Heterogeneous Phenotypes of Acute Respiratory Distress Syndrome after Major Trauma

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Abstract

Rationale: Acute respiratory distress syndrome (ARDS) is a heterogeneous syndrome that can develop at various times after major trauma.

Objectives: To identify and characterize distinct phenotypes of ARDS after trauma, based on timing of syndrome onset.

Methods: Latent class analyses were used to identify patterns of ARDS onset in a cohort of critically ill trauma patients. Identified patterns were tested for associations with known ARDS risk factors and associations were externally validated at a separate institution. Eleven plasma biomarkers representing pathophysiologic domains were compared between identified patterns in the validation cohort.

Measurements and Main Results: Three patterns of ARDS were identified; class I (52%) early onset on Day 1 or 2, class II (40%) onset on Days 3 and 4, and class III (8%) later onset on Days

4 and 5. Early-onset ARDS was associated with higher Abbreviated Injury Scale scores for the thorax ($P < 0.001$), lower lowest systolic blood pressure before intensive care unit admission ($P = 0.003$), and a greater red blood cell transfusion requirement during resuscitation ($P = 0.030$). In the external validation cohort, early-onset ARDS was also associated with a higher Abbreviated Injury Scale score for the thorax ($P = 0.001$) and a lower lowest systolic blood pressure before intensive care unit enrollment ($P = 0.006$). In addition, the early-onset phenotype demonstrated higher plasma levels of soluble receptor for advanced glycation end-products and angiotensin-2.

Conclusions: Degree of hemorrhagic shock and severity of thoracic trauma are associated with an early-onset phenotype of ARDS after major trauma. Lung injury biomarkers suggest a dominant alveolar–capillary barrier injury pattern in this phenotype.

Keywords: acute respiratory distress syndrome; critical illness; phenotype; trauma; hemorrhagic shock

(Received in original form August 22, 2013; accepted in final form February 18, 2014)

Supported by National Institutes of Health grants HL60290, HL079063, HL007891, HL081332, HL112656, HL103836, HL115354.

Author Contributions: Conception and design: J.P.R., S.B., M.G.S.S., N.J.M., P.N.L., L.B.W., J.D.C.; acquisition of data: M.G.S.S., R.G., N.J.M., P.N.L., S.K., D.N.H., A.K.M., L.B.W., J.D.C.; analysis and interpretation of data: J.P.R., S.B., M.G.S.S., N.J.M., P.N.L., D.N.H., A.K.M., L.B.W., J.D.C.; drafting or revising the manuscript for important intellectual content: all authors; final approval of the version to be published: all authors.

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

Ann Am Thorac Soc Vol 11, No 5, pp 728–736, Jun 2014

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DOI: 10.1513/AnnalsATS.201308-280OC

Internet address: www.atsjournals.org

Acute respiratory distress syndrome (ARDS) is estimated to affect more than 190,000 patients annually in the United States, with a mortality exceeding 35% (1). In 1994, the American–European Consensus Conference (AECC) developed

standardized criteria for diagnosing acute lung injury and ARDS that are now widely used both in research and clinically (2). Acute lung injury was defined as acute-onset bilateral pulmonary infiltrates on chest radiograph, a ratio of PaO_2 to fraction of

inspired oxygen (FiO_2) less than or equal to 300, and the absence of left atrial hypertension. More recently, experts have modified the AECC criteria to improve the validity and reliability of the ARDS definition, using The “Berlin definition” (3).

The establishment of clear definitions of ARDS has led to significant advances in the standardization of populations in research studies; however, a number of studies have shown significant heterogeneity within the population of patients meeting consensus criteria for ARDS (4, 5). Heterogeneity has been described on the basis of predisposing insult, such as sepsis or trauma, and mechanism of injury, such as direct or indirect pulmonary injury (6, 7). Within trauma populations, different patterns of ARDS onset with distinct ARDS risk factors have been empirically described and may have different pathogenesis (8–11). We hypothesized that there are clinically distinct phenotypes of ARDS based on the timing of ARDS onset after severe trauma with distinct clinical risk factors and pathogenesis.

Latent class analysis (LCA) is a statistical method used to identify unobserved (latent) patterns underlying the observed heterogeneity in a population. The latent class approach assumes that the disease of interest represents a mixture of distinct subgroups that are not directly observed but can be determined on the basis of variables of interest, allowing for an empiric derivation of phenotypes of disease rather than using prespecified groups. Latent class models have been applied to identify various syndrome phenotypes, including classes of asthma (12–15).

