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We conducted analyses in 3 cohorts of critically ill trauma and sepsis patients \( n = 3710 \) genotyped on genome-wide platforms to determine the association of the A1 blood type genotype with ARDS risk. We subsequently determined whether associations were present in FUT2-defined nonsecretors who lack ABO antigens on epithelium, but not endothelium. In a patient subgroup, we determined the associations of blood type with plasma levels of endothelial glycoproteins and disseminated intravascular coagulation (DIC). Lastly, we tested whether blood type A was associated with less donor lung injury recovery during human ex vivo lung perfusion (EVLP).

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The ABO histo-blood group, endothelial activation, and acute respiratory distress syndrome risk in critical illness

John P. Reilly,1,2 Nuala J. Meyer,1,2 Michael G.S. Shashaty,1,2,3 Brian J. Anderson,1,2 Caroline Ittner,1 Thomas G. Dunn,1,2 Brian Lim,1 Caitlin Forker,1 Michael P. Bonk,1 Ethan Kotloff,1 Rui Feng,3 Edward Cantu,2,4 Nilam S. Mangalmurti,1,2 Carolyn S. Calfee,1,6 Michael A. Matthay,5,6 Carmen Mikacenic,7 Keith R. Walley,8 James Russell,8 David C. Christiani,8 Mark M. Wurfel,7 Paul N. Lanken,1 Muredach P. Reilly,9,10 and Jason D. Christie1,2,3

1Division of Pulmonary, Allergy, and Critical Care, 2Center for Translational Lung Biology, 3Center for Clinical Epidemiology and Biostatics, and 4Division of Cardiovascular Surgery, Department of Surgery, University of Pennsylvania, Perelman School of Medicine, Philadelphia, Pennsylvania, USA. 5Department of Medicine and 6Department of Anesthesia and Cardiovascular Research Institute, University of California, San Francisco, San Francisco, California, USA. 7Division of Pulmonary, Critical Care, and Sleep Medicine, University of Washington, Seattle, Washington, USA. 8Centre for Heart Lung Innovation, University of British Columbia, Vancouver, British Columbia, Canada. 9T.H. Chan School of Public Health, Harvard University, Boston, Massachusetts, USA. 10Irving Institute for Clinical and Translational Research, Columbia University Irving Medical Center, New York, New York, USA.

BACKGROUND. The ABO histo-blood group is defined by carbohydrate modifications and is associated with risk for multiple diseases, including acute respiratory distress syndrome (ARDS). We hypothesized that genetically determined blood subtype A1 is associated with increased risk of ARDS and markers of microvascular dysfunction and coagulation.

METHODS. We conducted analyses in 3 cohorts of critically ill trauma and sepsis patients (n = 3710) genotyped on genome-wide platforms to determine the association of the A1 blood type genotype with ARDS risk. We subsequently determined whether associations were present in FUT2-defined nonsecretors who lack ABO antigens on epithelium, but not endothelium. In a patient subgroup, we determined the associations of blood type with plasma levels of endothelial glycoproteins and disseminated intravascular coagulation (DIC). Lastly, we tested whether blood type A was associated with less donor lung injury recovery during human ex vivo lung perfusion (EVLP).

RESULTS. The A1 genotype was associated with a higher risk of moderate to severe ARDS relative to type O in all 3 populations. In sepsis, this relationship was strongest in nonpulmonary infections. The association persisted in nonsecretors, suggesting a vascular mechanism. The A1 genotype was also associated with higher DIC risk as well as concentrations of thrombomodulin and von Willebrand factor, which in turn were associated with ARDS risk. Blood type A was also associated with less lung injury recovery during EVLP.

CONCLUSION. We identified a replicable association between ABO blood type A1 and risk of ARDS among the critically ill, possibly mediated through microvascular dysfunction and coagulation.

FUNDING. NIH HL122075, HL125723, HL137006, HL137915, DK097307, HL115354, HL101779, and the University of Pennsylvania McCabe Fund Fellowship Award.

