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ORIGINAL ARTICLE

Biological Subphenotypes of Acute Respiratory Distress Syndrome Show Prognostic Enrichment in Mechanically Ventilated Patients without Acute Respiratory Distress Syndrome

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Rationale: Recent studies showed that biological subphenotypes in acute respiratory distress syndrome (ARDS) provide prognostic enrichment and show potential for predictive enrichment.

Objectives: To determine whether these subphenotypes and their prognostic and potential for predictive enrichment could be extended to other patients in the ICU, irrespective of fulfilling the definition of ARDS.

Methods: This is a secondary analysis of a prospective observational study of adult patients admitted to the ICU. We tested the prognostic enrichment of both cluster-derived and latent-class analysis (LCA)–derived biological ARDS subphenotypes by evaluating the association with clinical outcome (ICU-day, 30-day mortality, and ventilator-free days) using logistic regression and Cox regression analysis. We performed a principal component analysis to compare blood leukocyte gene expression profiles between subphenotypes and the presence of ARDS.

Measurements and Main Results: We included 2,499 mechanically ventilated patients (674 with and 1,825 without ARDS). The cluster-derived "reactive" subphenotype was, independently of ARDS, significantly associated with a higher probability of ICU mortality, higher 30-day mortality, and a lower probability of successful extubation while alive compared with the "uninflamed" subphenotype. The blood leukocyte gene expression profiles of individual subphenotypes were similar for patients with and without ARDS. LCA-derived subphenotypes also showed similar profiles.

Conclusions: The prognostic and potential for predictive enrichment of biological ARDS subphenotypes may be extended to mechanically ventilated critically ill patients without ARDS. Using the concept of biological subphenotypes for splitting cohorts of critically ill patients could add to improving future precision-based trial strategies and lead to identifying treatable traits for all critically ill patients.

Keywords: acute respiratory distress syndrome; phenotypes; critically ill; personalized medicine

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This article has a related editorial.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

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At a Glance Commentary

Scientific Knowledge on the Subject: Biological subphenotypes identified in acute respiratory distress syndrome (ARDS) allow for prognostic enrichment and show potential for predictive enrichment. Identification of these subphenotypes relies on the measurement of plasma markers of inflammation, coagulation, and endothelial injury. To date, it remains unclear whether these subphenotypes also allow for prognostic enrichment and show potential for predictive enrichment in patients not fulfilling the definition of ARDS.

What This Study Adds to the Field: We show that biological subphenotypes identified in ARDS may be extended to mechanically ventilated critically ill patients without ARDS. The association between subphenotype and outcome remained, irrespective of the presence of ARDS, providing strong evidence for prognostic enrichment. More importantly, blood leukocyte gene expression profiles clustered together per individual subphenotype irrespective of the presence of ARDS. Taken together, these results suggest that the concept of splitting cohorts of critically ill patients through biological subphenotyping can be extended outside of ARDS. This could improve subphenotype-aware intervention studies and result in treatable traits for all critically ill patients.

Acute respiratory distress syndrome (ARDS) can be triggered by a multiplicity of conditions, without a singular underlying pathophysiologic mechanism that is present in all patients (1). This is reflected in its biological heterogeneity, which has been used to inform the differentiation of biological subphenotypes that might serve as treatable traits (2). Cluster analysis distinguished a "reactive" and "uninflamed" subphenotype (3), whereas latent-class analysis (LCA), revealed a "hyperinflammatory" and "hypoinflammatory" subphenotype (4). These subphenotypes can be identified based on parsimonious models that include plasma markers of coagulation, inflammation, and endothelial injury (3, 5).

The "reactive" and "hyperinflammatory" subphenotype was associated with a poor outcome, which was independent from clinically relevant factors like Acute Physiology and Chronic Health Evaluation (APACHE) IV score (3–5), providing possibilities for selection of higher-risk patients (prognostic enrichment) (6). Previous work has shown that a third of the genes in blood leukocytes were differentially expressed between cluster-derived subphenotypes (7). These differentially expressed genes could be traced back to different pathways, supporting the biological heterogeneity of patients with ARDS and possibly providing a precision-based therapeutic angle (7). Indeed, biological subphenotypes have been suggested to provide predictive enrichment (i.e., selecting patients more likely to respond to a given therapy) (6). Patients with a "hyperinflammatory" subphenotype seemed to respond differently to the application of high positive end-expiratory pressure (PEEP), conservative fluid management, and simvastatin administration (4, 8, 9).

The host responses used to classify patients are not unique to ARDS (10). Similar subphenotypes have also been identified in patients with acute kidney injury and acute respiratory failure (10–12). Based on these observations, the question arises whether these subphenotypes are limited to ARDS. We, therefore, hypothesized that biological subphenotypes, as identified in ARDS, can be extended to patients not fulfilling the definition of ARDS. Specifically, the cluster-derived "reactive" and LCA-derived "hyperinflammatory" biological subphenotypes will retain the association with ICU mortality, 30-day mortality, and ventilator-free days (prognostic enrichment), irrespective of the presence of ARDS. In addition, we postulate that blood leukocyte gene expression profiles are linked to the biological subphenotypes independently from ARDS, which would be suggestive of a shared immunological endotype.

