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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

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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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ARDS is by definition heterogenous, 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 significant 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 (Pao_2/Fio_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/Fio_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); angiopoietin-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