

## SYSTEMATIC REVIEW

# Precision medicine in Acute Respiratory Distress Syndrome

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## 1. Introduction

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

cornerstone of care for ARDS [4].

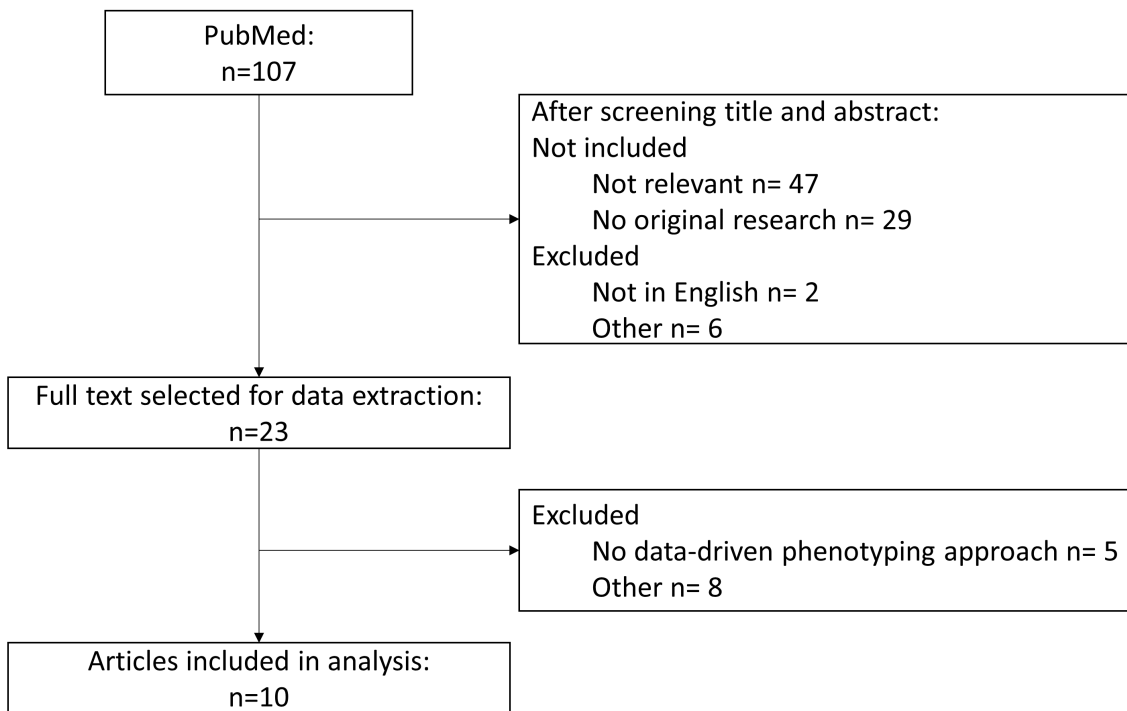
One reason for this failure could be the heterogeneity of the syndrome, which makes a “one-size fits all” approach insufficient [4, 5]. Precision medicine is defined as “*treatments targeted to the need of individual patients on the basis of genetic, biomarker, phenotypic, or psychosocial characteristics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

