ORIGINAL CLINICAL REPORT

OPEN

Plasma Metabolites in Early Sepsis Identify Distinct Clusters Defined by Plasma Lipids

Angela J. Rogers, MD, MPH[1]
Aleksandra Leligdowicz, MD, PhD[2,3]
Kévin Contrepois, PhD[4]
Alejandra Jauregui, BSc[2]
Kathryn Vessel, BSc[2]
Thomas J. Deiss, BSc[2]
Annika Belzer, BSc[2]
Tom Liu, MD[2]
Matthew Lippi, MD, MPH[2]
Serena Ke, BSc[2]
Erin Ross, BSc[2]
Hanjing Zhou, MSc[2]
Carolyn Hendrickson, MD, MAS[2]
Antonio Gomez, MD[2]
Pratik Sinha, MD, PhD[2]
Kirsten N. Kangelaris, MD[2]
Kathleen D. Liu, MD, PhD[2]
Carolyn S. Calfee, MD, MAS[2]
Michael A. Matthay, MD[2]

OBJECTIVES: Unbiased global metabolomic profiling has not been used to identify distinct subclasses in patients with early sepsis and sepsis-associated acute respiratory distress syndrome. In this study, we examined whether the plasma metabolome reflects systemic illness in early sepsis and in acute respiratory distress syndrome.

DESIGN: Plasma metabolites were measured in subjects with early sepsis.

SETTING: Patients were admitted from the emergency department to the ICU in a plasma sample collected within 24 hours of ICU admission. Metabolic profiling of 970 metabolites was performed by Metabolon (Durham, NC). Hierarchical clustering and partial least squares discriminant clustering were used to identify distinct clusters among patients with early sepsis and sepsis-associated acute respiratory distress syndrome.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: Among critically ill patients with early sepsis ($n = 197$), three metabolically distinct subgroups were identified, with metabolic subtype driven by plasma lipids. Group 1, with 45 subjects (23% of cohort), had increased 60-day mortality (odds ratio, 2; 95% CI, 0.99–4.0; $p = 0.04$ for group 1 vs all others). This group also had higher rates of vasopressor-dependent shock, acute kidney injury, and met Berlin acute respiratory distress syndrome criteria more often (all $p < 0.05$). Conversely, metabolic group 3, with 76 subjects (39% of cohort), had the lowest risk of 60-day mortality (odds ratio, 0.44; 95% CI, 0.22–0.86; $p = 0.01$) and lower rates of organ dysfunction as reflected in a lower Simplified Acute Physiology Score II ($p < 0.001$). In contrast, global metabolomic profiling did not separate patient with early sepsis with moderate-to-severe acute respiratory distress syndrome ($n = 78$) from those with sepsis without acute respiratory distress syndrome ($n = 75$).

CONCLUSIONS: Plasma metabolomic profiling in patients with early sepsis identified three metabolically distinct groups that were characterized by different plasma lipid profiles, distinct clinical phenotypes, and 60-day mortality. Plasma metabolites did not distinguish patients with early sepsis who developed acute respiratory distress syndrome from those who did not.

KEY WORDS: acute respiratory distress syndrome; clustering; metabolomics; phenotype; sepsis

Using "genomics" (omics) to identify clinically relevant disease subtypes of patients has led to breakthroughs in personalized therapy for multiple diseases. Examples of successful personalized therapies include the immune checkpoint inhibitor-based therapy targeting the programmed cell death 1 pathway in patients with cancer (1) or asthma therapy based on T helper 2 cell–associated inflammatory disease (2). The need to identify clinically important and biologically distinct clusters in critically ill patients with sepsis and the acute respiratory distress syndrome (ARDS) is increasingly recognized (3, 4). In critically

DOI: 10.1097/CCE.0000000000000478

ill patients with sepsis, whole blood gene expression and plasma proteins have been used to classify subjects into differential mortality groups (4–7). In patients with ARDS, plasma proteins have been used to categorize patients into "hyperinflammatory" and "hypoinflammatory" classes. The hyperinflammatory classification is associated with a higher risk of mortality and a differential response to therapy in several randomized clinical trials and prospective clinical cohorts (8–11).

Metabolomics is a promising profiling technique to study the marked metabolic changes that occur during critical illness (12, 13). Plasma lactate, a marker of a transition to anaerobic metabolism, is the most widely used biomarker in sepsis. An elevated plasma lactate level is used to define septic shock (14), and serial lactate levels are used to monitor response to therapy (15). Advances in metabolomics enable profiling of a much greater proportion of human plasma metabolites at decreasing cost. Recently, the task force for the Research Committee of the Surviving Sepsis Campaign identified five key basic and translational science research priorities (16). The first two priorities include: 1) elucidating mechanisms that underlie sepsis and 2) how sepsis alters metabolism. Metabolomic profiling has the potential to address both of these poorly understood biological mechanisms of injury in sepsis.

We and others have reported that the plasma metabolomic profile of critically ill patients differs between survivors and nonsurvivors (17, 18). However, it is unknown to what extent plasma metabolic profiling in critically ill patients reflects the biology of early sepsis and ARDS. At least four prior groups have carried out plasma metabolic profiling in ARDS cohorts (19–22). The study design has varied widely, and studies were limited by a small sample size as well as control populations comprised of healthy controls (20) or mechanically ventilated postoperative surgical patients (19). In addition, metabolomic profiling techniques differed between studies (i.e., nuclear magnetic resonance or mass spectrometry [MS], targeted profiling of known metabolites vs nontargeted profiling). As such, potentially significant metabolic pathways in patient with early sepsis who develop ARDS are unknown.

