

## REVIEW | Translational Physiology

# Evolution of ARDS biomarkers: Will metabolomics be the answer?

 Sayed M. Metwaly,<sup>1</sup> Andreanne Cote,<sup>1</sup> Sarah J. Donnelly,<sup>1</sup> Mohammad M. Banoei,<sup>1</sup> Ahmed I. Mourad,<sup>1</sup> and  Brent W. Winston<sup>1,2</sup>

<sup>1</sup>Department of Critical Care Medicine, University of Calgary, Calgary, Alberta, Canada; and <sup>2</sup>Departments of Medicine and Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada

Submitted 12 February 2018; accepted in final form 11 June 2018

**Metwaly SM, Cote A, Donnelly SJ, Banoei MM, Mourad AI, Winston BW.**

Evolution of ARDS biomarkers: will metabolomics be the answer? *Am J Physiol Lung Cell Mol Physiol* 315: L526–L534, 2018. First published June 28, 2018; doi:10.1152/ajplung.00074.2018.—To date, there is no clinically agreed-upon diagnostic test for acute respiratory distress syndrome (ARDS): the condition is still diagnosed on the basis of a constellation of clinical findings, laboratory tests, and radiological images. Development of ARDS biomarkers has been in a state of continuous flux during the past four decades. To address ARDS heterogeneity, several studies have recently focused on subphenotyping the disease on the basis of observable clinical characteristics and associated blood biomarkers. However, the strong correlation between identified biomarkers and ARDS subphenotypes has yet to establish etiology; hence, there is a need for the adoption of other methodologies for studying ARDS. In this review, we will shed light on ARDS metabolomics research in the literature and discuss advances and major obstacles encountered in ARDS metabolomics research. Generally, the ARDS metabolomics studies focused on identification of differentiating metabolites for diagnosing ARDS, but they were performed to different standards in terms of sample size, selection of control cohort, type of specimens collected, and measuring technique utilized. Virtually none of these studies has been properly validated to identify true metabolomics biomarkers of ARDS. Though in their infancy, metabolomics studies exhibit promise to unfold the biological processes underlying ARDS and, in our opinion, have great potential for pushing forward our present understanding of ARDS.

acute respiratory distress syndrome; alveolar epithelial cells; ARDS; biomarkers; metabolomics; vascular endothelium

## INTRODUCTION

Acute respiratory distress syndrome (ARDS) is a serious cause of morbidity and mortality in the intensive care unit (ICU; 9). ARDS is a frequently encountered condition with an incidence of 78.9 cases per 100,000 person years (56). According to the recent Large Observational Study to Understand the Global Impact of Severe Acute Respiratory Failure (LUNG SAFE), worldwide ARDS represents 10.4% of all ICU admissions with an overall mortality rate of 35.3% (5). In Canada, the average total hospital cost per patient with ARDS was \$128,860 (2002 Canadian dollars), with the majority of this cost generated in the ICU (\$97,810; 6, 27). Despite decades of ongoing ARDS research, there is still a battery of unmet challenges that are contributing to the perplexity of the condition. A better mechanistic understanding of ARDS may enable further improvement in the diagnosis, classification, and management of this complex

syndrome. This will be potentially achieved by identification of biomarkers early in the disease process that reflect the underlying alveolocapillary membrane injury, whether the pattern is epithelial injury, endothelial injury, or a combination.

Recently, there has been a growing interest in addressing ARDS heterogeneity, and consequently, several studies have adopted the approach of subgrouping patients with ARDS on the basis of clinical characteristics and the associated levels of a panel of blood biomarkers (10, 14, 15, 23). The identified phenotypes have generally been reproducible and correlated well with clinical aspects of ARDS such as ventilator-free days, organ failure-free days, response to fluid management, and mortality (14, 23). However, some of the limitations encountered in these studies include the broad variations in the populations recruited, variable timing of the measurement of the biomarkers from one study to another, and very few biomarkers being longitudinally compared in the same patients (32). Additionally, the subgrouping biomarkers and their consequent ARDS subtypes have not focused on pathophysiologic changes, and they need to be prospectively studied for biomarker validation (36, 37).

Address for reprint requests and other correspondence: B. W. Winston, Dept. of Critical Care Medicine, Univ. of Calgary, Health Research Innovation Center, Rm. 4C64, 3280 Hospital Dr., N.W., Calgary, AB, T2N 4Z6, Canada (e-mail: bwinston@ucalgary.ca).

Interestingly, research findings suggest that the differences between direct lung injury-induced ARDS and indirect lung injury-induced ARDS are not just limited to clinically observable characteristics, but rather extend to the core histopathology (17, 29, 46, 50). In light of such evidence, other holistic methodologies capable of highlighting the full complement of changes that occur at a molecular level may be better suited for studying ARDS. These include systems biology approaches, most notably, metabolomics, for example. In this review, we will shed light on the potential of using metabolomics in ARDS to identify biomarkers of disease and demonstrate the rich details this technology can reveal about the biological mechanisms involved in ARDS.

### WHAT IS METABOLOMICS?

Metabolomics is an emerging field of “omics” studies that uses a systems biology approach to examine metabolism and metabolites. Contrary to routine clinical chemistry, which relies on the measurement of single metabolites (such as glucose or lactate), metabolomics allows for the simultaneous evaluation of a large set of metabolites/biological compounds in a single sample (2), thus providing an integrative snapshot view of biological systems (38). Although the use of biological fluid metabolites for diagnosis of medical conditions has been in existence since ancient times, modern

metabolomics analysis began in the mid-1980s with the development of higher-sensitivity nuclear magnetic resonance spectrometers (47). Metabolites are small molecules up to ~1,000 Da and include a spectrum of compounds such as organic acids, amino acids, carbohydrates, peptides, vitamins, steroids, and xenobiotics, among others (24). The fundamental premise in metabolomics analyses is that changes, whether physiological or pathological, cause alterations in the metabolome that are detected as variations in metabolite concentrations. Commonly used metabolomics platforms include nuclear magnetic resonance spectroscopy (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS), and increasingly, studies tend to incorporate multiple techniques. A brief explanation of the typical workflow of a metabolomics study is provided in Fig. 1. Data analysis is the most challenging step in metabolomics experiments, and this is further compounded by the increasing complexity of data sets generated as the machinery improves (45). Detection, quantification, analysis, and careful interpretation of a combination of small molecules generates a metabolic “biopattern,” “profile,” or “fingerprint” that can potentially serve as a biomarker for the underlying condition. Given the scope of this review, only a cursory overview of metabolomics has been provided. More comprehensive information can be found elsewhere in the literature (2, 4, 40, 41).

