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Endothelial Biomarkers Are Associated With Indirect Lung Injury in Sepsis-Associated Pediatric Acute Respiratory Distress Syndrome

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Objectives: Acute respiratory distress syndrome occurring in the setting of direct versus indirect lung injury may reflect different pathobiologies amenable to different treatment strategies. We sought to test whether a panel of plasma biomarkers differed between children with sepsis-associated direct versus indirect acute respiratory distress syndrome. We hypothesized that a biomarker profile indicative of endothelial activation would be associated with indirect acute respiratory distress syndrome.

Design: Observational cohort.

Setting: Academic PICU.

Subjects: Patients less than 18 years old with sepsis-associated direct (pneumonia, $n = 52$) or indirect (extrapulmonary sepsis, $n = 46$) acute respiratory distress syndrome.

Interventions: None.

Measurements and Main Results: Of 58 biomarkers examined, 33 differed by acute respiratory distress syndrome subtype. We used classification and regression tree methodology to examine associations between clinical and biochemical markers and acute respiratory distress syndrome subtype. The classification and regression tree model using only clinical variables (age, sex, race, oncologic comorbidity, and Pediatric Risk of Mortality-III score) performed worse than the classification and regression tree model using five clinical variables and 58 biomarkers. The best classification and regression tree model used only four endothelial biomarkers, including elevated angiotensin-2/angiotensin-1 ratio, vascular cell-adhesion molecule, and von Willebrand factor, to identify indirect acute respiratory distress syndrome. Test characteristics were 89% (80–97%) sensitivity, 80% (69–92%) specificity, positive predictive value 84% (74–93%), and negative predictive value 86% (76–96%).

Conclusions: Indirect lung injury in children with acute respiratory distress syndrome is characterized by a biomarker profile indicative of endothelial activation, excess inflammation, and worse outcomes. A model using four biomarkers has the potential to be useful for more precisely identifying patients with acute respiratory distress syndrome whose pathobiology may respond to endothelial-targeted therapies in future trials.

Key Words: acute respiratory distress syndrome; children; endothelium; lung injury; pediatric; sepsis

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Acute respiratory distress syndrome (ARDS) that occurs in the setting of infection can result from indirect (e.g., extrapulmonary sepsis) or direct (i.e., pneumonia) lung injury. Etiology of lung injury is not always considered in trials of ARDS, which may have contributed to negative trials of ARDS therapies. This issue is exacerbated in children, as pediatric ARDS

management is often extrapolated from adults, despite possessing a distinct epidemiology (1). There are a paucity of studies examining the differences in biologic mechanism of pediatric ARDS, and improved understanding of the pathobiology of lung injury may allow an evaluation of differential response to therapies stratified by biologic subtypes.

Studies of adults with ARDS suggest differences in disease pathogenesis of ARDS arising from indirect versus direct lung injury (2), with indirect ARDS characterized by increased activation of the vascular endothelium and direct ARDS is characterized by epithelial injury (2–4). Our prior study suggested a potential role of endothelial activation in indirect ARDS occurring in children with extrapulmonary sepsis; however, this study excluded children with direct ARDS (5). Pneumonia, which reflects direct lung injury, is the most common cause of ARDS in children (1), and it remains unknown how its biomarker profile differs from that of indirect ARDS due to extrapulmonary infection.

Prior reports examining the pathobiologic mechanism of indirect ARDS in children, adults, and animal models have documented altered blood levels of biomarkers involved in regulation of vascular permeability (2, 4–7), neutrophil adhesion and chemotaxis (5, 8), platelet activation (5, 9, 10), and inflammation (3, 11). Prior reports examining the pathobiology of direct ARDS in adults (2) and mice (12) have documented increased circulating markers of lung epithelial injury. Few reports of indirect ARDS and no reports of direct ARDS included children, and the pathobiology of indirect versus direct ARDS has never been directly compared in children. Furthermore, these limited studies have interrogated relatively few biomarkers, thus providing only a partial view of endothelial activation and epithelial injury, and thus may miss an optimal combination of biomarkers to differentiate indirect from direct ARDS, and findings require external validation. Improved understanding of the pathobiology of lung injury in indirect versus direct ARDS could better inform our understanding of expected disease course (13) or response to therapies (14).

