Host-Response Subphenotypes Offer Prognostic Enrichment in Patients With or at Risk for Acute Respiratory Distress Syndrome*

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Objectives: Classification of patients with acute respiratory distress syndrome into hyper- and hypoinflammatory subphenotypes using plasma biomarkers may facilitate more effective targeted therapy. We examined whether established subphenotypes are present not only in patients with acute respiratory distress syndrome but also in patients at risk for acute respiratory distress syndrome (ARFA) and then assessed the prognostic information of baseline subphenotyping on the evolution of host-response biomarkers and clinical outcomes.

Design: Prospective, observational cohort study.

Setting: Medical ICU at a tertiary academic medical center.

Patients: Mechanically ventilated patients with acute respiratory distress syndrome or ARFA.

Interventions: None.

Measurements and Main Results: We performed longitudinal measurements of 10 plasma biomarkers of host injury and inflammation. We applied unsupervised latent class analysis methods utilizing baseline clinical and biomarker variables and

demonstrated that two-class models (hyper- vs hypoinflammatory subphenotypes) offered improved fit compared with one-class models in both patients with acute respiratory distress syndrome and ARFA. Baseline assignment to the hyperinflammatory subphenotype (39/104 [38%] acute respiratory distress syndrome and 30/108 [28%] ARFA patients) was associated with higher severity of illness by Sequential Organ Failure Assessment scores and incidence of acute kidney injury in patients with acute respiratory distress syndrome, as well as higher 30-day mortality and longer duration of mechanical ventilation in ARFA patients ($p < 0.0001$). Hyperinflammatory patients exhibited persistent elevation of biomarkers of innate immunity for up to 2 weeks postintubation.

Conclusions: Our results suggest that two distinct subphenotypes are present not only in patients with established acute respiratory distress syndrome but also in patients at risk for its development. Hyperinflammatory classification at baseline is associated with higher severity of illness, worse clinical outcomes, and trajectories of persistently elevated biomarkers of host injury and inflammation during acute critical illness compared with hypoinflammatory patients. Our findings provide strong rationale for examining treatment effect modifications by subphenotypes in randomized clinical trials to inform precision therapeutic approaches in critical care. (*Crit Care Med* 2019; 47:1724–1734)

Key Words: bacterial infections; endophenotypes; inflammation; pneumonia; sepsis; respiratory distress syndrome, adult

Biologic and clinical heterogeneity in sepsis and acute respiratory distress syndrome (ARDS) result in heterogeneous responses to investigational therapies, making it challenging to predict which patients are likely to derive benefit (1–4). It is thus a major research priority to identify subsets of critically ill patients (commonly referred to as "subphenotypes") who either have higher risk of disease-related outcome (*prognostic enrichment*) or differential responses to therapy (*predictive enrichment*) that could enable precision medicine trials in critical care (4–6).

Recent subphenotyping work in ARDS has consistently demonstrated the presence of two distinct subsets of patients (hyper- and hypoinflammatory subphenotypes) (5). These subphenotypes emerged from independent unsupervised examinations of clinical trial populations with latent class analysis (LCA) involving clinical and biomarker variables (7–10), as well as in an observational cohort study with cluster analysis of biomarker data only (11). Hyperinflammatory ARDS patients had higher mortality (5, 12) and differential responses to positive end-expiratory pressure levels, conservative fluid management, and statin treatment (8–10).

For clinical application of ARDS subphenotyping, a parsimonious predictive model with three biomarkers has been proposed (soluble tumor necrosis factor receptor [TNFR]-1, interleukin [IL]-8, and bicarbonate) (10). However, it remains unknown whether the subphenotypes discovered in ARDS may also be present in broader critically ill populations, such as patients with severe pneumonia or extrapulmonary sepsis, who do not meet diagnostic criteria but are at risk for ARDS (ARFA). Furthermore, given that patient clusters are discriminated by nonspecific biomarkers of innate immune function (i.e., TNFR1 and IL-8), our understanding of the potential molecular pathways involved in the determination of subphenotypes is limited.

We sought to determine whether there is evidence of distinct subphenotypes in an independent observational cohort of mechanically ventilated patients, including not only patients with ARDS but also ARFA, and in that case, to examine whether subphenotypic classification offers prognostic enrichment in heterogeneous critically ill populations beyond just patients with ARDS.

MATERIALS AND METHODS

Extensive methods are provided in the **Supplement** (Supplemental Digital Content 1, http://links.lww.com/CCM/E995).

Clinical Cohort

From October 2011 to January 2018, we prospectively enrolled a convenience sample of adult patients with acute respiratory failure, who were intubated and mechanically ventilated in the medical ICU at the University of Pittsburgh Medical Center. Exclusion criteria included inability to obtain informed consent, presence of tracheostomy, or mechanical ventilation for more than 72 hours prior to enrollment. The study was approved by the University of Pittsburgh Institutional Review Board (protocol PRO10110387), and written informed consent was provided by all participants or their surrogates.

From enrolled subjects, we collected serial blood samples for up to 2 weeks during their ICU stay at the following intervals: "baseline" (within 48 hr of intubation), "middle" (days 3–6 from intubation), "late" (days 7–10), and "very late" (days 11–14) interval. We prospectively collected baseline demographics, comorbidities, physiologic, mechanical ventilation and laboratory variables, and calculated Sequential Organ Failure Assessment (SOFA) scores.

Biomarker Analyses

Plasma levels of 10 biomarkers with validated associations with ARDS were measured with a customized Luminex assay (R&D Systems, Minneapolis, MN) (13) and classified into the following categories: 1) "innate immune responses" (IL-6, IL-8, IL-10, TNFR1, suppression of tumorigenicity [ST]-2, and fractalkine) (9, 14–16); b) "epithelial injury" (receptor of advanced glycation end products [RAGEs]) (17); c) "endothelial injury" (angiopoietin-2) (18); and d) "host-response to bacterial infections" (procalcitonin and pentraxin-3) (19, 20).