In this study, we obtained plasma samples from patients with early sepsis and carried out plasma metabolomic profiling of over 900 metabolites. The first aim was to determine whether there are metabolically distinct subgroups of patients with early sepsis and examine variation in clinical outcomes among these groups. Our second aim was to determine whether plasma metabolites can distinguish critically ill patients with early sepsis who develop ARDS from those who do not. Our third aim was to compare previously identified ARDS-associated metabolites with the severity of illness, the plasma metabolite subgroup, and ARDS status in our study population.

METHODS

Population

Patients included in this retrospective cohort study comprised 197 patients prospectively enrolled in the early assessment of renal and lung injury (EARLI) cohort between November 13, 2008, and December 14, 2016. Briefly, patients were recruited from the emergency departments of the University of California San Francisco Moffitt-Long Hospital and San Francisco General Hospital and were eligible for enrollment if admitted to the ICU, as described previously (7). Plasma samples were collected within 24 hours of ICU admission. ARDS was adjudicated using two-person review of chest radiographs and presence of ARDS by American-European Consensus Conference (23) and Berlin (24) criteria. The study was approved by the institutional review board at the University of California, San Francisco, approval number 310987.

Patient selection for the retrospective cohort study was based on the presence of sepsis as a risk factor for ARDS (**Supplemental Fig. 1**, http://links.lww.com/CCX/A730). A detailed explanation of patients included in aims 1 and 2 is provided in the online **Supplemental Methods** (http://links.lww.com/CCX/A730).

Metabolic Profiling Strategy

One-hundred fifty microliters aliquots of citrated plasma were profiled by Metabolon (Durham, NC) on a nontargeted metabolome platform capable of quantifying thousands of identified metabolites. Briefly, proteins were precipitated with methanol, and the resulting extracts were analyzed with three complementary methods: ultrahigh performance liquid chromatography/tandem mass spec (UHLC)/MS/MS2 for basic species, UHLC/MS/MS2 for acidic species, and UHLC/MS/MS2 for lipids. Data analysis was performed with Metabolon's software and included peak

peaking, retention time alignment, quantification, data curation, and normalization. Peaks were identified by matching against an in-house library of authentic standards and routinely detected unknown compounds. To assess technical and process variability, a quality control sample was used which consisted of individual samples combined into a single aliquot.

Data Processing and Statistical Analyses

Phenotypic differences between groups were tested using Fisher exact test for categorical variables, Wilcoxon rank-sum for binary continuous variables, and analysis of variance for differences among three groups. Analysis was performed using Metaboanalyst 3.0 and R v 3.0.1 (25, 26). Prior to analysis, metabolomic data were $\log_2$-transformed and auto scaled (27), and metabolites without variability across samples were removed from analysis.

Global metabolic differences were assessed using hierarchical clustering with Euclidean distance in the hclust package in R. To identify clusters based on plasma metabolomic profiling in patients with early sepsis, principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were used (28). PCA reduces the dimensionality of the data in an unbiased and unsupervised fashion to visualize the structure of the data. In contrast, PLS-DA is a supervised method that maximizes the separation between groups and is used to reveal discriminant metabolites. To test the model fit, R^2 reflects the degree of fit of the model to the data, whereas Q^2 is a measure of model performance on cross-validation (28, 29). To ensure that between-group differences were robust to overfitting, Q^2 value with leave-one-out partitioning greater than or equal to 0.6 was required for significance.

Individual metabolites were tested using logistic regression, adjusting for age, gender, race, pneumonia, and kidney function (glomerular filtration rate) (30). Analytes with a $\log_2$-fold change of greater than 0.3 and a false discovery rate (FDR) p value of less than 0.20 were considered significant. All metabolites that met the univariate significance threshold with a confirmed Human Metabolome Database Identifier were assessed for pathway overrepresentation using the hypergeometric test in the Pathway Analysis mbrole 2.0 (31). Node importance was assigned using relative betweenness centrality. Pathways with FDR p value of less than 0.05 were considered significant. Metabolic differences were assessed between subjects who had early sepsis with moderate-to-severe ARDS and those without ARDS.

RESULTS

Global Metabolic Profiling of Sepsis Patients Identifies High Mortality Cluster

Nine-hundred seventy metabolites were identified using targeted metabolomics profiling of plasma from 197 subjects with early sepsis collected within 24 hours of admission. Global metabolic profiling revealed three groups of critically ill patients with distinct metabolic profiles (**Fig. 1**). This separation was highly reproducible (Q^2 value on leave-one-out cross-validation of > 0.8, and $p < 0.001$ on thousand-fold permutation testing) (**Supplemental Fig. 2**, http://links.lww.com/CCX/A730).