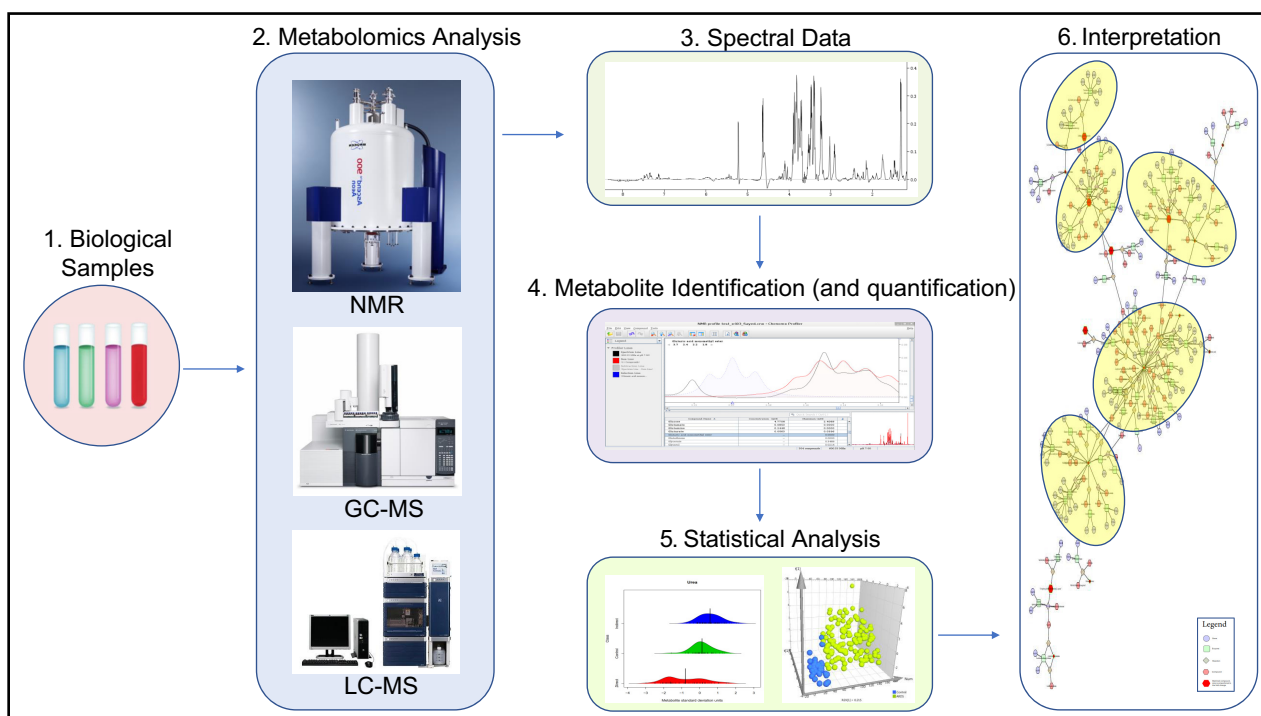


Fig. 1. Typical workflow cycle of a metabolomics study (steps 1–6). 1) Metabolomics studies start with the selection of adequate samples. 2) Commonly used analytical methods include NMR, GC-MS, and/or liquid chromatography-mass spectrometry (LC-MS). These methods may be employed for either global screening of all possible metabolites (untargeted approach) or selected measurement of specific metabolites (targeted approach). 3) Analysis of resultant spectral data. 4) Putative metabolite identification using compound libraries. NMR results are quantitative whereas GC-MS/LC-MS results can be quantitative only in targeted approaches. 5) Univariate and multivariate statistical analysis. The most commonly used multivariate analyses include principal component analysis and partial least squares analysis; however, there is a plethora of methods that are becoming increasingly reported in literature, e.g., orthogonal partial least squares analysis, random forest analysis, support vector machines analysis, and K-means clustering. 6) Interpretation and, if applicable, identification of relevant pathways involved. Instruments shown in step 2 from top down are as follows: Bruker Ascend 900 Aeon NMR (courtesy of Bruker BioSpin Group), Agilent 7200B GC/Q-TOF (© Agilent Technologies, 2014; reproduced with permission, courtesy of Agilent Technologies Incorporated), and Hitachi ChromasterUltra Rs Ultra-High Performance Liquid Chromatograph (courtesy of Hitachi High-Tech Science Corporation).

## WHY IS METABOLOMICS WELL SUITED FOR STUDYING ARDS?

Metabolomics and other multidimensional omics technologies make no assumptions about what is important in a particular disease and therefore are generally unbiased tools for the discovery of old and new disease pathways and processes (18). However, unlike other omics technologies, there are significantly fewer metabolites (~5,000–10,000 identified in humans) than proteins (~1,000,000), transcripts (~100,000), or genes (~25,000; 55). This renders metabolomics data more readily analyzable and compilable into unique biopatterns compared with other omics fields (33, 55). Additionally, metabolomics targets a level downstream in the biological cascade (55), and thus it is closer to phenotype, more reflective of the real-time perturbations in the biological processes, and more responsive to environmental influences among all the omics technologies (38). On the other hand, the inherent nature of ARDS, which requires an environmental trigger for the condition to manifest, is suboptimally suited for certain omics studies. For example, in ARDS genomic studies, individuals with a potential ARDS genetic susceptibility who have not sustained an adequate environmental trigger would not manifest ARDS and could very well be assigned to the control group thereby reducing the statistical power (53).

Furthermore, a vast array of biological samples are amenable to metabolomics analysis without the need for special preparations, unlike other omics, e.g., epigenomics (41); thus it is more technically feasible to use metabolomics for studying ARDS. Serum, plasma, urine, bronchoalveolar lavage fluid (BALF), tissue samples, and even exhaled breath condensate (EBC) can be used for metabolomics detection after simple preparation (41). The sample types commonly utilized in metabolomics studies of ARDS, their recommended preparation, and their advantages and disadvantages are summarized in Table 1. It can be argued that circulating metabolites (e.g., obtained from serum or plasma) represent the summation of a complex interplay of diverse biological systems and thus fall short of local lung metabolites (e.g., obtained from BALF or EBC) in studying ARDS. Although this argument may be intuitively sound, the local lung sampling techniques provide samples that are scarce in metabolites, often diluted with high concentration of salt, less standardized, and less suitable for prospective follow-up, and the sampling technique (i.e., bronchoscopy) can be overly invasive in the context of critically ill patients. Moreover, as a patchy disease that occurs in consequence to both pulmonary and extrapulmonary causes, ARDS may be more amenable to systemic compartment sampling (i.e., serum/plasma) that allows monitoring of extrapulmonary processes and provides an average estimate of processes occurring in the lungs.

Classically, the interpretation of metabolomics data was fraught with obstacles that hampered the utilization of this technology. Metabolomics results are characterized by high dimensionality (the number of measured metabolites is multiple orders of magnitude larger than the number of observations/samples), multicollinearity (several metabolites can be highly correlated because of both technical reasons, where a metabolite may have more than one signature, and biological reasons, where metabolites belonging to the same biological network are interconnected; 8), and high variability (due to

analytical deviations; for example, deviations in LC-MS may be related to column degradation, sample carryover, or small fluctuations in room temperature and mobile phase pH; 49). Such challenges explain the extreme uncertainty that once shrouded metabolomics results; however, the growing computational power in the past decade coupled with bioinformatics advances have made tangible progress in this regard (49).

Our judgement of the potential of metabolomics as an ARDS research tool does not rely solely on the theoretical capabilities of metabolomics but also, similarly, on the practical success metabolomics has proven in other related lung diseases. Metabolomics has been increasingly used for studying a number of lung conditions, especially asthma (21, 26, 66) and chronic obstructive pulmonary disease (44, 64, 65). Similar to ARDS, these conditions are heterogenous, and the application of metabolomics research helped in redefining their phenotypes (1, 19). Additionally, the application of metabolomics in pneumonia, whether in animal models (59, 60) or clinical studies (39), demonstrated the ability of this technology to provide early diagnosis of inflammatory lung conditions (12).