Clinical Classifications

A consensus committee reviewed clinical and radiographic data to retrospectively classify subjects into three distinct

clinical categories without knowledge of biomarkers: a) "ARDS" per Berlin criteria (21); b) "ARFA," based on the presence of an identifiable lung injury risk factor on enrollment, but not fulfilling ARDS criteria (22); and c) "patients not at risk for ARDS" (hereafter referred to as "Controls"), including patients intubated for airway protection or hypoxemia from congestive heart failure for whom no lung injury risk factor was identified.

Subphenotypic Classifications

We performed subphenotypic classifications separately in the ARDS and ARFA subgroups. Given that subphenotyping has not been previously applied in our patient population, we first estimated the optimal number of classes that best fit our cohort by applying LCA models similarly to previous descriptions (9, 10). We considered clinical and biomarker variables in LCA models similar to the ones previously used in the ARDS clinical trials, as well as variables not previously examined, such as procalcitonin and fractalkine (**Table S1**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). We did not apply LCA modeling in the Control group due to small sample size.

To examine whether a previously published, parsimonious model (10) can accurately predict subphenotypic assignments by LCA in our cohort, we obtained predicted probabilities for classification to the hyperinflammatory versus hypoinflammatory subphenotype from the following three-variable regression model using baseline values:

$$\text{Subphenotype} = 2.25 - 1.97 \times (\text{IL} - 8) + 1.71 \times (\text{bicarbonate}) - 1.71 \times (\text{TNFR1}).$$

We evaluated the agreement of subphenotypic classifications between the LCA and the predictive model with Gwet agreement coefficient (23) and area under the curve statistic. We considered the LCA-derived subphenotypes in our primary analyses with clinical outcomes.

Outcomes

We followed patients prospectively for occurrence rate of acute kidney injury (AKI) (24) or shock (defined as need for vasopressors) within the first week from enrollment, ICU length of stay, ventilator-free days (VFDs) (25), time-to-liberation from mechanical ventilation, and 30- and 90-day mortality.

Statistical Analyses

We performed LCA in STATA v.15 (StataCorp LLC, College Station, TX) and all other analyses in R v.3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Clinical groups (ARDS, ARFA, or Controls) and subphenotypes were compared with Wilcoxon tests and Fisher exact tests for continuous and categorical variables, respectively. We graphically examined the discriminatory continuous variables for the LCA-derived subphenotypes by plotting their standardized values (z-scaled to mean of 0 and SD of 1). As further validation for the significant associations of both continuous and categorical variables with subphenotypes, we performed network analyses with Probabilistic Graphical Models (26, 27). We then selected the variables that were independently associated with the subphenotype classification variable (i.e., feature selection of first neighbors) to derive parsimonious logistic regression models for subphenotype classifications separately for patients with ARDS and ARFA in our cohort. For 30- and 90-day mortality, we constructed logistic regression models and calculated adjusted odds ratios (ORs) for the effects of baseline subphenotypes. For survival and time-to-liberation, we also performed time-to-event analyses using Kaplan-Meier curves and Cox proportional hazard models. Finally, we evaluated the trajectories of plasma biomarkers over time with mixed linear regression models with random patient intercepts. Details on statistical models used are provided in the Supplement (Supplemental Digital Content 1, http://links.lww.com/CCM/E995).

RESULTS

Cohort Description

We enrolled 272 patients (104 with ARDS, 108 ARFA, and 60 Controls; **Table 1**; and **Table S3**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995) comprising a total of 597 longitudinal samples for analyses (**Fig. S2**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). Pneumonia, aspiration, and extrapulmonary sepsis were the most common lung injury risk factors in ARDS and ARFA patients. Compared with ARFA patients, those with ARDS had higher frequency of pneumonia, worse hypoxemia, higher plateau pressures, and experienced longer ICU stay and fewer VFDs (all $p < 0.01$).

Subphenotype Classifications and Associated Baseline Variables

LCA applied separately in ARDS and ARFA patients provided evidence that two-class models offered an improved fit compared with one-class models in both patient groups (**Table S4** and **Fig. S3**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). Thirty-eight percent of ARDS patients were assigned to class 2, characterized by elevated levels of RAGE, creatinine, TNFR1, and reduced bicarbonate (**Fig. 1**, ***A*** and ***B***), similarly to the previously described hyperinflammatory subphenotype (5), in terms of frequency (27–37% in previous studies) and associated discriminatory variables. Furthermore, 28% of ARFA patients were also assigned to class 2 (Fig. 1*A*) with a similar distribution of discriminatory variables from class 1 (**Fig. 1*C***; and Fig. S3, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). Thus, LCA models revealed that the hyperinflammatory class 2 (for consistency hereafter referred to as *hyperinflammatory subphenotype*) is present not only in ARDS patients but also in a clinically significant proportion of ARFA patients. Predicted subphenotypic assignments from the parsimonious three-variable regression model showed good agreement with the LCA-derived subphenotypes (Gwet agreement coefficients, 0.83–0.86; Fig. 1*A*; and **Fig. S4**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995).

TABLE 1. Baseline Characteristics and Clinical Outcomes of Enrolled Patients, Categorized as Acute Respiratory Distress Syndrome (ARDS), at Risk for ARDS (ARFA), and Controls Not ARFA