Methods

Study Design and Ethical Considerations

This study was a secondary analysis of the MARS (Molecular Diagnosis and Risk Stratification of Sepsis) study (ClinicalTrials.gov identifier NCT01905033). From 2011 to 2013, two university-based tertiary care hospitals (the Academic Medical Center in Amsterdam and the University Medical Center Utrecht in Utrecht, both in the Netherlands) performed this prospective observational cohort study at their mixed ICUs. The study protocol and opt-out consent method used for this study were approved by the Institutional Review Board of both study centers (IRB: 10-056C). Parts of the methods and data were previously described in reports about biological subphenotypes in ARDS and their link with blood leukocyte gene expression profiles (3, 7).

Population

The original MARS study included consecutive adult patients admitted to the ICU between 2011 and 2013, with an expected length of stay longer than 24 hours. For every admitted patient, the plausibility of infection was assessed using a four-point scale (ascending from none, possible, probable, to definite), as described previously (13, 14). The current analysis is scoped to a subset of patients *1*) both invasively ventilated and noninvasively ventilated (e.g., noninvasive positive-pressure ventilation), and *2*) of whom blood biomarkers were available and collected within 24 hours of ICU admission. Sepsis was defined as the presence of infection diagnosed within 24 hours after ICU admission with a probable or definite likelihood in combination with

Author Contributions: All authors contributed to the study concept and design. L.D.J.B. performed the data collection. N.F.L.H. and L.D.J.B. performed the data analysis and wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

A complete list of the MARS Consortium members may be found before the beginning of the References.

the sepsis-3 criteria for sepsis (13–15). A dedicated team of trained researchers screened for the presence of ARDS on a daily basis during the patient's ICU admission. ARDS was initially scored according to the American–European consensus criteria (16). In reevaluation, all cases were scored based on the current Berlin definition (17) as described previously (3).

Sample Collection

Blood from all included patients was collected *1*) in a plastic vacuum container filled with ethylenediaminetetraacetic acid for biological subphenotype characterization and *2*) in PAXgene Blood RNA tubes (Becton-Dickinson) for blood leukocyte gene expression. Both samples were obtained within 24 hours of ICU admission.

Plasma Biomarker–based Biological Subphenotypes

The collected ethylenediaminetetraacetic acid blood sample was centrifuged directly (1,500 G for 15 min), after which the plasma was frozen at −80°C for batch-wise analysis. IL-6, IL-8, and IFN-γ were determined in the plasma samples using cytometric bead analysis (Flex Set multiplex assay; BD Biosciences) according to the manufacturer's instructions. Plasminogen activator inhibitor-1, protein-C, ANG-1 (angiopoietin-1), and ANG-2 (angiopoietin-2) were measured with Luminex (Biorad) (13) according to the manufacturer's instructions.

The biological subphenotypes were distinguished based on the two parsimonious models previously proven to split ARDS patients into two groups (3, 4). First, a cluster-based model distinguishing an "uninflamed" and "reactive" subphenotype based on plasma concentrations of IL-6, IFN-γ, ANG-1, ANG-2, and PAI-1 (plasminogen activator inhibitor-1) (3). Second, an LCA model revealing a "hypoinflammatory" and "hyperinflammatory" subphenotype using plasma concentrations of IL-8, protein-C, and bicarbonate (4, 5).

Microarray Analysis of Blood Leukocyte Gene Expression

The gene expression profiles in all blood samples were generated using Human Genome U219 96-array plates and the GeneTitan instrument (Affymetrix) (18, 19). MARS gene expression data are available in the Gene Expression Omnibus, via accession number GSE65682. Data were preprocessed as described previously (7). In addition to the MARS study cohort, blood samples were also collected from healthy control subjects to serve as an additional control group.

Statistical Analysis

First, the ARDS subphenotype of each patient was identified based on plasma biomarkers, as described previously (3, 5). Second, demographic and clinical patient characteristics were compared between subphenotypes stratified for the presence of ARDS. Differences between groups were tested with the Student's *t* test, the Mann-Whitney *U* test, and the chi-squared test, as appropriate. Third, logistic regression analysis (*"lrm"* package) was used to assess the prognostic enrichment of the biological subphenotypes, with 30-day mortality as the primary outcome. The model was adjusted for selected clinically relevant variables: age, sex, diagnosis of ARDS, and APACHE IV. In addition, time-to-event analysis (*"survival," "survminer"* package) was performed to assess the ICU mortality and the probability of successful extubation while alive. Univariable and multivariable Cox proportional hazard models of patients with and without ARDS were performed with ICU mortality or ventilator-free days (adjudicated 28 d after admission) as the primary outcome and stratified per subphenotype. In the multivariable analysis, the primary outcome was adjusted for age, sex, and APACHE IV. Fourth, blood leukocyte gene expression profiles were assessed using principal component (PC) analysis. For this analysis, we used the set of top differentially expressed genes in blood leukocytes between the "uninflamed" and "reactive" biological subphenotypes in ARDS, as identified previously by Bos and colleagues (7). This gene set projected onto PCs to reduce the dimensionality using the same loading factors as before. The first two PCs, respectively explaining 56% and 8% of the variance, were used in this analysis, and means were compared by Tukey's "Honest Significant Difference" (*"stats"* package) between the five groups (no ARDS: uninflamed; no ARDS: reactive; ARDS: uninflamed; ARDS: reactive; and healthy control subjects). Fifth, a sensitivity analysis was performed with only invasively ventilated patients. Finally, each analysis was performed for both cluster-derived subphenotypes and LCA-derived subphenotypes. This was to ensure consistency and generate generalizable results regardless of the chosen phenotyping method. A $P = 0.05$ was considered statistically significant. All analyses were performed in R version 3.6.2. using the R-studio interface.

