Metabolomics of sepsis-induced acute lung injury: a new approach for biomarkers

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Metabolomics is a rapidly expanding new field of -omics that is gaining attention in numerous areas of biomedical research. It is a novel approach that generates a “snapshot” of all small-molecule chemicals and metabolites that are present in selected compartments in the body, and provides a viable alternative to genomics, transcriptomics, and proteomics. Metabolite detection and quantification has substantial potential for biomarker discovery through unique insights into metabolic changes with disease. Metabolomics (also known as metabolomics) is intimately coupled with other -omics sciences providing information downstream from genomics, which detects DNA, or transcriptomics/proteomics, which identify messenger RNA or protein.

The key concept in metabolomics is that changes in the proteome, transcriptome, or genome, are reflected in the metabolome as alterations in metabolite concentrations in biological fluids and tissues. This is not a new notion, as metabolite changes have been used since ancient times to determine the qualitative measures of color, taste, and smell of urine in diagnosis of numerous diseases by physicians. A well-known example of this is the diagnosis of diabetes mellitus based on the sweet taste of urine from patients with Type I diabetes, caused by excessive urinary excretion of the small metabolite glucose. Analytical tools developed more than 100 years ago, and still in use today, have been used to measure small molecule metabolites, and have assisted medical doctors in disease diagnosis. This has led to the formation of clinical chemistry as a major diagnostic tool in medical science. However, routine clinical chemistry tests available in most hospital laboratories rely on techniques that measure only a single small metabolite in patient samples, and many times only qualitatively. These tests are typically not sensitive or specific to any one particular disease, and results usually have to be taken into consideration together with other clinical measurements. A major difference in metabolomics is that there have been significant advances in computational technologies coupled with improvements in the application of small molecule detection tools to allow the measurement of complex metabolic profiles in biological fluids.

A range of metabolite-detecting technologies are available for characterizing small chemicals in patient samples. These include gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR). In particular, NMR is widely used for “classic” metabolic studies, because this approach has an exceptional capacity to rapidly identify and quantify multiple metabolites in biological fluids. Other approaches involving mass spectrometry are limited in their ability to identify more than a few small molecules in complex mixtures and are unable to quantify metabolites accurately. NMR is a non-destructive technique that requires little or no sample preparation and is particularly useful in recognizing as well as quantifying compounds that are less amenable for analysis by GC-MS or LC-MS, such as sugars, amino acids, and other relatively nonreactive compounds. Using NMR, small metabolites generated from a host of biochemical pathways in human tissues can be measured in plasma, serum, tissue, and urine samples and linked back to changes in the proteome, transcriptome, and genome. The main limitation of NMR is that it is relatively insensitive to very small amounts of metabolites, as it requires concentrations of ~1–2 mM in comparatively large samples (~0.5 ml). However, recent advances have allowed the measurement of smaller levels of metabolites in reduced sample sizes using NMR, making sensitivity less of a concern.

Now, by coupling NMR or mass spectrometry to the quantitative measurement of small molecule metabolites in patient samples, it is possible to associate changes in multiple metabolites to the diagnosis or characterization of disease processes in a way that has never been possible before. More chemicals can be rapidly identified and quantified in a single patient sample, allowing researchers to detect subtle changes in metabolites and metabolite families, and to develop biomarkers of disease based on these profiles. Because metabolomics has the capacity to identify and quantify hundreds, and potentially thousands, of small molecules, it greatly enhances our ability to characterize patterns of metabolites correlating with disease. In addition, it is possible to obtain numerous metabolic fingerprints to show the progression of disease from a single patient’s samples over time without the need for time-consuming techniques associated with genomics and proteomics. Thus, quantitative metabolomics provides a powerful new tool for the development of biomarkers for numerous diseases, particularly those with a rapid onset and high mortality rate, such as acute lung injury (ALI). The possibility that metabolomics may be applied to biomarker discovery in sepsis-induced ALI has been explored in a new report by Stringer et al. (10).