Introduction

Acute respiratory distress syndrome (ARDS) is a common complication of critical illness characterized by lung inflammation, epithelial and endothelial dysfunction, alveolar capillary leak, and microthrombosis (1, 2). Clinically, it is recognized as diffuse noncardiogenic pulmonary edema and severe hypoxemia leading to acute respiratory failure in the setting of a precipitating illness (3). It is estimated that ARDS affects at least 190,000 people in the United States annually, and mortality ranges from 30% to 50% (4). Currently, ARDS therapies are largely supportive, including lung-protective mechanical ventilation, fluid restrictive management, and prone positioning (5-7). Despite many clinical trials, however, no pharmacological therapy aimed at its pathogenesis has been consistently shown to either prevent ARDS or reduce mortality (8-11). One potential explanation for this failure of translation from promising preclinical therapeutics to human disease is pathogenic heterogeneity of ARDS, in which genetic factors may play a role (12-16).
We previously identified an association between ABO histo-blood type A and an increased risk of ARDS in trauma and sepsis populations (17). The ABO histo-blood group is genetically determined by the ABO gene, which encodes a family of glycosyltransferases that catalyze specific carbohydrate modifications on glycans and glycoproteins (18). In addition to RBCs, the ABO glycans are present on the surface of platelets, endothelium, epithelium, circulating solubilized glycoproteins, and epithelial secretions. The genetic diversity seen in the ABO gene has been shaped by evolutionary host-pathogen interactions that resulted in selective advantages over infectious diseases, particularly malaria, but may now contribute to altered risk for inflammatory conditions such as ARDS (19–21). More recently, the ABO locus has been identified to be among the most pleiotropic loci in the genome, influencing circulating blood levels of a host of glycoproteins (22–25). These include endothelium-derived proteins such as von Willebrand factor (vWF), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble thrombomodulin (sTM) (26–31). Plasma vWF, sICAM-1, and sTM are elevated in critical illnes and are markers of processes implicated in ARDS pathogenesis (32–35). Convergent with our findings in ARDS, ABO blood type A has also been associated with increased risk of vascular diseases, including myocardial infarction and venous thromboembolism (36, 37).

Although we previously identified an association between ABO blood type A and ARDS, routine antigen testing does not reflect the full antigen complexity of the ABO histo-blood group. Within blood type A, heterozygotes (A/O) express lower A antigen density relative to (A/A) homozygotes (38, 39). Additionally, there are 2 common genetically determined A subtypes, A1 and A2, which are distinguished by a 30–50-fold difference in A transferase activity. We reasoned that identifying a dose-response relationship of A antigen density with ARDS would provide stronger evidence of a causal link. Higher ARDS risk in A/A homozygotes compared with A/O heterozygotes and in patients with the A1 versus A2 genotype would provide evidence of such a relationship. Furthermore, the related FUT2 “secretor” gene encodes a fucosyltransferase that is necessary for the expression of ABO(H) antigens on epithelium and in secretions, but is not required for expression on endothelium, RBCs, or platelets. A common loss-of-function mutation in FUT2 results in the absence of ABO(H) antigens compared with blood bank–determined blood type (Sup -
is provided in Supplemental Table 6. The possession of an A1 allele was again independently associated with an increased risk of moderate or severe ARDS relative to the genetically inferred O blood type (OR 1.51; 95% CI 1.01–2.27; \( P = 0.044 \)). Similar to MESSI and PETROS, this association was attenuated by the addition of mild ARDS cases (OR 1.28; 95% CI 0.99–1.65; \( P = 0.056 \)).