Focusing on children with pulmonary and extrapulmonary sepsis, the most common causes of ARDS in children (1), we sought to determine if alterations in a comprehensive panel of plasma biomarkers differed in children with sepsis-associated indirect versus direct ARDS. We hypothesized that alterations in specific biomarkers would differ by ARDS subtype. We further hypothesized that a biomarker profile consistent with endothelial activation would be associated with indirect ARDS.

MATERIALS AND METHODS

Study Design and Population

This study was approved with a waiver of informed consent by the Institutional Review Board at the Children's Hospital of Philadelphia (CHOP). We conducted a secondary analysis of prospectively collected plasma from a cohort of pediatric patients with ARDS admitted to the PICU of a single academic children's hospital (CHOP). Participants had been enrolled in one of two prospective observational parent studies of pediatric sepsis (15) and ARDS (4, 16) between May 2014 and January 2018.

The parent sepsis study included patients less than 18 years old with severe sepsis or septic shock defined as: 1) greater than or equal to two systemic inflammatory response syndrome criteria, 2) suspected or confirmed systemic infection, and 3) greater than or equal to two organ system dysfunctions or cardiovascular dysfunction (17), and it excluded patients with WBC count less than $0.5 \times 10^3/\mu\text{L}$, known mitochondrial disorder, or unrepaired cyanotic congenital heart disease. The parent ARDS study included intubated patients greater than 1 month and less than 18 years old with ARDS defined by Berlin criteria, which were in use when the first patient was enrolled, including: 1) acute respiratory failure within 7 days of known risk factor requiring mechanical ventilation, 2) $\text{PaO}_2/\text{FiO}_2 \leq 300$ in two consecutive arterial blood gas samples drawn at least 2 hours apart with the patient receiving invasive positive end-expiratory pressure at least 5 cm H_2O , and 3) bilateral infiltrates on chest x-ray (18), and it excluded patients with chronic invasive mechanical ventilation, respiratory failure from primarily cardiac failure, or unrepaired cyanotic congenital heart disease.

Patients eligible for the current study had blood collected as part of their participation in the parent study, had sufficient volume of residual blood to permit biomarker analysis, and had consented for residual blood to be used for future research. This analysis was limited to patients who met criteria for sepsis (17) within 72 hours of meeting criteria for ARDS (18).

The primary site of infection was determined to be pulmonary (i.e., pneumonia) or extrapulmonary using established criteria (19, 20) with three-person adjudication of cases in which the site of infection was ambiguous after initial review. Patients with "direct ARDS" had infectious pneumonia and patients with "indirect ARDS" had extrapulmonary sepsis.

Biomarkers

Blood was collected in an EDTA or lithium heparin tube less than or equal to 72 hours of meeting criteria for ARDS. Samples were centrifuged within 30 minutes of collection at $3,000 \times g$ for 10 minutes and aliquoted plasma was stored at -80°C . Plasma was thawed once at the time of biomarker measurement, which was performed at the Penn Center for Cellular Immunotherapies.

Biomarker panel components were defined a priori based on prior reports suggesting utility in sepsis, ARDS, or pathways distinguishing these syndromes including inflammatory biomarkers (C-reactive protein, endocan, interleukins, interferons, monocyte chemoattractant protein, monocyte induced by interferon γ , macrophage inflammatory protein-1 α/β , serum glycoprotein 130, soluble receptor for advanced glycation end-products [sRAGE], and tumor necrosis factor [TNF]- α/β [4, 21–23]) and endothelial biomarkers, including: angiopoietin family biomarkers (Ang-1, Ang-2, Ang-2/-1 ratio, and Tie-2 [5, 6, 24]), cell adhesion molecules (intracellular adhesion molecule, vascular cell-adhesion molecule [VCAM], E-selectin, and endocan [25, 26]), vascular endothelial growth factor (VEGF) family biomarkers (VEGF, soluble fms-like tyrosine kinase, VEGF-R1, VEGF-R2, and VEGF-R3 [5, 27]), prothrombotic (von Willebrand factor [vWF] and thrombomodulin [9, 28]), chemotactic proteins (eotaxin, interferon gamma-induced protein-10, cluster of differentiation [CD]-14, CD-30,

and CD-163 [29]), and growth factors (epidermal growth factor, fibroblast growth factor, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and hepatocyte growth factor [1, 30]). Biomarkers were measured using a combination of commercially available assays (Life Technologies, Carlsbad, CA; EMD Millipore; Darmstadt, Germany), custom multiplex panels (R&D Systems, Minneapolis, MN), and enzyme-linked immunosorbent assay (ELISA) with human-specific reagents. Samples were run in duplicate, and standard curves for each analyte were required to have $R^2 > 95\%$.