Variable	ARDS (*n* = 104)	ARFA (*n* = 108)	Controls (*n* = 60)	*p* ARDS vs ARFA	*p* ARDS vs Controls
Age, median (IQR), yr	56.0 (43.9–64.4)	59.7 (47.3–68.5)	55.8 (45.4–65.5)	**0.04**	0.61
Males, *n* (%)	53 (51.0)	65 (60.2)	36 (60.0)	0.23	0.34
Body mass index, median (IQR)	30.0 (24.9–35.2)	29.6 (24.9–36.5)	28.2 (25.2–33.7)	0.93	0.61
History of chronic disease, *n* (%)					
Diabetes	29 (27.9)	43 (39.8)	22 (36.7)	0.09	0.32
Chronic obstructive pulmonary disease	21 (20.2)	32 (29.6)	8 (13.3)	0.15	0.37
Immunosuppression	20 (19.2)	15 (13.9)	14 (23.3)	0.39	0.67
Alcohol use	15 (14.4)	18 (16.7)	12 (20.0)	0.79	0.48
Risk factors for ARDS					
Pneumonia, *n* (%)	70 (67.3)	45 (41.7)	0 (0.0)	**< 0.01**	**< 0.01**
Sepsis, *n* (%)	25 (24.0)	40 (37.0)	0 (0.0)	0.06	**< 0.01**
Aspiration, *n* (%)	19 (18.3)	31 (28.7)	0 (0.0)	0.1	**< 0.01**
Lung injury prediction score, median (IQR)	6.0 (5.0–7.0)	5.5 (4.9–7.0)	3.0 (2.0–5.0)	0.19	**< 0.01**
Severity of illness					
Sequential Organ Failure Assessment score, median (IQR)[a]	7.0 (5.0–9.0)	7.0 (4.8–9.0)	5.0 (4.0–8.0)	0.24	**0.01**
Pao_2:Fio_2 ratio, median (IQR), mm Hg	132.5 (84.8–186.2)	168.0 (117.0–222.8)[b]	182.5 (136.8–225.2)	**< 0.01**	**< 0.01**
Hemodynamics					
Heart rate, median (IQR), beats/min	90.5 (78.0–107.2)	89.0 (78.0–105.0)	85.5 (75.0–95.2)	0.49	**0.02**
Systolic blood pressure, median (IQR), mm Hg	111.0 (101.8–125.2)	115.5 (101.0–130.2)	121.5 (105.8–145.5)	0.12	**< 0.01**
Shock, *n* (%)	66 (63.5)	60 (55.6)	22 (36.7)	0.3	**< 0.01**
Laboratory parameters, median (IQR)					
pHa	7.4 (7.3–7.4)	7.4 (7.3–7.4)	7.4 (7.3–7.4)	0.26	0.05
WBC, × 10^9/L	12.9 (9.3–17.7)	13.6 (9.9–17.9)	10.8 (7.8–15.7)	0.56	0.07
Platelets, × 10^9/L	186.5 (113.2–261.0)	179.0 (124.5–237.8)	174.5 (119.8–230.2)	0.55	0.43
Creatinine, mg/dL	1.4 (0.8–2.5)	1.5 (0.8–2.7)	1.1 (0.7–2.1)	0.48	0.36
Serum carbon dioxide, mEq/L	23.5 (20.0–27.0)	23.0 (20.0–26.0)	24.0 (22.0–26.0)	0.35	0.34
Mechanical ventilation parameters, median (IQR)					
Respiratory rate, 1/min	24.0 (20.0–28.0)	20.0 (16.0–24.0)	20.0 (16.0–23.2)	**< 0.01**	**< 0.01**
Positive end-expiratory pressure, cm	10.0 (5.0–12.0)	5.0 (5.0–8.5)	5.0 (5.0–5.0)	**< 0.01**	**< 0.01**
Plateau pressure, cm	24.5 (21.0–29.0)	19.0 (17.0–23.0)	18.5 (15.0–22.0)	**< 0.01**	**< 0.01**
Tidal volume (per kg of predicted body weight), mL/kg	6.4 (5.9–7.3)	6.8 (6.0–7.6)	6.7 (6.0–7.4)	0.12	0.45

(*Continued*)

TABLE 1. (*Continued*). Baseline Characteristics and Clinical Outcomes of Enrolled Patients, Categorized as Acute Respiratory Distress Syndrome (ARDS), at Risk for ARDS (ARFA), and Controls Not ARFA

Variable	ARDS (*n* = 104)	ARFA (*n* = 108)	Controls (*n* = 60)	*p* ARDS vs ARFA	*p* ARDS vs Controls
Outcomes					
Acute kidney injury, *n* (%)	81 (77.9)	77 (71.3)	30 (50.0)	0.35	**< 0.01**
Duration of mechanical ventilation, median (IQR), d	8.0 (5.0–15.0)	6.0 (4.0–11.0)	4.0 (2.8–7.0)	**< 0.01**	**< 0.01**
ICU length of stay, median (IQR), d	12.0 (8.0–21.0)	8.0 (5.0–13.0)	5.0 (4.0–9.2)	**< 0.01**	**< 0.01**
Ventilator-free days, median (IQR), d	12.0 (0.0–21.0)	19.0 (0.0–24.0)	23.5 (8.8–25.0)	**< 0.01**	**< 0.01**
30-d mortality, *n* (%)	31 (29.8)	29 (26.9)	12 (20.0)	0.75	0.23
90-d mortality, *n* (%)	38 (36.5)	30 (27.8)	13 (21.7)	0.22	0.07

ARDS = acute respiratory distress syndrome, ARFA = at risk for ARDS, IQR = interquartile range.

[a]Sequential Organ Failure Assessment (SOFA) score calculation does not include the neurologic component of SOFA score because all patients were intubated and receiving sedative medications, impairing our ability to perform assessment of the Glasgow Coma Scale in a consistent and reproducible fashion.

[b]Ninety-four (87%) of at risk for ARDS (ARFA) patients had hypoxemic respiratory failure (Pao_2:Fio_2 ratio, < 300) but did not meet radiographic ARDS criteria.

Data are presented as median (with interquartile ranges) for continuous variables and *n* (%) for categorical variables. *p* values for comparisons between ARDS versus ARFA and ARDS versus Controls, obtained from Wilcoxon test for continuous variables and Fisher test for categorical variables. Statistically significant *p* values ($p < 0.05$) are highlighted in bold. Of the 104 patients with ARDS, 99 (95%) met ARDS diagnostic criteria in the baseline interval (within 48 hr of intubation) and the remaining five (5%) in the middle interval (3–6 d from intubation).