The three metabolic groups were characterized by both a markedly different clinical phenotype and clinical outcome (**Table 1**). Group 1, with 45 subjects (23% of cohort), had increased 60-day mortality (odds ratio [OR] 2; 95% CI, 0.99–4.0; $p = 0.04$ for group 1 vs all others). This group also had higher rates of vasopressor-dependent shock, acute kidney injury, and met Berlin ARDS criteria more often (all $p < 0.05$). Conversely, metabolic group 3, with 76 subjects (39% of cohort), had the lowest risk of 60-day mortality (OR, 0.44; 95% CI, 0.22–0.86; $p = 0.01$) and lower rates of organ dysfunction as reflected in a lower Simplified Acute Physiology Score (SAPS) II ($p < 0.001$). Group 2 was an intermediate risk phenotype.

Plasma lipids were the key metabolites driving between group differences. The top metabolites ranked by PLS-DA Variable Importance in Projection score are shown in **Table 2**, with the complete results for all 970 metabolites available in **Supplemental Table 1** (http://links.lww.com/CCX/A731). These are notable for low lipid levels among the group 1 (high mortality group) and high lipid levels in group 3 (lowest mortality group). In fact, 173 of the 317 metabolites that distinguish group 1 subjects from all others were lipids. This is more than expected by chance (OR, 1.8; 95% CI, 1.4–2.4; $p < 0.001$). Of the 173 plasma lipids, 170 are lower in group 1 subgroup ($p < 0.001$). These lipids represent fatty acid metabolism pathways, lysophospholipids, sphingolipids, and phosphatidylcholines. Pathway analysis showed significant enrichment for alpha-linolenic acid and linoleic acid metabolism in

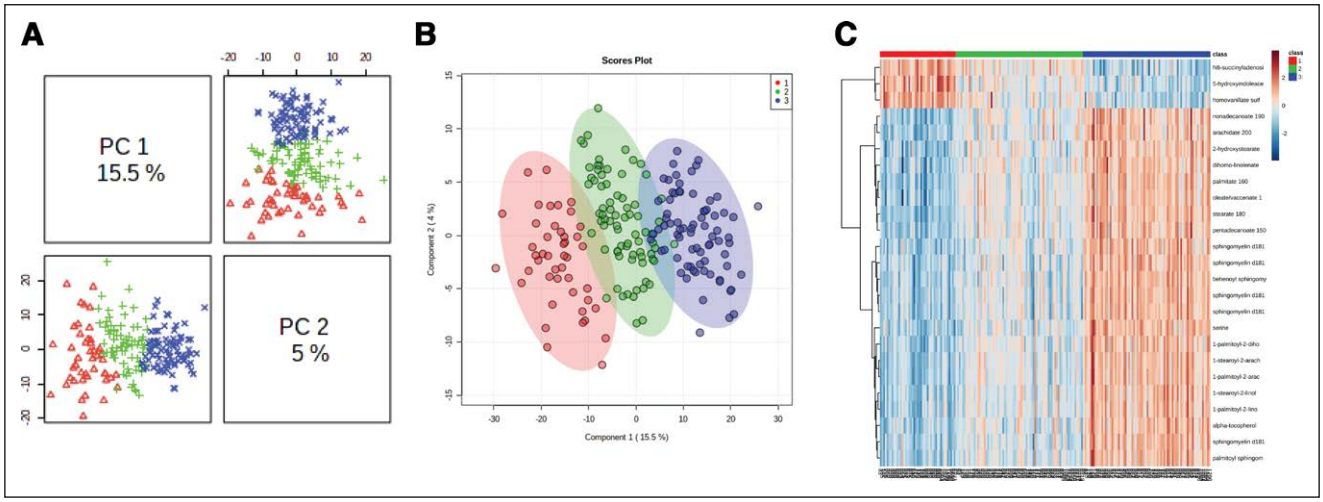

Figure 1. A, Principal components (PCs) analysis of whole cohort ($n = 197$) using hierarchical clustering of plasma metabolites reveals three distinct patient subgroups. **B**, Partial least squares discriminant analysis (PLS-DA) separates subjects into three subgroups by hierarchical clustering. **C**, Heat map of $\log_2$-fold change of the top 25 metabolites that differ between groups ranked by PLS-DA Variable Importance in Projection score. Complete names of all 970 metabolites measured are available in Supplemental Table 1 (http://links.lww.com/CCX/A731).

group 1 relative to all other subjects (FDR $p = 0.0013$). Levels of other metabolite classes were more evenly distributed, except for xenobiotics (**Supplemental Table 2**, http://links.lww.com/CCX/A730).

Plasma Metabolites Do Not Differ Between Early Sepsis Patients With ARDS and Without ARDS

We next tested whether global metabolic profiling could distinguish patients with early sepsis who have moderate-to-severe ARDS from those at-risk of the syndrome but who did not develop ARDS (please refer to Supplemental Fig. 1, http://links.lww.com/CCX/A730 for patient selection). In this cohort, patients who developed ARDS ($n = 78$) differed from patients with early sepsis at-risk of ARDS ($n = 75$) in that they were more severely ill, with higher baseline SAPS II, vasopressor-dependent shock, and mortality (**Supplemental Table 3**, http://links.lww.com/CCX/A730).