**Abstract**

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

**Keywords**

Precision medicine; Phenotypes; ARDS



**FIGURE 1. Flowchart of article selection.**

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

## 2. Search strategy and selection criteria

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

## 3. Definitions in precision medicine

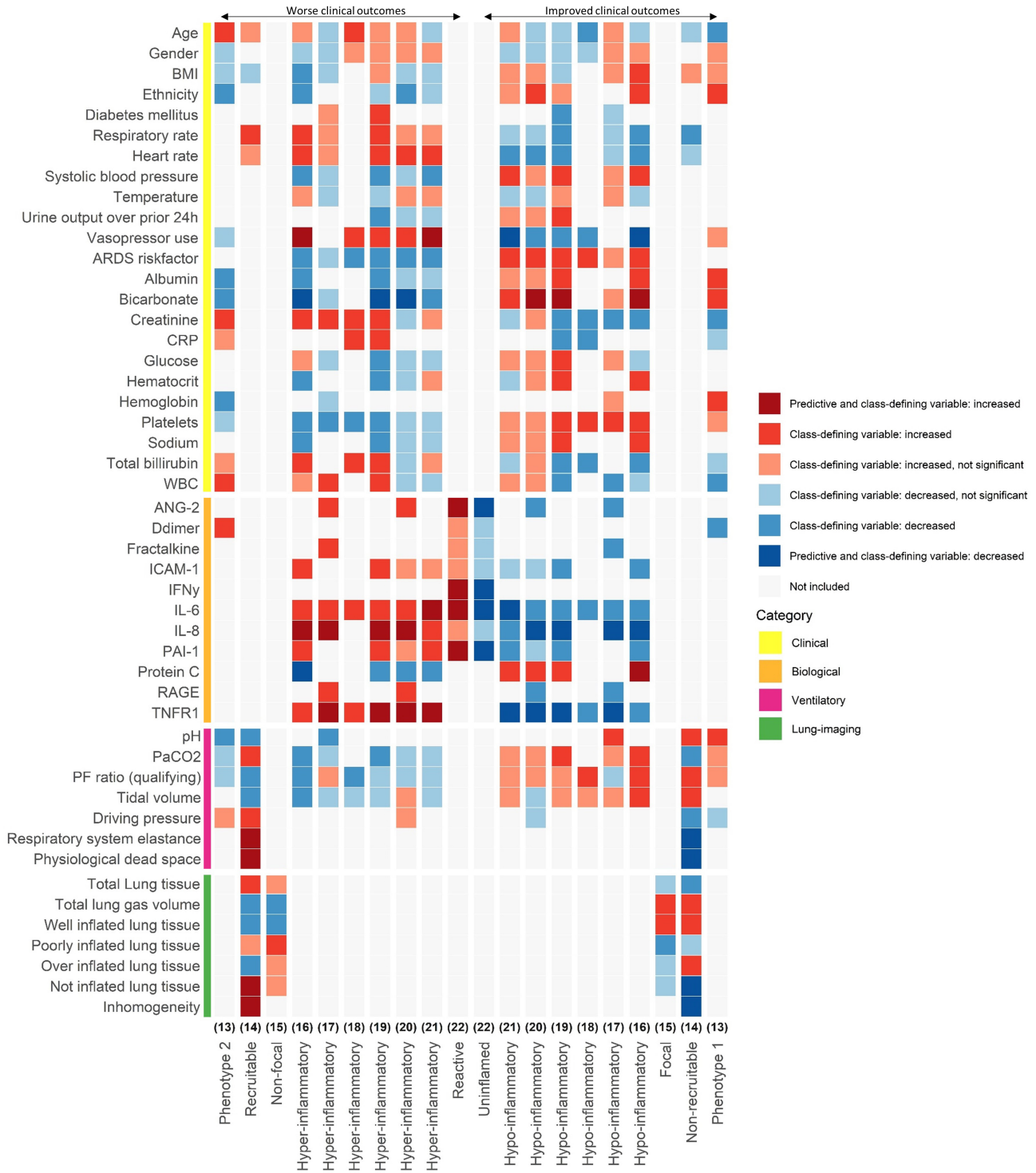
Recently, definitions have been proposed to standardize the terminology used in the search for targetable (sub) phenotypes in the critically ill and associated broad defined syndromes, like ARDS.