Hyperinflammatory ARDS and ARFA patients (as defined by the LCA models) were similar in terms of demographics, comorbid conditions, lung injury risk factors, and mechanical ventilation parameters compared with hypoinflammatory patients, with the exception of higher incidence of extrapulmonary sepsis in hyperinflammatory ARFA patients ($p = 0.02$) (**Table S5**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). In both ARDS and ARFA groups, hyperinflammatory patients had higher leukocytosis and creatinine levels, lower platelet counts and bicarbonate levels (all $p < 0.05$), as well as markedly higher levels of all 10 measured biomarkers (all $p < 0.01$) (Table S5, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). Similar results were obtained when we used the parsimonious model predicted subphenotypes (**Table S6**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). In order to delineate which of these differentially distributed clinical and biomarker variables were independently associated with the LCA-derived subphenotypes, we used Probabilistic Graphical Model analysis (**Fig. S5**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). We identified a small subset of biomarkers (RAGE, TNFR1, and fractalkine) and clinical variables (creatinine, temperature, bicarbonate, and arterial pH) that were independently informing on the subphenotype variable (first neighbors) in ARDS patients. These first neighbor variables were the ones with the largest standardized differences between the LCA-derived subphenotypes (extremes of the distribution of variables in Fig. 1*B*). Similarly, in ARFA patients, four biomarkers were first neighbors of subphenotypes: TNFR1, IL-10, fractalkine, and angiopoietin-2 (Fig. S5, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). With these first neighbor variables as predictors, we derived parsimonious logistic regression models that showed high accuracy for predicting LCA-derived subphenotypic assignments in our cohort (93.2% and 98.0% for ARDS and ARFA patients, respectively; **Table S7**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). Thus, Probabilistic Graphical Models further underscored the importance of small set of discriminatory variables identified by the LCA models (Fig. 1, *B* and *C*).

Severity of Illness and Clinical Outcomes by Subphenotypes

In univariate analyses, hyperinflammatory ARDS or ARFA patients had significantly higher SOFA scores and occurrence rate of AKI compared with hypoinflammatory patients ($p < 0.01$) (**Table 2**). Hyperinflammatory ARDS patients also showed a trend toward fewer VFDs ($p = 0.09$) and had a numerically higher absolute 90-day mortality (44% versus 32%), although these differences were not statistically significant. Hyperinflammatory ARFA patients had significantly fewer VFDs and higher 90-day mortality (53% vs 18%; $p < 0.01$), both in univariate (Table 2) and multivariate analyses (adjusted OR for 90-d mortality, 6.3; 95% CI, 2.0–19.7; **Table S8**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). These associations were also corroborated by time-to-event analyses for survival and time-to-liberation from mechanical ventilation (**Fig. 2**; and **Table S9**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995).

Overall, the associations of subphenotypes with clinical outcomes were similar when we used the assignments from the parsimonious predictive model (10) instead of the LCA in both patient groups (ARDS and ARFA; Table S6, Supplemental

