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Journal

CHEST Journal, 147(6)

ISSN

0012-3692

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Publication Date

2015-06-01

DOI

10.1378/chest.14-2454

Peer reviewed

Distinct Molecular Phenotypes of Direct vs Indirect ARDS in Single-Center and Multicenter Studies

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BACKGROUND: ARDS is a heterogeneous syndrome that encompasses lung injury from both direct and indirect sources. Direct ARDS (pneumonia, aspiration) has been hypothesized to cause more severe lung epithelial injury than indirect ARDS (eg, nonpulmonary sepsis); however, this hypothesis has not been well studied in humans.

METHODS: We measured plasma biomarkers of lung epithelial and endothelial injury and inflammation in a single-center study of 100 patients with ARDS and severe sepsis and in a secondary analysis of 853 patients with ARDS drawn from a multicenter randomized controlled trial. Biomarker levels in patients with direct vs indirect ARDS were compared in both cohorts.

RESULTS: In both studies, patients with direct ARDS had significantly higher levels of a biomarker of lung epithelial injury (surfactant protein D) and significantly lower levels of a biomarker of endothelial injury (angiopoietin-2) than those with indirect ARDS. These associations were robust to adjustment for severity of illness and ARDS severity. In the multicenter study, patients with direct ARDS also had lower levels of von Willebrand factor antigen and IL-6 and IL-8, markers of endothelial injury and inflammation, respectively. The prognostic value of the biomarkers was similar in direct and indirect ARDS.

CONCLUSIONS: Direct lung injury in humans is characterized by a molecular phenotype consistent with more severe lung epithelial injury and less severe endothelial injury. The opposite pattern was identified in indirect lung injury. Clinical trials of novel therapies targeted specifically at the lung epithelium or endothelium may benefit from preferentially enrolling patients with direct and indirect ARDS, respectively. CHEST 2015; 147(6):1539-1548

Manuscript received October 6, 2014; revision accepted November 13, 2014; originally published Online First December 11, 2014.

ABBREVIATIONS: Ang-2 = angiopoietin-2; APACHE = Acute Physiology and Chronic Health Evaluation; RAGE = receptor for advanced glycation end products; SP-D = surfactant protein D; VALID = Validation of Biomarkers in Acute Lung Injury Diagnosis; vWF = von Willebrand factor antigen

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FUNDING/SUPPORT: This work was supported by contracts with the National Heart, Lung, and Blood Institute (NHLBI) [NO1-HR-46046-64 and NO1-HR-16146-54]. Dr Calfee was supported by the National Institutes of Health (NIH) [HL090833 and HL110969]. Dr Janz was supported by the NIH [T32 HL087738]. Dr Kangelaris was supported by the NHLBI [1K23 HL116800-01]. Dr Matthay was supported by the NIH [HL51856]. Dr Ware was supported by the NIH [HL103836 and HL112656].

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DOI: 10.1378/chest.14-2454

ARDS is by definition heterogeneous, encompassing lung injury in the setting of underlying illnesses that may cause either direct injury to the lung (eg, pneumonia, aspiration of gastric contents) or indirect injury to the lung (eg, nonpulmonary sepsis, massive transfusion, pancreatitis).¹ Although the pathogenesis of ARDS is characterized by severe injury to both the lung epithelium and the vascular endothelium, leading to increased permeability of the alveolar-capillary membrane, animal models suggest that direct lung injury begins with an insult to the lung epithelium and consequently leads to more severe lung epithelial injury compared with indirect lung injury.² Conversely, indirect lung injury in experimental models originates with lung and systemic endothelial damage induced by intravascular inflammatory mediators.³ Despite strong experimental evidence for these differences in pathogenesis in animal models, whether these differences are relevant to human ARDS remains unknown.

In 1992, the committee charged with generating the first consensus definition of ARDS at the American-European Consensus Conference recognized that the pathogenesis of ARDS is likely different in direct vs indirect lung injury.⁴ Although some human studies demonstrated differences in clinical phenotype between these subgroups,^{5,6} findings are inconsistent, and more recent consensus definitions of ARDS have not drawn signifi-

cant distinctions based on direct or indirect lung injury.⁷ As a result, most clinical trials of novel ARDS therapies, including those of new therapies specifically targeted to the lung epithelium or vascular endothelium, have focused on broad samples of patients with a mixture of direct and indirect ARDS risk factors.⁸ If significant differences in pathogenesis are present in human direct vs indirect ARDS, this heterogeneity may obscure treatment effects evident only in subgroups and may contribute to the many negative pharmaceutical trials in ARDS.

