Chapter

Serum Metabolomics as a Powerful Tool in Distinguishing Trauma from Other Critical Illness Conditions

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Abstract

Critical illness is highly variable, complicating patient care and recovery. We have previously used metabolomics to investigate several causes of intensive care unit admission, seeking to assess changes in metabolism occurring with each condition. We present a meta-analysis of these serum metabolomes, exploring how the metabolomes differ with each condition. We also present how mass spectrometry-based metabolomics could be used for predictive monitoring. Serum metabolites were previously quantified using nuclear magnetic resonance spectroscopy in patients with traumatic injury, respiratory failure, pancreatitis, and combat trauma. Healthy controls are also included. Spectral features were analyzed with principal component analysis (PCA) to explore patterns in patients' underlying conditions. PCA suggests trauma metabolic profiles, particularly combat casualties, differ from other conditions. Principal components 2 and 3, accounting for 16% of the variation in the model, distinguish samples obtained from trauma patients. Metabolomics is a powerful tool for quantifying variability in critical illness, highlighting trauma as separate from other conditions. This observation is in line with the -omics literature, which has described a massive global "genomic storm" in response to severe injury. Mass spectrometry highlights this extreme variability, which occurs in ICU patients but not healthy controls. With new technology, metabolomics could be used to bring faster, individualized patient care to the ICU.

Keywords: metabolomics, NMR, ICU, critical illness, biomarker, traumatic injury, combat casualty, mass spectrometry

1. Introduction

Critical illness encompasses a wide variety of life-threatening conditions, often requiring intensive monitoring and sophisticated life support, such as dialysis, mechanical ventilation, and nutritional support. Patients are cared for in intensive care units (ICUs), staffed by specialists. Because patients' conditions can change quickly over time, ICU staff are highly trained and nurses regularly care for only one or two patients at a time. Because of these factors, critical illness carries a high cost burden. It has been estimated that anywhere from 17 to 39% of hospital costs in

the United States are due to critical illness. Total costs, including 1 year of care after discharge are estimated at $121–263 billion, or 5–11% of United States health care expenditures [1]. The cost burden is difficult to estimate, due in part to the complicated recovery process.

Recently, post-intensive care syndrome (PICS) has been identified as a constellation of cognitive, psychological, and physical impairments that result from critical illness [2], occurring with increased prevalence due to the increased survivability of critical illness [3]. ICU-acquired delirium and mechanical ventilation are among the risk factors for PICS, and the effects can be long-lasting. An estimated 90% of patients report ICU-acquired weakness lasting 2–5 years from ICU discharge, and 74% of patients with acute respiratory distress syndrome report cognitive impairments at discharge. Approximately a quarter of these patients report effects lasting as long as 6 years [4].

While survivability from critical illness has increased, it has been difficult to make further advances in patient care and outcomes due to the heterogeneity of the patient population. Respiratory disorders requiring mechanical ventilation, acute myocardial infarction, intracranial hemorrhage, percutaneous cardiovascular procedure with drug-eluting stent, and septicemia are the leading causes of ICU admission, but gastrointestinal disorders, renal disorders, and trauma are also frequent causes of ICU admission [5]. To further complicate matters, as many as 1/3 of ICU patients have multiple co-morbidities. Homogenous patient populations can be difficult to identify, let alone study, in the ICU. As such, a "one-size-fits-all" approach to patient care can lead to unpredictable results. To cope with these hallmarks of critical illness, modern ICU clinicians argue for precision medicine approaches to critical care as a way to improve patient care [6–8].

Metabolomics, which reflects the phenome more closely than other -omics disciplines, may be a key to this endeavor. This terrain has been largely unexplored, save for a few studies. Targeted metabolomics has been used to discriminate non-infectious systemic inflammatory response syndrome (SIRS) from infections SIRS [9]. Untargeted approaches have identified significant, severe metabolic derangements that are associated with mortality [10, 11].

This chapter presents efforts to use metabolomics to explore this difficult-to-study space. Namely, critical illness is highly variable and affects diffuse organ systems in a heterogeneous patient population that may have multiple co-morbidities. Since the metabolome is closest to the phenome, it is more likely to reflect the individual patient's state at any given time than other -omes. As others have pointed out, issues of heterogeneity and variability make biomarker studies problematic [6, 10]. A first step is to examine how metabolic profiles differ with different underlying diseases and with illness severity to get a better sense of this variability. This chapter touches on current efforts in this direction.