In this review, we use the following: (1) Phenotype — “A set of clinical features in a group of patients who share

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

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

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



**FIGURE 2. Heatmap of all included class-defining variables and identified predictive variables in ARDS subphenotyping models.** Only variables significant in at least one study are depicted. Class-defining variables were used for identifying subphenotypes. Predictive variables were used to classify the subphenotypes using fewer variables. The increased class-defining variables: (1) gender implicates a higher percentage of males and (2) ethnicity implicates a higher percentage of white people present in that subphenotype, (3) source of infection is pre-dominantly the thorax, and (4) ARDS risk factor is pre-dominantly pneumonia. Subphenotypes depicted on the left side of the graph were associated with worse clinical outcomes compared to the subphenotypes depicted on the right side of the graph. The numbers above the subphenotypes refer to the original study and correspond to the reference bibliography number. BMI, body mass index; CRP, C-reactive protein; WBC, white blood cell count; ANG-2, angiotensin-2; ANG2/1, angiotensin 2 and 1 ratio; ICAM-1, intercellular adhesion molecule-1; IFN $\gamma$ , interferon gamma; IL, interleukin; PAI-1, plasminogen activator inhibitor 1; RAGE, receptor for advanced glycation end products; TNFR1, tumor necrosis factor receptor 1; PF ratio, PaO $_2$ /FiO $_2$  ratio.

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

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

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

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

#### 4. Identified ARDS subphenotypes

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

##### 4.1 Clinically-derived subphenotypes

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

##### 4.2 Physiology and morphology-derived subphenotypes

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

Three radiological subphenotypes of ARDS were described: lobar attenuations (‘LA’); diffuse attenuations (‘DA’) and patchy attenuations (‘PA’). These were later redefined as ‘focal’ ARDS (LA subphenotype) and ‘non-focal’ ARDS (DA and PA subphenotype) [15, 23]. It is important to note that these subphenotypes were not the result of data-driven evaluation of the CT images, but rather the result of systematic

evaluation of these scans by human operators. Non-focal lung morphology is characterized by diffuse and patchy lung aeration loss (increased inhomogeneity) and distinct lung mechanics including decreased total lung gas volume, a lower compliance of the respiratory system and a higher amount of recruitable lung compared with focal ARDS. This subphenotype has also been associated with an increased ICU-mortality in a more recent study [23].

##### 4.3 Biology-derived subphenotypes

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

##### 4.4 Subphenotypes based on combined variables

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

#### 5. Evidence for heterogeneity of treatment effect

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

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

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

The first and currently only prospective evaluation of a precision medicine by subphenotypes in a RCT is the LIVE-trial: Lung Imaging for Ventilator Settings in ARDS [30]. They tested whether personalized mechanical ventilation strategies

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

## 5.1 Subphenotype classification using parsimonious models and time-related changes

Most of the cluster and latent class analyses algorithms are not suitable for clinical classification of patients at the bedside due to the number of variables required as input. Therefore, predictive models containing fewer variables have been identified to classify patients with high accuracy (Fig. 2; Table 2, Ref. [14, 16, 17, 19–21, 31]). This also provides guidance in developing classifying tests suitable for clinical practice, like IL-6 and TNFR1 point-of-care tests (ClinicalTrials.gov Identifier: NCT04009330). Awaiting these point-of-care tests for specific plasma markers, researchers were recently able to classify patients in hypo- and hyperinflammatory subphenotypes using readily available clinical data including demographics variables (e.g., age, sex, ARDS risk factor), respiratory variables (e.g.,  $\text{PaO}_2/\text{FiO}_2$  ratio,  $\text{PaCO}_2$ ), vital signs (e.g., temperature, heart rate, respiratory rate), and laboratory variables (e.g., hematocrit, white cell count, platelets, sodium) with high accuracy (AUC: 0.95; 95% CI 0.94–0.96; Table 2) [31]. Although this classification was performed in highly selected study populations, this finding is very promising. Together with previous results, this provides multiple opportunities to enable classification in clinical practice.

**TABLE 2. Predictive models classifying ARDS subphenotypes.**

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

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

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

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

## 5.2 Underlying processes captured by subphenotypes

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

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

when a wide range of variables were used in the derivation phase.