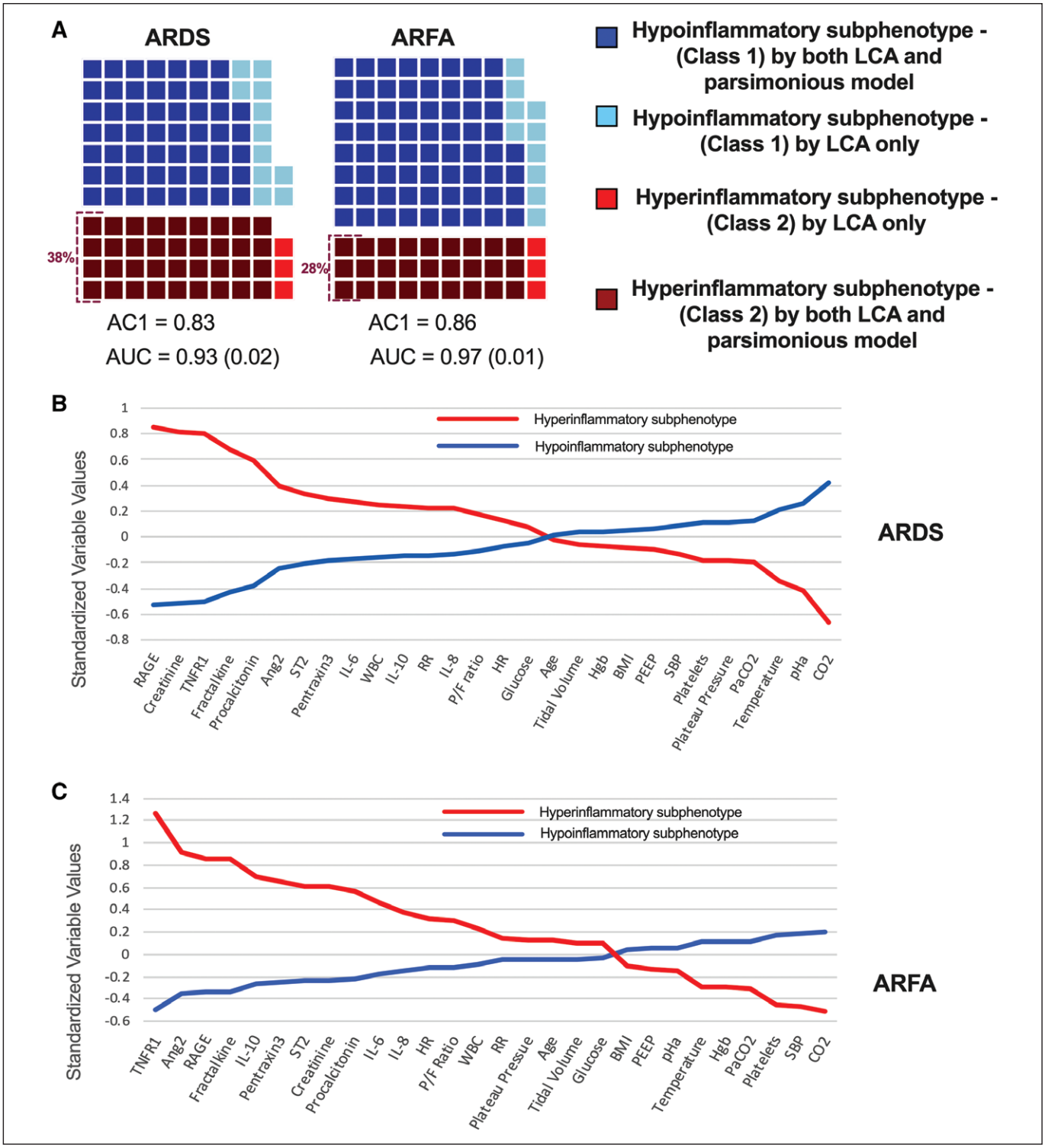

Figure 1. Distribution of subphenotypes derived by the latent class analysis (LCA) and the three-variable parsimonious predictive models in patients with acute respiratory distress syndrome (ARDS) and at risk for ARDS (ARFA) (**A**), and differences in standardized values of each continuous variable by LCA subphenotypes in ARDS (**B**) and ARFA patients (**C**). **A**, The two waffle graphs illustrate the distribution of the hyperinflammatory versus hypoinflammatory patients in patients with ARDS and ARFA, as well as the agreement of subphenotypic assignments by the two methods used. Hyperinflammatory patients defined by both the LCA and the parsimonious model are shown in *dark red boxes*, whereas hyperinflammatory patients defined only by the LCA method are shown in *light red* (with the same depictions in *blue color* for hypoinflammatory patients). *Light blue* and *red color boxes* represent patients misclassified by the parsimonious model if we consider the LCA method as the reference standard in our cohort. The two methods had good agreement by Gwet agreement coefficient 1 (AC1) and area under the curve (AUC with SD) statistics. **B**–**C**, The variables are sorted on the basis of the degree of separation between the subphenotypes from maximum positive separation on the left (i.e., hyperinflammatory higher than hypoinflammatory). All variables were standardized by mean scaling to zero and SD to 1. Ang2 = angiopoietin-2, BMI = body mass index, Hgb = hemoglobin, HR = heart rate, IL = interleukin, PEEP = positive end expiratory pressure, P/F ratio = Pao_2/Fio_2 ratio, RAGE = receptor of advanced glycation end-product, RR = respiratory rate, SBP = systolic blood pressure, ST-2 = suppression of tumorigenicity, TNFR1 = tumor necrosis factor receptor.

TABLE 2. Severity of Illness and Clinical Outcomes Between Latent Class Analysis–Derived Subphenotypes in Patients With Acute Respiratory Distress Syndrome (ARDS) and at Risk for ARDS

	ARDS			At Risk for ARDS		
Variable	Hypoinflammatory	Hyperinflammatory	*p*	Hypoinflammatory	Hyperinflammatory	*p*
n	65	39		78	30	
Sequential Organ Failure Assessment score, median (IQR)[a]	6.0 (4.0–8.0)	9.0 (7.5–11.0)	**< 0.01**	6.0 (4.0–8.0)	9.0 (7.0–12.0)	**< 0.01**
Pao_2:Fio_2 ratio, median (IQR), mm Hg	120.0 (84.0–178.0)	158.0 (93.5–203.5)	0.09	164.0 (110.0–205.0)	201.5 (155.0–274.2)	**0.04**
Shock, *n* (%)	38 (58.5)	28 (71.8)	0.25	35 (44.9)	25 (83.3)	**< 0.01**
Acute kidney injury, *n* (%)	43 (66.2)	38 (97.4)	**< 0.01**	49 (62.8)	28 (93.3)	**< 0.01**
Duration of mechanical ventilation, median (IQR), d	9.0 (6.0–15.0)	7.0 (5.0–13.5)	0.38	6.0 (4.0–9.8)	6.5 (4.2–12.8)	0.37
ICU length of stay, median (IQR), d	13.0 (9.0–21.0)	11.0 (7.0–19.0)	0.39	8.0 (5.0–12.8)	8.5 (5.2–14.0)	0.7
Ventilator-free days, median (IQR), d	14.0 (0.0–21.0)	0.0 (0.0–21.0)	0.09	22.0 (14.5–24.0)	0.0 (0.0–18.0)	**< 0.01**
30-d mortality, *n* (%)	16 (24.6)	15 (38.5)	0.2	13 (16.7)	16 (53.3)	**< 0.01**
90-d mortality, *n* (%)	21 (32.3)	17 (43.6)	0.34	14 (17.9)	16 (53.3)	**< 0.01**

ARDS = acute respiratory distress syndrome, IQR = interquartile range.

[a]Sequential Organ Failure Assessment (SOFA) score calculation does not include the neurologic component of SOFA score because all patients were intubated and receiving sedative medications, impairing our ability to perform assessment of the Glasgow Coma Scale in a consistent and reproducible fashion.

Data are presented as median (with IQR) for continuous variables and *n* (%) for categorical variables. *p* values for comparisons between hyperinflammatory and hypoinflammatory patients were obtained from Wilcoxon test for continuous variables and Fisher test for categorical variables. Statistically significant *p* values ($p < 0.05$) are highlighted in bold.

Digital Content 1, http://links.lww.com/CCM/E995). In a post hoc examination of outcomes by subphenotypic assignments by the parsimonious model in the Controls not at risk for ARDS, hyperinflammatory patients had much higher SOFA scores (median, 10.0 vs 5.0) and incidence of shock ($p = 0.01$), but due to low numbers in this patient subgroup, the numerically higher risk of mortality and AKI was not statistically significant (Table S6, Supplemental Digital Content 1, http://links.lww.com/CCM/E995).