TABLE 1.
Clinical Characteristics in Clusters Identified by Plasma Metabolomic Profiling in 197 Patients With Early Sepsis

Clinical Characteristics	Group 1, *N* = 45	Group 2, *N* = 76	Group 3, *N* = 76	*p*
Age, median (interquartile range)	73 (60–83)	70 (60–77)	66 (55–73)	0.07
Gender (male), *n* (%)	26 (58)	47 (62)	34 (44)	0.09
Race (White), *n* (%)	21 (47)	37 (49)	46 (61)	0.22
Pneumonia, *n* (%)	18 (40)	46 (61)	49 (65)	0.02
Acute respiratory distress syndrome, *n* (%)	24 (53)	33 (43)	21 (28)	0.01
Simplified Acute Physiology Score II, median (interquartile range)	71 (51–85)	55 (42–72)	42 (31–59)	< 0.001
Shock[a], *n* (%)	26 (58)	37 (49)	32 (42)	0.25
Glomerular filtration rate (modification of diet in renal disease), median (interquartile range)	18 (10–28)	38 (27–54)	83 (62–112)	< 0.001
60-d mortality, *n* (%)	23 (51)	31 (41)	20 (26)	0.02

[a]Shock is defined by need for vasopressors at enrollment.
p by analysis of variance for continuous variables, Fisher exact for categorical variables.

TABLE 2.

Top Plasma Metabolites Distinguishing Patients in Group 1 or Group 3 Relative to All Other Subjects With Early Sepsis in the Early Assessment of Renal and Lung Injury Cohort

Metabolites	Super Pathway	Subpathway	Log_2 FC Group 1 vs Other	Log_2 FC Group 3 vs Other	Partial Least Squares Discriminant Analysis Variable Importance in Projection Score
Stearate (18:0)	Lipid	Long chain fatty acid	−1.6	1.3	2.3
Palmitate (16:0)	Lipid	Long chain fatty acid	−1.7	1.3	2.2
Serine	Amino acid	Glycine, serine, and threonine metabolism	−1.2	1.3	2.1
Sphingomyelin (d18:1/14:0, d16:1/16:0)[a]	Lipid	Sphingolipid metabolism	−1.1	1.1	2.1
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	Lipid	Phosphatidylcholine	−1.2	1.1	2.1
1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6)	Lipid	Phosphatidylcholine	−1.1	1.1	2.1
1-palmitoyl-2-dihomo-linolenoyl-GPC (16:0/20:3n3 or 6)[a]	Lipid	Phosphatidylcholine	−1.3	1.2	2.1
Palmitoyl sphingomyelin (d18:1/16:0)	Lipid	Sphingolipid metabolism	−1.0	1.1	2.1
Pentadecanoate (15:0)	Lipid	Long chain fatty acid	−1.3	1.1	2.1
Behenoyl sphingomyelin (d18:1/22:0)[a]	Lipid	Sphingolipid metabolism	−1.2	1.1	2.1

FC = fold change, GPC = glycerophosphocholine.

[a]Metabolites are ranked by Partial Least Squares Discriminant Analysis Variable Importance in Projection score.

No clustering was observed among the subgroup of patients with early sepsis and moderate-to-severe ARDS and those at-risk of the syndrome (**Fig. 2*A***). Although PLS-DA appears to partially separate patients with and without ARDS (**Fig. 2*B***), the Q^2 value is less than 0.2 on leave-one-out cross-validation (**Supplemental Fig. 3**, http://links.lww.com/CCX/A730), suggesting that the separation likely represents noise rather than true biological significance.

Individual ARDS-Associated Metabolites Were Related to the Severity of Illness

To reveal potential biologic mechanisms of ARDS, we next tested whether individual metabolites differ between patients with early sepsis with ARDS relative to those without ARDS. After adjustment, only two metabolites met our predefined threshold for significance: rocuronium and 17alpha-hydroxypregnenolone 3-sulfate, a sulfated steroid metabolite whose functional significance is not known.

To increase the potential for identification of key pathways, we used a more liberal FDR threshold of p value of less than 0.1. This identified 43 metabolites (**Supplemental Table 4**, http://links.lww.com/CCX/A732) and included drugs that may relate to intubation and mechanical ventilation (i.e., rocuronium, pantoprazole), which were higher in patients with ARDS. The other differential metabolites involved numerous classes, including nucleotides (purine and pyrimidine) and lipid metabolites.

Given the higher SAPS II and vasopressor-dependent shock in patients with ARDS, we next assessed whether individual metabolites associated with ARDS were related to global severity of illness rather than ARDS. Indeed, of the 43 metabolites, 29 were correlated with the nonpulmonary SAPS II (Pearson's correlation $\geq |0.2|$ and FDR-adjusted