2. Metabolomics methodology and previous work

The NMR-based metabolomics studies we performed were pilot studies seeking to characterize metabolic profiles in combat injury [12], civilian traumatic injury [13], acute pancreatitis [14], and respiratory failure [15]. Healthy controls were also profiled [12, 13].

The use of the same protocol to process serum samples, collect NMR spectra, and quantify metabolites allows for a meta-analysis comparing the metabolic profiles from each study.

Briefly, samples were filtered using a 3 kDa ultracentrifuge filter to remove large molecules such as proteins that bind to the internal standard. Filtrate is mixed in equal parts with 200 mM sodium phosphate buffer and with 50 microliters of the internal standard 3-(trimethylsilyl)propionic acid. A 1D Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used to collect spectra, and metabolites were identified and quantified using Chenomx software [16]. Full experimental details can be found in the original research articles [12–15].

Metabolic profiles were limited to the 41 metabolites identified and quantified in all four studies. Metabolite concentrations (millimoles per liter or mM) were log-transformed and auto-scaled before principal component analysis (PCA) was performed with R software [17]. PCA scores were colored by underlying diagnosis/patient group (combat trauma, civilian trauma age 21–40, civilian trauma age 65 and older, acute pancreatitis, respiratory failure, healthy controls age 21–40, and healthy controls age 65 and older).

For the purposes of visualizing the diagnosis groups in this meta-analysis, some simplifications were made based on the previously published results. Because no clear difference was seen between patients in respiratory failure regardless of underlying cause, patients with chronic obstructive pulmonary disease (COPD) exacerbation, heart failure, and pneumonia were combined into the "respiratory failure" group [15]. Non-hospitalized patients who did not develop pancreatitis [14] and non-hospitalized patients with stable COPD [15] were excluded from this analysis to facilitate visualization.

3. Meta-analysis results

In total, 291 serum samples were analyzed with principal component analysis. Most of these were from trauma patients. The number of samples studied in each diagnosis group is presented in **Table 1**.

Principal component analysis scores (**Figures 1** and **2**) and loadings (**Figures 3** and **4**) are shown for the first three components. Each dot in the scores plot represents a serum sample, which is colored according to the diagnosis or condition. The loadings plots show how the metabolites profiled contribute to the model. The first three components account for 51% of the variability in the data. Component 1 accounts for 35% of the variation; components 2 and 3 account for 9.8 and 6.6% of the variation, respectively. A three-dimensional biplot (**Figure 5**) helps visualize all the information.

Interestingly, the most meaningful pattern in the PCA scores is observed in **Figure 2**, the plot of component 2 vs. component 3. A clear line can be drawn along PC2 and PC3, demarcating the samples from trauma patients (red,

Condition	Number of samples
Combat Trauma	111
Civilian Trauma (age 21-40)	36
Civilian Trauma (age 65+)	42
Respiratory Failure	23
Acute Pancreatitis (hospitalized)	30
Healthy Control (age 21-40)	23
Healthy Control (age 65+)	26

Table 1.
Number of samples profiled with NMR-based metabolomics per condition studied.