## METABOLOMICS STUDIES OF ARDS

Experimental preclinical animal models of ARDS generally have not fared well in representing the disease in humans (62); therefore, there is a scarcity of metabolomics research in animal models. Devising cultured human cell models of ARDS is not feasible given the complex array of biological tissues involved (lung epithelium, vascular endothelium, immune cells, fibroblasts, etc.). However, some of the cellular responses that occur in ARDS can be inferred from studying cultured human cell models. For example, the effect of bacterial infection on the metabolome of A549 cultured human airway epithelial cells (mimics an infection-related direct lung injury) demonstrated an increase in extracellular secretion of glutamate and pyruvate (28). The study also revealed that concentrations of glycine, aspartate, and alanine were increased in the extracellular space suggesting a reduction in the cellular usage of these amino acids with consequent intracellular overflow and secretion (28).

In one of the earliest clinical ARDS metabolomics studies identified in the literature, Schubert et al. used a targeted GC-MS study to compare metabolites in the exhaled breath of 19 ventilated patients with ARDS and 18 ventilated surgical ICU patients (57). Nine metabolites were profiled, and a significantly decreased level of isoprene was present in the patients with ARDS compared with ventilated ICU controls [ $9.8 (8.2 \pm 21.6)$  vs.  $21.8 (13.9 \pm 41.4)$  nmol·m<sup>-2</sup>·min<sup>-1</sup>, medians (95% confidence intervals),  $P = 0.04$ ; 57]. Isoprene is a by-product of cholesterol synthesis through the mevalonate pathway (34). Additionally, isoprene is the most prevalent hydrocarbon in breath (34) though it exhibits a highly variable concentration. The exact physiological role of isoprene remains unclear although it is postulated that it may exert a thermoprotective function in cells exposed to heat stress and may have a protective effect against reactive oxygen species (35). Of note, the small sample size, the lack of exhaled breath follow-up validation studies, and the difficulties associated with reproducibility of GC-MS results make it hard to extrapolate the results to the extremely heterogeneous groups of patients with ARDS.

Table 1. *Sample types commonly used for ARDS metabolomics studies*

Sample Type	Preparation	Advantages	Disadvantages	Recommendations
BALF/mini-BALF	<ul style="list-style-type: none"> <li>● Centrifuge to remove cells and debris (800 g at 4°C for 10 min)</li> <li>● Remove supernatant</li> <li>● Aliquot and freeze (−80°C) until the time of assay</li> </ul>	<ul style="list-style-type: none"> <li>● Collected from specific area of the lung (BALF only)</li> <li>● Collected under clinical and reproducible conditions</li> </ul>	<ul style="list-style-type: none"> <li>● Invasive and not well tolerated in patients with severe ARDS</li> <li>● Diluted (typically 100X); thus not all metabolites can be assessed</li> <li>● Difficult to accurately normalize for sample dilution</li> <li>● Concentration process is often required leading to variability</li> <li>● Cannot be used easily for longitudinal sampling</li> <li>● Mini-BALF is less standardized compared with BALF</li> </ul>	<ul style="list-style-type: none"> <li>● First-cycle lavage is preferred</li> <li>● Correct for dilution with urea ratio (e.g., BALF: plasma)</li> <li>● Consider buffer exchange to remove salt and methanol or acetone precipitation for protein separation</li> </ul>
Exhaled breath condensate	<ul style="list-style-type: none"> <li>● Collect during tidal breathing using a nose clip and a saliva trap</li> <li>● Define cooling temperature and collection time</li> <li>● Use inert material for condenser</li> <li>● Do not use resistor and do not use filter between the subject and the condenser</li> </ul>	<ul style="list-style-type: none"> <li>● Noninvasive</li> <li>● Safe</li> <li>● Suitable for analysis of nonvolatile components</li> <li>● Suitable for longitudinal study</li> <li>● Feasible in children</li> </ul>	<ul style="list-style-type: none"> <li>● Very diluted</li> <li>● Difficult to standardize</li> <li>● Often requires concentration steps leading to variability</li> <li>● Potential variability due to differences in droplet dilution</li> <li>● Samples the entire airway; difficult to localize changes</li> <li>● Difficult to normalize metabolites for the total content</li> <li>● High variability in sample quality</li> </ul>	<ul style="list-style-type: none"> <li>● Consider commercial equipment, such as EcoScreen or RTube</li> <li>● Collection time of 10 min generally sufficient to obtain 1–2 ml of sample and is well tolerated by patients</li> </ul>
Plasma/serum	<ul style="list-style-type: none"> <li>● Collect blood by direct venipuncture, if possible, into a Vacutainer tube</li> <li>● For plasma, make sure the Vacutainer tube contains either EDTA or sodium heparin; immediately invert the tube several times to ensure mixture with anticoagulant</li> <li>● For serum, make sure the Vacutainer tube has no additive; allow the blood to clot at room temperature for at least 30 min</li> <li>● After 30 min of blood collection, centrifuge balanced tubes (15 min at 1,300 g) with no brake to ensure proper separation</li> <li>● After centrifugation, use the upper layer (clear and pale yellow in color) and avoid disturbing other layer(s)</li> <li>● Carefully aliquot and freeze (−80°C) in Cryovial</li> </ul>	<ul style="list-style-type: none"> <li>● Minimally invasive</li> <li>● Easy to collect with standardized protocols</li> <li>● Widely used in metabolomics studies</li> <li>● Composition relatively well documented</li> <li>● Relatively consistent and easy to define protocol in multicenter studies</li> <li>● Contains a large number of potential targets</li> </ul>	<ul style="list-style-type: none"> <li>● Plasma is not well suited for NMR especially if filters are used</li> <li>● Lipid composition dominated by lipoproteins, possibly masking minor components</li> <li>● Distant from the tissue of interest, so there is potential bias toward systemic changes in disease</li> </ul>	<ul style="list-style-type: none"> <li>● Refrigeration before or during plasma centrifugation is recommended</li> </ul>

ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid. [Adapted from Bowler et al. (12) and Wheelock et al. (68).]

Bos et al. performed a similar study, using GC-MS analysis of exhaled breath to compare 23 ventilated patients with ARDS and 20 ventilated ICU control patients (11). Significant differences were seen between ARDS and control patients in three volatile organic compounds: octane, acetaldehyde, and 3-methylheptane; however, as a composite biomarker, the diagnostic accuracy was not particularly high (area under the curve = 0.80, 95% confidence interval = 0.66–0.92; 11). Such a metabolomics signature denotes oxidative stress (62), and in fact, octane is the end product of lipid peroxidation (52). The findings were validated using a cohort of 19 patients with ARDS and 27 controls with moderate diagnostic accuracy (area under the curve = 0.78, 95% confidence interval =

0.65–0.91; 11). However, the study was not able to show a difference between patients with mild and moderate/severe ARDS ( $P = 0.21$ ), and the identified volatile organic compounds were not correlated with the arterial partial pressure of oxygen-to-fraction of inspired oxygen ratio (Spearman's correlation  $r = 0.18$ ,  $P = 0.27$ ; 11). Additionally, the study could not differentiate between direct and indirect ARDS ( $P = 0.24$ ). Collectively, these findings suggested a limited clinical utility of EBC GC-MS metabolomics analysis in ARDS.