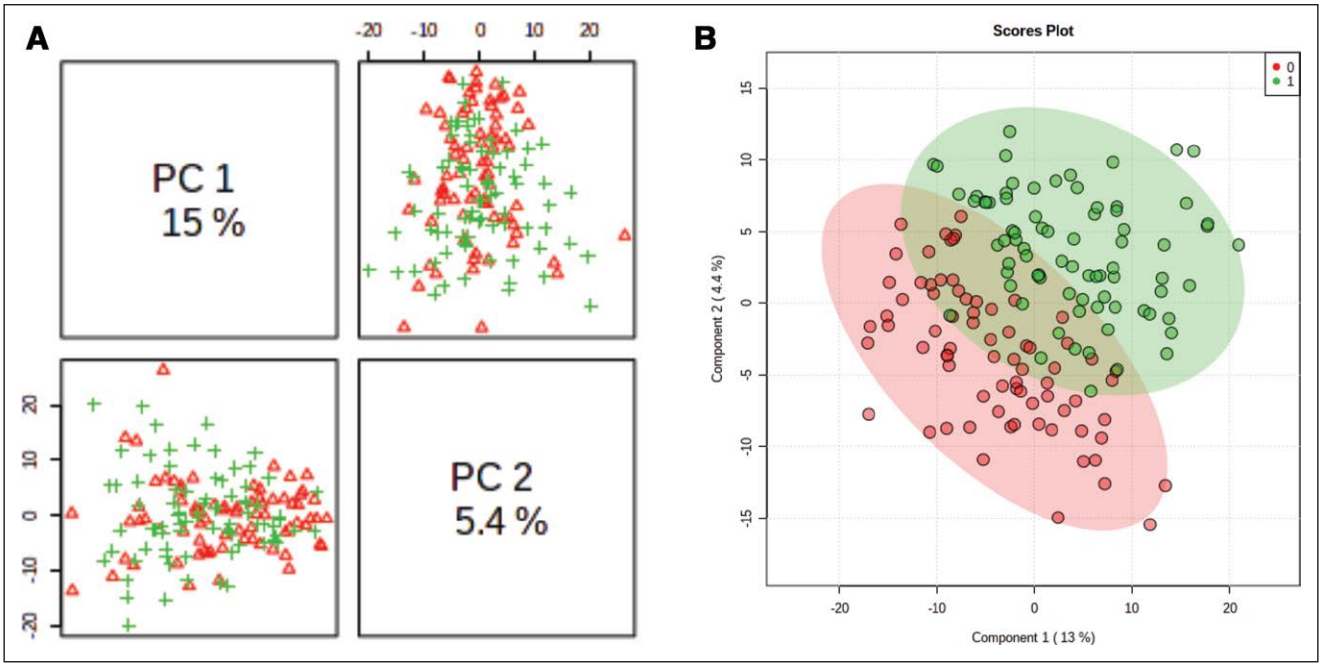

Figure 2. A, Principal components (PCs) analysis of subject with sepsis and moderate-severe acute respiratory distress syndrome (ARDS) ($n = 78$) relative to subjects with sepsis without ARDS ($n = 75$), showing a lack of separation between these patient subgroups. **B**, Partial least squares discriminant analysis poorly discriminates patients with sepsis with compared with without ARDS.

$p < 0.1$). After adjustment for the nonpulmonary SAPS II, renal function, age, race, and gender, only rocuronium and pantoprazole remained significantly associated with ARDS.

Comparison With Prior ARDS Metabolomic Literature

Several prior studies examined plasma metabolites in cohorts of patients with ARDS (19–22). These studies varied by the sample size, the control population, and the method of metabolomic profiling (**Table 3**). As such, the identified key metabolites varied widely. Twelve of the previously reported ARDS-associated metabolites were also quantified using our metabolomic profiling platform. This enabled testing whether these metabolites are also related to 1) severity of illness (SAPS II), 2) the plasma metabolite group, or 3) ARDS status in our patient cohort.

Of the 12 metabolites associated with ARDS status in previous studies and measured in the EARLI cohort, six were associated with the SAPS II. In addition, seven were nominally associated with plasma metabolite group 1 status, and three remained significantly associated after FDR adjustment for multiple comparisons (sphingomyelin, glutamate, phenylalanine). In comparison, only sphingomyelin was nominally associated with ARDS status after adjustment, but the association was weaker than associations of sphingomyelin with SAPS II and group 1 status and did not withstand multiple comparisons testing for these 12 metabolites (FDR $p = 0.13$) (Table 3).

DISCUSSION

In this large-scale plasma metabolomics profiling study of early sepsis using unbiased hierarchical clustering, three metabolically distinct subgroups were identified. The groups differed most notably in plasma lipid levels across numerous superclasses and were associated with marked phenotypic differences including widely differing rates of mortality. Patients with the lowest lipid levels (group 1) had the highest baseline SAPS II, renal dysfunction, vasopressor-dependent shock, and 60-day mortality. This study adds important insights regarding the potential for plasma metabolites to identify pathobiology-driven subgroups among patients with early sepsis.

These findings add to the literature on the importance of lipidomic changes in sepsis. Although our study is to our knowledge the first to examine hundreds of lipids across several lipid subclasses in a large

TABLE 3.

Associations of Previously Reported Acute Respiratory Distress Syndrome Metabolites in the Early Assessment of Renal and Lung Injury Cohort