Figure 4A provides the OR (95% CIs) for the association of ABO genotypes grouped by estimated A1 antigen density and ARDS relative to the genetically inferred O blood type. The ABO and ARDS association is driven by nonpulmonary sources of sepsis. Having validated the genetic association in 3 critically ill populations, we next sought to determine whether this association was present in both pulmonary and nonpulmonary sepsis. There is significant evidence for “ARDS heterogeneity” in sepsis based on the source of infection. Specifically, nonpulmonary (nonpneumonia) sepsis has been associated with higher levels of endothelial biomarkers, suggesting a more significant systemic vascular insult relative to pulmonary sepsis (50, 51). Given this evidence, we a priori planned to test for a statistical interaction in the association between ABO genotype and moderate to severe ARDS by source of sepsis. In MESSI and iSPAAR, the association between the A1 genotype and higher risk of ARDS relative to the O genotype was only present in patients with a nonpulmonary source of sepsis.
The P values for statistical interaction were 0.023 and 0.082 for MESSI and iSPAAR, respectively. Given that these P values were less than 0.10, we additionally present the results of the association between the A1 genotype relative to O and ARDS stratified by source of sepsis (Table 2; Figure 3, B and C; Figure 4, B and C).

FUT2-determined secretor status does not modify the ABO and ARDS association. Nonsecretors do not have ABO(H) antigens on epithelium or in secretions and can be identified using the rs601338 SNP in the FUT2 gene (40, 52). In order to determine whether epithelial secretor status was relevant to the ABO and ARDS association, we tested the association between ABO genotype and ARDS among genetically determined nonsecretors. Given the autosomal dominant inheritance of secretor status, subjects homozygous for the minor allele were FUT2-null and thus nonsecretors. In order to have sufficient power, we combined the nonsecretors from the PETROS and nonpulmonary sepsis MESSI cohorts who represented 25% and 24% of the overall cohorts, respectively. The A1 allele relative to the O allele was still associated with increased ARDS risk (OR 2.15; 95% CI 1.18–3.92; P = 0.012; n = 432) in nonsecretors adjusting for age, sex, population stratification, and sepsis versus trauma. This finding suggests that the presence of ABO antigens on epithelium is not necessary to influence ARDS risk. Supplemental Table 7 provides the association of the A1 allele versus O allele with moderate or severe ARDS risk, individually in MESSI stratified by sepsis source and PETROS among nonsecretors.

**ABO blood type is associated with biomarkers of endothelial activation in sepsis and trauma.** In ambulatory patients, ABO blood type A is associated with higher plasma levels of vWF and lower levels of sTM, sICAM-1, and E-selectin (26–28, 30–35, 41). However, critical illness is an evoked phenotype whereby plasma concentrations of endothelium-derived glycoproteins increase dramatically and are associated with the development of ARDS. We measured plasma concentrations of these 4 endothelium-derived glycoproteins in more than 500 PETROS and MESSI subjects. The 4 endothelium-derived glycoproteins demonstrated mild to moderate correlation with each other (Supplemental Table 8).

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race, and transplant center, ABO blood type A was associated with a significantly reduced likelihood of lung injury recovery relative to blood type O (OR 0.20; 95% CI 0.06, 0.64; \( P = 0.007 \)). Additionally, the last measured partial pressures of arterial oxygen on EVLP were statistically higher in blood type O relative to A (Table 4).

**Discussion**

In this study, we identified a consistent association between genetically determined ABO blood type A and higher ARDS risk in 3
critically ill populations, including a total of 3710 closely phenotyped patients. This relationship was driven by the A1 subtype of blood type A, which confers a significantly higher density of A antigens than the A2 subtype. In sepsis, subjects with a nonpulmonary source of infection drove the ABO and ARDS association. We also demonstrated that the association between ABO blood type A and ARDS was present in FUT2-determined nonsecretors, suggesting that the presence of A antigens on epithelium is not required to alter ARDS risk.

ABO blood type A was also associated with higher plasma levels of endothelial and coagulation biomarkers, sTM, vWF, and sICAM-1. Plasma levels of sTM and vWF were also associated with ARDS risk, providing evidence that endothelial activation and microvascular coagulation is important in the ABO-ARDS relationship. ABO blood type A was also associated with a higher risk of clinically determined DIC, which in turn conveyed a significantly higher risk of ARDS. Lastly, we demonstrated in an EVLP model that acutely injured lungs suboptimal for transplantation were less likely to recover their lung injury during EVLP if the donor blood type was A. Taken together, our study provides strong evidence that the ABO blood type A glycan confers a higher ARDS risk relative to blood type O mediated through microvascular activation and coagulation, suggesting a population that may particularly benefit from therapies targeting plasma glycoproteins such as sTM and vWF.