Data Collection

Clinical data including demographics, comorbidities, and primary site of infection were collected using the Research Data Capture system (31) by one investigator (J.E.W.), who reviewed all patient charts and was blinded to biomarker measurements. Oxygenation index (OI [$\text{FiO}_2 \times \text{mean airway pressure}$]/ Pao_2) and $\text{Pao}_2/\text{FiO}_2$ were calculated to reflect ARDS severity at the time of biomarker measurement. In the absence of arterial blood gas data, we computed oxygen saturation index ($\text{FiO}_2 \times \text{mean airway pressure}/\text{Spo}_2$), and $\text{Spo}_2/\text{FiO}_2$ ratio then converted these values to OI and $\text{Pao}_2/\text{FiO}_2$ ratios (32), using accepted cutoffs (1). Illness severity less than 12 hours of PICU admission was summarized using the Pediatric Risk of Mortality (PRISM)-III score (33).

Outcomes

The main outcomes were biomarker concentrations by ARDS subtypes.

Confounders

Clinical variables were chosen a priori as potential confounders based on biologic plausibility of an association with the exposure or outcome, providing they were absent in the causal pathway linking exposure and outcome (34). We considered five confounders: age, sex, race, oncologic comorbidity, and PRISM-III score.

Statistical Analyses

Analyses were performed using Stata version 14 (StataCorp, College Station, TX) and SAS (SAS Institute, Cary, NC). We reported median and interquartile range for continuous variables and proportions for categorical variables. We compared continuous variables using Wilcoxon rank sum and proportions using chi-squared or Fisher exact test.

First, we determined the association between biomarkers and ARDS subtype (indirect/direct) using univariate logistic regression. When used as predictors, biomarkers were expressed as log-transformed units of standard deviation to facilitate comparison with other arrays. Next, we generated two models to determine the association between clinical or biochemical predictor variables and ARDS subtype using the classification and regression tree (CART [35]). Compared with traditional regression approaches, CART provides flexibility to allow for nonlinear effects and interaction effects of predictors, and it generates optimal cutoffs for continuous predictors (35).

We also performed a supplementary analysis using elastic net (EN) as a comparator method, since it takes into account variable

grouping, in order to account for potential biomarker clusters that naturally associated. We used CART rather than EN as the primary analytic method, because we did not know if the relationship between biomarkers and ARDS type would be linear, CART does not require selection of tuning parameters, and the optimal cutoff values required for EN, which represent the probability of ARDS type per patient, are subject to bias.

The first CART model used only the five clinical variables (age, sex, race, oncologic comorbidity, and PRISM-III score) as predictors, and the second model used all 58 biomarkers and the five clinical variables. Each model resulted in a decision tree in which each branchpoint was determined by a predictor variable and a cutoff value, and each end node contains a predictor for the outcome variable. We used five-fold cross-validation to mitigate overfitting. We reported the test characteristics for each decision tree, and the models were compared using a paired likelihood regression method (36).

The EN model used 10-fold cross validation to select optimal tuning parameters α and λ for penalized logistic regression models. We applied EN using the selected α and λ to select a set of predictors from all biomarkers. The final models were refitted using the selected variables. We used individual predicted probabilities to generate the receiver-operating-characteristic curve (ROC) and reported the area under the ROC curve (AUC). We chose a probability cutoff optimized for combined sensitivity and specificity.

RESULTS

Among 332 potentially eligible patients, 98 met all inclusion criteria, including 46 with indirect ARDS and 52 with direct ARDS (**Supplemental Fig. 1**, <http://links.lww.com/CCX/A443>). Fifteen cases (15%) required three-person adjudication to determine the primary site of infection. Patient characteristics are shown in **Table 1**. Patients with direct ARDS had a higher prevalence of pulmonary comorbidity ($p = 0.006$). PRISM-III score was higher in patients with indirect ARDS ($p = 0.007$). Fifteen patients (15%) died prior to hospital discharge, including 11 with indirect and four with direct ARDS ($p = 0.046$).