Results

Patient Population

A total of 2,499 patients were included in the analysis, of whom 674 (27%) fulfilled the definition of ARDS and 1,825 (73%) did not. Of these totals, 1,159 patients without ARDS (63.5%) had a cluster-derived "uninflamed" subphenotype, whereas 666 (36.5%) had a "reactive" subphenotype. Patients without ARDS with the "reactive" subphenotype were more severely ill on admission than the "uninflamed" subphenotype. Notably, they had a higher APACHE IV score, a higher sequential organ failure assessment score on the day of admission, and lower compliance of the respiratory system (*see* Table 1 and Figures E1A and E2A in the online supplement). Despite the different characteristics of patients with and without ARDS (Table E1), the subphenotype-associated differences seen in patients without ARDS were comparable to those of patients with ARDS split into a "reactive" and "uninflamed" subphenotype (Table E2). The LCA-derived subphenotypes showed similar subphenotype-associated differences to the cluster-derived subphenotypes (Tables 1 and E2 and Figures E1B and E2B). When looking at overlap between both subphenotyping methods, 369 of all patients with a "hyperinflammatory" subphenotype without ARDS had a "reactive" subphenotype, but 51 matched an "uninflamed" subphenotype (55.4% and 4.4% of respectively "reactive" and "uninflamed" patients). As for the "hypoinflammatory" subphenotype, 1,097 patients had an "uninflamed" subphenotype and 294 had a "reactive" subphenotype (94.7% and 44.1% of respectively "uninflamed" and "reactive" patients) (*see* Tables E3 and E4).