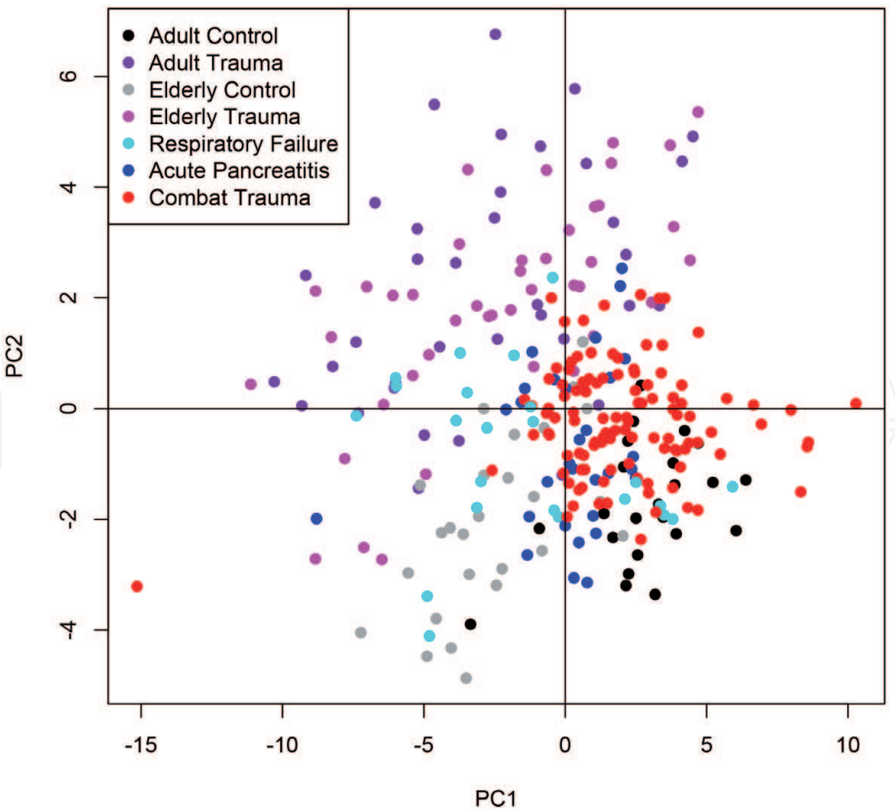

Figure 1.
Scores plot of PC1 vs. PC2 for serum samples described in ***Table 1.*** *Samples are colored by diagnosis.*

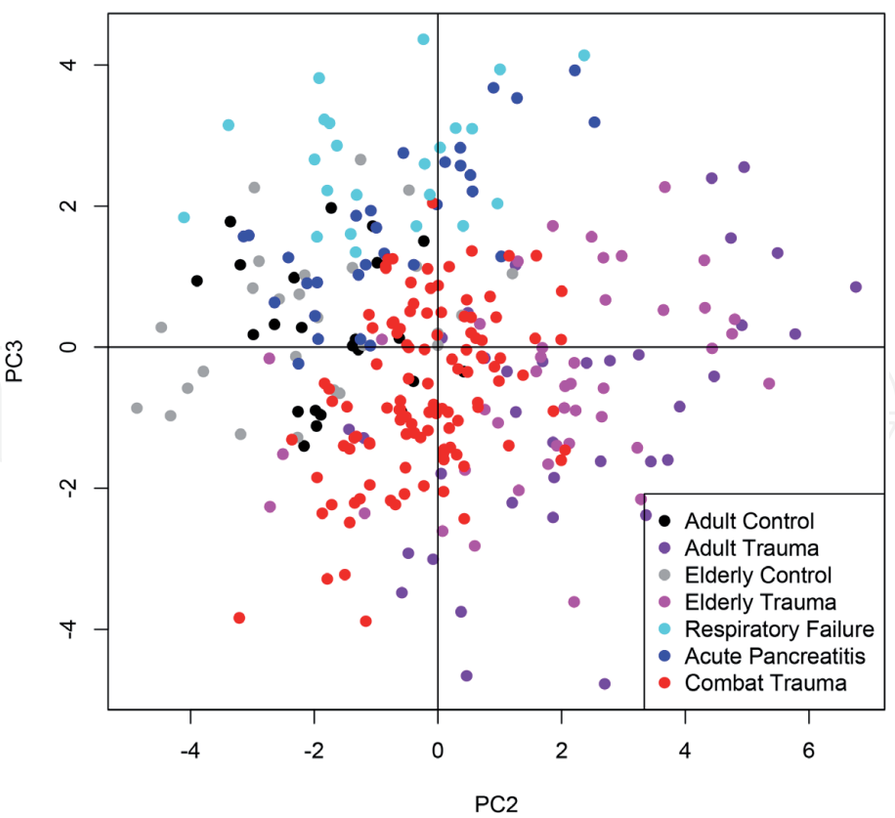

Figure 2.
Scores plot of PC2 vs. PC3 for serum samples described in ***Table 1.*** *Samples are colored by diagnosis. These two principal components most clearly distinguish trauma samples from non-trauma samples.*