References	Sample Type	Cases/ Controls	Control Population	No. of Metabolites Profiled	ARDS-Associated Metabolites	Human Metabolome Database Identifier	Simplified Acute Physiology Score II[a] (*N* = 197)	Group 1 vs Other[b] (*N* = 45 vs *N* = 152)	ARDS Status[b] (*N* = 78 vs *N* = 75)
Stringer et al (20)	Plasma	13/8	Healthy	40	Glutathione adenosine	00058	0.6	0.9	0.24
					Phosphatidylserine	10163	**0.007**	0.12	0.11
					Sphingomyelin	12087	**< 0.001**	**0.001**[d]	**0.01**[e]
Singh et al (21)	Serum	26/19	Ventilated ICU	> 100	Multiple lipids				
					Creatinine	00562	0.05	0.05	0.11
					Lactate	00190	**0.001**	0.9	0.1
					Formate				
					Glutamate	00148	**0.001**	**0.01**[d]	0.82
Izquierdo-Garcia et al (22)	Serum	25/28[c]	H1N1 without ARDS	> 100	Methylhistidine	00001	0.11	0.14	0.22
					Phenylalanine glucose	00159	**0.03**	**0.003**[d]	0.42
					Glutamine	00122	0.66	**0.02**	0.21
					Methylguanidine	00641	**0.009**	0.7	0.52
					Alanine	01522	0.24	**0.02**	0.1
						00161	0.73	0.39	0.73
Viswan et al (19)	Serum	197/68	Ventilated after elective surgery	Nuclear magnetic resonance > 500	Achieve separation with orthogonal projections to latent structures discriminant analysis	NA	NA	NA	NA
					No metabolites highlighted				

ARDS = acute respiratory distress syndrome, NA = not available.

[a]Simplified Acute Physiology Score II *p* is for Pearson's correlation.

[b]*p* values for association with metabolic group and ARDS are adjusted for age, sex, race, and glomerular filtration rate but not for multiple comparisons.

[c]Analyzed as a derivation (12 ARDS/18 controls) and validation (13 ARDS/10 controls; 3 controls removed) set.

[d]Metabolites associated with metabolic Group 1 membership after false discovery rate (FDR) adjustment for 12 metabolites, with FDR $p < 0.05$.

[e]FDR $p = 0.13$ after adjustment for 12 metabolites.

Boldface values indicate $p < 0.05$.

critically ill population, hypocholesterolemia has been described in multiple critically ill cohorts, including those with sepsis (32, 33). Cholesterol may play a role in host defense in sepsis, as suggested by the observation that circulating lipids and lipoproteins bind and neutralize endotoxin (lipopolysaccharide [LPS]) (34). Furthermore, increasing low-density lipoprotein (LDL) clearance by lowering proprotein convertase subtilisin/

kexin type 9 levels may be beneficial in sepsis. The mechanism underlying this benefit may be mediated by increasing LDL receptor levels and thus also promoting LPS clearance (35, 36). Additionally, reduced plasma-free fatty acid levels in patients with acute lung injury were the rationale behind the ARDS Network OMEGA trial (omega-3 fatty acid, gamma-linolenic acid, and anti-oxidant supplementation in the management of acute lung injury or ARDS), which was a negative trial (37). Our cross-sectional study cannot determine whether low levels of plasma lipids are a pathogenic finding, a reflection of greater severity of illness, or of higher microbial burden and LPS levels. Therefore, it remains uncertain whether interventions to raise plasma lipids could offer potential therapeutic benefit.

The EARLI cohort with its detailed clinical phenotyping was also ideal to assess whether plasma metabolites can distinguish other critical illness syndromes, including ARDS, among patients with early sepsis. This differs from prior plasma metabolomics studies evaluating ARDS that compared patients with ARDS to healthy controls (20) or to postsurgical intubated patients (19). In the current study, plasma metabolites did not distinguish patients with moderate-to-severe ARDS from patients with early sepsis who never developed the syndrome. Patients with early sepsis who developed ARDS were almost twice as likely to belong to the high-risk metabolic group 1 (although this was not statistically significant), which reflected their higher baseline severity of illness, including a higher nonpulmonary SAPS II. As such, in our cohort of critically ill patients with early sepsis, plasma metabolites reflect severity of illness and do not distinguish ARDS among this population. In addition, as shown in Table 3, previously identified ARDS-associated metabolites were not associated with ARDS status in our cohort, but rather with severity of illness and metabolite group assignment.

Not only does global metabolic profiling fail to separate patients with early sepsis who develop ARDS from those who do not, but individual plasma metabolites associated with ARDS identified by other studies (none of which were adjusted for systemic illness) were also not associated with ARDS in our cohort after this correction. The exceptions to this were an association with rocuronium, a reflection of treatment rather than biology, and a single metabolite, 17alpha-hydroxypregnenolone 3-sulfate, a sulfated steroid metabolite that is found in most human tissues that is produced by the adrenal gland, but without known human biologic function. In this cohort, some of the previously reported ARDS-associated metabolites were related to metabolic subgroup assignment and severity of illness, supporting the observation that plasma metabolites reflect systemic inflammation and severity of illness rather than lung injury.

Our prior meta-analysis of all publicly available whole blood gene expression datasets showed key similarities to this study, notably, 1) an inability to identify a plasma gene expression signal specific to ARDS to enable building a robust ARDS classifier and 2) the whole blood gene expression signal was driven by systemic inflammation (38). Taken together, these studies suggest that a plasma 'omics may not be an ideal method for discovering novel lung-specific biology in critically ill patients or that ARDS per se is not a useful syndromic rubric for capturing a specific biologic phenotype. Instead, lung injury–specific biospecimen such as bronchoalveolar lavage (39, 40), pulmonary edema fluid (41), or heat moisture exchange filter fluid (42) may provide more proximal and more biologically relevant material that is less confounded by a systemic signal present in plasma and whole blood.