In a pilot study, Stringer et al. used plasma  $^1\text{H}$ -NMR to assess metabolic differences between 13 patients with sepsis-induced acute lung injury (ALI; mild ARDS) and 6 healthy controls (63). They identified significant increases in the levels

of total glutathione, adenosine, and phosphatidylserine and a decreased sphingomyelin level in patients with sepsis-induced ALI compared with healthy controls (63). This metabolomics fingerprint reflects the complex array of biological processes involved in ARDS pathogenesis, namely, oxidative stress (glutathione), energy metabolism (adenosine), apoptosis (phosphatidylserine), and disruption of the endothelial barrier (sphingomyelin; 7, 13, 42). The study demonstrated a very weak association between acute physiology score, myoinositol, and total glutathione ( $r_s = 0.53$ ,  $q = 0.25$ ,  $P = 0.05$  and  $r_s = 0.56$ ,  $q = 0.25$ ,  $P = 0.04$ , respectively), yet suggested they may be used for determination of disease severity. Given the research evidence of the impact of mechanical ventilation on metabolites (31, 48) and the fact that the majority of patients with ARDS have some form of respiratory support, the use of healthy nonventilated controls is a potentially important confounder in the Stringer et al. (63) study.

Stringer and colleagues later performed a follow-up serum  $^1\text{H-NMR}$  study in 2014 comparing 14 patients with ARDS and 33 unventilated patients with sepsis (61). The study showed an association between ARDS and a metabolomics profile of increased concentrations of phosphatidylserine, total lipids, total methylene lipids, and total cholines at the time of presentation to the emergency department (61). The concentrations of identified metabolites further increased in the ARDS group after 72 h of admission (61). Increased methylene lipids ( $-\text{CH}_2$ )<sub>n</sub>, total cholines, and phosphatidylserine have been linked to apoptosis-induced disintegration of cell membranes (61).

Evans et al. performed a metabolomics study using BALF and untargeted LC-MS to assess metabolic differences between 18 patients with ARDS (sepsis-induced, pneumonia-induced, and aspiration-induced) and 8 healthy controls (22). The study found increased levels of guanosine, xanthine, hypoxanthine, and lactate and a decreased level of phosphatidylcholine in patients with ARDS (22). Phosphatidylcholine is the most abundant phospholipid in the lung surfactant, and its level inversely correlates with lung injury caused by inflammatory cells (62). Remarkably, several uric acid precursor metabolites were profoundly increased (hypoxanthine 41-fold, xanthine 19-fold, and guanosine 4-fold). Uric acid further aggravates the lung injury by inducing acute inflammation, let alone its production by xanthine oxidase enzyme, which intensifies the oxidative stress by liberating  $\text{O}_2^-$  (62).

Rai et al. used nonbronchoscopic mini-BALF samples analyzed by high-resolution 800-MHz  $^1\text{H-NMR}$  to compare 21 patients with ARDS (10 ARDS and 11 ALI) and 9 ventilated ICU control patients (51). This pilot study showed significant differences in isoleucine, valine, lysine, leucine, lactate, threonine, alanine, betaine, arginine, choline, ethanol, and proline concentrations between controls versus ALI/ARDS cohort ( $R^2Y = 0.89$  and  $Q^2 = 0.84$ ). Increased lactate concentration in the ALI/ARDS cohort ( $P = 0.001$ ) denotes lung inflammation and anaerobic metabolism (70).

In 2014, Singh and colleagues performed another high-resolution 800-MHz  $^1\text{H-NMR}$  pilot study to compare the serum of 26 patients with ARDS (sepsis, pneumonia, malaria, chronic alcoholism, and acute pancreatitis) and 19 ICU controls ventilated for nonrespiratory reasons (neuromuscular diseases, Guillain-Barre syndrome, and pancreatitis). The study demonstrated a panel of metabolites composed of lipids,

branched-chain amino acids (BCA), alanine, acetate, *N*-acetyl glycoproteins, glutamate, glutamine, acetoacetate, creatinine, histidine, formate, and lactate that could differentiate patients with ARDS from control patients (58). The increase in BCA levels has been attributed to protein catabolism that is associated with lung injury and infections (58).

Rogers et al. sought to prove ARDS metabolic heterogeneity and hypothesized the presence of a subset of ARDS with a distinct metabolic profile (54). They compared the edema fluid of 16 patients with ARDS and 13 controls with hydrostatic pulmonary edema using untargeted metabolomics (ultrahigh-performance liquid chromatography-tandem mass spectrometry for basic species, acidic species, and lipids). The study identified 6 patients with “hypermetabolic subtype” ARDS (6/16, 38%) mostly caused by nonpulmonary sepsis who had higher levels of 235 metabolites and were different from the rest of the patients with ARDS (10/16, 62%). The group compared their findings with Calfee et al.’s results (14); however, because of lack of plasma samples a conclusion was not possible (54). The study could not differentiate between patients with ARDS and hydrostatic pulmonary edema controls (54). Pathway analysis of the 235 significant metabolites revealed a statistically significant overrepresentation of a single pathway (alanine, aspartate, and glutamate metabolism). The strong suggestion of ARDS subgroups defined by metabolites makes this study particularly interesting.

Finally, Viswan et al. sought to better characterize ARDS severity using high-resolution 800-MHz  $^1\text{H-NMR}$ . Mini-BALF samples ( $n = 36$ ) were collected from patients with mild ARDS ( $n = 13$ ) and moderate/severe ARDS ( $n = 23$ ). A total of 29 metabolites were identified in the mini-BALF samples. A final predictive model consisting of six metabolites (proline, lysine/arginine, taurine, threonine, and glutamate) was constructed to separate mild and moderate/severe ARDS (accuracy = 0.91,  $R^2Y = 0.72$ , and  $Q^2 = 0.60$ ; 67). Pathway analysis indicated the dysregulation of arginine and proline metabolism; lysine biosynthesis and degradation; aminoacyl-tRNA biosynthesis; taurine and hypotaurine metabolism; glycine, serine, and threonine metabolism; D-glutamine and D-glutamate metabolism; and alanine, aspartate, and glutamate metabolism (67). Interestingly, an increased level of taurine has been linked to the severity of lung inflammation (69). Taurine regulates epithelial cells’ osmotic balance and plays a role in the protection against oxidative damage caused by neutrophil myeloperoxidase (16). It is noteworthy that despite the good metrics of the final ARDS severity predictive model, the initial unsupervised principal component analysis scatterplot shows a complete overlap between mild and moderate/severe ARDS.