We designed the current study to test the hypothesis that direct ARDS is characterized by more severe lung epithelial injury and less severe endothelial injury in humans compared with indirect ARDS. We tested this hypothesis in two cohorts of patients with ARDS: (1) a single-center observational cohort study in 100 patients with ARDS and severe sepsis and (2) a multicenter sample of 853 patients with ARDS enrolled in a randomized controlled trial of fluid management strategies. We measured lung epithelial and endothelial injury and inflammation using a panel of plasma biomarkers with an established value for pathogenesis and prognosis in ARDS.⁹⁻¹¹ As a secondary objective, we determined whether the prognostic value of these biomarkers differed based on direct vs indirect lung injury. Some of these findings have been published previously in abstract form.^{12,13}

Materials and Methods

Single-Center Study

Patients were drawn from the Validation of Biomarkers in Acute Lung Injury Diagnosis (VALID) study, a prospective cohort of critically ill patients at Vanderbilt University Medical Center, a tertiary care medical center. The inclusion and exclusion criteria for VALID have been described previously and are summarized in e-Appendix 1. Patients were enrolled in VALID on ICU day 2.¹⁴ The study was approved by the Vanderbilt University Institutional Review Board (#051065).

Patients were followed for 4 days for development of ARDS (P_{aO_2}/F_{iO_2} ratio < 300 by American-European Consensus Conference definition) using a two-physician review of chest radiographs and clinical data.⁴ If an arterial blood gas result was not available, then the oxygen saturation as measured by pulse oximetry/ F_{iO_2} ratio was used to assess hypoxemia.¹⁵

For this substudy within VALID, we used 100 patients who met criteria for ARDS on at least 2 of the first 4 days of study enrollment and had severe pulmonary or nonpulmonary sepsis at enrollment. Risk factors for ARDS were categorized as sepsis, pneumonia, or aspiration as adjudicated by the study principal investigator.¹⁶ Sepsis was defined by consensus criteria.¹⁷ Patients with sepsis due to pneumonia or aspiration were categorized as having direct lung injury ($n = 44$). Patients with nonpulmonary sepsis were categorized as having indirect lung injury ($n = 56$).

Multicenter Study

This study was designed as a secondary analysis of clinical data and biologic specimens collected by the NIH NHLBI ARDS Network from

the FACTT (Fluid and Catheter Treatment Trial).^{18,19} This trial used a factorial design to compare (1) the use of pulmonary arterial vs central venous catheters and (2) fluid liberal vs fluid conservative management strategies in patients with ARDS enrolled within 48 h of meeting ARDS criteria. All patients provided informed consent; inclusion and exclusion criteria have been previously described.^{18,19} Risk factors for ARDS were adjudicated by site investigators. For this analysis, we included patients with a primary ARDS risk factor of pneumonia or aspiration (direct lung injury; $n = 620$) or nonpulmonary sepsis (indirect lung injury; $n = 233$); patients with other primary ARDS risk factors were excluded.

Biosamples

Enzyme-linked immunosorbent assays were used to measure the biomarkers in plasma from study enrollment day in both studies (prior to randomization in FACTT). Surfactant protein D (SP-D), a marker of lung epithelial injury (Yamasa Corporation); soluble receptor for advanced glycation end products (RAGE), a marker of lung epithelial injury and innate immune response (R&D Systems, Inc); angiotensin-2 (Ang-2), a marker and mediator of endothelial injury (R&D Systems, Inc); and IL-6 and IL-8, markers of inflammation (Meso Scale Diagnostics, LLC) were measured in both studies. In the multicenter study, von Willebrand Factor antigen (vWF), a marker of endothelial injury (Diagnostica Stago, Inc), was also measured.

Statistical Analysis

Statistical analysis was performed with Stata/SE 12 software (StataCorp LP). Additional details are included in e-Appendix 1. To test whether associations between biomarker levels and direct vs indirect ARDS were confounded by severity of illness or lung injury, we carried out logistic regression using direct vs indirect ARDS as the outcome and

biomarker level as the predictor, adjusting for severity of illness (Acute Physiology and Chronic Health Evaluation [APACHE] scores) or severity of lung injury ($\text{PaO}_2/\text{FiO}_2$ ratio). To avoid collinearity, APACHE score and $\text{PaO}_2/\text{FiO}_2$ were not included in the same models because APACHE contains arterial blood gas data. To test whether the prognostic value

of biomarkers for mortality differed based on type of lung injury, we conducted logistic regressions stratified by direct vs indirect ARDS. If the stratified ORs appeared different by $> 15\%$, we formally tested for the presence of multiplicative interaction by adding an interaction term to the regression.