Table 1. Demographics and Clinical Characteristics of Patients without ARDS

		Cluster Subphenotypes			LCA Subphenotypes		
		Uninflamed (*n* = 1,159)	Reactive (*n* = 666)	*P* Value	Hypoinflammatory (*n* = 1,391)	Hyperinflammatory (*n* = 420)	*P* Value
Patient characteristics							
Sex, M, *n* (%)		750 (64.7)	395 (59.3)	0.03	899 (64.6)	238 (56.7)	0.004
Age in years, median (IQR)		62 (50–71)	64 (54–73])	0.002	62 (51–71)	64 (54–72)	0.03
Admission type, *n* (%)	Medical	774 (66.8)	414 (62.2)	0.007	914 (65.7)	262 (62.4)	<0.001
	Surgical elective	196 (16.9)	102 (15.3)	—	247 (17.7)	50 (11.9)	—
	Surgical emergency	189 (16.3)	149 (22.4)	—	230 (16.5)	108 (25.7)	—
	Unknown	0 (0.0)	1 (0.2)	—	0 (0.0)	0 (0.0)	—
Comorbidities, *n* (%)	COPD	154 (13.3)	78 (11.7)	0.37	191 (13.7)	41 (9.8)	0.04
	Diabetes mellitus	200 (17.3)	136 (20.4)	0.12	239 (17.2)	94 (22.4)	0.02
	Immunodeficiency	140 (12.1)	121 (18.2)	<0.001	170 (12.2)	90 (21.4)	<0.001
	Liver cirrhosis	15 (1.3)	29 (4.4)	<0.001	22 (1.6)	22 (5.2)	<0.001
	Hematologic malignancy	28 (2.4)	45 (6.8)	<0.001	42 (3.0)	31 (7.4)	<0.001
	Metastatic malignancy	35 (3.0)	22 (3.3)	0.84	38 (2.7)	18 (4.3)	0.15
	Nonmetastatic solid tumor	126 (10.9)	76 (11.4)	0.78	149 (10.7)	53 (12.6)	0.32
Admission characteristics							
APACHE IV, mean (SD)		70 (25.2)	89 (29.50)	<0.001	72 (25.85)	92.47 (30.31)	<0.001
SOFA score on day of admission, median (IQR)		6 (3–8)	9 (6–11)	<0.001	6 (4–8)	9 (7.0–12.0)	<0.001
Infection, *n* (%)		612 (52.8)	460 (69.1)	<0.001	766 (55.1)	298 (71.0)	<0.001
Source of infection*, *n* (%)	Abdominal	98 (16.0)	136 (29.6)	<0.001	120 (15.7)	114 (38.3)	<0.001
	Cardiovascular	62 (10.1)	71 (15.4)	—	99 (12.9)	33 (11.1)	—
	Central nervous system	26 (4.2)	8 (1.7)	—	30 (3.9)	4 (1.3)	—
	Respiratory	341 (55.7)	145 (31.5)	—	397 (51.8)	84 (28.2)	—
	Skin	15 (2.5)	33 (7.2)	—	22 (2.9)	25 (8.4)	—
	Urological	40 (6.5)	48(10.4)	—	57 (7.4)	31 (10.4)	—
	Other	30 (4.9)	19 (4.1)	—	41 (5.4)	7 (2.3)	—
Sepsis-3 criteria*, *n* (%)		605 (98.9)	460 (100)	0.06	763 (99.6)	298 (100)	0.66
Septic shock, *n* (%)		64 (5.5)	179 (26.9)	<0.001	100 (7.2)	143 (34.0)	<0.001
SOFA score: circulatory, median (IQR)		2 (0.0–4.0)	4 (1.0–4.0)	<0.001	3 (1.0–4.0)	4 (3.0–4.0)	<0.001
Bicarbonate, median (IQR)		21.9 (19.0–25.3)	18.2 (14.9–21.8)	<0.001	22.0 (19.2–25.2)	15.6 (12.8–18.3)	<0.001
Lactate, median (IQR)		2.1 (1.4–3.2)	3.6 (2.2–6.5)	<0.001	2.2 (1.4–3.4)	4.1 (2.4–8.1)	<0.001
CRP, median (IQR)		55.0 (8.0–150.5)	94.5 (28.0–194.8)	<0.001	60.0 (10.0–153.0)	101.0 (32.3–197.8)	<0.001
Creatinine, median (IQR)		83.0 (64.0–121.3)	141.0 (93.0–214.0)	<0.001	88.0 (66.0–130.0)	155.0 (107.8–231.3)	<0.001
Bilirubin, median (IQR)		11.0 (7.0–17.0)	16.0 (9.0–30.5)	<0.001	11.0 (7.0–18.0)	18.0 (10.5–34.0)	<0.001
Respiratory variables							
Invasively ventilated, *n* (%)		893 (77.0)	546 (82.0)	0.01	1,091 (78.4)	343 (81.7)	0.17
PEEP in cm H_2O, median (IQR)		5.0 (5.0–8.0)	7.0 (5.0–10.0)	<0.001	5.0 (5.0–8.0)	8.0 (5.0–10.0)	<0.001
ΔP, median (IQR)		12.0 (8.0–15.0)	14.0 (10.0–18.0)	<0.001	12.0 (9.0–16.0)	15.0 (11.0–19.0)	<0.001
Pa_{O_2}/FI_{O_2} ratio, median (IQR)		260.0 (197.6–343.8)	232.5 (172.5–322.9)	<0.001	251.5 (193.8–338.2)	237.1 (167.5–332.9)	0.03
Crs in ml/cm H_2O, median (IQR)		40.3 (29.2–61.6)	34.0 (24.5–52.6)	<0.001	39.6 (28.7–60.2)	31.0 (23.1–46.6)	<0.001
Outcome							
Ventilator-free days, median (IQR)		25.0 (17.0–27.0)	22.0 (0.0–27.0)	<0.001	25.0 (12.8–27.0)	19.5 (0.0–27.0)	<0.001
ICU length of stay, d, median (IQR)		4.0 (2.0–7.0)	5.0 (2.0–10.0)	<0.001	4.0 (2.0–8.0)	5.0 (2.0–9.0)	0.01
ICU mortality, *n* (%)		103 (8.9)	168 (25.2)	<0.001	145 (10.4)	124 (29.5)	<0.001
30-d mortality, *n* (%)		189 (16.3)	199 (29.9)	<0.001	236 (17.0)	149 (35.5)	<0.001

Definition of abbreviations: APACHE IV = Acute Physiology and Chronic Health Evaluation score IV; ARDS = acute respiratory distress syndrome; COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein; Crs = compliance of the respiratory system; ΔP = driving pressure; IQR = interquartile range; LCA = latent-class analysis; PEEP = positive end-expiratory pressure; SOFA = sequential organ failure assessment score on day of admission.

Bicarbonate: min 24 hours. Lactate: max 24 hours. CRP: first 24 hours. Creatinine: max 24 hours. Bilirubin: max 24 hours. Cluster subphenotypes as described by Bos and colleagues (3); LCA subphenotypes model 3 as described by Sinha and colleagues (5).

*Source of infection and sepsis-3 criteria depict the number and percentage of the patients who also had an infection. The number and percentage of septic shock refer to all included patients.

Table 2. Predictors of ICU Mortality in Critically Ill Patients without Acute Respiratory Distress Syndrome

	Univariable		Cluster Subphenotypes Multivariable		LCA Subphenotypes Multivariable	
Predictor	**Hazard Ratio (95% CI)**	***P* Value**	**Hazard Ratio (95% CI)**	***P* Value**	**Hazard Ratio (95% CI)**	***P* Value**
Reactive subphenotype	2.43 (1.90–3.11)	<0.001	1.66 (1.28–2.16)	<0.001	—	—
Hyperinflammatory subphenotype	2.54 (2.00–3.24)	<0.001	—	—	1.69 (1.31–2.19)	<0.001
Age, yr	1.02 (1.01–10.3)	<0.001	1.01 (1.00–1.02)	0.08	1.01 (1.00–1.02)	0.13
Sex, M	1.05 (0.82–1.34)	0.71	1.10 (0.86–1.41)	0.47	1.11 (0.86–1.42)	0.43
APACHE IV	1.02 (1.02–1.03)	<0.001	1.019 (1.015–1.023)	<0.001	1.019 (1.015–1.023)	<0.001
SOFA	1.13 (1.09–1.17)	<0.001	—	—	—	—
Presence of septic shock	2.00 (1.54–2.60)	<0.001	—	—	—	—
$Pa_{O_2}/F_{I_{O_2}}$ ratio	1.00 (0.998–1.001)	0.46	—	—	—	—

Definition of abbreviations: APACHE IV = Acute Physiology and Chronic Health Evaluation score IV; CI = confidence interval; LCA = latent-class analysis; SOFA = sequential organ failure assessment score on day of admission.
Cluster subphenotypes as described by Bos and colleagues (3); LCA subphenotypes model-3 as described by Sinha and colleagues (5).