Although the ABO histo-blood group was first discovered more than 100 years ago, its role in human biology outside of transfusion medicine is incompletely understood (54). The antigenic diversity of the ABO histo-blood group has been linked to susceptibility and outcomes in a host of inflammatory and vascular diseases ranging from infections (e.g., severe malaria and Vibrio cholerae) to thrombotic disorders (e.g., myocardial infarction, venous thromboembolism, and stroke) (20, 21, 55). Evolutionary host-pathogen
interactions are believed to underlie the genetic diversity seen in the human ABO gene, likely resulting in altered risk to historic infections as well as modern inflammatory diseases, such as ARDS.

In our study, sTM and vWF were the proteins most strongly associated with both ABO blood type and ARDS risk in the setting of critical illness. Although our study cannot definitely prove causality, it is possible that these proteins mechanistically link ABO blood type and ARDS. Therefore, biological pathways that include sTM and/or vWF may be therapeutic targets in a critical illness endotype defined by ABO blood type A and high risk for ARDS. VWF is a large multimeric glycoprotein that is produced predominately by endothelial cells and released into circulation alongside other proteins from the Weibel-Palade bodies (30). The protein has a major role in microvascular coagulation by binding factor VIII and localizing it to sites of injury as well as coupling platelets to endothelial surfaces. Although it is not definitively known whether ABO antigens affect vWF secretion, function, or clearance, ABO(H) antigens are known to be present on the vWF molecule and are believed to alter the ability of ADAMTS13 to degrade vWF multimers. It is possible future therapies aimed at vWF, such as recombinant ADAMTS13, will be proven effective in ARDS if aimed at an endotype defined by blood type. Thrombomodulin is a glycoprotein present on the cell surface of endothelial cells and serves as a receptor for thrombin and accelerates thrombin-induced activation of protein C, leading to an anticoagulation effect (56). In the setting of critical illness, thrombomodulin is cleaved from the endothelial cell surface and released into circulation. The mechanisms linking sTM to ABO blood type remain unclear; however, recombinant human thrombomodulin was recently studied in a large randomized clinical trial in sepsis-associated coagulopathy (57). In this study, coagulopathy was defined as an elevated international normalized ratio (INR) or thrombocytopenia believed to be secondary to sepsis. The clinical

Figure 5. Box-and-whisker plots comparing the median concentrations of vWF, sTM, sICAM-1, and sE-selectin of ABO blood type A to O separately in trauma and sepsis. The box-and-whisker plots display the median value as a line within the boxes, the bounds of the box representing the IQR, and the whiskers representing the range of the data. P values are for the unadjusted comparison of ABO blood type A to O using the Wilcoxon rank-sum test. Blood type A and O sample sizes for each biomarker are vWF: 450 trauma, 465 sepsis; sTM: 451 trauma, 465 sepsis; sICAM-1: 449 trauma, 454 sepsis; sE-selectin: 451 trauma, 453 sepsis.
The Association between ABO Blood Type and COVID-19 Disease

ABO blood type and COVID-19 disease.

Our study was conducted prior to the COVID-19 pandemic and did not include COVID-19 patients. Future research is needed to determine whether our findings are relevant to the association between ABO blood type and COVID-19 disease.

We also identified an association between ABO blood type A and lower concentrations of plasma E-selectin in sepsis and trauma. Plasma E-selectin concentration was not associated with ARDS risk in our cohorts. In ambulatory patients, ABO blood type A is consistently associated with lower concentrations of plasma E-selectin (26, 59, 60). We hypothesize that the associations between ABO blood type and plasma E-selectin level identified in our cohorts represent the baseline relationship between blood type and E-selectin, rather than an evoked phenotype with relevance to ARDS. These findings suggest a specific endothelial injury pattern relevant to certain endothelium-derived glycoproteins, including vWF and sTM, but not all markers of endothelial activation.