Results

Patient Characteristics

Table 1 compares the patient characteristics in the two cohorts, stratified by direct vs indirect ARDS. In the single-center cohort, there were no significant differences in demographics between patients with direct and indirect ARDS; however, there were significant differences in severity of illness, with higher proportions of patients with indirect ARDS receiving vasopressors. Likewise, there was a trend toward higher APACHE II scores in indirect ARDS. Although the $\text{PaO}_2/\text{FiO}_2$ ratio was lower on average in patients with direct lung injury, this difference was not statistically significant. Findings were largely similar in the multicenter cohort; specifically, patients with indirect ARDS were more likely to be receiving vasopressors and had higher APACHE scores than those with direct ARDS.

In the single-center study, 32 of the 44 patients with direct ARDS were given a primary diagnosis of pneumonia; all

56 patients with indirect ARDS had nonpulmonary sepsis as their primary ARDS risk factor. In the multicenter study, 471 of the patients with direct ARDS had pneumonia, and 149 had aspiration as their primary ARDS risk factor; all 233 patients with indirect ARDS had nonpulmonary sepsis as their primary ARDS risk factor.

Single-Center Study

Biomarker levels in the 100 patients in the single-center study, stratified by direct vs indirect ARDS, are summarized in Figure 1. Levels of biomarkers of lung epithelial injury (SP-D, RAGE) were significantly higher in direct ARDS than in indirect ARDS. Conversely, levels of Ang-2, a biomarker of endothelial injury, were significantly lower in direct ARDS than in indirect ARDS. Levels of two biomarkers of inflammation (IL-6, IL-8) were similar between the two groups.

Multicenter Study

Biomarker levels in the multicenter study patients, stratified by direct vs indirect ARDS, are summarized in

TABLE 1 Patient Characteristics in the Single-Center and Multicenter Studies

Characteristic	Single Center			Multicenter		
	Direct (n = 44)	Indirect (n = 56)	P Value	Direct (n = 620)	Indirect (n = 233)	P Value
Age, y	55 ± 14	58 ± 11	.32	51 ± 15	51 ± 17	.49
Male sex	25 (57)	27 (48)	.39	324 (52)	120 (52)	.84
Race			.18			.01
White	42 (95)	47 (84)		403 (65)	146 (63)	
Black	2 (5)	8 (14)		145 (23)	43 (19)	
Other	0 (0)	1 (2)		72 (12)	44 (19)	
On vasopressors on study day 1	14 (32)	30 (54)	.03	186 (30)	114 (49)	< .001
AIDS	0 (0)	0 (0)	...	60 (10)	8 (4)	.002
Chronic liver disease	3 (7)	10 (18)	.10	26 (4)	5 (2)	.15
Diabetes	10 (23)	14 (25)	.79	104 (17)	52 (23)	.07
APACHE II score	27 ± 7	29 ± 6	.12
APACHE III score	94 ± 31	103 ± 31	.0002
$\text{PaO}_2/\text{FiO}_2$ ratio	128 ± 82	158 ± 77	.11	128 ± 60	136 ± 64	.08
Died ^a	14 (32)	17 (30)	.88	177 (29)	82 (35)	.06
Ventilator-free days	21 (1-24)	17 (4-25)	.95	17 (0-23)	13.5 (0-22)	.007

Data are presented as mean ± SD, No. (%), or median (interquartile range) unless otherwise indicated. APACHE = Acute Physiology and Chronic Health Evaluation.

^aMortality at hospital discharge in the single-center cohort, 90 d in multicenter cohort.