Prognostic Enrichment

Patients not fulfilling the definition of ARDS with a cluster-derived "reactive" subphenotype had a higher ICU mortality (25.2%) and 30-day mortality (29.9%) than the "uninflamed" subphenotype (respectively: 8.9% and 16.3%) (Tables 1 and Figures E3A and E4A). A "reactive" subphenotype was associated with higher 30-day mortality (odds ratio [OR], 1.48; 95% confidence interval [CI], 1.16–1.89; $P = 0.002$), independently from ARDS (interaction term: OR, 1.30; 95% CI, 0.84–2.00; $P = 0.24$) and after adjusting for age, sex, diagnosis of ARDS, and APACHE IV score, compared with the "uninflamed" subphenotype. Univariable analysis showed that the cluster-derived "reactive" subphenotype was associated with a higher probability of ICU mortality (ARDS: hazard ratio [HR], 2.34; 95% CI, 1.66–3.23; $P < 0.001$; without ARDS: HR, 2.43; 95% CI, 1.90–3.11; $P < 0.001$) (Table 2 and Figure 1A) and a lower probability for successful extubation while alive compared with the "uninflamed" subphenotype in both patients with ARDS (HR, 0.57; 95% CI, 0.47–0.69; $P < 0.0001$) and without ARDS (HR, 0.66; 95% CI, 0.59–0.74; $P < 0.0001$) (Table 3 and Figure 2A). The association between subphenotype and the probability of both ICU mortality and successful extubation while alive remained significant when adjusted for age, sex, and APACHE IV score (Tables 2 and 3).

Similar associations were found in LCA-derived subphenotypes: the "hyperinflammatory" subphenotype was associated with a higher ICU mortality probability, higher 30-day mortality, and lower probability for successful extubation while alive, compared with the "hypoinflammatory" subphenotype (Tables 2 and 3 and Figures 1B and 2B).

Blood Leukocyte Gene Expression Profiles

Blood leukocyte gene expression profiles of 719 patients were available for analysis (Table E7). Patients with a "reactive" and "uninflamed" subphenotype clustered together after PC analysis of gene expression profiles irrespective of the presence of ARDS (Figure 3A). This was reflected by a nonsignificant difference between patients with and without ARDS for PC2 within the "reactive" subphenotype ($P = 0.50$) and the "uninflamed" subphenotype ($P = 0.21$) (Figure 3A). Furthermore, gene expression profiles did significantly differ between patients with a "reactive" and "uninflamed" subphenotype, irrespective of ARDS status (no ARDS: $P < 0.001$; ARDS: $P < 0.001$) (Table E7). These results were confirmed in the LCA-derived subphenotypes (Figure 3B). Both "hyperinflammatory" and "hypoinflammatory" subphenotypes did not significantly differ between patients with ARDS and without ARDS for PC2 ($P = 0.54$; $P = 0.35$) (Figure 3B).

Sensitivity Analysis

A sensitivity analysis that only included invasively ventilated patients confirmed the previously mentioned associations (*see* online supplement: sensitivity analysis).

Discussion

The prognostic enrichment of biological subphenotypes as identified in ARDS also applies to critically ill ICU patients not