The primary goal of this study was to define clinically meaningful subgroups within the diagnosis of ARDS by first applying a latent class modeling approach to a cohort of major trauma patients with ARDS based on the certainty of the ARDS diagnosis each day over 5 days. Second, we aimed to characterize the construct validity of resulting latent classes of ARDS by testing differences in both clinical ARDS risk factors and biomarkers known to be associated with ARDS risk and outcomes. Some of the results of this study were previously reported in the form of abstracts (16, 17).

Methods

Please see the online supplement for further details of the study methods.

Derivation Population

A cohort study of acutely injured trauma patients was used to derive ARDS classes.

Subjects were enrolled from 1999 to 2002 and from 2005 to 2008 at the Hospital of the University of Pennsylvania (Philadelphia, PA) (18, 19). All trauma patients presenting to the emergency department (ED) and admitted to the surgical intensive care unit (ICU) were screened. Inclusion criteria included age greater than 13 years, Injury Severity Score (ISS) greater than or equal to 16 (20), and traumatic injury within 24 hours of presentation. Outside hospital transfers were included only if they were transferred from another ED on the day of acute trauma. Exclusion criteria included discharge or death within 24 hours of admission, current or past evidence of congestive heart failure, and isolated head trauma. Patients with isolated head trauma were excluded because neurogenic pulmonary edema is believed to have a distinct pathogenesis from trauma-related ARDS (21), and the early mortality of patients with isolated head trauma and an ISS greater than or equal to 16 is extremely high, resulting in patients dying before developing ARDS.

All patients identified for the cohort were prospectively screened for ARDS and had clinical data collected during the first 5 days after admission. We monitored subjects for ARDS development for 5 days to capture ARDS cases directly related to acute incident trauma (22). Using our previous published methods, patients were classified during each 24-hour period from the time of admission as (1) definite ARDS, (2) equivocal, or (3) definite non-ARDS (23). Patients classified as definite ARDS met all of the AECC defining criteria for acute lung injury while intubated and receiving mechanical ventilation. The criteria include acute-onset confluent infiltrates on chest radiograph consistent with pulmonary edema, a $\text{PaO}_2/\text{FiO}_2$ ratio less than or equal to 300, and absence of left atrial hypertension (2). All available chest radiographs and arterial blood gases ordered for clinical purposes were evaluated. The timing of ARDS onset was determined by identifying the time when both arterial blood gas and chest radiograph criteria had been met, according to the consensus definition. Chest radiograph classification was determined by two physician-investigators, blinded to all data and each other's interpretations, who reviewed all available chest radiographs with adjudication by a third reviewer if necessary. Chest radiographs were

considered “positive” if bilateral confluent opacities consistent with pulmonary edema were present, “negative” if pulmonary opacities were not consistent with pulmonary edema or were only unilateral, and “equivocal” if reviewers could not confidently classify the chest radiograph (e.g., the infiltrates were difficult to distinguish from atelectasis or effusion, or the chest radiograph was technically deficient) (23). Patients who had a definitive diagnosis of ARDS on at least one of the first 5 days, defined as 24-hour periods from admission, were considered for inclusion in the LCA.

Validation Population

To enhance clinical usefulness, the classes resulting from the derivation population LCA were simplified as early- and late-onset ARDS with a 48-hour cutoff, based on data inspection. This 48-hour cutoff for early and late onset was used for validation in a separate cohort of patients admitted to an ICU at Vanderbilt University Medical Center (Nashville, TN) from 2006 to 2011 (the VALID [Validating Acute Lung Injury Biomarkers for Diagnosis] Study). Patients were eligible if they were at least 18 years of age, suffered an acute traumatic injury within 24 hours of ED presentation, and were admitted to the ICU for at least 48 hours, regardless of ISS. Subjects were enrolled on the morning after ICU admission. Details of this cohort have been previously described (24, 25). Eleven biomarkers representing various domains of ARDS pathogenesis (see Table E1 in the online supplement) were measured in plasma samples obtained from a subgroup of 112 subjects in the validation cohort at the time of enrollment (26–28).

The diagnosis of ARDS was determined in the validation cohort daily, using all available chest radiographs and arterial blood gases in the prior 24 hours obtained at the discretion of the treating physicians (24, 25). Two physician-investigators uninvolved in the patients' care interpreted the chest radiographs by consensus. Patients were first evaluated for ARDS at enrollment in the ICU and then again daily for three subsequent days. Similar to the derivation cohort, the timing of ARDS onset was defined as the time that both arterial blood gas and chest radiograph criteria were met.

The Institutional Review Boards of the University of Pennsylvania and Vanderbilt

University approved the studies with waivers of informed consent.