Individual Biomarkers and ARDS Subtype

Figure 1 shows the unadjusted odds of indirect ARDS for each biomarker. **Supplemental Table 1** (<http://links.lww.com/CCX/A445>) shows biomarker values by ARDS subtype. Thirty-three biomarkers differed by ARDS subtype; 31 were higher in indirect ARDS, whereas Ang-1 and regulated on activation, normal T cell expressed and secreted (RANTES) were higher in direct ARDS.

Biomarker Clusters and ARDS Subtype

In the first CART model (model 1), which used five clinical variables (age, sex, nonwhite/white race, oncologic comorbidity, and PRISM-III) as predictors and ARDS subtype as the outcome, a tree using age and PRISM-III score discriminated indirect from direct ARDS (**Supplemental Fig. 2**, <http://links.lww.com/CCX/A444>).

In the second CART model (model 2), which used all biomarkers plus five clinical variables as predictors, elevated Ang-2/Ang-1 ratio, VCAM, and vWF concentration identified indirect ARDS (**Fig. 2**).

The positive likelihood ratio (LR+) of model 1 was 2.03, the LR+ of model 2 was 4.52, and the ratio of the two was 0.45 (95% CI,

TABLE 1. Patient Demographics and Clinical Characteristics by Group Are Shown

Patient Characteristic	Indirect ARDS (n = 46)	Direct ARDS (n = 52)	p ^a
Age (yr)	5.1 (3.6–13.5)	5.9 (2.5–13.2)	0.83
Sex, n (%)			
Female	21 (46)	23 (44)	0.53
Male	25 (54)	29 (56)	
Race			
Black/African American	10 (22)	15 (29)	0.04^a
Other	8 (17)	18 (35)	
White	28 (61)	19 (37)	
Comorbidities ^b			
None	9 (20)	10 (19)	0.58
Cardiac	9 (20)	10 (19)	0.58
Pulmonary	10 (22)	26 (50)	0.006^a
Gastrointestinal	19 (41)	18 (35)	0.54
Endocrine	7 (15)	10 (19)	0.40
Renal	1 (2)	0 (0)	0.47
Rheumatologic	3 (7)	0 (0)	0.10
Immunologic	3 (7)	4 (8)	0.57
Hematologic	4 (9)	2 (4)	0.28
Oncologic	11 (24)	4 (8)	0.03^a
Musculoskeletal	7 (15)	6 (12)	0.41
Dermatologic	1 (2)	1 (2)	0.72
Neurologic	18 (39)	28 (54)	0.11
Immunosuppressed ^c	16 (35)	9 (17)	0.40
Oxygenation index	7.6 (5.1–11)	8 (5.1–13.4)	0.52
Pao ₂ /Fio ₂ ratio	241 (166–291)	225 (153–291)	0.50
Pediatric Risk of Mortality-III score	15 (10–24)	9 (6–15)	0.007^a
Sepsis source			
Respiratory	0 (0)	52 (100)	
Blood	19 (41)	0 (0)	
Gastrointestinal	12 (26)	0 (0)	
Urinary	5 (11)	0 (0)	
CNS	1 (2)	0 (0)	
Skin	2 (4)	0 (0)	
Musculoskeletal	1 (2)	0 (0)	
Unknown	6 (13)	0 (0)	

ARDS = acute respiratory distress syndrome.

^ap value reflects difference between indirect versus direct ARDS. Significance is defined as $p < 0.05$.

^bCategories are not mutually exclusive as some patients had more than one baseline comorbidity.

^cImmunosuppressed patients included those with immune deficiencies and those receiving immunosuppressive therapies.

Data are presented as median (interquartile range) or n (%). Boldface values indicate race, pulmonary and oncologic comorbidity, and Pediatric Risk of Mortality-III score differed by ARDS type.

0.25–0.80, $p = 0.007$). The negative likelihood ratio (LR⁻) of model 1 was 0.20, the LR⁻ for model 2 was 0.14, the ratio of the two was 1.42 (95% CI, 0.55–3.70, $p = 0.5$). Both ratios suggest that model 2 is significantly better for discriminating ARDS subtype than model 1. Diagnostics for both models are presented in **Table 2**. Compared with model 1, model 2 had similar sensitivity (89% [80–97%] vs 89% [77–78%]), superior specificity (80% [69–92%] vs 57% [41–71%]), positive predictive value (84% [74–93%] vs 70% [57–80%]), and negative predictive value (86% [76–96%] vs 81% [64–93%]).