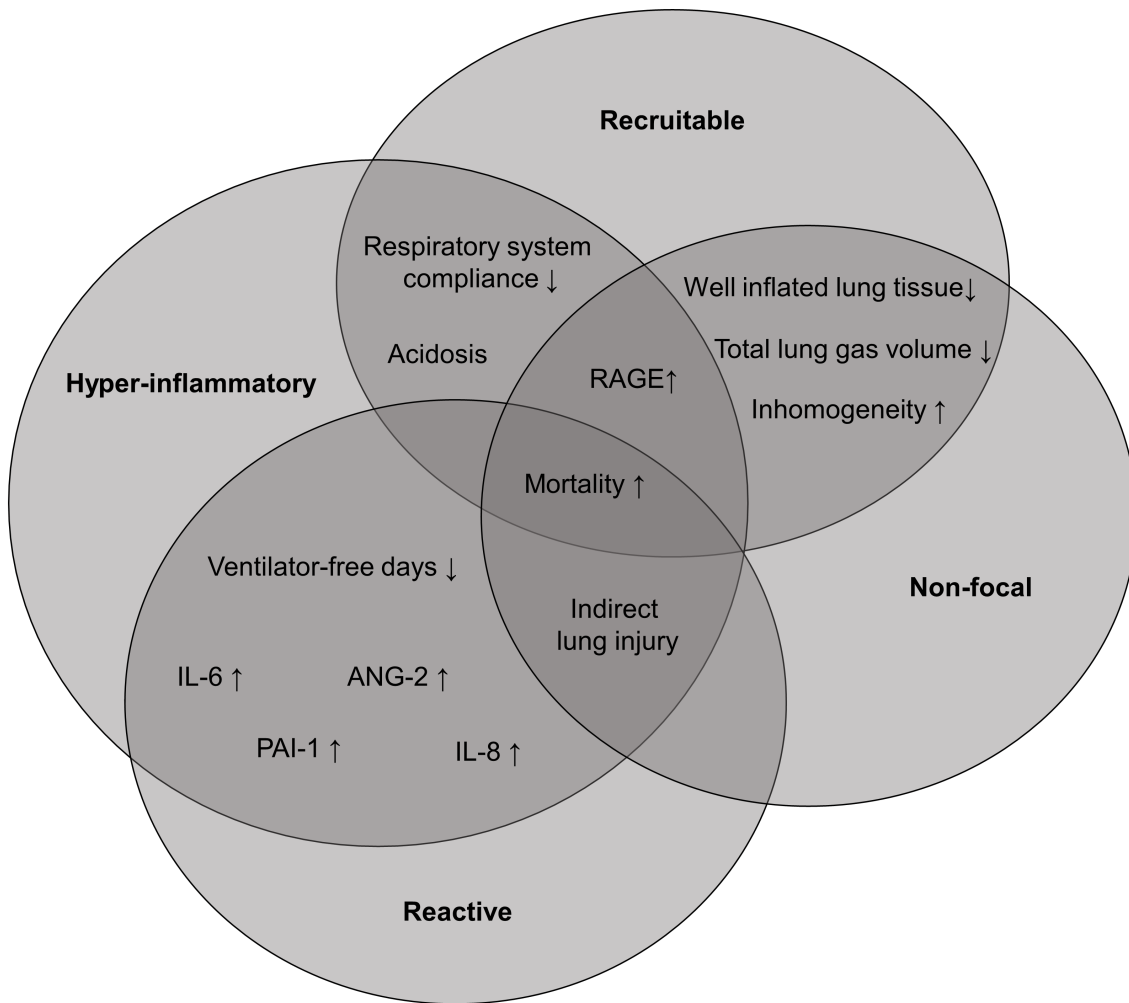
Given the multiple subphenotypes described in this review, it is possible that we end up with a multi-layered system just like in asthma, where stratification is based on age of symptom onset, lung function (FEV1), allergic status and type of airway inflammation [9]. In ARDS, the following layers could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

## 6. Towards precision medicine in ARDS

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

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





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

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

## 7. Conclusions

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

## AUTHOR CONTRIBUTIONS

NFLH, DCJJB, MJS, LDJB contributed to the study concept and design. NFLH performed the data collection and wrote the

first draft of the manuscript. DCJJB, MJS, LDJB commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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