Statistical Analysis

A latent class analysis was applied in the derivation cohort by modeling the daily ARDS status (definite ARDS, equivocal, definite non-ARDS) over the first five hospital admission days. LCA is a statistical analysis method useful in defining groups on the basis of similarities of observed outcome patterns over time (29, 30). Analyses were limited to subjects who were definite ARDS cases on at least one of the five hospital days of interest. As such, for each study participant, LCA produced a predicted probability of class membership for each hypothesized latent class. In this instance, the latent classes were based on the timing of ARDS onset and possible resolution for each subject. Final class membership was determined by assigning subjects to the class in which they had the highest predicted probability of membership. Therefore, each subject was assigned to a single class where classes are discrete and mutually exhaustive. The three-level ARDS outcome for the 5 days after traumatic injury was used to generate the latent classes among those subjects who were diagnosed with ARDS on at least one of the five hospital days. We assessed model fit and determined the number of latent classes,

using the Bayesian information criterion and entropy (29, 30).

Once classes were identified, we examined construct validity of these classes by evaluating differences in frequencies of known risk factors for ARDS between the classes. In the validation cohort, we simplified the derived classes into early- and late-onset ARDS groups for simplicity and clinical usefulness and compared the simplified classes across clinical variables and the panel of 11 biomarkers. Variables were compared using *t* tests, Pearson's chi-square, Fisher's exact, analysis of variance, Wilcoxon rank-sum or Kruskal-Wallis test, as appropriate. LCA was performed with the SAS add-on LCA procedure (31) and other statistical analyses were performed with Stata/IC 12.0 (StataCorp LP, College Station, TX).

Results

Derivation Cohort Characteristics

Of 636 subjects enrolled, 189 (30%) developed ARDS within the first 5 days of admission and were included in the derivation cohort LCA (Figure 1). Baseline characteristics comparing patients who did not develop ARDS and patients who did develop ARDS are consistent with previous described ARDS cohorts (Table 1). Subjects with ARDS had a median age of

37 (interquartile range [IQR], 23–50), a median Injury Severity Score of 25 (IQR, 20–29), and were approximately equally split between African Americans and whites. The mechanism of injury was blunt in 73% of patients with ARDS and penetrating in 27%. Seven of the patients who developed ARDS were transferred from an outside hospital on the day of acute injury.

Phenotype Identification

LCA identified three patterns of ARDS after major trauma, using the Bayesian information criterion to identify the number of classes that best fit the data. Figure 2 displays the certainty of the diagnosis of ARDS over time for each of the three classes. Class I was characterized by early onset of ARDS on the first or second day and contained 98 of the 189 patients with ARDS (52%) (Figure 2A). By 48 hours from presentation, patients categorized in class I met the diagnostic criterion for definite ARDS. Class II was characterized by a later onset ARDS and consisted of 76 patients (40%). Although patients in the second class were nearly universally free of definite ARDS by the end of the second study day, many were characterized by the “equivocal” classification before the diagnosis of definitive ARDS. Class II members' probability of ARDS then increased daily after the second study

Figure 1. Derivation and validation trauma cohorts. In the derivation cohort, subjects with acute respiratory disease syndrome (ARDS) were classified by latent class analysis into three classes. These classes were then collapsed into the simplified early- and late-onset ARDS based on a 48-hour cutoff. The 48-hour cutoff was used to categorize ARDS cases in the validation cohort into early- and late-onset ARDS.

Table 1. Baseline characteristics of derivation and validation cohorts by acute respiratory distress syndrome diagnosis

Characteristic	Derivation Cohort			Validation Cohort		
	ARDS (n = 189; 30%)	Not ARDS (n = 447; 70%)	P Value	ARDS (n = 205; 34%)	Not ARDS (n = 404; 66%)	P Value
Demographics						
Age, yr	37 (23–50)	33 (24–48)	0.741	44 (27–58)	43 (28–56)	0.922
Male sex	148/188 (79%)	348/447 (78%)	0.808	149/205 (73%)	297/404 (74%)	0.827
Race			0.567			0.808
African American	88/186 (47%)	234/441 (53%)		26/205 (13%)	59/404 (15%)	
White	94/186 (51%)	197/441 (45%)		171/205 (83%)	329/404 (81%)	
Other race	4/186 (2%)	10/441 (3%)		8/205 (4%)	16/404 (4%)	
Severity of illness						
ISS	25 (20–29)	22 (18–27)	0.003	33 (25–41)	26 (19–34)	<0.001
Etiology of injury						
Blunt	138/189 (73.0%)	298/446 (66.8%)	0.124	188/205 (92%)	339/404 (84%)	0.008
Penetrating	51/189 (27.0%)	148/446 (33.2%)		17/205 (8%)	65/404 (16%)	
Transfusions						
RBC, units	2 (0–7)	0 (0–4)	<0.001	2 (0–6)	1 (0–4)	0.003
FFP, units	0 (0–4)	0 (0–2)	<0.001	2 (0–4)	0 (0–4)	0.015
Mortality	45/189 (23.8%)	29/447 (6.5%)	<0.001	26/205 (13%)	45/404 (11%)	0.575