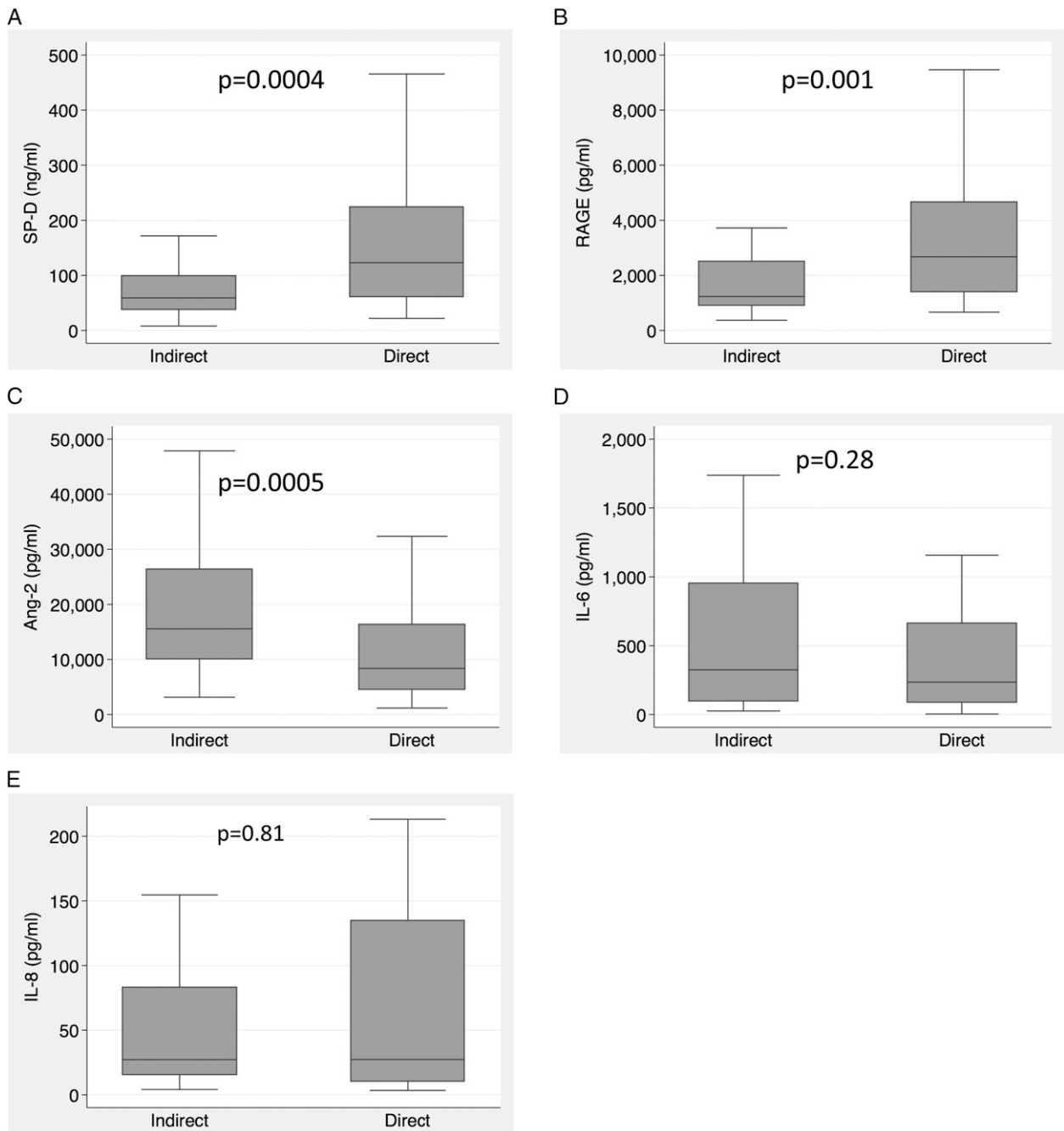


Figure 1 – A-E, Biomarker levels in the single-center study (n = 100). Box plots showing median, interquartile range (box), and upper and lower adjacent values (bars) for biomarker levels stratified by direct (n = 44) vs indirect (n = 56) lung injury. Biomarkers depicted are SP-D (A), RAGE (B), Ang-2 (C), IL-6 (D), and IL-8 (E). Ang-2 = angiopoietin-2; RAGE = receptor for advanced glycation end products; SP-D = surfactant protein D.

Figure 2. As in the single-center study, levels of SP-D, a biomarker of lung epithelial injury, were significantly higher in direct than in indirect ARDS; however, in contrast to the single-center study, there was no difference in plasma RAGE levels between the two groups. As in the single-center study, levels of Ang-2, a biomarker of endothelial injury, were significantly lower in direct ARDS than in indirect ARDS. One additional biomarker of endothelial injury, vWF, was measured in this cohort

as well; in parallel with the Ang-2 data, vWF levels were significantly lower in direct ARDS than in indirect ARDS. In this larger sample, patients with direct ARDS had significantly lower levels of inflammatory biomarkers (IL-6 and IL-8) than patients with indirect ARDS.