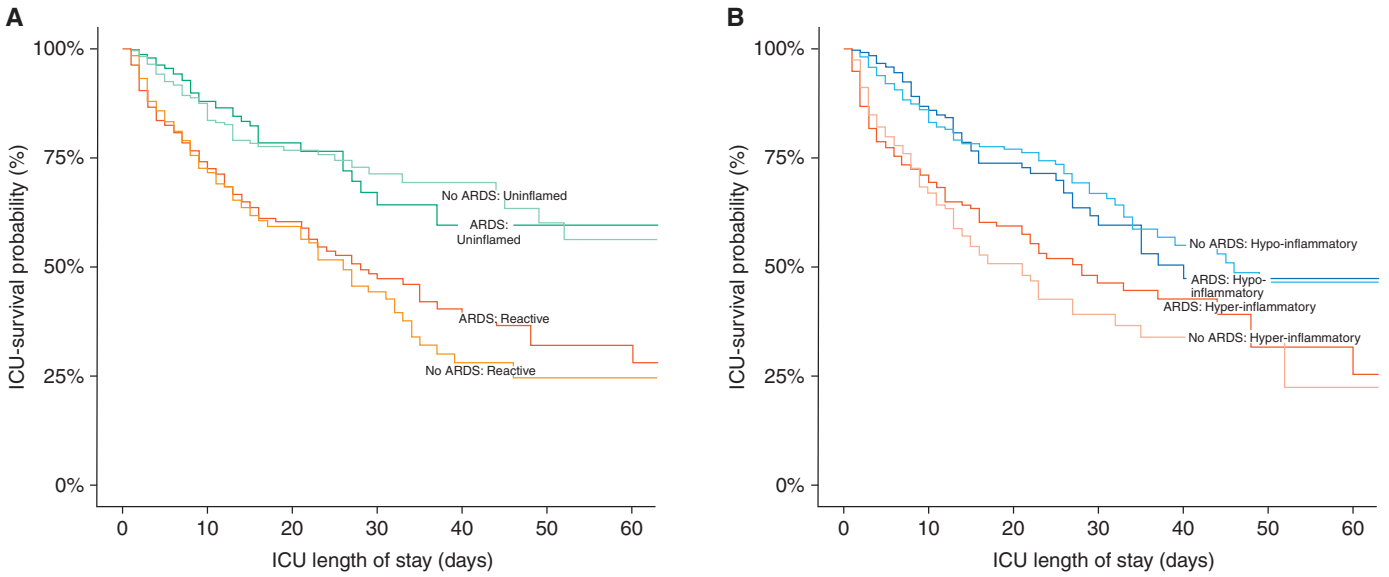

Figure 1. ICU survival probability for ICU length of stay by cluster subphenotype (*A*) and by latent-class analysis subphenotype (*B*). Both figures depict the univariable Cox proportional hazard modeling. Cluster subphenotypes as described by Bos and colleagues (3) and latent-class analysis subphenotypes model 3 as described by Sinha and colleagues (5). ARDS = acute respiratory distress syndrome.

Table 3. Predictors of Successful Extubation while Alive in Critically Ill Patients without Acute Respiratory Distress Syndrome

	Univariable		Cluster Subphenotypes Multivariable		LCA Subphenotypes Multivariable	
Predictor	**Hazard Ratio (95% CI)**	***P* Value**	**Hazard Ratio (95% CI)**	***P* Value**	**Hazard Ratio (95% CI)**	***P* Value**
Reactive subphenotype	0.66 (0.59–0.74)	<0.001	0.86 (0.76–0.97)	0.01	—	—
Hyperinflammatory subphenotype	0.62 (0.54–0.70)	<0.001	—	—	0.82 (0.71–0.94)	0.005
Age, yr	0.99 (0.99–1.00)	<0.001	1.000 (0.996–1.003)	0.94	1.000 (0.997–1.003)	0.98
Sex, M	0.97 (0.87–1.08)	0.59	0.93 (0.83–1.03)	0.17	0.92 (0.83–1.03)	0.15
APACHE IV	0.98 (0.98–0.99)	<0.001	0.99 (0.98–0.99)	<0.001	0.99 (0.98–0.99)	<0.001
SOFA	0.92 (0.91–0.93)	<0.001	—	—	—	—
Presence of septic shock	0.54 (0.46–0.64)	<0.001	—	—	—	—
Pa_{O_2}/FI_{O_2} ratio	1.002 (1.001–1.002)	<0.001	—	—	—	—

Definition of abbreviations: APACHE IV = Acute Physiology and Chronic Health Evaluation score IV; CI = confidence interval; LCA = latent-class analysis; SOFA = sequential organ failure assessment score on the day of admission.
Cluster subphenotypes as described by Bos and colleagues (3); LCA subphenotypes model-3 as described by Sinha and colleagues (5).
A hazard ratio below 1 indicates a lower probability of successful extubation while alive.

fulfilling the definition of ARDS, with similar ORs for mortality, irrespective of ARDS status. In addition, the subphenotypes showed similar profiles of blood leukocyte gene expression, irrespective of the presence of ARDS. Thus, biological subphenotypes identified in ARDS might be generalizable to other critically ill patients in the ICU.

In this study, we used two different parsimonious models to identify previously validated subphenotypes of ARDS in patients without ARDS. Although the cluster-derived and LCA-derived subphenotypes show similar clinical characteristics (3, 5, 20) they do not identify exactly the same groups. Almost all patients with the "hyperinflammatory" subphenotype were also classified as having the "reactive"