Definition of abbreviations: ARDS = acute respiratory distress syndrome; FFP = fresh frozen plasma; ISS = Injury Severity Score; RBC = red blood cells. Values represent median and interquartile range or number and percentage. Distributions of continuous variables were compared using a Wilcoxon rank-sum test and categorical variables using a Pearson chi-square test.

day (Figure 2B). Class III, the smallest class identified by the latent class model, was characterized mostly by a late deterioration and increased probability of ARDS on post-admission Days 4 and 5 (Figure 2C). This class consisted of 15 patients (8%) who developed an increasing probability of ARDS 3 or 4 days after the acute trauma.

Construct Validity of Identified Classes in the Derivation Cohort

Clinical and demographic characteristics of each of the three identified classes are provided in Table 2, including known risk factors for ARDS development. Subjects with early-onset ARDS (class I) had higher

median Abbreviated Injury Score (AIS) for the thorax, lower systolic blood pressure in the ED or operating room before ICU admission, and a greater median red blood cell transfusion requirement before ICU admission. Patients were similar regarding demographic characteristics including age, sex, and race. In-hospital mortality was similar across all three classes of ARDS (23.5% for class I, 25% for class II, and 20% for class III; $P = 0.966$).

Because the third class consisted of only 8% of the cohort and did not appear different from the second class, the identified classes were simplified to two phenotypes of ARDS, “early onset” and

“late onset,” based on a cutoff of 48 hours. This division was the most logical cutoff based on the ARDS patterns (Figure 2); however, it resulted in a small number of subjects in class I being classified as late onset ($n = 4$), and a small number of patients in classes II and III being classified as early onset ($n = 14$) (Figure 1). Using the simplified classification system, 108 (57%) patients were classified as early onset and 81 (43%) as late onset. Comparisons of characteristics across the simplified classes were similar to comparisons between the first two classes identified in the LCA and are provided in Table 3. The clinical factors that discriminate early and late

Figure 2. Latent class modeling estimated daily probabilities of acute respiratory distress syndrome (ARDS) for the identified (A) latent class I, (B) latent class II, and (C) latent class III. The graphs represent the estimated probability of being categorized as definite ARDS, equivocal, or definite non-ARDS on each study day for 5 days. The y axis indicates the conditional probabilities determined by the latent class model on a given day. The x axis represents the study day, with Day 1 being the day of presentation. The probability of definite ARDS among subjects in each latent class is given by solid diamonds connected by a solid black line. The probability of being categorized as equivocal or definite non-ARDS is given by the squares connected by a dashed line and gray triangles connected by a gray line, respectively.

Table 2. Clinical characteristics of latent class analysis–defined patterns of acute respiratory distress syndrome onset