Table 2 summarizes all the human ARDS studies discussed above. We have run an integrative pathway analysis using 27 significant metabolites (acetate, alanine, lysine, aspartate, glutathione, formate, arginine, glutamine, acetaldehyde, choline, leucine, histidine, proline, phosphatidylcholine, acetoacetate, valine, lactate, threonine, adenosine, hypoxanthine, xanthine, guanosine, isoleucine, ethanol, sphingomyelin, betaine, and phosphatidylserine) identified in the studies that compared ARDS and controls. The pathways were plotted using Cytoscape v.3.6.0 software (MetScape plugin v.3.1.3; Supplemental Fig. S1; Supplemental Material for this article is available online at the *American Journal of Physiology Lung Cellular and Molecular Physiology* website). Comprehensive details of

Table 2. *Human ARDS metabolomics studies identified in the literature*

Authors	Year	Cases, <i>n</i>	Controls, <i>n</i>	Sample Type	Analytical Platform	Metabolites Profiled	ARDS-Associated Metabolites
Schubert et al. (57)	1998	19 ARDS	18 Ventilated SICU	Exhaled breath	GC-MS	9	Isoprene
Stringer et al. (63)	2011	13 Sepsis-induced ALI	6 Healthy	Plasma	<sup>1</sup> H-NMR	40	Total glutathione, adenosine, phosphatidylserine, and sphingomyelin
Rai et al. (51)	2012	21 ARDS	9 Ventilated ICU	Mini-BALF	<sup>1</sup> H-NMR	>100	BCA, arginine, glycine, aspartic acid, succinate, glutamate, lactate, ethanol, acetate, and proline
Evans et al. (22)	2014	18 ARDS	8 Healthy	BALF	LC-MS	>500	Guanosine, xanthine, hypoxanthine, lactate, and phosphatidylcholines
Bos et al. (11)	2014	42 ARDS	59 Ventilated ICU	Exhaled breath	GC-MS	>500 (Untargeted for test group); 5 for training and validation groups	3-Methylheptane, octane, and acetaldehyde
Singh et al. (58)	2014	26 ARDS	19 Ventilated non-ARDS	Serum	<sup>1</sup> H-NMR	>100	<i>N</i> -acetyl glycoproteins, acetoacetate, lactate, creatinine, histidine, formate, and BCA
Stringer et al. (61)	2014	14 ARDS	33 Unventilated sepsis	Serum	<sup>1</sup> H-NMR	51	Phosphatidylserine, total lipids, total methylene lipids, and total cholines (in ARDS compared with sepsis)
Rogers et al. (54)	2017	16 ARDS	13 Hydrostatic pulmonary edema	Pulmonary edema fluid	UHLC/MS/MS2 for basic species, acidic species, and lipids	760	In a subset of 6 patients with ARDS (hypermetabolic), 235 were significantly higher
Viswan et al. (67)	2017	36 ARDS (23 moderate/severe ARDS and 13 mild ARDS)	None	Mini-BALF	<sup>1</sup> H-NMR	29	A proposed biomarker composed of 6 metabolites was identified; proline, lysine/arginine, taurine, and threonine were correlated to moderate/severe ARDS whereas glutamate was found characteristic of mild ARDS

Here, *n* = no. of patients. ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; ICU, intensive care unit; LC-MS, liquid chromatography-mass spectrometry; SICU, surgical ICU; UHLC/MS/MS2, ultrahigh-performance liquid chromatography-tandem mass spectrometry.

the metabolites used are provided in Supplemental Table S1. Despite the apparent discrepancies between the studies' results, the plot demonstrates an overall biological relatedness among the identified metabolites. This may be explained by the multicollinear nature of the metabolites along with differences in sample types, sample preparations, and analytical sensitivities that collectively can favor the selection of one or another metabolite among a group of biologically interconnected metabolites. The plot generally reflects a biological profile of deranged energy metabolism (activation of glycolysis and gluconeogenesis), enhanced collagen synthesis and fibrosis (arginine and proline metabolism), negative nitrogen balance (urea cycle), inflammation (glycerophospholipid metabolism), and disturbed cellular turnover (purine and pyrimidine metabolism) that is commonly perceived in patients with ARDS. Interestingly, higher levels of BCA (essential amino acids comprising leucine, isoleucine, and valine) were reported in the sera and mini-BALF of patients with ARDS by Singh et al. (58) and Rai et al. (51), respectively. BCA elevation has been consistently linked to insulin resistance (25, 30, 71). Whether the elevation of BCA levels in ARDS is related to protein catabolism, preexisting type II diabetes mellites, or the possibility of an unidentified ARDS-associated insulin resistance needs further investigation to get a clear insight into this

relation especially when recent research suggests that diabetes mellites may confer a protection against mortality in direct ARDS (43). The plethora of biological details that can be drawn from interpretation of metabolomics biomarkers serves as a showcase for the ability of metabolomics to provide new insights into pathogenesis and its prospects for advancing our understanding of the biology of ARDS (3, 55). However, the complex nature of the metabolite interactions and the analytical variability require validation before metabolomics fingerprints can be used as valid clinical biomarkers.

#### SYNTHESIS AND FUTURE DIRECTIONS

Despite the immense potential, ARDS metabolomics research is still in its infancy. So far, metabolomics studies of ARDS have mostly focused on differentiating ARDS from control subjects and deriving a metabolic signature of the disease. Yet careful examination of the results reveals disagreements. Many factors contribute to these disagreements, such as the variability in study population and control group, the variability in the types and timing of samples, and the differences in the analytical methods used. This emphasizes the critical need for standardizing the recruitment of patients, inclusion of a proper ARDS study cohort representative of the

spectrum of the condition, inclusion of ventilated ICU controls, careful matching between the controls and the study cohort, and carefully standardizing the sampling and preparation techniques. Additionally, it is crucially important to increase the studies' sample size in general, verify findings in independent validation studies, and run prospective longitudinal studies to ascertain enough statistical power, ensure the reproducibility of the results, and track the disease development process, respectively. There is a lack of consensus as to which sample type is most appropriate for ARDS metabolomics studies. We think that readily available noninvasive sample types, e.g., plasma and serum, are suitable choices in critically ill patients, though only good-quality studies and time will tell.

On the other hand, the very nature of identified metabolites (which can be highly correlated) together with the variability in data-processing steps (normalization, transformation, and centering) and statistical methods used may also be contributing to the disagreements in results. Again, this stresses the importance of standardization, comparison, and consensus building in this area (12).

In a notoriously heterogeneous condition such as ARDS, devising a disease subclassification system that is deeply ingrained on the underlying pathobiological molecular mechanisms (so-called "endotyping") will greatly improve our understanding of the disease. Out of the aforementioned ARDS studies, only that of Rogers et al. (54) followed this approach using metabolomics. Further studies along this line can be improved by applying strategies for increasing statistical power and standardization. Endotyping is a major change in the thought process about ARDS that will potentially reformulate the present definition of the condition and allow proper selection of uniform patient subsets for further research, and it may even help in discovering specific therapies.

## CONCLUSIONS

The quest to identify useful ARDS diagnostic and prognostic biomarkers has continued for four decades, yet this endeavor has been constantly hampered by the inherent heterogeneity of the disease, the inclusion of small sample sizes, the suboptimal selection of controls, the lack of validation of findings, and the absence of prospective longitudinal studies. Following major breakthroughs achieved in examining similarly heterogeneous disorders, such as asthma (18, 20), the approach of subclassifying ARDS according to the driving molecular mechanisms rather than empirical clinical features could improve several aspects of care. The nascent field of metabolomics has a promising capability for biomarker discovery as demonstrated in several preliminary small ARDS studies. This type of work should be encouraged and supported in the future. Proper validation of any of the findings is critical in metabolomics-based studies in the future.