In sepsis, the associations between genetically determined ABO blood type A1 and ARDS risk were only present in nonpulmonary sepsis. Additionally, the statistical tests of interaction between pulmonary and nonpulmonary sepsis were significant for ABO blood type A1 and ARDS risk. Possible reasons for this difference include differences in the distribution of the ABO blood type A subtypes between races, which were determined in this study but not our previous study; increased power in our current study; adjustment for population stratification; or an increased severity of illness observed in our current study populations.

Recently, a large genome-wide association study identified an association between the genetic variation that determines ABO blood type A and increased risk of severe COVID-19, the current global disease pandemic resulting from SARS-CoV-2 infection (61). These findings are consistent with a small study published in 2005 reporting an association between ABO blood type A and increased risk of infection with SARS-CoV (55). ARDS is the primary manifestation of COVID-19 in the critically ill, and a large number of patients who did not survive COVID-19 manifested overt DIC in addition to severe ARDS (62, 63). Although COVID-19 is a pulmonary form of ARDS, autopsy reports demonstrate profound microvascular coagulation, suggesting a vascular mechanism of disease that may overlap with other subtypes of nonpulmonary ARDS (64).

Our study was conducted prior to the COVID-19 pandemic and did not include COVID-19 patients. Future research is needed to determine whether our findings are relevant to the association between ABO blood type and COVID-19 disease.

Our study has several strengths. First, we utilized a large critically ill population that was extensively phenotyped for sepsis, systemic markers of endothelial activation and microvascular coagulation in nonpulmonary sepsis and our data linking ABO blood type A to higher vWF and sTM (50, 51). The interaction finding is also consistent with our data demonstrating an association between ABO blood type and ARDS even among nonsecretors who lack ABO(H) antigens on epithelium but not in the vasculature. In our previous study, the association between clinically determined ABO blood type and ARDS was only present in individuals of European ancestry and not African ancestry (17). In our current study using genetically determined blood type, individuals of African ancestry also demonstrated an association between ABO blood type A1 and ARDS. Possible reasons for this difference include differences in the distribution of the ABO blood type A subtypes between races, which were determined in this study but not our previous study; increased power in our current study; adjustment for population stratification; or an increased severity of illness observed in our current study populations.

### Table 3. Adjusted association of ABO blood type with plasma endothelial biomarker concentrations and plasma endothelial biomarker concentrations with ARDS in the combined MESSI and PETROS cohort

<table>
<thead>
<tr>
<th>Plasma marker</th>
<th>Association with blood type A vs. O</th>
<th>Association with ARDS risk</th>
<th>Association with ARDS risk, excluding pulmonary sepsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>vWF</td>
<td>β (95% CI) = 0.11 (0.04, 0.19)</td>
<td>OR (95% CI) = 1.22 (0.93, 1.62)</td>
<td>OR (95% CI) = 1.43 (1.00, 2.05)</td>
</tr>
<tr>
<td>sTM</td>
<td>β (95% CI) = 0.06 (0.01, 0.13)</td>
<td>OR (95% CI) = 1.90 (1.39, 2.60)</td>
<td>OR (95% CI) = 1.98 (1.35, 2.90)</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>β (95% CI) = 0.13 (0.04, 0.23)</td>
<td>OR (95% CI) = 1.13 (0.92, 1.40)</td>
<td>OR (95% CI) = 1.18 (0.91, 1.54)</td>
</tr>
<tr>
<td>E-selectin</td>
<td>β (95% CI) = -0.35 (–0.49, –0.21)</td>
<td>OR (95% CI) = 1.00 (0.86, 1.16)</td>
<td>OR (95% CI) = 0.95 (0.80, 1.12)</td>
</tr>
</tbody>
</table>

The interaction between ABO blood type A versus O and each plasma biomarker concentration measured early in critical illness was determined using multivariable linear regression adjusting for age, sex, race, and sepsis versus trauma. Biomarker concentrations were log-transformed to approximate normality. The association between plasma biomarker concentration and moderate or severe ARDS risk was determined using multivariable logistic regression adjusting for age, sex, race, and sepsis versus trauma. Odds ratios are for each log increase in plasma biomarker concentration.