Trajectories of Host-Response Biomarkers by Subphenotype

For patients who survived in the ICU during our study sampling period (up to 14 d postintubation), we examined the longitudinal evolution of the plasma biomarkers from available samples in the follow-up intervals, stratified by baseline interval subphenotypes. Hyperinflammatory ARDS patients in the baseline interval had persistently higher levels of innate immunity biomarkers (TNFR1, fractalkine, and ST-2) and procalcitonin at all follow-up intervals, with similar trajectory overtime compared with hypoinflammatory patients (**Table S10**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995; and **Fig. 3**). For the biomarkers of endothelial (angiopoietin-2) and epithelial injury (RAGE), the significant differences between subphenotypes at the baseline interval were attenuated over time, with evidence of significant reduction in RAGE only in the hyperinflammatory subphenotype. In ARFA patients, baseline hyperinflammatory subphenotype was associated with persistently elevated levels of biomarkers belonging to all four major pathways over the follow-up period (TNFR1, fractalkine, ST-2, RAGE, angiopoietin-2, procalcitonin) (**Table S11** and **Fig. S6**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995). Conversely, when we ignored the subphenotypic classifications and examined longitudinal trajectories of biomarkers stratified by clinical diagnosis of ARDS versus ARFA, no significant differences were seen (**Fig. S7**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995), thereby highlighting the prognostic enrichment offered by baseline subphenotyping beyond clinical diagnoses.

DISCUSSION

In an observational cohort of mechanically ventilated patients with acute respiratory failure, we employed unsupervised classification methods and demonstrated the presence of two distinct subphenotypes both in patients with ARDS and in those who remained at risk for the syndrome. Using LCA models, we considered multiple baseline clinical and biomarker variables for subphenotype derivation, and then identified a small subset of biomarkers of host injury and inflammation that

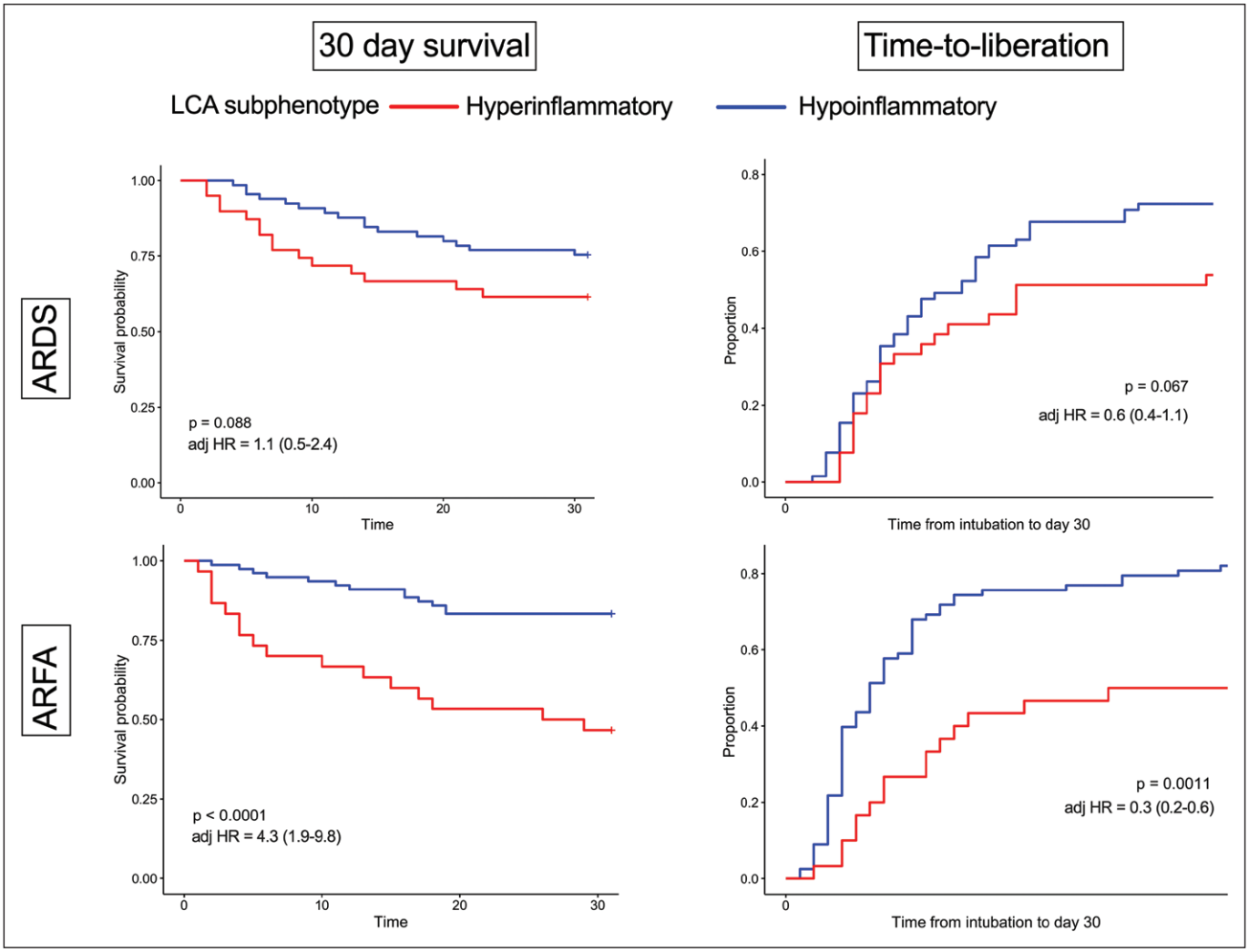

Figure 2. Kaplan-Meier curves for the outcomes of 30-day survival and time-to-liberation from mechanical ventilation for patients with acute respiratory distress syndrome (ARDS) (*top row*) or at risk for ARDS (ARFA) (*bottom row*), stratified by latent class analysis (LCA)-derived subphenotypes. *p* values for differences between subphenotypes were obtained with a log-rank test. Adjusted hazard ratios (HRs) with their 95% CIs for the effects of the hyperinflammatory subphenotype were obtained from multivariate Cox proportional hazards models. For 30-day survival, Cox models were adjusted for age, Pao_2:Fio_2 ratio, and Sequential Organ Failure Assessment (SOFA) scores. For time-to-liberation, Cox models were adjusted for Pao_2:Fio_2 ratio, SOFA scores, and positive end-expiratory pressure levels. Ninety-day survival data were very similar to 30-day and are not shown.

were mostly informative for subphenotypic assignments. Importantly, subphenotype predictions based on a previously validated, parsimonious three-variable regression model showed good agreement with our de novo subphenotype classifications, further supporting the external validity of subphenotype predictions beyond the index populations of clinical trials. Baseline hyperinflammatory classification was associated with organ dysfunction and severity of illness in patients with ARDS, as well as higher mortality and longer time-to-liberation from mechanical ventilation in ARFA patients. Hyperinflammatory patients exhibited higher levels of several biomarkers of injury and inflammation throughout their ICU stay.