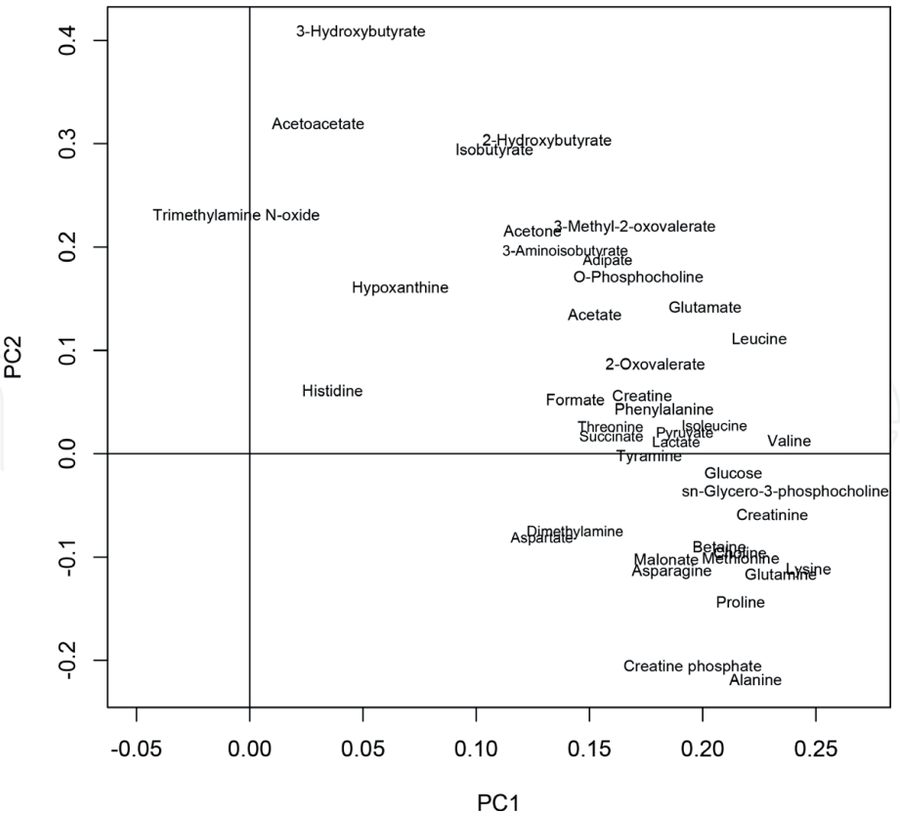

Figure 3.
Loadings plot of PC1 vs. PC2 for serum samples described in ***Table 1.*** *Loadings values are shown in* ***Table 2.***

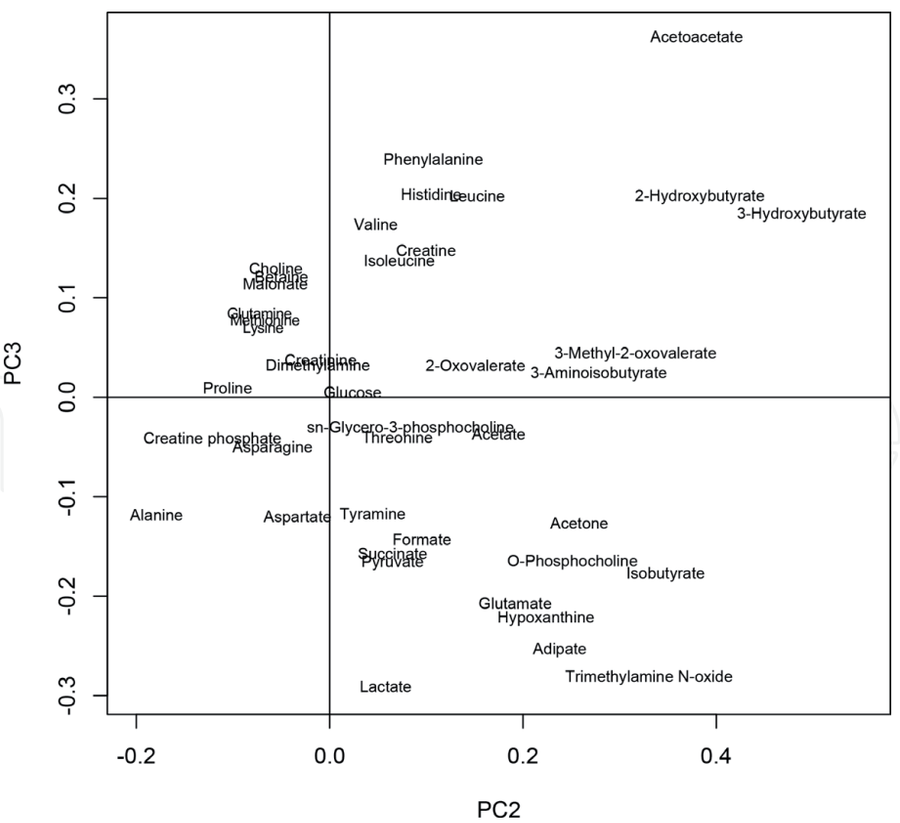

Figure 4.
Loadings plot of PC2 vs. PC3 for serum samples described in ***Table 1.*** *Loadings values are shown in* ***Table 2,*** *and the magnitude of the loadings vector spanned by PC2 and PC3 is calculated. Metabolites most associated with trauma occupy the lower right quadrant.*