## ACKNOWLEDGMENTS

We thank Prof. Hans J. Vogel for critical reading of this manuscript.

## GRANTS

This work was supported by an Emerging Team grant from Faculty of Medicine, University of Calgary, Alberta Health Services, and Alberta's Health Research Innovation Strategy to B. W. Winston and Hans J. Vogel and by a team grant from Alberta Innovates-Health Solutions to the Alberta Sepsis Network.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

S.M.M. and B.W.W. conceived and designed research; S.M.M., A.C., M.M.B., and A.I.M. analyzed data; S.M.M. prepared figures; S.M.M., A.C., S.J.D., and M.M.B. drafted manuscript; S.M.M. and B.W.W. edited and revised manuscript; B.W.W. approved final version of manuscript.

## REFERENCES

- Adamko DJ, Nair P, Mayers I, Tsuyuki RT, Regush S, Rowe BH. Metabolic profiling of asthma and chronic obstructive pulmonary disease: a pilot study differentiating diseases. *J Allergy Clin Immunol* 136: 571–580, 2015. doi:10.1016/j.jaci.2015.05.022.
- Banoei MM, Donnelly SJ, Mickiewicz B, Weljie A, Vogel HJ, Winston BW. Metabolomics in critical care medicine: a new approach to biomarker discovery. *Clin Invest Med* 37: E363–E376, 2014. doi:10.25011/cim.v37i6.22241.
- Barnett N, Ware LB. Biomarkers in acute lung injury: marking forward progress. *Crit Care Clin* 27: 661–683, 2011. doi:10.1016/j.ccc.2011.04.001.
- Beckonert O, Keun HC, Ebbels TM, Bundy J, Holmes E, Lindon JC, Nicholson JK. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. *Nat Protoc* 2: 2692–2703, 2007. doi:10.1038/nprot.2007.376.
- Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, Gattinoni L, van Haren F, Larsson A, McAuley DF, Ranieri M, Rubenfeld G, Thompson BT, Wrigge H, Slutsky AS, Pesenti A; LUNG SAFE Investigators; ESICM Trials Group. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. *JAMA* 315: 788–800, 2016. doi:10.1001/jama.2016.0291.
- Bice T, Cox CE, Carson SS. Cost and health care utilization in ARDS: different from other critical illness? *Semin Respir Crit Care Med* 34: 529–536, 2013. doi:10.1055/s-0033-1351125.
- Biswas SK, Rahman I. Environmental toxicity, redox signaling and lung inflammation: the role of glutathione. *Mol Aspects Med* 30: 60–76, 2009. doi:10.1016/j.mam.2008.07.001.
- Blaise BJ, Correia G, Tin A, Young JH, Vergnaud AC, Lewis M, Pearce JT, Elliott P, Nicholson JK, Holmes E, Ebbels TM. Power analysis and sample size determination in metabolic phenotyping. *Anal Chem* 88: 5179–5188, 2016. doi:10.1021/acs.analchem.6b00188.
- Blank R, Napolitano LM. Epidemiology of ARDS and ALI. *Crit Care Clin* 27: 439–458, 2011. doi:10.1016/j.ccc.2011.05.005.
- Bos LD, Schouten LR, van Vught LA, Wiewel MA, Ong DSY, Cremer O, Artigas A, Martin-Loeches I, Hoogendijk AJ, van der Poll T, Horn J, Juffermans N, Calfee CS, Schultz MJ; MARS Consortium. Identification and validation of distinct biological phenotypes in patients with acute respiratory distress syndrome by cluster analysis. *Thorax* 72: 876–883, 2017. doi:10.1136/thoraxjnl-2016-209719.
- Bos LD, Weda H, Wang Y, Knobel HH, Nijssen TM, Vink TJ, Zwinderman AH, Sterk PJ, Schultz MJ. Exhaled breath metabolomics as a noninvasive diagnostic tool for acute respiratory distress syndrome. *Eur Respir J* 44: 188–197, 2014. doi:10.1183/09031936.00005614.
- Bowler RP, Wendt CH, Fessler MB, Foster MW, Kelly RS, Lasky-Su J, Rogers AJ, Stringer KA, Winston BW; American Thoracic Society Workgroup on Metabolomics and Proteomics. New strategies and challenges in lung proteomics and metabolomics. An official American Thoracic Society Workshop report. *Ann Am Thorac Soc* 14: 1721–1743, 2017. doi:10.1513/AnnalsATS.201710-770WS.
- Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, Davies NA, Cooper CE, Singer M. Association between mitochondrial dysfunction and severity and outcome of septic shock. *Lancet* 360: 219–223, 2002. doi:10.1016/S0140-6736(02)09459-X.
- Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA; NHLBI ARDS Network. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir Med* 2: 611–620, 2014. doi:10.1016/S2213-2600(14)70097-9.
- Calfee CS, Janz DR, Bernard GR, May AK, Kangelaris KN, Matthay MA, Ware LB. Distinct molecular phenotypes of direct vs indirect ARDS in single-center and multicenter studies. *Chest* 147: 1539–1548, 2015. doi:10.1378/chest.14-2454.