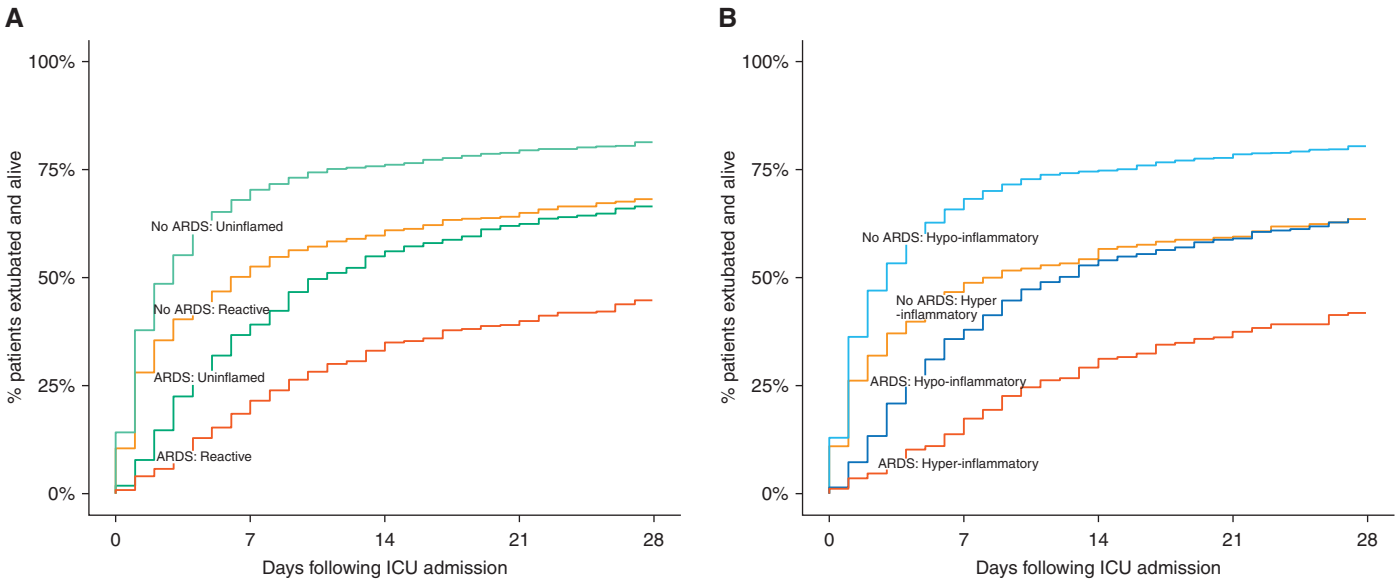

Figure 2. Percentage of patients extubated and alive over time by cluster subphenotype (*A*) and by latent-class analysis subphenotype (*B*). Both figures depict the univariable Cox proportional hazard modeling. Cluster subphenotypes as described by Bos and colleagues (3) and latent-class analysis subphenotypes model 3 as described by Sinha and colleagues (5). ARDS = acute respiratory distress syndrome.

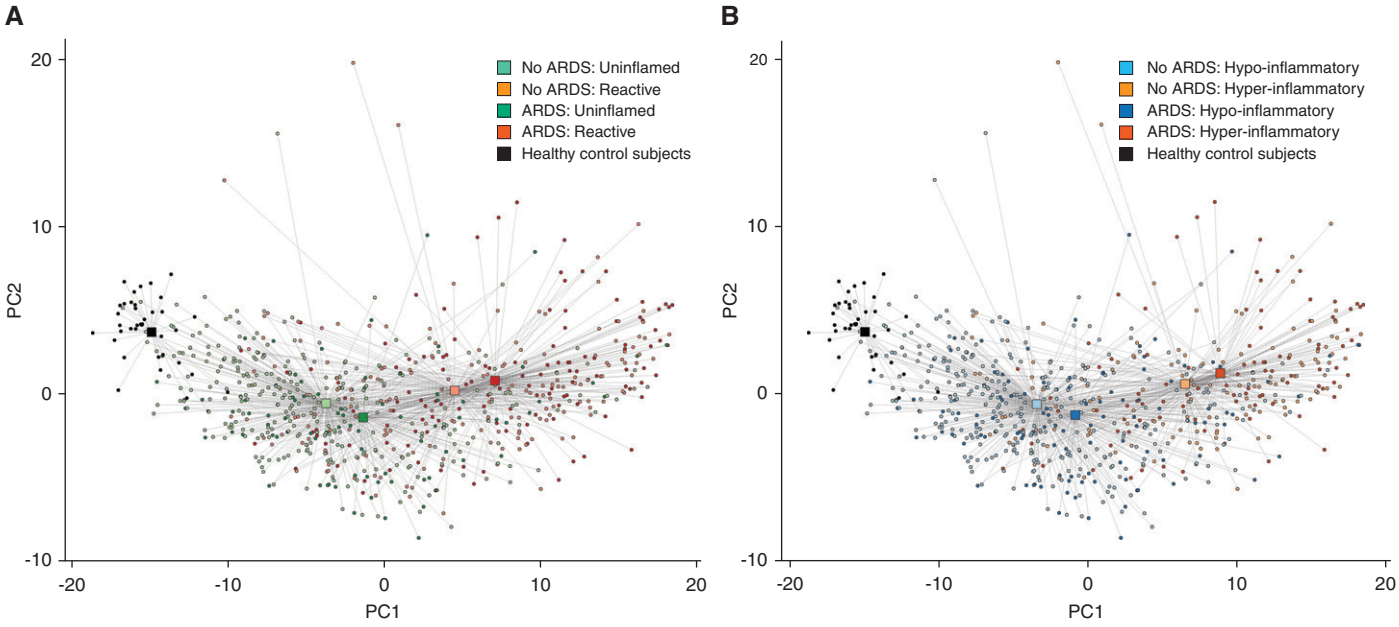

Figure 3. Principal component (PC) analysis by cluster subphenotype (*A*) and by latent-class analysis subphenotype (*B*). On the *x*-axis PC1 explaining approximately 56% of the variance and on the *y*-axis PC2 explaining approximately 8% of the variance in the most differentially expressed genes. The colors identify groups, the rectangles represent the centroid per group, and the dots represent individual patients. Cluster subphenotypes are as described by Bos and colleagues (3); LCA subphenotypes model 3 are as described by Sinha and colleagues (5). ARDS = acute respiratory distress syndrome.

subphenotype, but there was a considerable subset of patients with the "reactive" subphenotype who were classified as "hypoinflammatory." Yet, the gene expression profiles were remarkably similar between the two classifiers, and the same patterns were observed. Further studies are needed to compare clustering and latent-class methods and the utility of either in identifying subphenotypes of critical illness.