Characteristic	Class I: Early Onset (n = 98)	Class II: Late Onset (n = 76)	Class III: Latest Onset (n = 15)	P Value
Demographics				
Age, yr	38 (22–50)	34 (23–48)	38 (21–53)	0.986
Sex, % male	77/97 (79%)	58/76 (76%)	12/15 (80%)	0.926
Race				0.567
African American	41/97 (42%)	38/74 (51%)	7/15 (47%)	
White	53/97 (55%)	35/74 (47%)	7/15 (47%)	
Asian	2/97 (2%)	1/74 (1%)	1/15 (7%)	
Other race	1/97 (1%)	0/74 (0%)	0/15 (0%)	
Severity of illness				
ISS	25 (20–32)	25 (19–29)	21 (18–29)	0.419
AIS thorax (0–5)	3 (2–4)	3 (0–3)	2 (0–3)	<0.001
AIS head and neck (0–5)	2 (0–3)	2 (1–4)	3 (0–4)	0.272
AIS face (0–5)	0 (0–2)	0 (0–2)	1 (0–2)	0.817
AIS abdomen (0–5)	2 (0–3)	2 (0–4)	0 (0–3)	0.308
AIS extremities (0–5)	2 (1–3)	2 (1–3)	1 (0–3)	0.551
AIS external (0–5)	1 (0–1)	1 (0–1)	0 (0–1)	0.118
APACHE III w/o ABG	59 (50–71)	58 (47–73)	58 (48–74)	0.821
Etiology of injury				
Blunt	72/98 (74%)	56/76 (74%)	10/15 (67%)	0.846
Penetrating	26/98 (26%)	20/76 (26%)	5/15 (33%)	
Pulmonary contusion	39/98 (40%)	24/75 (32%)	3/15 (20%)	0.276
Substance history				
Alcohol use	12/74 (16%)	9/60 (15%)	3/14 (21%)	0.846
Current smoker	23/58 (40%)	21/54 (39%)	5/13 (39%)	0.995
Initial assessment				
Lowest SBP, ED or OR	78 (68–98)	90 (80–104)	90 (80–102)	0.003
Temp max, ED, °C	36.3 (35.5–36.8)	36.5 (35.9–36.6)	35.9 (35.0–36.6)	0.740
Initial Hgb, mean	12.2 ± 2.1	12.4 ± 2.2	12.7 ± 2.1	0.586
Initial WBC	12 (8.4–18.8)	12.5 (8.2–16.6)	16.2 (9.7–21.4)	0.579
Initial Cr	1.1 (0.9–1.3)	1.1 (0.9–1.3)	1.0 (0.9–1.3)	0.756
Interventions				
Intravenous fluids, L	5.0 (2.3–8.0)	3.5 (2.0–7.5)	5.9 (3.0–8.1)	0.384
RBC, units*	4 (0–9)	2 (0–6)	1 (0–6)	0.011
RBC, transfusion*	70/98 (71%)	42/76 (55%)	8/15 (53%)	0.062
FFP, units	1 (0–4)	0 (0–3)	2 (0–4)	0.470
Mortality	23/98 (24%)	19/76 (25%)	3/15 (20%)	0.966

Definition of abbreviations: ABG = arterial blood gas; AIS = Abbreviated Injury Scale; APACHE = Acute Physiology and Chronic Health Evaluation; Cr = creatinine; ED = emergency department; FFP = fresh frozen plasma; Hgb = hemoglobin; ISS = Injury Severity Score; OR = operating room; RBC = red blood cells; SBP = systolic blood pressure; Temp max = maximal temperature; WBC = white blood cells; w/o = without.

Values represent median and interquartile range, mean ± standard deviation, or number and percentage. Distributions of continuous variables were compared using a Kruskal–Wallis test or analysis of variance test as appropriate, and categorical variables were compared using a Pearson chi-square test or Fisher's exact test.

*Packed RBC transfusion was considered a continuous variable based on number of units transfused during resuscitation and a dichotomous categorical variable distinguishing subjects transfused from those who required no transfusion.

ARDS phenotypes remained systolic blood pressure, number of early blood transfusions, and thoracic injury severity. Therefore, the simplified early- and late-onset classes based on a 48-hour cutoff were used in the validation cohort.

Construct Validity in the Validation Cohort

The validation cohort included 205 (34%) patients who developed ARDS out of a total of 609 trauma patients (Figure 1). The clinical characteristics of the trauma population and those patients who

developed ARDS were similar to the derivation cohort (Table 1). Patients with ARDS in the validation cohort had a median age of 44 (IQR, 28 to 56) and a median ISS of 33 (IQR, 24 to 41). Unlike the derivation cohort, 83% of subjects with ARDS in the validation cohort were white and 92% had a blunt mechanism of trauma.

Using the simplified classification, 115 (56%) patients who developed ARDS were classified as early onset and 90 (44%) were classified as late onset, a distribution similar to that in the derivation cohort.

Comparisons of clinical characteristics across classes are provided in Table 3. Consistent with the analysis of the derivation cohort, patients in the early-onset ARDS class had a higher median AIS thorax score and a lower SBP before enrollment. The validation cohort failed to demonstrate a difference in transfusions between classes. The early-onset class was also older and included a higher percentage of subjects with a pulmonary contusion. In-hospital mortality was again similar by class (11% in the early-onset class, 14% in the late-onset class; $P = 0.503$).

Table 3. Clinical characteristics of early- versus late-onset acute respiratory distress syndrome in the derivation and validation cohorts