### Table 4. Number of suboptimal donor lungs transplanted after ex vivo lung perfusion by ABO blood type in the NOVEL trial

<table>
<thead>
<tr>
<th>Blood type</th>
<th>Transplanted</th>
<th>Not transplanted</th>
<th>Last PaO2 (mmHg)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12 (38%)</td>
<td>20 (62%)</td>
<td>419 (342, 470)</td>
<td>0.024</td>
</tr>
<tr>
<td>O</td>
<td>39 (67%)</td>
<td>19 (33%)</td>
<td>460 (399, 505)</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>311 (106, 515)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>5 (50%)</td>
<td>5 (50%)</td>
<td>491 (444, 542)</td>
<td></td>
</tr>
</tbody>
</table>

ABO blood type was compared between donor lungs that went on to transplantation and those that did not using the Fisher exact test. Median and IQR of the last measured partial pressure of oxygen while on 100% inspired oxygen via EVLP. ABO blood type A and O compared via the Wilcoxon rank-sum test.
severe trauma, and ARDS outcome. Second, we genotyped the ABO gene to determine ABO blood type, including the A1 and A2 subtypes that are predicted to have distinct A antigen expression. Third, our analyses were adjusted for genetic population stratification via principal component analyses rather than patient-reported race. Fourth, we supported our primary genetic findings with data suggesting vWF and sTM may underlie the association between ABO blood type and ARDS, and that ABO blood type may affect lung recovery on EVL. Finally, we used clinical data to phenotype DIC in the MESSI population and identified a potentially novel association between ABO blood type A and increased risk of sepsis-associated DIC.

Our study also has several limitations. First, despite providing strong evidence to direct future research, we cannot definitively identify the mechanism linking ABO blood type A and increased ARDS risk. The lack of a mouse model of ABO glyobiology, since mice lack ABO expression, significantly limits our ability to closely study the mechanism in animals, leaving our human epidemiological research as an important first step. Future research should focus on in vitro cell models aimed at refining mechanistic associations. Second, despite including 3 cohorts with over 3000 closely phenotyped patients, we are underpowered to compare all individual genotypes, including the rarer blood type B genotypes. Third, the ARDS phenotype is difficult to identify consistently, and it is possible some degree of misclassification of our outcome occurred; however, each individual patient was closely phenotyped by trained clinician investigators with adjudication for disagreements. Additionally, the DIC phenotype relied on labs drawn for clinical purposes with missing lab values assumed to be normal, possibly introducing misclassification and an underestimate of the true incidence of DIC. However, the DIC outcome misclassification is likely nondifferential by ABO blood type and therefore would bias us to the null.

In summary, we have demonstrated a strong reproducible association between genetically determined ABO blood type A1 and increased risk of ARDS mediated through microvascular dysfunction and coagulation. Our findings suggest that ABO blood type A defines a unique endotype of critical illness that may benefit from therapies targeting the microvasculature.

Methods

Study populations. Three study populations were included in the reported analyses (Supplemental Table 1). PETROS and the MESSI cohort studies are ongoing prospective cohort studies enrolling at the University of Pennsylvania (13, 17, 43–45). iSPAAR is a multicenter case-control study (45, 46). Of the PETROS and MESSI patients included in this study, 39% and 41% were also enrolled in our prior nongenetic study (45, 46). Of the PETROS and MESSI cohort, which included a critically ill at-risk population. For the purposes of this study, only subjects with sepsis as their primary ARDS risk factor were included. Other at-risk populations enrolled in iSPAAR, such as aspiration- and transfusion-related acute lung injury, were excluded.