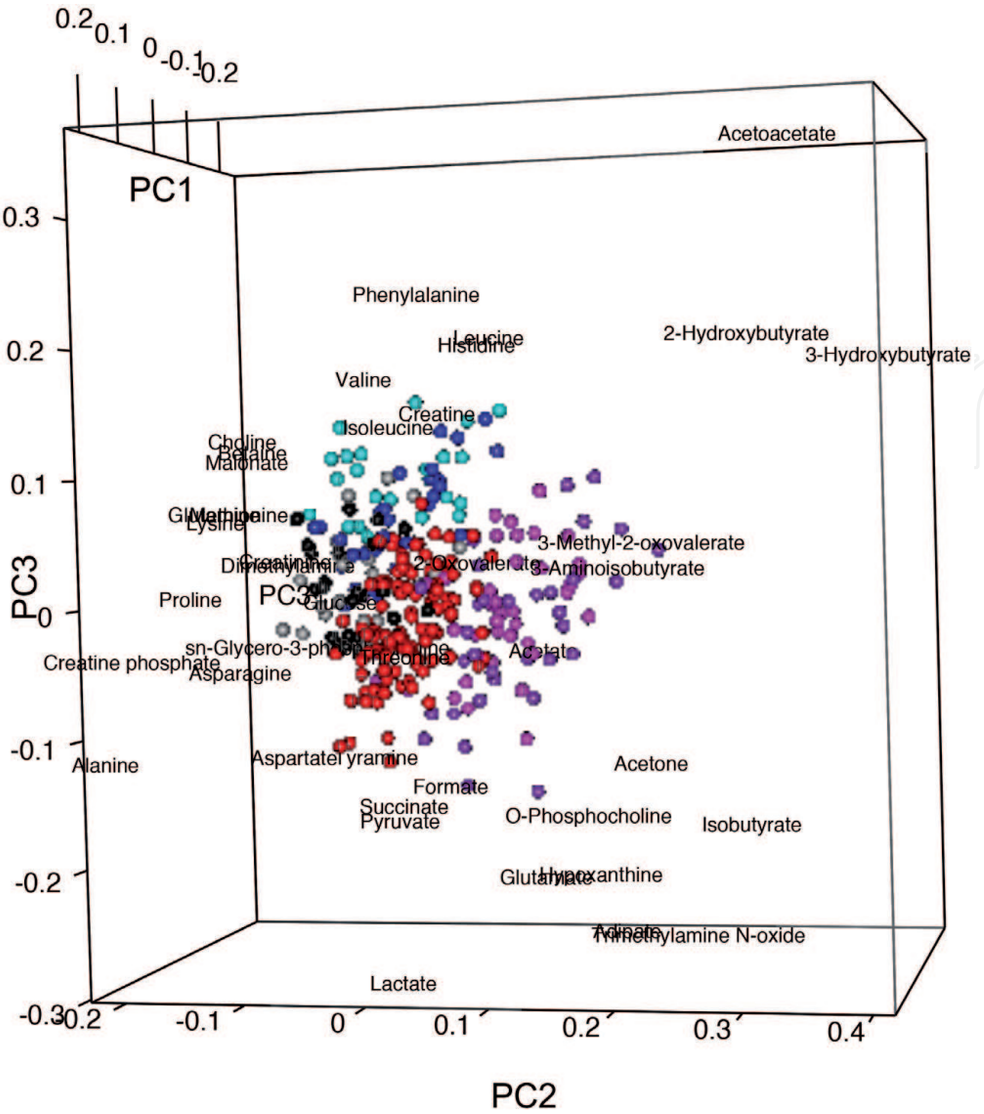

Figure 5.
Biplot of the first three principal components for serum samples described in ***Table 1.*** *Samples are colored by diagnosis.*

purple, or magenta) from the healthy controls (gray or black) and the patients with other conditions (blue or light blue).