Multivariable Models

Because severity of illness as measured by APACHE III differed between patients with direct and indirect ARDS

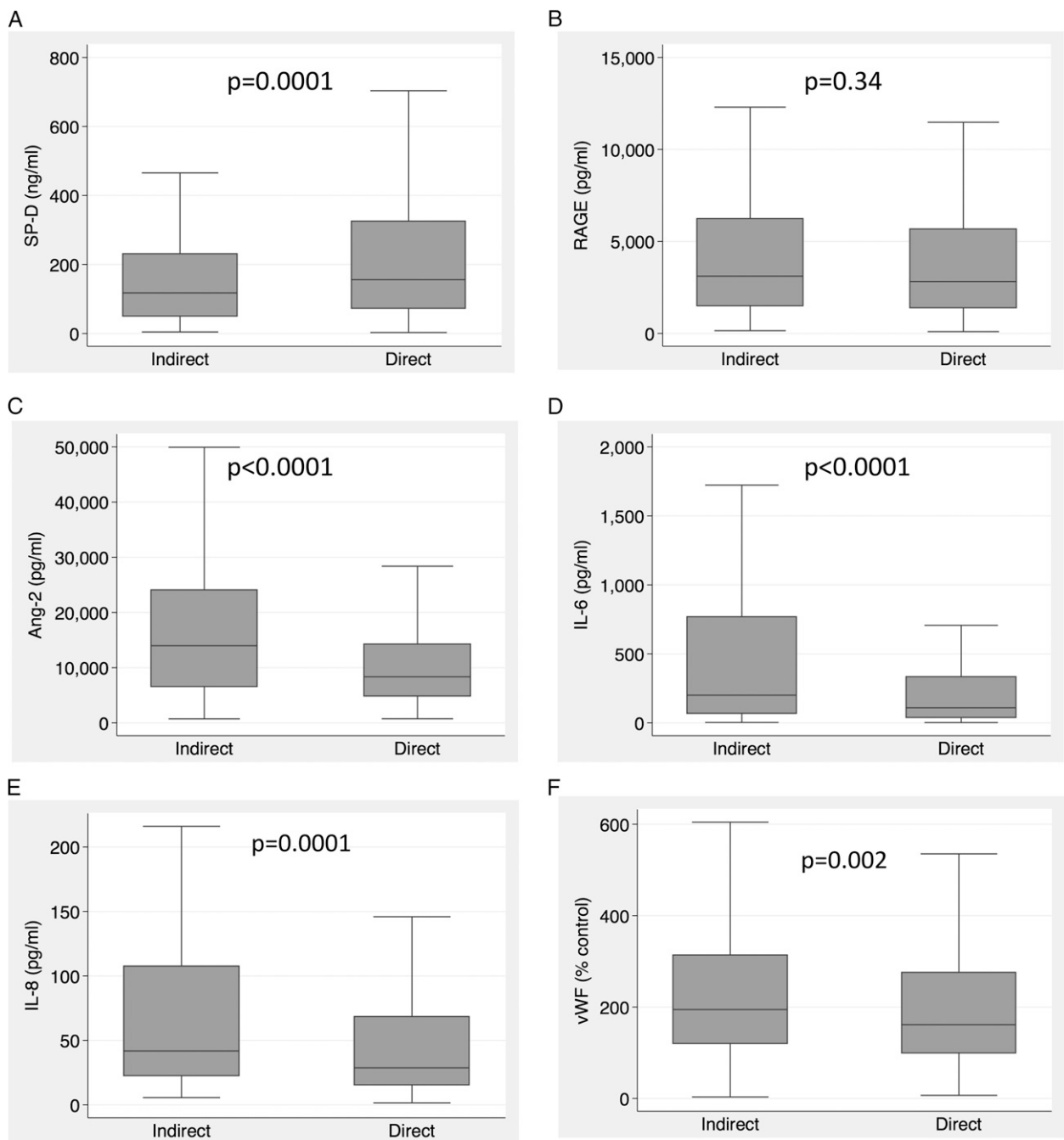


Figure 2 – A-F, Biomarker levels in the multicenter study (n = 853). Box plots showing median, interquartile range (box), and upper and lower adjacent values (bars) for biomarker levels stratified by direct (n = 620) vs indirect (n = 233) lung injury. Biomarkers depicted are SP-D (A), RAGE (B), Ang-2 (C), IL-6 (D), IL-8 (E), and vWF (F). vWF = von Willebrand factor antigen. See Figure 1 legend for expansion of other abbreviations.

in the multicenter cohort and because there were trends toward differences in the $\text{PaO}_2/\text{FIO}_2$ ratio in the two groups, we created logistic regression models to determine whether these differences were confounding biomarker comparisons (Table 2) as described in the Materials and Methods section. In the single-center study, adjustment for APACHE II score had no appreciable effect on the associations between the biomarkers

and the odds of direct vs indirect ARDS. Similarly, adjustment for severity of ARDS ($\text{PaO}_2/\text{FIO}_2$) did not significantly change the biomarker pattern described, although the association between Ang-2 and indirect ARDS was mildly attenuated.