Classification into the "reactive" and "hyperinflammatory" subphenotype increased the odds of mortality and longer mechanical ventilation in patients without ARDS similarly to patients with ARDS. This

extends previous studies that showed prognostic enrichment of subphenotypes in specific subgroups of critically ill patients (3, 5, 19, 21) and suggests that biological variation in one syndrome may translate to patients without that syndrome. Indeed, two subgroups of critical illness with different survival rates were recently identified based on whole-blood transcriptomics and derived immune cell fractions, without preselecting for a singular etiology of critical illness (10). Interestingly, pathways traced back to the identified differentially expressed genes did not differ statistically significant between the various etiologies of the included critically ill patients, suggesting that the underlying immune processes are possibly shared across disease etiologies in critical illness. Taken together, the data suggest that there are shared systemic immune processes that provide prognostic enrichment in a heterogeneous cohort of critically ill patients without a singular syndrome diagnosis.

The studied leukocyte gene expression profiles were more dependent on subphenotypes classification than the presence or absence of ARDS. The differentially expressed genes between the cluster-derived subphenotypes have previously been traced back to the corresponding pathways (7). The "reactive" subphenotype was characterized by upregulation of individual neutrophil related genes and enrichment of canonical pathways related to oxidative phosphorylation and mitochondrial dysfunction. Our data suggest that these gene expression profiles are by no means unique to ARDS and syndrome overlapping biological processes seem to be captured by the subphenotypes. Interestingly, a systemic neutrophilic response seems to be predictive for increased mortality throughout datasets (10).

Although generalizability of subphenotypes to patients without ARDS would be promising, translation into pathway-specific pharmacologic interventions is not trivial (20, 22). The insights gained increase current knowledge about the subphenotypes identified in ARDS but also revealed knowledge gaps. These gaps emphasize the need to elucidate the natural history of biological heterogeneity, the overlap between and stability of both subphenotyping strategies, the beneficial and harmful aspects of host response within each subphenotype, and the biological progression or resolution during the evolution of the underlying condition, to be able to move toward precision-based trial strategies and biologically tailored precision therapies.

This study has several strengths. This is the first study to investigate the extension of both prognostic and potential for predictive enrichment of ARDS subphenotypes to other critically ill patients within the ICU. The main strength of this study is the consistent alternation in leukocyte gene expression between subphenotypes, irrespective of the presence of ARDS. It is noteworthy that the biological subphenotypes still added value in prognostic analyses even after correcting for APACHE IV. Furthermore, both cluster-derived and LCA-derived subphenotypes and plasma biomarkers and blood leukocyte expression profiles showed similar results, suggesting a strong and consistent underlying biological signal. This is remarkable because cluster-derived subphenotypes were differentiated based on only biomarkers and LCA-derived subphenotypes on both biomarker and clinical data. However, it has not yet been validated that the subphenotypes, respectively identified in an observational study and randomized controlled trials, capture the same biologic variation as suggested by our data and this requires further confirmation. It should be noted that the prevalence of the "reactive" subphenotype in ARDS was higher than that of the "hyperinflammatory" subphenotype.

Several limitations should be taken into account. Although the data suggest an underlying host response being captured by the ARDS subphenotypes, possibly enabling the generalizability, our results are based on observational data. We can only speculate about possible explanations and no definitive conclusions can be reached. Further research is needed to unravel the underlying biological processes and potential clinical value. This should include the development of clinically useful bedside tests to facilitate future studies (ClinicalTrial.gov: NCT04009330) (23). Second, although our dataset was sufficiently large for the analyses required to answer our research questions, our findings need additional external validation. Third, as we did not repeat *de novo* clustering or LCA, it is important to be clear that we do not provide statistical evidence that a two subphenotype approach is a "better" way to describe patients without ARDS than the conventional methods. This remains an unanswered question for future research. Finally, in the original MARS study, plasma biomarkers were only available for a selected set of patients (2,499 out of 5,920 patients in the cohort). Plasma biomarkers were preferentially measured in patients with an infection likelihood of probable or definite and matching noninfectious populations. For example, all patients undergoing major elective gastrointestinal surgery were previously used as a control population for abdominal sepsis (24, 25). Therefore, selection bias could have occurred. Yet, the characteristics of the patients included in this study are similar to those of other populations of unselected patients in the ICU, with the possible exception of a lower percentage of patients after elective surgery in this analysis (26–28).

Conclusions

Our findings show that the prognostic enrichment of biological subphenotypes as identified in ARDS can be extended to mechanically ventilated critically ill patients not fulfilling the definition of ARDS and that the blood leukocyte gene expression profiles are similar between these subphenotypes. In the long run, generalizing the concept of biological subphenotypes could add to improving precision-based trial strategies in the general ICU population by splitting and lead to identifying treatable traits for all critically ill patients. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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