16. Cantin AM. Taurine modulation of hypochlorous acid-induced lung epithelial cell injury in vitro. Role of anion transport. *J Clin Invest* 93: 606–614, 1994. doi:10.1172/JCI117013.
17. Capelozzi VL. What have anatomic and pathologic studies taught us about acute lung injury and acute respiratory distress syndrome? *Curr Opin Crit Care* 14: 56–63, 2008. doi:10.1097/MCC.0b013e3282f449de.
18. Chung KF, Adcock IM. How variability in clinical phenotypes should guide research into disease mechanisms in asthma. *Ann Am Thorac Soc* 10, Suppl: S109–S117, 2013. doi:10.1513/AnnalsATS.201304-087AW.
19. Comhair SA, McDunn J, Bennett C, Fettig J, Erzurum SC, Kalhan SC. Metabolomic endotype of asthma. *J Immunol* 195: 643–650, 2015. doi:10.4049/jimmunol.1500736.
20. Corren J. Asthma phenotypes and endotypes: an evolving paradigm for classification. *Discov Med* 15: 243–249, 2013.
21. Esther CR Jr, Boysen G, Olsen BM, Collins LB, Ghio AJ, Swenberg JW, Boucher RC. Mass spectrometric analysis of biomarkers and dilution markers in exhaled breath condensate reveals elevated purines in asthma and cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol* 296: L987–L993, 2009. doi:10.1152/ajplung.90512.2008.
22. Evans CR, Karnovsky K, Kovach MA, Standiford TJ, Burant CF, Stringer KA. Untargeted LC-MS metabolomics of bronchoalveolar lavage fluid differentiates acute respiratory distress syndrome from health. *J Proteome Res* 13: 640–649, 2014. doi:10.1021/pr4007624.
23. Famous KR, Delucchi K, Ware LB, Kangelaris KN, Liu KD, Thompson BT, Calfee CS. ARDS subphenotypes respond differently to randomized fluid management strategy. *Am J Respir Crit Care Med* 195: 331–338, 2017. doi:10.1164/rccm.201603-0645OC.
24. Fernier B, Blasco H, Godat E, Bocca C, Moëne-Loccoz J, Emond P, Andres CR, Laffon M, Ferrandière M. Specific metabolome profile of exhaled breath condensate in patients with shock and respiratory failure: a pilot study. *Metabolites* 6: 26, 2016. doi:10.3390/metabo6030026.
25. Fiehn O, Garvey WT, Newman JW, Lok KH, Hoppel CL, Adams SH. Plasma metabolomic profiles reflective of glucose homeostasis in non-diabetic and type 2 diabetic obese African-American women. *PLoS One* 5: e15234, 2010. doi:10.1371/journal.pone.0015234.
26. Fitzpatrick AM, Park Y, Brown LA, Jones DP. Children with severe asthma have unique oxidative stress-associated metabolomic profiles. *J Allergy Clin Immunol* 133: 258–61.e8, 2014. doi:10.1016/j.jaci.2013.10.012.
27. Fudala R, Krupa A, Matthay MA, Allen TC, Kurdowska AK. Anti-IL-8 autoantibody:IL-8 immune complexes suppress spontaneous apoptosis of neutrophils. *Am J Physiol Lung Cell Mol Physiol* 293: L364–L374, 2007. doi:10.1152/ajplung.00179.2006.
28. Gierok P, Harms M, Methling K, Hochgräfe F, Lalk M. *Staphylococcus aureus* infection reduces nutrition uptake and nucleotide biosynthesis in a human airway epithelial cell line. *Metabolites* 6: 41, 2016. doi:10.3390/metabo6040041.
29. Hoelz C, Negri EM, Lichtenfels AJ, Conceição GM, Barbas CS, Saldiva PH, Capelozzi VL. Morphometric differences in pulmonary lesions in primary and secondary ARDS. A preliminary study in autopsies. *Pathol Res Pract* 197: 521–530, 2001. doi:10.1078/0344-0338-00122.
30. Huffman KM, Shah SH, Stevens RD, Bain JR, Muehlbauer M, Slentz CA, Tanner CJ, Kuchibhatla M, Houmard JA, Newgard CB, Kraus WE. Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. *Diabetes Care* 32: 1678–1683, 2009. doi:10.2337/dc08-2075.
31. Izquierdo-García JL, Naz S, Nin N, Rojas Y, Erazo M, Martínez-Carol L, García A, de Paula M, Fernández-Segoviano P, Casals C, Esteban A, Ruiz-Cabello J, Barbas C, Lorente JA. A metabolomic approach to the pathogenesis of ventilator-induced lung injury. *Anesthesiology* 120: 694–702, 2014. doi:10.1097/ALN.0000000000000074.
32. Janz DR, Ware LB. Plasma biomarkers in acute respiratory distress syndrome: a work in progress\*. *Crit Care Med* 42: 755–756, 2014. doi:10.1097/CCM.0000000000000060.
33. Kiehntopf M, Nin N, Bauer M. Metabolism, metabolome, and metabolomics in intensive care: is it time to move beyond monitoring of glucose and lactate? *Am J Respir Crit Care Med* 187: 906–907, 2013. doi:10.1164/rccm.201303-0414ED.
34. King J, Koc H, Unterkofler K, Mochalski P, Kupferthaler A, Teschl G, Teschl S, Hinterhuber H, Amann A. Physiological modeling of isoprene dynamics in exhaled breath. *J Theor Biol* 267: 626–637, 2010. doi:10.1016/j.jtbi.2010.09.028.
35. King J, Mochalski P, Unterkofler K, Teschl G, Klieber M, Stein M, Amann A, Baumann M. Breath isoprene: muscle dystrophy patients support the concept of a pool of isoprene in the periphery of the human body. *Biochem Biophys Res Commun* 423: 526–530, 2012. doi:10.1016/j.bbrc.2012.05.159.
36. Knottnerus JA, Muris JW. Assessment of the accuracy of diagnostic tests: the cross-sectional study. *J Clin Epidemiol* 56: 1118–1128, 2003. doi:10.1016/S0895-4356(03)00206-3.
37. Koulman A, Lane GA, Harrison SJ, Volmer DA. From differentiating metabolites to biomarkers. *Anal Bioanal Chem* 394: 663–670, 2009. doi:10.1007/s00216-009-2690-3.
38. Lacy P. Metabolomics of sepsis-induced acute lung injury: a new approach for biomarkers. *Am J Physiol Lung Cell Mol Physiol* 300: L1–L3, 2011. doi:10.1152/ajplung.00375.2010.
39. Laiakis EC, Morris GA, Fornace AJ, Howie SR. Metabolomic analysis in severe childhood pneumonia in the Gambia, West Africa: findings from a pilot study. *PLoS One* 5: e12655, 2010. doi:10.1371/journal.pone.0012655.
40. Lei Z, Huhman DV, Sumner LW. Mass spectrometry strategies in metabolomics. *J Biol Chem* 286: 25435–25442, 2011. doi:10.1074/jbc.R111.238691.
41. León Z, García-Cañaveras JC, Donato MT, Lahoz A. Mammalian cell metabolomics: experimental design and sample preparation. *Electrophoresis* 34: 2762–2775, 2013. doi:10.1002/elps.201200605.
42. Lucas R, Verin AD, Black SM, Catravas JD. Regulators of endothelial and epithelial barrier integrity and function in acute lung injury. *Biochem Pharmacol* 77: 1763–1772, 2009. doi:10.1016/j.bcp.2009.01.014.
43. Luo L, Shaver CM, Zhao Z, Koyama T, Calfee CS, Bastarache JA, Ware LB. Clinical predictors of hospital mortality differ between direct and indirect ARDS. *Chest* 151: 755–763, 2017. doi:10.1016/j.chest.2016.09.004.
44. McClay JL, Adkins DE, Isern NG, O'Connell TM, Wooten JB, Zedler BK, Dasika MS, Webb BT, Webb-Robertson BJ, Pounds JG, Murrelle EL, Leppert MF, van den Oord EJ. <sup>1</sup>H nuclear magnetic resonance metabolomics analysis identifies novel urinary biomarkers for lung function. *J Proteome Res* 9: 3083–3090, 2010. doi:10.1021/pr1000048.
45. Misra BB, van der Hooft JJ. Updates in metabolomics tools and resources: 2014–2015. *Electrophoresis* 37: 86–110, 2016. doi:10.1002/elps.201500417.
46. Negri EM, Hoelz C, Barbas CS, Montes GS, Saldiva PH, Capelozzi VL. Acute remodeling of parenchyma in pulmonary and extrapulmonary ARDS. An autopsy study of collagen-elastic system fibers. *Pathol Res Pract* 198: 355–361, 2002. doi:10.1078/0344-0338-00266.
47. Nicholson JK, Lindon JC. Systems biology: metabolomics. *Nature* 455: 1054–1056, 2008. doi:10.1038/4551054a.
48. Owens RL, Stigler WS, Hess DR. Do newer monitors of exhaled gases, mechanics, and esophageal pressure add value? *Clin Chest Med* 29: 297–312, 2008. doi:10.1016/j.ccm.2008.02.001.
49. Patti GJ, Yanes O, Siuzdak G. Metabolomics: the apogee of the omics trilogy. *Nat Rev Mol Cell Biol* 13: 263–269, 2012. doi:10.1038/nrm3314.
50. Peres e Serra A, Parra ER, Eher E, Capelozzi VL. Nonhomogeneous immunostaining of hyaline membranes in different manifestations of diffuse alveolar damage. *Clinics (São Paulo)* 61: 497–502, 2006. doi:10.1590/S1807-59322006000600002.
51. Rai RK, Azim A, Sinha N, Sahoo JN, Singh C, Ahmed A, Saigal S, Baronia AK, Gupta D, Gurjar M, Poddar B, Singh RK. Metabolic profiling in human lung injuries by high-resolution nuclear magnetic resonance spectroscopy of bronchoalveolar lavage fluid (BALF). *Metabolomics* 9: 667–678, 2012. doi:10.1007/s11306-012-0472-y.
52. Riely CA, Cohen G, Lieberman M. Ethane evolution: a new index of lipid peroxidation. *Science* 183: 208–210, 1974. doi:10.1126/science.183.4121.208.
53. Rogers AJ. GWAS in ARDS: finding the needle in the haystack to advance our understanding of ARDS. *Am J Respir Crit Care Med* 197: 1373–1374, 2018. doi:10.1164/rccm.201801-0098ED.
54. Rogers AJ, Contrepois K, Wu M, Zheng M, Peltz G, Ware LB, Matthay MA. Profiling of ARDS pulmonary edema fluid identifies a metabolically distinct subset. *Am J Physiol Lung Cell Mol Physiol* 312: L703–L709, 2017. doi:10.1152/ajplung.00438.2016.
55. Rogers AJ, Matthay MA. Applying metabolomics to uncover novel biology in ARDS. *Am J Physiol Lung Cell Mol Physiol* 306: L957–L961, 2014. doi:10.1152/ajplung.00376.2013.
56. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, Hudson LD. Incidence and outcomes of acute lung injury. *N Engl J Med* 353: 1685–1693, 2005. doi:10.1056/NEJMoa050333.

