available are in dark grey and the ones included in the model in yellow. For continuous variables positive values are red, negative in blue(standardized mean variable values were extracted from graphs and tables in the respective publications),ones not available in dark grey and ones available but not referenced with mean values are marked as if categorical. The variables included in the 3- or 4-variable parsimonious models are marked with *.

Of note, the pattern in the peripheral blood cytokines, combined with respiratory acidosis and vasopressor requirements is consistently present throughout, however the respective weighting differed between studies, especially when considering the clinical variables.

Abbreviations: COPD – chronic obstructive pulmonary disease, PF ratio – ratio of partial pressure of oxygen in arterial blood over inspired partial pressure of oxygen, paCO2 – arterial partial pressure of CO2, CRP – C-reactive protein, WBC – white blood count, ang2 – angiopoietin2, ICAM1 – intercellular adhesion molecule 1, IL6 – interleukin 6, IL8 –interleukin 8, PAI1 – plasminogen activator inhibitor 1, RAGE - receptor for advance glycation end products, SPD– surfactant protein D, sTNFr1 – soluble tumour-necrosis factor receptor-1, vWF– von Willebrand factor.

Figure 1

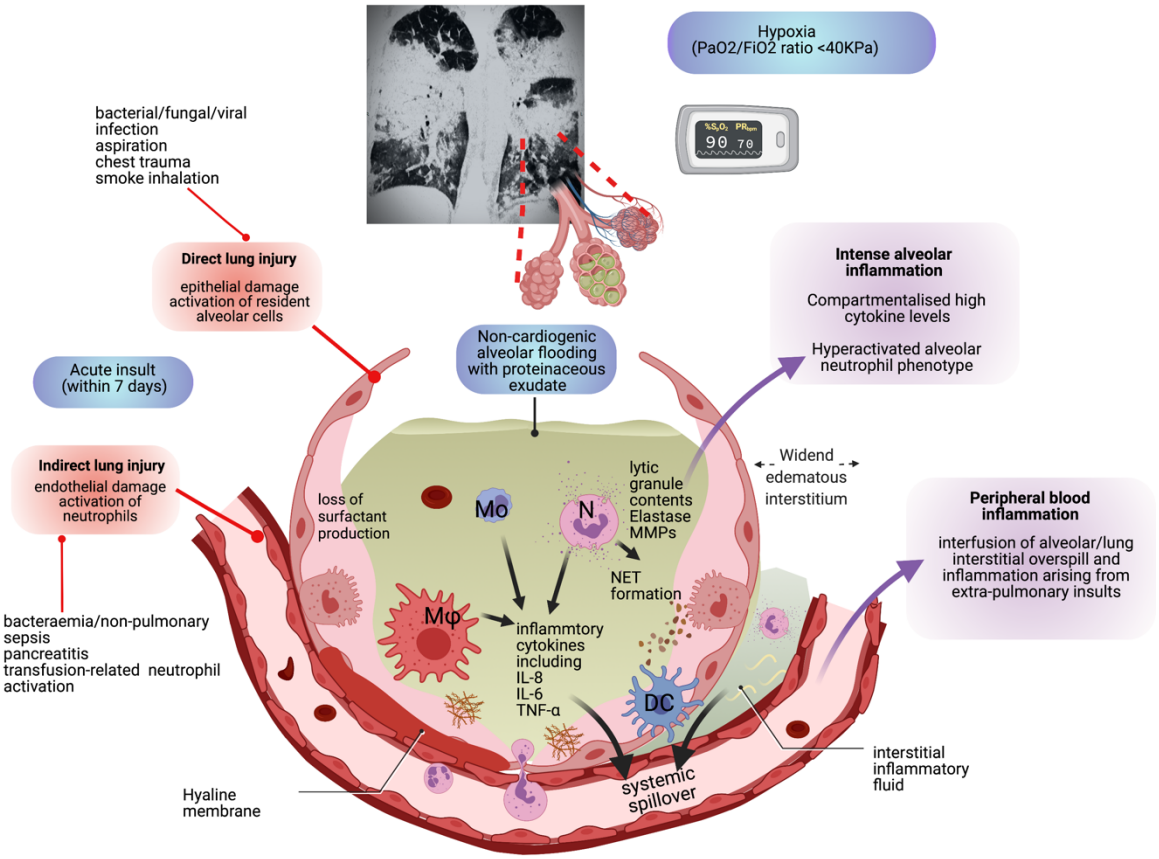
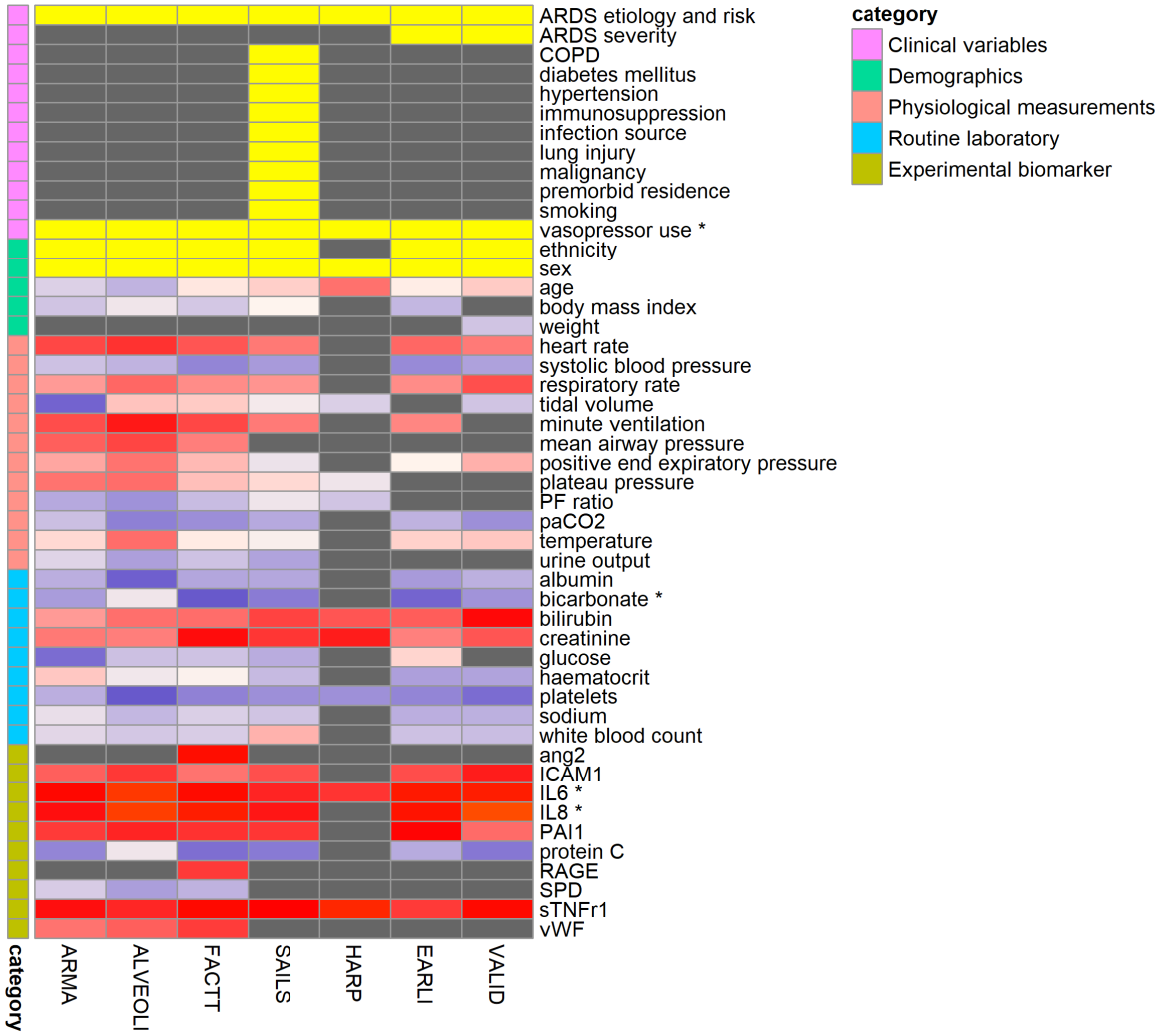