We acknowledge some important limitations to this work. First, although it is one of the largest plasma metabolomics studies of early sepsis performed to date, it was carried out at a single center. Although our findings were robust based on leave-one-out cross-validation and permutation testing, the identification of three metabolically distinct subsets of sepsis requires external validation in independent populations. Second, samples were collected up to 24 hours after ICU admission, and therefore some metabolites may reflect treatment. Although collecting samples at an even earlier time point (i.e., at the time of presentation to the emergency department) may be ideal in future studies, this will be challenging to achieve. Third, treatment-related factors may influence metabolic profiling (i.e., administration of propofol or glucose-containing products that alter metabolism from the fasting state). However, the lipid metabolites identified in our study were long chain fatty acids, whereas drugs such as propofol are small lipophilic hydrocarbons, suggesting our analysis was less likely affected by this drug. Finally, metabolomics is one of many 'omics technologies to identify biologically distinct subsets of sepsis with different outcomes and potentially differential treatment

responses (4). Future studies will be needed to determine whether the same subgroups of patients can be identified by different methods. Ultimately, a minimal set of features across different 'omics technologies may be necessary to enable accurate and rapid subgroup identification for the enrichment of future sepsis clinical trials.

In summary, hierarchical clustering of plasma metabolites in early sepsis identified three clusters that are characterized by distinct lipidomic differences that were associated with markedly different severity of illness and mortality. Global metabolic profiling of plasma reflects systemic illness in early sepsis but did not distinguish ARDS from at-risk critically ill controls.

ACKNOWLEDGMENTS

We are grateful to the patients and their families for participation in the early assessment of renal and lung injury cohort, as well as to the bedside clinical nurses and providers.

1 *Division of Pulmonary and Critical Care, Department of Medicine, Stanford, CA.*

2 *Departments of Medicine and Anesthesia, Cardiovascular Research Institute, University of California, San Francisco, CA.*

3 *Interdepartmental Division of Critical Care Medicine, Department of Medicine, University of Toronto, Toronto, ON, Canada.*

4 *Department of Genetics, Stanford University School of Medicine, Stanford, CA.*

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (http://journals.lww.com/ccejournal).

Dr. Rogers was supported by K23 HL125663 and HL 152083. Dr. Leligdowicz was supported by the University of Toronto Clinician Investigator Program and the Clinician Scientist Training Program. Dr. Kangelaris was supported by K23HL116800, Dr. Liu by HL140026, Dr. Calfee by HL140026, and Dr. Matthay by HL51856. The remaining authors have disclosed that they do not have any conflicts of interest.

For information regarding this article, E-mail: ajrogers@stanford.edu

REFERENCES

1. Havel JJ, Chowell D, Chan TA: The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. *Nat Rev Cancer* 2019; 19:133–150
2. Lloyd CM, Hessel EM: Functions of T cells in asthma: More than just T(H)2 cells. *Nat Rev Immunol* 2010; 10:838–848
3. Sinha P, Calfee CS: Phenotypes in acute respiratory distress syndrome: Moving towards precision medicine. *Curr Opin Crit Care* 2019; 25:12–20
4. Leligdowicz A, Matthay MA: Heterogeneity in sepsis: New biological evidence with clinical applications. *Crit Care* 2019; 23:80
5. Sweeney TE, Perumal TM, Henao R, et al: A community approach to mortality prediction in sepsis via gene expression analysis. *Nat Commun* 2018; 9:694
6. Rogers AJ, Guan J, Trtchounian A, et al: Association of elevated plasma interleukin-18 level with increased mortality in a clinical trial of statin treatment for acute respiratory distress syndrome. *Crit Care Med* 2019; 47:1089–1096
7. Sweeney TE, Khatri P: Generalizable biomarkers in critical care: Toward precision medicine. *Crit Care Med* 2017; 45:934–939
8. Sinha P, Calfee CS: Peeking under the hood of acute respiratory distress syndrome phenotypes: Deeper insights into biological heterogeneity. *Am J Respir Crit Care Med* 2019; 200:4–6
9. Calfee CS, Delucchi K, Parsons PE, et al; NHLBI ARDS Network: Subphenotypes in acute respiratory distress syndrome: Latent class analysis of data from two randomised controlled trials. *Lancet Respir Med* 2014; 2:611–620
10. Calfee CS, Delucchi KL, Sinha P, et al; Irish Critical Care Trials Group: Acute respiratory distress syndrome subphenotypes and differential response to simvastatin: Secondary analysis of a randomised controlled trial. *Lancet Respir Med* 2018; 6:691–698
11. Famous KR, Delucchi K, Ware LB, et al; ARDS Network: Acute respiratory distress syndrome subphenotypes respond differently to randomized fluid management strategy. *Am J Respir Crit Care Med* 2017; 195:331–338
12. Serkova NJ, Standiford TJ, Stringer KA: The emerging field of quantitative blood metabolomics for biomarker discovery in critical illnesses. *Am J Respir Crit Care Med* 2011; 184:647–655
13. Englert JA, Rogers AJ: Metabolism, metabolomics, and nutritional support of patients with sepsis. *Clin Chest Med* 2016; 37:321–331
14. Singer M, Deutschman CS, Seymour CW, et al: The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016; 315:801–810
15. Dellinger RP, Levy MM, Rhodes A, et al; Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup: Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 2013; 39:165–228
16. Deutschman CS, Hellman J, Ferrer Roca R, et al; Research Committee of the Surviving Sepsis Campaign: The surviving sepsis campaign: Basic/translational science research priorities. *Crit Care Med* 2020; 48:1217–1232
17. Langley RJ, Tsalik EL, van Velkinburgh JC, et al: An integrated clinico-metabolomic model improves prediction of death in sepsis. *Sci Transl Med* 2013; 5:195ra95
18. Rogers AJ, McGeachie M, Baron RM, et al: Metabolomic derangements are associated with mortality in critically ill adult patients. *PLoS One* 2014; 9:e87538