Our findings expand the patient populations in whom biomarker-based subphenotyping may offer prognostic enrichment. In the original study and validation in independent cohorts, ARDS subphenotypes were discriminated by levels of biomarkers involved in pathways not uniquely specific to lung injury (i.e., IL-8, TNFR1, IL-6, interferon-γ, angiopoietin-2, and plasminogen activator inhibitor-1) (5, 12). We, therefore, hypothesized that similar subphenotypes would offer prognostic information in other critically ill patient populations. We were nonetheless surprised by the strong, independent effect size by which subphenotypes were associated with clinical outcomes in the ARFA population, that is, a six-fold increased risk for death, a difference that far exceeded the predicted risk by median SOFA scores (9.0 versus 6.0; $p < 0.01$) (Table 2, expected increase ≈ 75% [28]), indicating that point-of-care subphenotypic information may enhance prognostication beyond the capacity of available clinical tools.

The hyperinflammatory classification in our ARDS subgroup identified a sicker population with significantly higher rates of organ dysfunction, and trends toward longer time-to-liberation and worse survival, effects that were not statistically significant, in contrast to the results of prior studies (8–11). Limited statistical power is a plausible explanation (Table S2, Supplemental Digital Content 1, http://links.lww.com/CCM/E995), given that

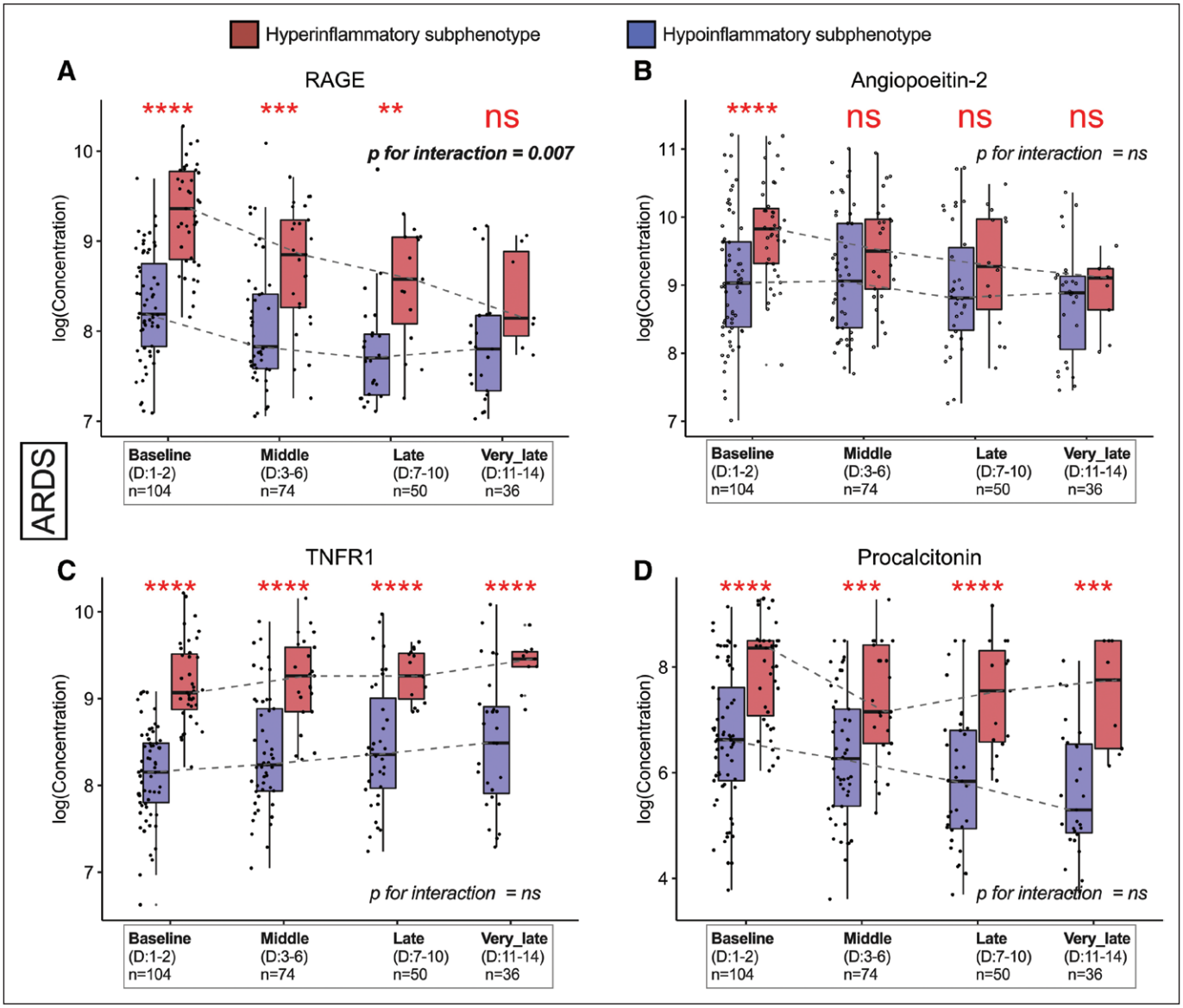

Figure 3. Patients with acute respiratory distress syndrome (ARDS) assigned to the hyperinflammatory subphenotype at baseline by latent class analysis had persistently higher levels of tumor necrosis factor receptor 1 (TNFR1) and procalcitonin compared with the hypoinflammatory subphenotype, whereas baseline differences in receptor of advanced glycation end-product (RAGE) and angiopoietin-2 were attenuated over time. Levels of statistical significance for between subphenotype comparisons obtained from Wilcoxon test at each follow-up time are shown with *red asterisks* (nonsignificant [ns] $p \geq 0.05$, * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and **** for $p < 0.0001$). *p* values for interaction were obtained from mixed linear regression models with random patient intercepts and inclusion of an interaction term subphenotype × follow-up interval. The significant *p* for interaction in the case of RAGE indicates that the trajectory of RAGE levels is different in the hyperinflammatory subphenotype (declining) compared with the hypoinflammatory subphenotype (no significant change over time).

the observed absolute differences in 90-day mortality between subphenotypes in our study were smaller than expected based on the Fluids and Catheters Treatment Trial (FACTT) subphenotype outcomes (12% vs 23%, respectively) (10). We noted that this diminished mortality difference in our cohort was accounted for by higher mortality in the hypoinflammatory ARDS patients compared with FACTT (32% vs 22%), whereas hyperinflammatory patients in both studies had similar mortality (44% vs 45%, respectively). Clinical differences in the enrolled populations could have contributed to the observed discrepancies, as our cohort included on average older patients with higher plateau pressures (**Table S12**, Supplemental Digital Content 1, http://links.lww.com/CCM/E995), and our observational study had very inclusive eligibility criteria, potentially enrolling moribund patients who would have been excluded in a clinical trial. Timing of sampling may have also played a role, given that we obtained baseline samples for subphenotypic assignment within 48 hours of intubation, whereas time to enrollment in FACTT was 48 hours from ARDS development (unknown time from intubation). Thus, some of the baseline subphenotypic assignments in FACTT could reflect time-points later in the course of ARDS evolution, which may have stronger associations with outcomes (29). Finally, the input clinical and biomarkers variables for our LCA models were only partially overlapping with

the variables considered in previous subphenotyping studies (7–11), and thus, the possibility for differential classifications between individual cohorts cannot be excluded.