In the multicenter study, adjustment for APACHE III score had no appreciable effect on the associations between direct causes of ARDS and lower IL-6 levels,

TABLE 2] Associations Between Plasma Biomarkers and Direct Etiology of ARDS in Single-Center and Multicenter Studies

Biomarker, per 1-Log Increment	Direct ARDS, ^a Unadjusted	P Value	Direct ARDS, Adjusted for APACHE Score ^a	P Value	Direct ARDS, Adjusted for PF Ratio ^a	P Value
Single center						
IL-8	1.01 (0.82-1.26)	.91	1.06 (0.84-1.33)	.61	1.00 (0.76-1.31)	.99
IL-6	0.88 (0.71-1.09)	.24	0.92 (0.73-1.16)	.48	0.98 (0.75-1.28)	.87
SP-D	2.45 (1.45-4.14)	.001	2.38 (1.41-4.02)	.001	2.46 (1.34-4.53)	.004
RAGE	2.11 (1.27-3.48)	.004	2.40 (1.40-4.12)	.002	2.24 (1.22-4.11)	.009
Ang-2	0.37 (0.21-0.67)	.001	0.36 (0.19-0.70)	.003	0.50 (0.27-0.95)	.04
Multicenter						
IL-8	0.87 (0.78-0.96)	.005	0.92 (0.83-1.03)	.15	0.86 (0.78-0.95)	.004
IL-6	0.81 (0.75-0.89)	<.001	0.84 (0.77-0.92)	<.001	0.80 (0.74-0.88)	<.001
SP-D	1.33 (1.16-1.52)	<.001	1.33 (1.15-1.52)	<.001	1.32 (1.15-1.51)	<.001
RAGE	0.92 (0.79-1.07)	.26	0.96 (0.83-1.12)	.62	0.89 (0.77-1.04)	.14
Ang-2	0.55 (0.45-0.68)	<.001	0.62 (0.50-0.77)	<.001	0.55 (0.45-0.67)	<.001
vWF	0.72 (0.58-0.90)	.003	0.81 (0.64-1.02)	.07	0.72 (0.58-0.90)	.004

Data are presented as OR (95% CI) unless otherwise indicated. Ang-2 = angiotensin-2; PF = Pao₂/Fio₂; RAGE = receptor for advanced glycation end products; SP-D = surfactant protein D; vWF = von Willebrand factor antigen. See Table 1 legend for expansion of other abbreviation.

^aReferent group in logistic regressions is indirect ARDS.

higher SP-D levels, and lower Ang-2 levels. This adjustment mildly attenuated the association between direct causes of ARDS and lower vWF levels and moderately attenuated the association with lower IL-8 levels. Adjustment for severity of ARDS (Pao₂/Fio₂) had no significant effect on the biomarker associations with direct ARDS.

Prognostic Value of Biomarkers

Because the biomarkers measured have all previously been associated with poor clinical outcomes in ARDS, we determined whether this prognostic value was maintained in these cohorts and whether it differed in direct vs indirect ARDS (Table 3). In the single-center study, IL-8, IL-6, RAGE, and Ang-2 were all associated with a significant increase in the odds of death, as in prior reports^{10,11,20}; however, SP-D was not associated with mortality. These findings were replicated in the multicenter study, although the specific ORs for each biomarker differed slightly. Of note, we did not detect any significant interactions between direct vs indirect ARDS and the prognostic value of any marker. There were modest differences in the stratified ORs for SP-D in the single-center study and for Ang-2 in the multicenter study, as described in Table 3, but these differences were not statistically significant.

Discussion

In this analysis of two distinct patient cohorts, we found that direct ARDS is characterized by more severe lung

epithelial injury compared with indirect ARDS, and conversely, that indirect ARDS is characterized by more severe endothelial injury and inflammation. With few exceptions, these findings were similar in both cohorts and were robust to adjustment for differences in severity of illness and severity of lung injury. These distinct molecular phenotypes of direct vs indirect lung injury provide strong evidence that the heterogeneity in ARDS pathogenesis observed in experimental models is relevant to human ARDS, a finding that may have important implications for clinical trials of novel therapies.