Characteristic	Derivation Cohort			Validation Cohort		
	Early Onset (n = 108; 57%)	Late Onset (n = 81; 43%)	P Value	Early Onset (n = 115; 56%)	Late Onset (n = 90; 44%)	P Value
Demographics						
Age, yr	38 (24–52)	33 (23–47)	0.728	48 (32–60)	38 (25–56)	0.015
Sex, % male	84/107 (79%)	63/81 (78%)	0.905	89/115 (77%)	60/90 (67%)	0.087
Race						
African American	44/107 (41%)	42/79 (53%)	0.098	15/115 (13%)	11/90 (12%)	0.528
White	61/107 (57%)	34/79 (43%)		94/115 (82%)	77/90 (86%)	
Other race	2/107 (2%)	3/79 (4%)		6/115 (5%)	2/90 (2%)	
Severity of illness						
ISS	25 (20–33)	24 (19–29)	0.248	34 (27–41)	29 (21–38)	0.063
AIS thorax (0–5)	3 (2–4)	3 (0–3)	<0.001	4 (3–5)	4 (3–4)	0.001
AIS head and neck (0–5)	2 (0–3)	2 (0–4)	0.194	3 (2–4)	3 (1–4)	0.329
AIS face (0–5)	0 (0–2)	0 (0–2)	0.564	2 (1–2)	2 (1–2)	0.232
AIS abdomen (0–5)	2 (0–3)	2 (0–3)	0.990	2 (2–3)	2 (1–4)	0.724
AIS extremities (0–5)	2 (1–3)	2 (0–3)	0.134	2 (2–3)	2 (1–3)	0.381
AIS external (0–5)	1 (0–1)	1 (0–1)	0.157	2 (2–3)	2 (1–3)	0.381
APACHE III w/o ABG	61 (51–73)	56 (45–70)	0.109	*	*	*
Etiology of injury						
Blunt	80/108 (74%)	58/81 (72%)	0.705	109/115 (95%)	79/90 (88%)	0.071
Penetrating	28/108 (26%)	23/81 (28%)		6/115 (5%)	11/90 (12%)	
Pulmonary contusion	42/108 (39%)	24/80 (30%)	0.207	57/115 (50%)	31/90 (34%)	0.030
Substance history						
Alcohol use	12/81 (15%)	12/67 (18%)	0.611	22/115 (19%)	20/90 (22%)	0.506
Current smoker	26/68 (38%)	23/59 (39%)	0.931	52/115 (45%)	41/90 (46%)	0.962
Initial assessment						
Lowest SBP	80 (70–97)	92 (80–105)	<0.001	85 (73–95)	92 (82–100)	0.006
Temp max, °C	36.3 (35.6–36.8)	36.4 (35.9–36.8)	0.684	38.1 (37.7–38.5)	38.0 (37.6–38.6)	0.498
Hgb or Hct [†]	12.3 ± 2.0	12.5 ± 2.3	0.392	30 (25–35)	30 (26–34)	0.792
WBC [‡]	11.7 (8.4–18.6)	12.5 (8.2–16.6)	0.810	16.7 (12.5–21.7)	17.4 (12.3–22.4)	0.486
Initial Cr	1.1 (0.9–1.3)	1.1 (0.9–1.3)	0.810	1.2 (0.8–1.4)	1.1 (0.9–1.3)	0.205
Interventions						
Intravenous fluids, L	5.4 (2.4–8.5)	3.5 (2.0–7.0)	0.077	8.0 (5.5–10.1)	6.6 (4.6–9.7)	0.087
RBC, units	4 (0–9)	1 (0–6)	0.009	2 (0–6)	2 (0–5)	0.548
Plasma, units	1 (0–4)	0 (0–4)	0.268	2 (0–4)	2 (0–6)	0.712
Mortality	23/108 (21%)	22/81 (27%)	0.349	13/115 (11%)	13/90 (14%)	0.503

Definition of abbreviations: ABG = arterial blood gas; AIS = Abbreviated Injury Scale; APACHE = Acute Physiology and Chronic Health Evaluation; Cr = creatinine; Hct = hematocrit; Hgb = hemoglobin; ISS = Injury Severity Score; RBC = red blood cells; SBP = systolic blood pressure; Temp max = maximal temperature; WBC = white blood cells; w/o = without.

Early-onset acute respiratory distress syndrome (ARDS) includes patients diagnosed within 48 hours of admission, whereas late-onset ARDS includes those diagnosed beyond 48 hours. Values represent median and interquartile range, mean ± standard deviation, or number and percentage. Distributions of continuous variables were compared using a Wilcoxon rank-sum test or Student *t* test as appropriate, and categorical variables were compared using a Pearson chi-square test.

*APACHE III w/o ABG data were unavailable in the validation cohort.

[†]Initial Hgb is provided for the derivation cohort and lowest Hct before enrollment is provided for the validation cohort.

[‡]WBC represents the initial white blood cell count in the derivation cohort and the highest before enrollment in the validation cohort.