In the EN model, which used all biomarkers, high Ang-2, interleukin (IL)-8, TNF-RII, and low RANTES identified indirect ARDS. Compared with CART model 2, EN had inferior sensitivity (65% [51–78%] vs 86% [80–97%]), similar specificity (83% [69–92%] vs 80% [69–92%]), positive predictive value (81% [66–91%] vs 84% [74–93%]), and inferior negative predictive value (68% [54–80%] vs 86% [76–96%]). The AUC was 0.84 and the optimal cutoff probability was 68%. Diagnostics are summarized in **Supplemental Table 2** (<http://links.lww.com/CCX/A446>).

DISCUSSION

In this cohort of children with sepsis-associated ARDS, 33 of 58 biomarkers differed by ARDS subtype, and a biomarker profile indicative of endothelial activation and inflammation was associated with indirect ARDS. Using CART analysis, a model using four endothelial biomarkers discriminated ARDS subtype better than a model using clinical variables. Using EN analysis, a model using four biomarkers (one endothelial and three inflammatory) discriminated ARDS subtype. Elevated Ang-2 was identified by both models to be associated with indirect ARDS. This result supports a role for endothelial activation and inflammation in the pathogenesis of indirect, but not direct, ARDS in children. Discrimination of disease subphenotypes according to pathobiology may permit investigation of therapies most likely to ameliorate sequelae of inflammation or endothelial activation (predictive enrichment) and may improve identification of patients at risk for worse outcomes (prognostic enrichment).

Elevated Ang-2 identified indirect ARDS by CART and EN. Ang-2 antagonism of the Tie-2 receptor causes weakened endothelial cell junctions, increased expression of leukocyte adhesion molecules (e.g., VCAM), and increased prothrombotic proteins at the endothelial surface (e.g., vWF) (37). Ang-1 promotes vascular quiescence (37), so increased Ang-2/Ang-1 ratio indicates a state of endothelial activation, which has been associated with poor outcomes in patients with sepsis and acute lung injury (5). VCAM promotes migration of leukocytes and endothelial progenitor cells to the source of infection and vascular damage (38). vWF causes platelet aggregation on the vessel wall (39) and is associated with microangiopathy in patients with sepsis (40, 41) and worse clinical outcomes in patients with ARDS (42).

Our finding that increased Ang-2/Ang-1 ratio is associated with indirect ARDS by CART is consistent with prior literature, including our prior finding of increased Ang-2 in patients with ARDS and indirect lung injury (4). Calfee et al (24) previously reported that low Ang-2 was associated with direct ARDS in septic adults,