The loadings vectors for the first three principal components are reported in **Table 2**. Since PC2 and PC3 can be used to discriminate trauma samples from non-trauma samples, we used the loadings of these components to identify the metabolites most associated with trauma. To do this, we calculated the magnitude of the vector spanned by PC2 and PC3, shown in column 4 of **Table 2**. The 10 metabolites with the largest magnitude in the PC loadings are acetoacetate, 3-hydroxybutyrate, trimethylamine N-oxide, 2-hydroxybutyrate, isobutyrate, adipate, lactate, hypoxanthine, glutamate, and alanine. These metabolites reflect disruptions to energy metabolism and oxidative stress.

4. Meta-analysis discussion

Our metabolomics studies can be united under a common theme: all were done in conditions that are common causes for admission to the ICU. Because sample preparation protocol is the same for all our serum-based NMR metabolomics

Metabolite	PC1 Loading	PC2 Loading	PC3 Loading	Magnitude of PC2 and PC3
Acetoacetate	0.00380019	0.31775843	0.36065228	0.48066671
3-Hydroxybutyrate	0.01446508	0.40722582	0.18306298	0.4464806
TMAO	-0.0488827	0.22963983	-0.2823471	0.36394273
2-Hydroxybutyrate	0.09672712	0.30185385	0.20091817	0.36260702
Isobutyrate	0.08468395	0.29280528	-0.1782326	0.34278536
Adipate	0.13815294	0.19626967	-0.2545485	0.32142918
Lactate	0.17015011	0.01694167	-0.2926651	0.29315501
Hypoxanthine	0.03908591	0.15958037	-0.2230033	0.2742196
Glutamate	0.17889476	0.13997017	-0.2089776	0.25152192
Alanine	0.20568069	-0.2205282	-0.1199601	0.25104403
Acetone	0.10609781	0.2141336	-0.1285256	0.24974391
Phenylalanine	0.15497467	0.0416933	0.23759456	0.24122501
O-phosphocholine	0.13681365	0.16962719	-0.1660462	0.23737041
Leucine	0.2067085	0.10994236	0.20055647	0.22871427
3-Methyl-2-Oxovalerate	0.12816517	0.21837494	0.04288388	0.22254582
Creatine Phosphate	0.15903242	-0.2070038	-0.0426857	0.21135904
Histidine	0.01717568	0.0594115	0.20228903	0.21083305
3-Aminoisobutyrate	0.10503272	0.19351667	0.02292365	0.19486969
Valine	0.22254027	0.01077726	0.17203364	0.17237089
Pyruvate	0.1708877	0.01863289	-0.166928	0.16796474
Choline	0.19856831	-0.0974704	0.12782132	0.1607444
Succinate	0.140404	0.01503279	-0.1588119	0.15952176
Creatine	0.15406358	0.05461516	0.14575958	0.15565562
Formate	0.12481118	0.05078229	-0.1446407	0.15329638
Malonate	0.16357974	-0.1040489	0.11232368	0.15311039
Betaine	0.18953171	-0.0917461	0.11954568	0.15069343
Aspartate	0.12217261	-0.0824399	-0.1216538	0.1469557
Proline	0.19982237	-0.1452455	0.00767188	0.14544797
Glutamine	0.21257371	-0.1182284	0.07247271	0.13867315
Acetate	0.13445786	0.13279824	-0.038851	0.13836464
Isoleucine	0.18346117	0.02099238	0.13570294	0.13731703
Lysine	0.23059301	-0.1130716	0.06653587	0.1311953
Asparagine	0.16265794	-0.1146923	-0.0516647	0.12579175
Methionine	0.19368617	-0.1028348	0.07213219	0.12561072
Tyramine	0.1557889	-0.0037425	-0.1187582	0.11881712
2-Oxovalerate	0.15110179	0.08529709	0.03026353	0.09050676
Dimethylamine	0.12591453	-0.0805601	0.03061979	0.08618293
Creatinine	0.20879239	-0.0609296	0.03552048	0.07052746
Sn-Glycero-3-phosphocholine	0.18485381	-0.0376795	-0.0318023	0.0493065
Threonine	0.14074561	0.01897372	-0.0422133	0.04628141
Glucose	0.19468583	-0.0203949	0.00310279	0.02062958

PC2 and PC3 show a clear separation between samples from trauma patients and samples from other research participants. The table is sorted according to the magnitude of the loadings vectors in PC2 and PC3 (column 4). Magnitude of PC2 and PC3 was calculated as follows: $[(PC2\ Loading)^2 + (PC3\ Loading)^2]^{(1/2)}$
PC2, principal component 2; PC3, principal component 3; TMAO, trimethylamine N-oxide.