19. Viswan A, Ghosh P, Gupta D, et al: Distinct metabolic endotype mirroring acute respiratory distress syndrome (ARDS) subphenotype and its heterogeneous biology. *Sci Rep* 2019; 9:2108
20. Stringer KA, Serkova NJ, Karnovsky A, et al: Metabolic consequences of sepsis-induced acute lung injury revealed by plasma ^{1}H-nuclear magnetic resonance quantitative metabolomics and computational analysis. *Am J Physiol Lung Cell Mol Physiol* 2011; 300:L4–L11
21. Singh C, Rai RK, Azim A, et al: Metabolic profiling of human lung injury by H-1 high-resolution nuclear magnetic resonance spectroscopy of blood serum. *Metabolomics* 2015; 11:166–174
22. Izquierdo-Garcia JL, Nin N, Jimenez-Clemente J, et al: Metabolomic profile of ARDS by nuclear magnetic resonance spectroscopy in patients with H1N1 influenza virus pneumonia. *Shock* 2018; 50:504–510
23. Bernard GR, Artigas A, Brigham KL, et al: The American-European consensus conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994; 149:818–824
24. Ferguson ND, Fan E, Camporota L, et al: The Berlin definition of ARDS: An expanded rationale, justification, and supplementary material. *Intensive Care Med* 2012; 38:1573–1582
25. Xia J, Sinelnikov IV, Han B, et al: MetaboAnalyst 3.0–making metabolomics more meaningful. *Nucleic Acids Res* 2015; 43:W251–W257
26. R Core Team: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing, 2018. Available at: https://www.R-project.org/
27. van den Berg RA, Hoefsloot HC, Westerhuis JA, et al: Centering, scaling, and transformations: Improving the biological information content of metabolomics data. *BMC Genomics* 2006; 7:142
28. Worley B, Powers R: Multivariate analysis in metabolomics. *Curr Metabolomics* 2013; 1:92–107
29. Szymańska E, Saccenti E, Smilde AK, et al: Double-check: Validation of diagnostic statistics for PLS-DA models in metabolomics studies. *Metabolomics* 2012; 8:3–16
30. Sekula P, Goek ON, Quaye L, et al: A metabolome-wide association study of kidney function and disease in the general population. *J Am Soc Nephrol* 2016; 27:1175–1188
31. López-Ibáñez J, Pazos F, Chagoyen M: MBROLE 2.0-functional enrichment of chemical compounds. *Nucleic Acids Res* 2016; 44:W201–W204
32. Wilson RF, Barletta JF, Tyburski JG: Hypocholesterolemia in sepsis and critically ill or injured patients. *Crit Care* 2003; 7:413–414
33. Fraunberger P, Schaefer S, Werdan K, et al: Reduction of circulating cholesterol and apolipoprotein levels during sepsis. *Clin Chem Lab Med* 1999; 37:357–362
34. Harris HW, Grunfeld C, Feingold KR, et al: Human very low density lipoproteins and chylomicrons can protect against endotoxin-induced death in mice. *J Clin Invest* 1990; 86:696–702
35. Boyd JH, Fjell CD, Russell JA, et al: Increased plasma PCSK9 levels are associated with reduced endotoxin clearance and the development of acute organ failures during sepsis. *J Innate Immun* 2016; 8:211–220
36. Walley KR, Thain KR, Russell JA, et al: PCSK9 is a critical regulator of the innate immune response and septic shock outcome. *Sci Transl Med* 2014; 6:258ra143
37. Rice TW, Wheeler AP, Thompson BT, et al; NIH NHLBI Acute Respiratory Distress Syndrome Network of Investigators; NHLBI ARDS Clinical Trials Network: Enteral omega-3 fatty acid, gamma-linolenic acid, and antioxidant supplementation in acute lung injury. *JAMA* 2011; 306:1574–1581
38. Sweeney TE, Thomas NJ, Howrylak JA, et al: Multicohort analysis of whole-blood gene expression data does not form a robust diagnostic for acute respiratory distress syndrome. *Crit Care Med* 2018; 46:244–251
39. Singh C, Rai RK, Azim A, et al: Mini-bronchoalveolar lavage fluid can be used for biomarker identification in patients with lung injury by employing H-1 NMR spectroscopy. *Crit Care* 2013; 17:430
40. Morrell ED, Radella F II, Manicone AM, et al: Peripheral and alveolar cell transcriptional programs are distinct in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2018; 197:528–532
41. Rogers AJ, Contrepois K, Wu M, et al: Profiling of ARDS pulmonary edema fluid identifies a metabolically distinct subset. *Am J Physiol Lung Cell Mol Physiol* 2017; 312:L703–L709
42. McNeil JB, Shaver CM, Kerchberger VE, et al: Novel method for noninvasive sampling of the distal airspace in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2018; 197:1027–1035