Sepsis-induced acute lung injury: biomarker development.
Sepsis is a serious systemic inflammatory state that is associated with the presence of a known or suspected infection, often with a rapid onset. Patients with sepsis are usually treated in the intensive care unit, administered intravenous fluids and antibiotics, and may require mechanical ventilation to support lung function. Unfortunately, progression to ALI occurs in a third of patients with sepsis. ALI is an extraordinarily complex lung-associated disorder characterized by a neutrophilic inflammatory response and associated with increased pulmonary vascular permeability. Its more severe form is acute respiratory distress syndrome (ARDS), which carries the risk of multiorgan failure. The development of ALI is associated with a high rate of morbidity and a variable mortality rate of 30–50% despite vigorous attempts to reduce injury and deterioration in
organ function. The overall pooled mortality rate for ALI/ARDS in several locations around the world is 43% from 72 separate clinical studies (11). In general, sepsis is considered the highest risk factor for progression to ALI or ARDS. Conversely, a large proportion of patients with ALI/ARDS die of sepsis-related multiorgan failure (1, 3, 4).

It is not possible to accurately predict the progression to ALI/ARDS in patients with sepsis. Furthermore, causes of ALI/ARDS are extremely heterogeneous, leading to difficulties in phenotyping patients and arriving at a consensus approach for monitoring and treatment of individual patients. While physiological indices are fairly limited in their predictive value for mortality, there are currently several promising cell-specific biomarkers that correlate with increased mortality from ALI. These include specific cytokines and their receptors (IL-6, IL-8, soluble tumor necrosis factor receptor I and II) and products of epithelial and endothelial injury [receptor for advanced glycation end-products (RAGE), surfactant protein D, ICAM-1, and von Willebrand factor antigen] as well as factors associated with coagulation (protein C and plasminogen activator inhibitor-1) (6).

However, while these biomarkers have helped to improve our understanding of the pathogenesis of ALI and have shown a correlation with mortality in large multicenter clinical trials of ALI, there are still no useful biomarkers that can predict progression to ALI in sepsis. Thus, there is a dire need for identification and characterization of new biomarkers that can predict progression to ALI/ARDS in sepsis, as well as for mortality in sepsis-induced ALI. Advances in genomics and proteomics have extended our understanding of early cellular processes in ALI and have also identified potential biomarkers for the development of ALI. Recently, there has been interest in applying metabolomics to the identification of novel biomarkers in ALI.

In the report by Stringer et al. (10), quantitative metabolomics is proposed as a useful approach for the discovery of clinically relevant biomarkers in sepsis-induced ALI. In this report, the authors obtained plasma samples from mechanically ventilated patients with sepsis-induced ALI or ARDS, as confirmed by the ERS/ATS consensus definition. Following a dual methanol-chloroform extraction of aqueous and organic phase components, samples were dissolved in either deuterated water (for detection of aqueous components) or a deuterated methanol:chloroform mixture (for detection of organic components) and transferred into NMR tubes (see Fig. 1). Data were collected from samples using an NMR spectrometer, and signals were collected on a computer for postprocessing spectral analysis and metabolite signal integration. From this, it was possible to generate a table of metabolites present in plasma samples from patients with sepsis-induced ALI and compare these values statistically with those of healthy normal controls. Using these approaches, the authors generated preliminary data suggesting a positive correlation with individual metabolites (myo-inositol and total glutathione as two examples) and the Acute Physiological Score (APS) for ALI. The pathways for these metabolites were described by bioinformatics analysis using the internet-based bioinformatics platform Cytoscape with

![Diagram of metabolomics process](http://ajplung.physiology.org/)

**Fig. 1.** Application of quantitative metabolomics to plasma samples from patients with sepsis-induced acute lung injury. Scheme showing the process of identification and quantification of multiple small molecule metabolites in a patient plasma sample in the study by Stringer et al. (10). Plasma samples were subjected to a chloroform:methanol extraction protocol to generate a clarified solution of aqueous or lipid molecules that were subsequently transferred to an NMR tube. The tube was analyzed in a 500 MHz NMR magnet, and, following signal collection, a spectrum was generated allowing identification of small metabolites. These are subjected to further analysis for quantification of each metabolite and tabulated as appropriate.
the Metscape plugin. These findings provide the first such insight into the metabolic profiles of plasma samples from patients with sepsis-induced ALI.

While these findings are promising, they are preliminary since the number of patients enrolled in the study is low (13 sepsis-induced ALI patients compared with 6 healthy controls). Clearly, more studies on larger sample sizes are needed to determine the significance of these findings in a broader patient population. An important advance would be to analyze metabolite concentrations for numerous patients, then compare the sample clustering of quantified metabolites through multivariate statistical approaches such as principal components analysis. This approach has been successful in determining the metabolite concentrations in urine samples for detection of pneumonia (8, 9). Further advances are anticipated in the visualization of complex metabolomics data using various bioinformatics platforms that were used in the current study (5).

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REFERENCES