Hypoxia
(PaO2/FiO2 ratio <40KPa)
bacterial/fungal/viral
infection
aspiration
chest trauma
smoke inhalation
Direct lung injury
epithelial damage
activation of resident
alveolar cells
Acute insult
(within 7 days)
Indirect lung injury
endothelial damage
activation of
neutrophils
bacteraemia/non-pulmonary
sepsis
pancreatitis
transfusion-related neutrophil
activation
Non-cardiogenic
alveolar flooding
with proteinaceous
exudate
Intense alveolar
inflammation
Compartmentalised high
cytokine levels
Hyperactivated alveolar
neutrophil phenotype
Widend
edematous
interstitium
Peripheral blood
inflammation
interfusion of alveolar/lung
interstitial overspill and
inflammation arising from
extra-pulmonary insults
loss of
surfactant
production
Mo
N
lytic
granule
contents
Elastase
MMPs
NET
formation
Mφ
inflammtory
cytokines
including
IL-8
IL-6
TNF-α
DC
systemic
spillover
interstitial
inflammatory
fluid
Hyaline
membrane

Figure 2


ARDS etiology and risk
ARDS severity
COPD
diabetes mellitus
hypertension
immunosuppression
infection source
lung injury
malignancy
premorbid residence
smoking
vasopressor use *
ethnicity
sex
age
body mass index
weight
heart rate
systolic blood pressure
respiratory rate
tidal volume
minute ventilation
mean airway pressure
positive end expiratory pressure
plateau pressure
PF ratio
paCO2
temperature
urine output
albumin
bicarbonate *
bilirubin
creatinine
glucose
haematocrit
platelets
sodium
white blood count
ang2
ICAM1
IL6 *
IL8 *
PAI1
protein C
RAGE
SPD
sTNFr1
vWF
category
ARMA
ALVEOLI
FACTT
SAILS
HARP
EARLI
VALID
category
Clinical variables
Demographics
Physiological measurements
Routine laboratory
Experimental biomarker