Table 2.
Principal component loadings for the first three components for all profiled metabolites.

studies, we performed a meta-analysis of the metabolic profiles obtained from these previously published studies [12–15]. Our results suggest that samples from trauma patients are distinguishable from healthy controls and patients with respiratory failure or acute pancreatitis. Principal components 2 and 3 can be used to separate trauma patients' samples from other samples, and highlight oxidative stress and disruptions to energy metabolism.

Traumatic injury is known to have a profound effect on molecular processes, impacting more than 80% of cellular functions and pathways, earning the moniker "genomic storm" [18]. In light of this, it is unsurprising that our unsupervised

analysis would separate trauma samples from non-trauma samples. In our own work evaluating metabolomes of trauma patients age 21–40 years and trauma patients older than 65 years, we found a clear difference between metabolic profiles of younger healthy controls and older healthy controls. However, the data forced us to reject our hypothesis that metabolomes of older trauma patients would be distinguishable from younger trauma patients [13]. One interpretation of these data is that trauma deals a massive insult to metabolism that completely overtakes any baseline differences in metabolism caused by age.

Trauma from unintentional injury is the most common cause of death for persons age 44 and under [19]. Treatment of traumatic injury remains limited to supportive care such as stopping any bleeding and giving fluids to resuscitate. Lacking specific therapies for traumatic injury, early treatment is a key to improving survival. Metabolomics has already been successfully used to identify succinate, an objective biomarker of mortality, to improve triage [20–22]. However, new technology needs to be developed to bring succinate detection and quantification to the clinic.

5. Improving patient monitoring with metabolomics

It may be surprising that NMR, with its relatively low resolution, can discriminate metabolic profiles of trauma patients from others. However, this technique does not reflect the extremely variable, highly individualized nature of critical illness. Improving the sensitivity of metabolite detection with mass spectrometry is required to highlight these features of critical illness.

In a preliminary study (manuscript in preparation), we used mass spectrometry to generate metabolic profiles of five ICU patients and five healthy controls. Samples were collected every 4 h for a period of 24 h. A standard methanol/acetone protocol was used to extract metabolites. A Q Exactive™ Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) was employed for mass analysis. Analysis was performed in positive mode over a mass range of 70–1050 m/z. Spectra were aligned and processed with Progenesis QI software (Nonlinear Dynamics, Durham, NC).

Spectral intensities of 15,000 features identified by Progenesis QI were log-transformed and principal components analysis was performed using R software. The resulting scores are shown in **Figure 6**. Scores were colored by participant. A single, relatively tight cluster of healthy controls is clearly visible in the upper left quadrant of **Figure 6** (HC01-HC05, colored blue, green, and pink). Strikingly, each ICU patient (ICU01-IC05, red, orange, and purple) is clearly visible, and each patient forms its own unique cluster. Interestingly, the ICU patient colored in red was demonstrably less sick than the other patients, with a lower APACHE II (acute physiology and chronic health evaluation) score and a shorter ICU length of stay. It is likely that the sampling frequency combined with the sensitivity of mass spectrometry allowed us to see such highly individualized patterns in the metabolic profiles.

Based on these data, we posit that mass spectrometry-based metabolomics offers a unique way to characterize the highly individual, highly variable nature of critical illness. The PCA scores in **Figure 6** further offer the tantalizing suggestion that metabolic profiles reflect severity of illness, since the scores of patient with the lower APACHE II score and shorter ICU stay were closest to the scores of the healthy controls. We further hypothesize that, tracked over time, principal component analysis of individual patients' metabolic profiles could offer insight