Although many previous studies have described the prognostic value of plasma biomarkers in ARDS, relatively few prior studies have tested for differences in plasma biomarkers related to a direct vs an indirect source of lung injury. Eisner et al²¹ reported that plasma SP-D and surfactant protein A levels were highest in patients with pneumonia as an ARDS risk factor compared with other ARDS risk factors and found that surfactant proteins were most strongly prognostic in patients with pneumonia and sepsis. In contrast to the present findings, Ware et al^{22,23} reported in two separate studies that plasma vWF levels were significantly lower at baseline in subjects with indirect ARDS than in subjects with direct ARDS, although discrepancies in severity of illness may have been responsible for this difference in at least one of the studies. In a novel recent report, Schmidt et al²⁴ reported different circulating glycosaminoglycan patterns (likely reflecting degradation

TABLE 3] Prognostic Value of Plasma Biomarkers in Single-Center and Multicenter Studies

Biomarker, per 1-Log Increment	Death	P Value
Single center		
IL-8	1.65 (1.25-2.17)	< .001
IL-6	1.81 (1.34-2.45)	< .001
SP-D ^a	1.33 (0.82-2.14)	.25
RAGE	1.98 (1.18-3.33)	.01
Ang-2	2.54 (1.38-4.68)	.003
Multicenter		
IL-8	1.41 (1.27-1.57)	< .001
IL-6	1.24 (1.14-1.35)	< .001
SP-D	1.09 (0.95-1.24)	.23
RAGE	1.16 (1.003-1.34)	.045
Ang-2 ^b	1.43 (1.19-1.73)	< .001
vWF	1.83 (1.46-2.30)	< .001

Data are presented as OR (95% CI). See Table 2 legend for expansion of abbreviations.

^aOR for mortality in indirect ARDS, 0.99 (95% CI, 0.52-1.91; $P = .98$); OR for mortality in direct ARDS, 2.26 (95% CI, 0.94-5.45; $P = .07$). Test of interaction $P = .14$. There was no evidence for interaction for any other biomarker in the single-center data.

^bOR for mortality in indirect ARDS, 1.17 (95% CI, 0.85-1.62; $P = .33$); OR for mortality in direct ARDS, 1.51 (95% CI, 1.19-1.91; $P = .001$). Test of interaction $P = .22$. There was no evidence for interaction for any other biomarker in the multicenter data.

of the endothelial glycocalyx) in patients with direct vs indirect lung injury. However, to our knowledge, the current study is the first to test a multipathway panel of biomarkers in two separate cohorts of patients with direct vs indirect ARDS.

What implications might distinct molecular phenotypes reflecting different patterns of cellular injury in patients with direct and indirect ARDS have for future research? For one, if ARDS in patients with direct lung injury is primarily characterized by lung epithelial injury, then novel trials of therapies designed to target the lung epithelium (eg, keratinocyte growth factor) may benefit from preferentially enrolling these patients.²⁵ Likewise, therapies focused on modulating endothelial function (eg, statins, recombinant angiopoietin-1) may benefit from targeting patients with indirect causes of ARDS such as nonpulmonary sepsis. Alternatively, stratified randomization and analysis strategies may be relevant for broader therapeutic studies or studies of novel therapeutics in which the precise mechanism of action is unknown. More broadly, these findings represent a step toward the identification of molecular phenotypes of ARDS. The identification of molecular subphenotypes

of other heterogenous syndromes, such as asthma and breast cancer, has revolutionized treatment of these conditions.²⁶⁻²⁸ For example, the recognition that asthma may be characterized by more or less T-helper 2-type inflammation has already demonstrated an impact in clinical trials and continues to be a major research focus.^{27,29} Identification of molecular phenotypes of critical illness has lagged behind these other areas and has the potential to greatly affect future research and clinical care.

Most of the literature identifying the pathologic and prognostic significance of the biomarkers studied in the current analysis used patient data and samples from the high tidal volume era.^{10,20-22,30} Although the prognostic value of the measured biomarkers was not the primary focus of this project, the finding that five of the six measured biomarkers (IL-6, IL-8, Ang-2, RAGE, and vWF) remain strongly predictive of clinical outcomes in the low tidal volume era is important, particularly because the current study included both a more-select group of patients enrolled in a randomized controlled trial and a broader group of patients enrolled in an observational cohort. These findings add to the previous literature supporting the value of these plasma biomarkers for prognosis, regardless of the etiology of ARDS. Of note, SP-D, which reflects lung epithelial injury, was the only plasma biomarker not associated with clinical outcomes in the present analyses, perhaps because the lower tidal volumes with which patients are now ventilated lead to less lung epithelial injury.