Baseline subphenotyping offered important prognostic information on the temporal trajectories of biomarkers for patients who survived the acute phase of their critical illness in the ICU. In contrast, clinical diagnosis of ARDS (vs ARFA) offered no measurable prognostic information for evolution of biomarkers over time. Thus, subphenotypic information appears to be more important than clinical diagnosis for predicting the evolution of injury and inflammation in critically ill patients.

Integrative analyses with graphical modeling allowed us to comprehensively investigate the independent associations of variables with subphenotypic assignments in our dataset and complemented the LCA findings (28). In ARDS patients, we confirmed the independent associations of the hyperinflammatory subphenotype with TNFR1 and RAGE (10), and identified a new link with fractalkine, a marker of monocyte recruitment (15). In ARFA patients, the hyperinflammatory subphenotype was strongly associated with TNFR1 and fractalkine as in ARDS patients, but also with angiopoietin-2, which was further linked to procalcitonin and ST-2. Given the higher rate of extrapulmonary sepsis in hyperinflammatory ARFA patients (57% vs 30%), these direct links of biomarkers of bacterial infection and endothelial injury (procalcitonin and angiopoietin-2) may be reflective of the host-responses to the infectious cause of extrapulmonary sepsis that resulted into a hyperinflammatory subphenotype classification. These hypothesis-generating findings can guide future studies of the mechanistic underpinnings of subphenotypes. We further demonstrated that these small subsets of clinical and biomarker variables can be combined in predictive models for subphenotypic assignments with high accuracy in our cohort. Nonetheless, validation in external cohorts is needed before wide use can be recommended.

Our study is limited by its single-center design and sample size. Thus, the LCA-based classification in two subphenotypes should be considered hypothesis generating, especially for the ARFA subgroup, because with a larger sample size, it is possible that a three- or four-class model might provide better fit (30). However, we compared our LCA-derived subphenotypes to the ones predicted by a published predictive model and showed good agreement on classifications and similar prognostic enrichment, thus supporting the generalizability of our findings. We detected large effect sizes in the ARFA cohort and robust significant associations with biomarker data, confirming the adequacy of statistical power for the examined associations in this patient subgroup. With regard to the examined trajectories of biomarkers by baseline subphenotypes, inferences have to be cautious because biomarker data missingness at follow-up intervals are not random (i.e., patients can be lost to follow-up due to either early mortality or due to rapid clinical improvement and discharge from the ICU). Additionally, the premorbid inflammatory state of hyperinflammatory patients is unknown, and it is possible that such patients could have higher baseline levels of inflammation due to other comorbid conditions, or that early insults of acute illness may have resulted in persistently higher transcriptional levels over the study period examined. Finally, our observational study design did not allow us to examine for predictive enrichment by subphenotypes, that is, differential response to treatments.

In summary, we demonstrate that biomarker-based subphenotyping of mechanically ventilated patients is relevant not only in patients with ARDS but also in those at risk for ARDS, thus broadening the applicability of these subphenotypes to a much wider patient population. The two subgroups also had markedly distinct trajectories of host-response profiles, for which we identified novel subsets of biomarkers that could be used for prediction and also provide insight into possible mechanisms of the subphenotypes. Furthermore, we demonstrated that subphenotypic predictions offered by a predictive model developed in a clinical trial population had good agreement and similar performance with de novo–derived subphenotypes in an observational cohort. Such parsimonious and user-friendly models may help us detect subphenotypes in clinical practice in diverse patient populations with sepsis, pneumonia, or ARDS if rapid measurement of blood biomarkers becomes available (31). Our findings provide strong rationale for future studies of existing or ongoing clinical trials for examination of treatment effect modifications by subphenotypes in order to inform precision therapeutic approaches in critical care.

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