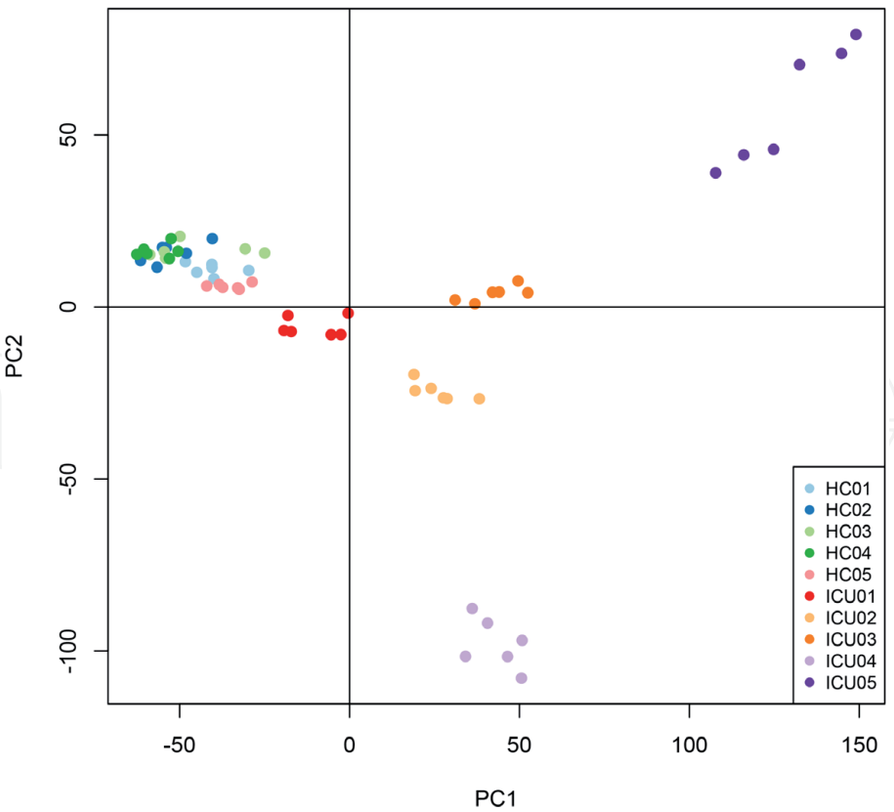

Figure 6.
Scores plot of the first two principal components, constructed from metabolic profiles of five ICU patients and five healthy controls. Samples are colored by individual study subject. Subjects HC01 through HC05 (top left quadrant) are healthy controls. Subjects ICU01-ICU05 are ICU patients. Samples were collected in each participant every 4 h for 24 h. PCA clearly illustrates the extreme variability and individuality of metabolic profiles in critical illness.

into their clinical courses, moving farther away from a "healthy" profile as conditions worsen and closer to a "healthy" profile as conditions improve. Others have used principal component analysis of circulating inflammatory cytokines in a similar way, to identify individual patients who go on to develop multiple organ dysfunction syndrome [23].

This line of inquiry is not without challenges. Collecting samples frequently around the clock from human study participants is a challenge, and a substantial sample bank will have to be obtained to establish a "healthy" metabolic profile. Mass spectrometry results in a large, extremely rich data set of features which are difficult to map to individual metabolites, so it is difficult to identify the set of metabolites that drive the patterns observed here.

Finally, patients will have to be monitored over time and their metabolic profiles will have to be mapped to their outcomes in order to link their "trajectories" to outcomes or adverse events. This may be a daunting task. However, others have successfully established continuous predictive analytics monitoring from physiologic data in neonatal ICUs [24]. Continuous predictive analytics monitoring allows ICU staff to follow patient trajectories that serve as an early-warning system for sepsis [25], allowing for earlier treatment before inflammation and infection worsen. Since, as with trauma, early intervention is a key to survival from sepsis, bringing predictive monitoring to the ICU is a clear way to improve patient outcomes.

New technology needs to be developed to bring metabolomics to the bedside if it is to be used to track patient trajectories in a clinically useful manner. In the meantime, much can be learned about critical illness from metabolomics.

6. Conclusions

Critical illness encompasses a variety of life-threatening conditions characterized by the need for frequent, intensive interventions. Patients are heterogeneous and may not respond to treatments in a predictable way; further, their conditions can change quickly over time. Metabolomics, reflective of the phenome, has great potential to impact patient care. NMR-based metabolomics highlights trauma as having a unique impact on the metabolome relative to healthy controls and other conditions. Mass spectrometry, with its increased sensitivity over NMR, highlights an extremely individualized variation in the metabolomes of ICU patients that does not exist in healthy controls. With technological innovations to bring metabolomics to the bedside, it may be used in the future to bring predictive analytics to the ICU, leading to faster and more appropriately individualized interventions, and improving patient care and outcomes.

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Conflict of interest

Dr. Lusczek is on the board of directors of the Society for Complex Acute Illness.

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