Although SP-D as a biomarker of lung epithelial injury was consistently higher in patients with direct ARDS in both cohorts, the higher levels of RAGE in direct ARDS in the single-center cohort were not observed in the multicenter cohort. Furthermore, RAGE levels were substantially higher across the board in the multicenter cohort than in the single-center cohort (Figs 1, 2). RAGE is a pattern recognition receptor expressed on multiple cell types but is constitutively expressed at its highest levels on alveolar type 1 epithelial cells. As such, it has been used in both experimental and clinical studies as a marker of alveolar epithelial injury.^{10,31,32} However, RAGE also plays an important role in the innate immune response.³³ The discrepancy in findings between the two cohorts may reflect the proinflammatory role of RAGE in innate immunity and suggests that it may be less useful than SP-D in discriminating direct from indirect ARDS. In concert with these differences, IL-6 and IL-8 levels, which were significantly higher in indirect ARDS in the multicenter cohort, did not differ in the single-center cohort between indirect and direct

ARDS. This discrepancy could be a result of greater statistical power in the larger cohort or may reflect other, unmeasured differences between the two studies. Importantly, in both cohorts, Ang-2 was a robust indicator of indirect ARDS, whereas SP-D was a consistent marker of direct ARDS, suggesting that these two biomarkers may be the most reliable indicators of the direct vs indirect molecular phenotype.

The current study has several strengths, including the use of two distinct cohorts to replicate findings, a diverse patient population drawn from multiple centers around the United States, the use of a broad panel of biomarkers designed to capture several different aspects of ARDS pathogenesis, and rigorous approaches to biomarker measurement using the same validated assays for both cohorts. The study also has some limitations. First, not all biomarkers previously associated with ARDS pathogenesis or prognosis were measured. Other biomarkers associated with lung epithelial cell injury (club cell 16, KL-6),^{34,35} endothelial injury (soluble intercellular adhesion molecule-1, circulating glycosaminoglycans),^{24,30} disordered coagulation and fibrinolysis (plasminogen activator inhibitor-1, protein C, thrombomodulin),^{36,37} and inflammation (IL-1/IL-1-receptor antagonist, soluble tumor necrosis factor receptor 1),³⁸ to name a few, might further enhance the molecular phenotypes of epithelial and endothelial injury in direct vs indirect ARDS. Second, the larger of the two patient samples used in these analyses came from a secondary analysis of a randomized controlled trial, which excluded many patients

at highest risk for mortality from ARDS and was not designed to test the hypothesis under study in this analysis. This could potentially limit generalizability and dampen the strength of the prognostic associations. This limitation is mitigated by the inclusion of the single-center sample from a cohort that had many fewer exclusions and in which findings were largely similar to the multicenter sample. Third, the etiology of ARDS was identified in the multicenter cohort by the investigative team at the local site in contrast to the single-center study in which a single investigator (L. B. W.) classified all ARDS risk factors. This inherent variability introduced by the multicenter design may partly explain why some of the biomarker differences are slightly less robust in the multicenter cohort. Finally, biomarker data were not available on all patients enrolled in the multicenter cohort. Biomarker data were missing for some patients, as further detailed in e-Appendix 1, because of the lack of plasma availability; however, no substantive differences were found between patients with and without plasma samples (data not shown).

In summary, we present data from two separate human studies demonstrating that direct ARDS is characterized by more severe lung epithelial injury than indirect ARDS and, conversely, that indirect ARDS is characterized by more severe endothelial injury and inflammation. These findings represent a significant step toward the identification of molecular phenotypes of ARDS and may have important implications for the design and conduct of future clinical trials in ARDS.

Acknowledgments

Author contributions: C. S. C. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. C. S. C. and L. B. W. contributed to the study design, data cleaning and analysis, data interpretation, drafting and revision of the manuscript, and final approval of the manuscript; D. R. J. and K. N. K. contributed to the data collection and cleaning, data interpretation, and critical revision and final approval of the manuscript; and G. R. B., A. K. M., and M. A. M. contributed to data interpretation and critical revision and final approval of the manuscript.

Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following conflicts of interest: Drs Calfee, Matthay, and Ware have previously served on advisory boards for GlaxoSmithKline plc, and Drs Calfee and Matthay have received grant funding from GlaxoSmithKline plc. Drs Janz, Bernard, May, and Kangelaris have reported that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

Role of sponsors: The sponsors had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

Additional information: The e-Appendix can be found in the Supplemental Materials section of the online article.

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