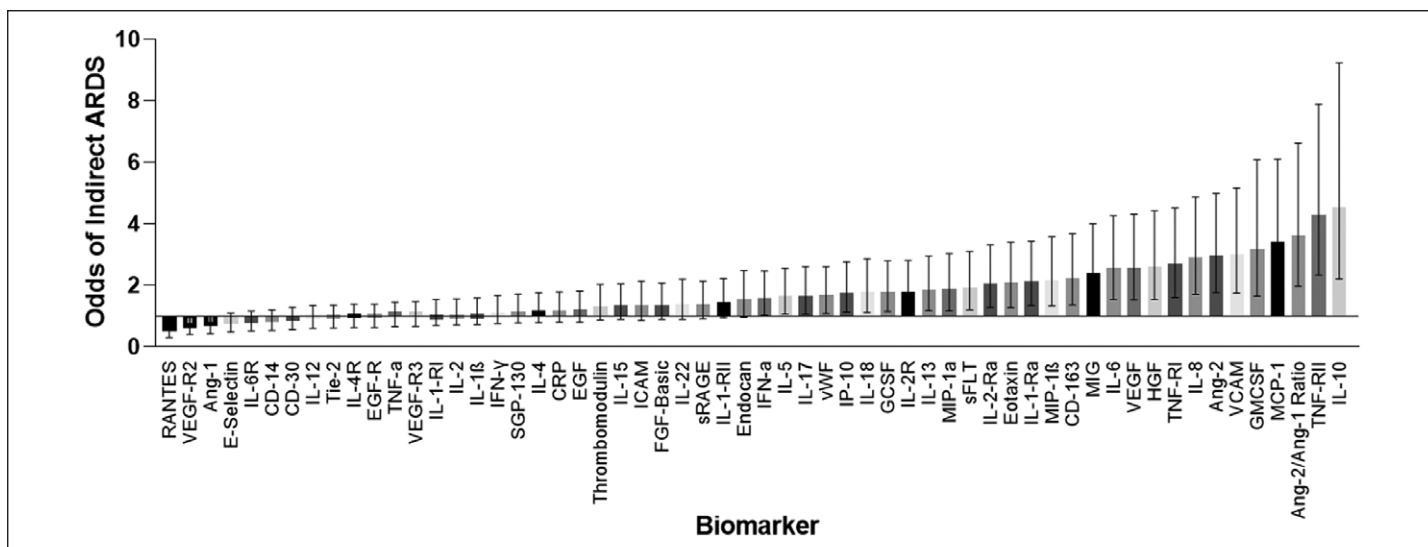


Figure 1. Odds of indirect acute respiratory distress syndrome (ARDS) by biomarker. Logistic regression was used to determine the association between biomarker and ARDS subtype. Odds ratio (OR) < 1 indicates an association with direct ARDS and OR > 1 indicates an association with indirect ARDS. Whiskers represent 95% CI. Ang = angiopoietin, ARDS = acute respiratory distress syndrome, CD = cluster of differentiation, CRP = C-reactive protein, EGF = epidermal growth factor, FGF = fibroblast growth factor, GCSF = granulocyte colony-stimulating factor, GMCSF = granulocyte-macrophage colony-stimulating factor, HGF = hepatocyte growth factor, ICAM = intracellular adhesion molecule, IFN = interferon, IL = interleukin, IP = interferon gamma-induced protein, MCP = monocyte chemoattractant protein, MIG = monocyte induced by interferon γ , MIP = macrophage inflammatory protein, -R = -receptor, RANTES = regulated on activation, normal T cell expressed and secreted, sFLT = soluble fms-like tyrosine kinase, SGP = serum glycoprotein, sRAGE = soluble receptor for advanced glycation end-products, Tie-2 = tyrosine kinase with immunoglobulin-like loop epidermal growth factor homology domain 2, TNF = tumor necrosis factor, VCAM = vascular adhesion molecule, VEGF = vascular endothelial growth factor, vWF = von Willebrand factor.

and Reilly et al (7) used causal inference methods to implicate Ang-2 in the development of ARDS in septic adults. Our finding that increased vWF is associated with indirect ARDS by CART is consistent with prior reports by Rubin (9), who documented high vWF in adults with ARDS secondary to indirect lung injury, and Calfee et al (24), who documented lower vWF in adults with direct compared with indirect ARDS. To our knowledge, VCAM has not previously been studied in sepsis-associated ARDS.

Several biomarkers measured in our cohort, which did not discriminate ARDS subtype, performed differently from those in prior reports. Ware et al (3) found sRAGE, IL-6, and IL-8 to be associated with development of ARDS in adults with sepsis. IL-6

and IL-8 were higher in our patients with indirect compared with direct ARDS (Supplemental Table 1, <http://links.lww.com/CCX/A445>), and elevated IL-8 was associated with indirect ARDS by EN. IL-6 and IL-8 levels were lower in our cohort than Ware et al's (3), possibly due to differences in timing of measurements, measurement method (i.e., multiplex versus singleplex ELISA), or pediatric versus adult physiology. Despite being suggested as a marker of alveolar epithelial damage (12), we found no difference in sRAGE between indirect and direct ARDS. However, sRAGE is also expressed in the endothelium and is associated with the strength of the immune response (43).

Supplementary EN analysis identified an association between high TNF-R2, consistent with prior literature showing elevated TNF-R2 in adult ARDS (44). TNF-R2 is an inflammatory biomarker but may indicate endothelial activation as it is expressed on endothelial cells (45). Few studies have demonstrated elevated RANTES in rodent models of ARDS (46), in contrast with our finding that low RANTES associated with indirect ARDS. One possible interpretation of our finding is that elevation in biomarkers associated with endothelial activation is more prominent than those associated with inflammation in indirect ARDS.