57. Schubert JK, Müller WP, Benzing A, Geiger K. Application of a new method for analysis of exhaled gas in critically ill patients. *Intensive Care Med* 24: 415–421, 1998. doi:10.1007/s001340050589.
58. Singh C, Rai RK, Azim A, Sinha N, Ahmed A, Singh K, Kayastha AM, Baronia AK, Gurjar M, Poddar B, Singh RK. Metabolic profiling of human lung injury by <sup>1</sup>H high-resolution nuclear magnetic resonance spectroscopy of blood serum. *Metabolomics* 11: 166–174, 2014. doi:10.1007/s11306-014-0688-0.
59. Slupsky CM, Cheyesh A, Chao DV, Fu H, Rankin KN, Marrie TJ, Lacy P. *Streptococcus pneumoniae* and *Staphylococcus aureus* pneumonia induce distinct metabolic responses. *J Proteome Res* 8: 3029–3036, 2009. doi:10.1021/pr900103y.
60. Slupsky CM, Rankin KN, Fu H, Chang D, Rowe BH, Charles PG, McGeer A, Low D, Long R, Kunimoto D, Sawyer MB, Fedorak RN, Adamko DJ, Saude EJ, Shah SL, Marrie TJ. Pneumococcal pneumonia: potential for diagnosis through a urinary metabolic profile. *J Proteome Res* 8: 5550–5558, 2009. doi:10.1021/pr9006427.
61. Stringer KA, Jones AE, Puskarich MA, Karnovsky A, Serkova NJ. <sup>1</sup>H-nuclear magnetic resonance (NMR)-detected lipids associated with apoptosis differentiate early acute respiratory distress syndrome (ARDS) from sepsis (Abstract). *Am J Respir Crit Care Med* 189, Suppl: A5000, 2014.
62. Stringer KA, McKay RT, Karnovsky A, Quémerais B, Lacy P. Metabolomics and its application to acute lung diseases. *Front Immunol* 7: 44, 2016. doi:10.3389/fimmu.2016.00044.
63. Stringer KA, Serkova NJ, Karnovsky A, Guire K, Paine R III, Standiford TJ. Metabolic consequences of sepsis-induced acute lung injury revealed by plasma <sup>1</sup>H-nuclear magnetic resonance quantitative metabolomics and computational analysis. *Am J Physiol Lung Cell Mol Physiol* 300: L4–L11, 2011. doi:10.1152/ajplung.00231.2010.
64. Ubhi BK, Cheng KK, Dong J, Janowitz T, Jodrell D, Tal-Singer R, MacNee W, Lomas DA, Riley JH, Griffin JL, Connor SC. Targeted metabolomics identifies perturbations in amino acid metabolism that sub-classify patients with COPD. *Mol Biosyst* 8: 3125–3133, 2012. doi:10.1039/c2mb25194a.
65. Ubhi BK, Riley JH, Shaw PA, Lomas DA, Tal-Singer R, MacNee W, Griffin JL, Connor SC. Metabolic profiling detects biomarkers of protein degradation in COPD patients. *Eur Respir J* 40: 345–355, 2012. doi:10.1183/09031936.00112411.
66. van de Kant KD, van Berkel JJ, Jöbsis Q, Lima Passos V, Klaassen EM, van der Sande L, van Schayck OC, de Jongste JC, van Schooten FJ, Derks E, Dompeling E, Dallinga JW. Exhaled breath profiling in diagnosing wheezy preschool children. *Eur Respir J* 41: 183–188, 2013. doi:10.1183/09031936.00122411.
67. Viswan A, Singh C, Rai RK, Azim A, Sinha N, Baronia AK. Metabolomics based predictive biomarker model of ARDS: A systemic measure of clinical hypoxemia. *PLoS One* 12: e0187545, 2017. [Erratum in *PLoS One* 13: e0193474, 2018.] doi:10.1371/journal.pone.0187545.
68. Wheelock CE, Goss VM, Balgoma D, Nicholas B, Brandsma J, Skipp PJ, Snowden S, Burg D, D'Amico A, Horvath I, Chaiboonchoe A, Ahmed H, Ballereau S, Rossios C, Chung KF, Montuschi P, Fowler SJ, Adcock IM, Postle AD, Dahlén SE, Rowe A, Sterk PJ, Auffray C, Djukanovic R; U-BIOPRED Study Group. Application of 'omics technologies to biomarker discovery in inflammatory lung diseases. *Eur Respir J* 42: 802–825, 2013. doi:10.1183/09031936.00078812.
69. Witko-Sarsat V, Delacourt C, Rabier D, Bardet J, Nguyen AT, Descamps-Latscha B. Neutrophil-derived long-lived oxidants in cystic fibrosis sputum. *Am J Respir Crit Care Med* 152: 1910–1916, 1995. doi:10.1164/ajrccm.152.6.8520754.
70. Wolak JE, Esther CR Jr, O'Connell TM. Metabolomic analysis of bronchoalveolar lavage fluid from cystic fibrosis patients. *Biomarkers* 14: 55–60, 2009. doi:10.1080/13547500802688194.
71. Yoon MS. The emerging role of branched-chain amino acids in insulin resistance and metabolism. *Nutrients* 8: 405, 2016. doi:10.3390/nu8070405.