To further evaluate discriminant validity, levels of plasma biomarkers drawn at the time of enrollment and known to be associated with ARDS risk and/or outcomes were compared between classes in 112 subjects from the validation cohort (Table 4). Levels of the soluble receptor for advanced glycation end-products (sRAGE) and angiotensin-2 (Ang-2) were significantly higher in the early-onset class relative to the late-onset class (Figure 2). The Ang-2 findings were robust to

a conservative Bonferroni adjustment for multiple comparisons (adjusted *P* = 0.022). All other biomarker levels tested did not show a statistically significant difference between the two onset classes.

Discussion

Increasingly, phenotypes of clinical syndromes including chronic obstructive pulmonary disease and asthma have been

identified to further understanding of the heterogeneity underlying these syndromes (32, 33). In this study, we used LCA to identify three subgroups of ARDS based on timing of onset and certainty of diagnosis in a cohort of critically ill trauma patients. The latent class model identified a cutoff point of approximately 48 hours after presentation separating the early- and late-onset subgroups of ARDS. Early-onset ARDS was associated with increased severity of thoracic trauma, more severe

Table 4. Plasma biomarker levels by acute respiratory distress syndrome onset class

Biomarker	Early-Onset ARDS (n = 66; 59%)	Late-Onset ARDS (n = 46; 41%)	P Value
IL-8, pg/ml	15.6 (15.6–58.2)	15.6 (15.6–85.5)	0.644
VWF, % control	231 (168–351)	248 (194–353)	0.219
SP-D, ng/ml	57 (37–88)	63 (42–80)	0.927
PAI-1, ng/ml	138 (40–266)	87 (40–197)	0.271
CC16, ng/ml	6.9 (4.1–12.4)	5.8 (4.4–8.3)	0.187
sRAGE, pg/ml	1,773 (949–3,227)	1,226 (773–1,944)	0.025
Ang-2, pg/ml	5,684 (4,190–7,773)	4,365 (2,957–5,647)	0.002
BNP, ng/ml	0.41 (0.31–0.67)	0.33 (0.26–0.56)	0.123
PCP III, ng/ml	3.5 (2.8–4.9)	3.2 (2.7–4.2)	0.249
IL-10, pg/ml	16 (9–86)	21 (9–70)	0.703
TNF- α , pg/ml	1.03 (0.61–3.47)	0.67 (0.61–4.83)	0.535

Definition of abbreviations: Ang-2 = angiopoietin-2; BNP = brain natriuretic peptide; PAI-1 = plasminogen activator inhibitor-1; PCP III = procollagen peptide type III; SP-D = surfactant protein D; sRAGE = soluble receptor for advanced glycation end-products; TNF- α = tumor necrosis factor- α ; VWF = von Willebrand factor.

Values represent medians and interquartile ranges. Classes were compared using the Wilcoxon rank sum test.

early hypotension, and increased red blood cell transfusion during the initial resuscitation when compared with the later onset subgroup. Using the identified cutoff of 48 hours, we validated our findings in a separate population of critically ill trauma patients. The proportions of patients with early- and late-onset ARDS was nearly identical in the two populations. The early-onset phenotype was again associated with a lower systolic blood pressure before ICU enrollment and severity of thoracic trauma; however, red blood cell transfusion during resuscitation did not differ by early- and late-onset ARDS. The divergent characteristics between classes suggest a unique early-onset phenotype of ARDS, possibly characterized by a more severe degree of hemorrhagic shock (as evidenced by early hypotension) and the type of traumatic injury suffered. In addition, the biomarkers sRAGE and Ang-2 were significantly higher in the serum of patients with early-onset ARDS while all other biomarkers including markers of systemic inflammation were similar between the two classes of ARDS, suggesting distinct molecular profiles early posttrauma.

The early-onset clinical phenotype derived from a purely statistical method in our study is consistent with prior observations. In 1999, Croce and colleagues conducted an observational study of posttraumatic patients with ARDS aimed at analyzing clinical variables based on the time interval from injury to diagnosis of ARDS (9). The authors concluded that the

onset of ARDS within 48 hours of presentation was associated with a higher degree of hemorrhagic shock. The cause of ARDS among the patients who developed later onset ARDS is less clear. We hypothesize that later onset ARDS is associated with progressive multiorgan system dysfunction, medical errors, or a complication arising after hospital admission, such as pneumonia, although these potential explanations will need to be formally evaluated in the future. The small size of the third, latest onset ARDS class limited our ability to draw meaningful conclusions regarding this class. We found no signal for a difference in mortality between the early- and late-onset ARDS groups in either the derivation or validation cohort, a finding that is consistent with previous studies of ARDS associated with trauma and sepsis (9, 34). Therefore, we conclude that although early- and late-onset ARDS may have heterogeneous pathogenesises, they do not have appreciably divergent outcomes in terms of mortality risk.