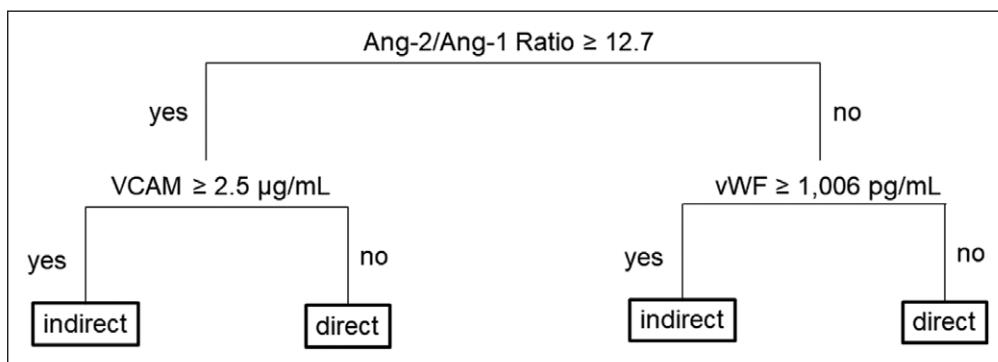


Figure 2. Decision tree from classification and regression analysis (CART) analysis using biomarkers and clinical variables. CART analysis using all 58 biomarkers and five clinical variables (age, sex, non-White/White race, oncologic comorbidity, and Pediatric Risk of Mortality-III score), only angiopoietin (Ang)-2/Ang-1 ratio, vascular adhesion molecule (VCAM), and von Willebrand factor (vWF) concentration-discriminated acute respiratory distress syndrome (ARDS) subtype. The decision tree shows branchpoints based on raw biomarker concentration determined by the model. In the first generation, the branchpoint is determined by Ang-2/Ang-1 ratio ≥ 12.7 (high) vs < 12.7 (low). In the second generation, branchpoints are determined by VCAM for patients with high Ang-2/Ang-1 ratio and by vWF for patients with low Ang-2/Ang-1 ratio. Indirect ARDS is associated with VCAM ≥ 2.5 $\mu\text{g/mL}$ and high Ang-2/Ang-1 ratio, and with vWF $\geq 1,006$ pg/mL and low Ang-2/Ang-1 ratio.

TABLE 2. Diagnostic Characteristics of Two Classification and Regression Analysis Models

Model	Indirect ARDS (n = 46)	Direct ARDS (n = 52)	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive Predictive Value % (95% CI)	Negative Predictive Value % (95% CI)
Model 1 (clinical variables)			89 (77–96)	57 (41–71)	70 (57–80)	81 (64–93)
Predicted indirect	26	6				
Predicted direct	20	46				
Model 2 (biomarkers and clinical variables)			89 (80–97)	80 (69–92)	84 (74–93)	86 (76–96)
Predicted indirect	37	6				
Predicted direct	9	46				

ARDS = acute respiratory distress syndrome.

misclassification bias. ARDS subtype was determined by a single author's review of the electronic medical record and a minority (15%) of cases required three-person adjudication to determine the primary site of infection. Second, our cohort was composed of patients who had been enrolled in either of two prospective parent studies with overlapping but distinct enrollment criteria, which may have contributed to selection bias and may limit generalizability. Third, our sample size provided adequate power to investigate our aims but precluded subgroup analyses, including the ability to investigate thoroughly the possibility of differences in endothelial activation associated with race or chemotherapy exposure given the relatively high proportion of oncologic comorbidity in the indirect ARDS group. Finally, although our selection of biomarkers was guided by prior literature, it was not exhaustive, and endothelial biomarkers were overrepresented. Results from CART analysis were supported but completely replicated by supplementary EN analysis. Future studies are needed to validate prospectively our findings.

Our study also has several strengths. This was the first study to use plasma biomarkers to compare indirect and direct ARDS in pediatric patients. We used a large panel of biomarkers, assembled based on previous adult, pediatric, and animal studies, to examine pathobiologic differences in pediatric ARDS subphenotypes. The model of four biomarkers that identified indirect ARDS demonstrated both biologic plausibility and good discriminative performance.

CONCLUSIONS

In a cohort of pediatric ARDS, biomarkers indicative of endothelial activation and inflammation were associated with indirect ARDS. Models incorporating endothelial and inflammatory biomarkers identified indirect ARDS with good sensitivity, specificity, and positive and negative predictive values. The association of biomarkers indicative of endothelial activation and inflammation with indirect, but not direct, ARDS suggests differences in underlying pathobiology. External validation is required. Prospective studies are warranted to investigate further biological differences in ARDS subtypes. Plasma biomarkers, as a tool for predictive enrichment, may aid in identification of patients with ARDS whose physiology may respond more favorably to targeted therapies in future studies.

This study was performed at the Children's Hospital of Philadelphia.

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