Genotyping. DNA was extracted from whole blood collected at the time of enrollment using standard methods in all 3 cohorts. In PETROS and MESSI, subjects were genotyped using the Affymetrix Axiom Txv1 array including 785,194 SNPs. In iSPAAR, subjects were genotyped with the Illumina Human610-Quad Bead array of more than 500,000 SNPs. Principal component analysis was performed using all genotyped SNPs that passed quality control. Quality control included filtering SNPs with a minor allele frequency less than 5%, with missing calls for more than 10% of the populations, and SNPs playing Hardy Weinberg disequilibrium (P < 10^-6). All SNPs used to infer genetically determined ABO blood type and FUT2 secretor status passed quality control.

ARDS outcome ascertainment. In PETROS and MESSI, ARDS was phenotyped within 6 days of presentation based on the Berlin definition with the added requirement for invasive mechanical ventilation (3, 70). Two physician investigators trained in identifying ARDS reviewed all chest radiographs ordered for clinical purposes, with consensus discussion for any disagreements. Arterial blood gases ordered for clinical purposes were used to determine the ratio of arterial partial pressure of oxygen to fraction of inhaled oxygen, with a ratio less than or equal to 300 constituting ARDS and less than or equal to 200 moderate to severe ARDS. If an arterial blood gas was unavailable within 24 hours of a positive chest radiograph, the ratio of the oxygen saturation to fraction of inhaled oxygen was used based on previously published methods (71).

In iSPAAR, ARDS cases were phenotyped based on the American European Consensus Criteria definition of ARDS given that the cases were drawn from clinical trials that predate the Berlin definition (72). Plasma biomarker measurements in PETROS and MESSI. Residual citrated plasma was collected from patients at emergency room/trauma bay presentation in patients admitted through the emergency department and at the point closest to ICU admission in patients transferred from the hospital ward. Plasma was centrifuged within 30 minutes of blood draw, used for clinical testing, and then refrigerated at 4°C. Residual plasma was then collected within 24 hours, placed in aliquots, and frozen at -80°C until biomarker testing was performed. This approach allowed us to obtain plasma from the earliest possible time point in patients’ presentation regardless of availability of research personnel. Plasma concentrations of sICAM-1, vWF, sTM, and E-selectin were then measured using commercially available ELISA assays developed for research use.
DIC phenotype. In the MESSI cohort, subjects were determined to have DIC based on an International Society of Thrombosis and Haemostasis (ISTH) score greater than or equal to 5 based on labs drawn for clinical purposes within the first 6 days of admission (53). The lowest platelet count and fibrinogen and highest prothrombin time and D-dimer for each ICU day were utilized for ISTH score calculation. Because fibrinogen and D-dimer are often only clinically measured if DIC is suspected, missing values were assumed to be normal. No subjects were missing platelet counts or prothrombin time. We excluded patients with a history of cirrhosis or hepatic failure to avoid misclassifying patients who cannot produce coagulation factors as patients with DIC who are consuming coagulation factors.

EVLP experiments. We tested the association between ABO blood type and recovery rate of suboptimal donor lungs for transplantation in a prospective cohort of donor lungs treated with EVLP as part of the 6-center NOVEL lung trial (73). In the NOVEL lung trial, EVLP was considered if the donor lungs had evidence of acute lung injury. Specifically, donor lungs demonstrated a PaO2/FiO2 less than or equal to 300 mmHg or a PaO2/FiO2 greater than 300 mmHg, but the donor had any of the following risk factors: multiple blood transfusions, pulmonary edema, donation after cardiac death, or the investigator deemed the donor lung quality as poor. Donor lungs with chronic lung disease, pneumonia, gastric acid aspiration, or significant barotrauma were excluded. After procurement, donor lungs were placed in a cold preservation solution and transported to a study site. Lung grafts were then placed on a mechanical ventilator and EVLP perfusion circuit and rewarmed. Lungs were perfused with an acellular perfusate, including balanced electrolytes and protein. EVLP was maintained for 4 hours and the grafts underwent serial assessments for improvement in physiological parameters. Once placed on EVLP, lungs were considered transplantable if they had 2 consecutive PaO2 greater than 350 on 100% FIO2 and had stable or improving pulmonary vascular resistance, compliance, and airway pressure. EVLP cases were performed on the XVIVO Perfusion System platform. ABO blood type was determined for each lung by blood bank typing of the donor because genetically determined ABO blood type was unavailable.