The two trauma cohorts in our study had several important clinical differences including demographics, years of cohort enrollment, and overall mortality. The differences likely influenced treatments patients received at the respective institutions, including the likely increased use of lung-protective ventilation in more recently enrolled patients. However, despite this clinical heterogeneity, the discriminating factors for early- versus

late-onset ARDS were similar, supporting the generalizability of our findings to diverse populations of severely injured trauma patients with ARDS (35).

ARDS has been recognized as a heterogeneous syndrome occurring after diverse environmental insults and involving various molecular pathways along with a general imbalance between injurious and reparative mechanisms (5–7). Uncertainty remains regarding how diverse initial environmental injuries result in a sequence of events culminating in the clinical syndrome of ARDS. On the basis of our findings of higher serum Ang-2 and sRAGE in patients with early-onset ARDS, we hypothesize that the early-onset endophenotype is characterized by a higher degree of early vascular injury and disrupted alveolar–capillary barrier integrity than the later onset group. We used a novel approach using validated biomarkers representing different pathways important in the pathophysiology of ARDS to identify a subgroup of patients who may benefit from targeted therapies. Ang-2, a protein ligand that is expressed when endothelial injury is present, promotes vascular permeability, neutrophil transmigration, and angiogenesis and is predictive of ARDS development (36–38). RAGE is a transmembrane receptor that binds glycoproteins resulting in a proinflammatory intracellular signal (39), and has been identified as a regulator of alveolar–capillary barrier integrity in experimental models (40, 41). The soluble secreted form of RAGE is believed to be a marker of lung type I epithelial damage and has also been recognized to correlate with markers of endothelial function, including Ang-2 (42, 43). Given the important function of these molecules in the pathogenesis of ARDS, therapeutics aimed at Ang-2 and RAGE targets are under active study (39, 44–48). Our study suggests that the trauma patients with early-onset ARDS may be an ideal population for evaluation of such therapeutics.

There are several important limitations to our study. The sample sizes of the derivation (n = 189) and validation (n = 205) cohorts limited statistical power to evaluate differences in low-prevalence clinical variables between classes. Therefore, we may have failed to identify additional important clinical differences between the identified classes.

We were limited by the availability of diagnostic data to studies ordered at the clinicians' discretion and defined ARDS onset as the time when both blood gas and chest radiograph criteria were met. This resulted in some uncertainty as to the exact timing of ARDS onset. However, despite this uncertainty we identified statistically significant differences between early- and late-onset classes. The investigated clinical variables were related to the acute injury and resuscitation rather than later ICU factors, limiting our ability to examine potentially relevant risk factors that developed later in the hospital course. In addition, potentially important clinical variables including ventilator data, initial lactate levels, and interventions performed in the field before hospital presentation were unavailable. Also, we cannot ensure the temporal relationship of clinical variables and ARDS; therefore, variables, such as lowest systolic blood pressure, may have occurred after the onset of ARDS. However, our objective was not to identify independent risk factors for ARDS; rather, it was to describe the construct validity

of our early- and late-onset ARDS classes. Furthermore, the enrollment and data collection techniques were somewhat different in each cohort; however, our clinical findings were similar despite these differences. Biomarker levels were available only early in the patients' hospital course and not necessarily on the day of ARDS development for all patients. Also, we could not ensure that the biomarkers were drawn before the development of ARDS in the early-onset class. Therefore, it is possible that differences in biomarker levels between the classes relate to differences in levels drawn concordant with ARDS and those drawn before the development of ARDS. However, if differences in biomarker levels represented solely the timing of the blood draw relative to the development of ARDS, we would expect differences to be identified across most or all of the biomarkers associated with ARDS. Unlike the clinical variable findings, biomarker measurements were performed only in one cohort and require further validation. Last, the ideal time from which to measure ARDS onset may be the time of

injury rather than the time of presentation. Data on the time of injury were not reliably collected in our study; however, all enrolled patients presented acutely after trauma.

In conclusion, latent class analysis based on timing and certainty of diagnosis in a cohort of critically ill trauma patients with ARDS identified three phenotypes of ARDS, with more than 90% of patients being represented by two classes: early onset and late onset. Degree of hemorrhagic shock and severity of thoracic trauma were associated with an early-onset phenotype of ARDS. In a validation cohort, early-onset ARDS was also associated with higher levels of the biomarkers sRAGE and Ang-2 compared with the later onset ARDS. Further work is necessary, but the distinction between early- and late-onset ARDS may be important in future mechanistic and therapeutic studies targeting ARDS after major trauma, as the divergent characteristics between classes suggest unique pathogenic pathways. ■

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

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