Statistics. Study characteristics were compared between patients who developed ARDS and those who did not using the x2 test for categorical variables and Wilcoxon rank-sum test for continuous variables. In the primary analyses, multivariable logistic regression was used to determine the association of genetically determined blood type A1 versus O with moderate or severe ARDS risk, adjusting for a prespecified list of potential confounders and population stratification. Population stratification was determined via principal component analyses and all multivariable models were adjusted for principal components 1 through 4. Our primary comparison was between ABO blood type A1 and O because it is unclear whether rarer blood types exhibit a phenotype closer to A1 or O, and we are underpowered to evaluate such differences. We used moderate to severe ARDS as the primary outcome because these patients are more likely to have diffuse alveolar damage, the pathological correlate to ARDS, as their underlying pathology type. We also prespecified secondary analyses stratified by source of sepsis in the 2 sepsis cohorts. We tested for statistical interaction by source of sepsis, race, and mechanism of trauma using the likelihood ratio test. Biomarker concentrations were compared between blood types first in MESSI and PETROS separately using the Wilcoxon rank-sum test. We then combined the cohorts to conduct adjusted analyses using multivariable linear regression. For regression models, biomarker concentrations were first log-transformed to approximate a normal distribution. Biomarker concentrations were then compared between ARDS and no ARDS using the Wilcoxon rank-sum test followed by multivariable logistic regression adjusting for potential confounders. Multivariable logistic regression was also used to test the association of blood type and DIC, as well as blood type and EVLP recovery. A P value less than 0.05 was considered significant.

Study approval. Participants enrolled in PETROS were enrolled under a waiver of informed consent granted with approval from the IRB of the University of Pennsylvania. Participants enrolled in MESSI were enrolled with a waiver of informed consent granted with approval from the IRB of the University of Pennsylvania. All subjects or their surrogates subsequently provided written informed consent.

Author contributions
JPR, NJM, MPR, and JDC designed the studies included in the manuscript. JPR, NJM, MGSS, BJ, CI, CF, PNL, and JDC designed and conducted the prospective cohort studies MESSI and PETROS at the University of Pennsylvania. JPR, NJM, MGSS, BJ, CI, TGD, BL, CF, MPB, EK, PNL, and JDC acquired data for MESSI and PETROS, including clinical data, outcome phenotypes, and plasma biomarker measurements. NJM, CSC, MAM, CM, KW, JR, DCC, MMW, and JDC analyzed data for the iSPAAR case-control study. EC acquired data from the NOVEL lungs study. JPR, NJM, MPB, RF, EC, NSM, and JDC analyzed data. JPR initially drafted the manuscript with all authors editing and approving the final version.

Acknowledgments
We would like to acknowledge our funding sources for this research including NIH grants HL122075 (to JPR), HL125723 (to JPR), HL137915 (to NJM), DK097307 (to MGSS), HL115354 (to JDC), HL101779 (to MMW), and the University of Pennsylvania McCabe Fund Fellowship Award (to JPR). We would also like to acknowledge the patients enrolled in the studies included in this manuscript and their families for their participation.

Address correspondence to: John P. Reilly, Division of Pulmonary, Allergy, and Critical Care, University of Pennsylvania, Perelman School of Medicine, 5011 Gibson Building, 3400 Spruce Street, Philadelphia, Pennsylvania 19104, USA. Phone: 215.662.2824; Email: John.Reilly@pennmedicine.upenn.edu. Or to: Jason D. Christie, Division of Pulmonary, Allergy, and Critical Care, University of Pennsylvania, Perelman School of Medicine, 873 Maloney Building, 3400 Spruce Street, Philadelphia, Pennsylvania 19104, USA. Phone: 215.662.6003; Email: jason.christie@pennmedicine.upenn.edu.


