Metabolomics as a Novel Approach for Early Diagnosis of Pediatric Septic Shock and Its Mortality

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Rationale: Septic shock is a significant cause of morbidity and mortality in the pediatric population. Early recognition of septic shock and appropriate treatment increase survival rate; thus, developing new diagnostic tools may improve patients' outcomes.

Objectives: To determine whether a metabolomics approach could be useful in the diagnosis and prognosis of septic shock in pediatric intensive care unit (PICUs).

Methods: Serum samples were collected from 60 patients with septic shock, 40 PICU patients with systemic inflammatory response syndrome (not suspected of having an infection), and 40 healthy children. Proton nuclear magnetic resonance spectroscopy spectra were analyzed and quantified using targeted profiling methodology.

Measurements and Main Results: Multivariate statistical analysis was applied to detect specific patterns in metabolic profiles and to highlight differences between patient samples. Supervised analysis afforded good predictive models and managed to separate patient populations. Some of the metabolite concentrations identified in serum samples changed markedly, indicating their influence on the separation between patient groups. These metabolites represent a composite biopattern of the pediatric metabolic response to septic shock and might be considered as the basis for a biomarker panel for the diagnosis of septic shock and its mortality in PICU.

Conclusions: Our results indicate that nuclear magnetic resonance metabolite profiling might serve as a promising approach for the diagnosis and prediction of mortality in septic shock in a pediatric population and that quantitative metabolomics methods can be applied in the clinical evaluations of pediatric septic shock.

Keywords: pediatric septic shock; biomarkers; metabolomics; proton nuclear magnetic resonance spectroscopy; mortality

In the 1980s the death rate from septic shock in children was around 50% (1, 2), but over the past few decades with better early diagnosis and therapy it has decreased to approximately 10% (3, 4). Unfortunately, in third world countries the mortality rate is still extremely high (5, 6). Moreover, every hour of septic

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Septic shock is a significant health problem and a leading cause of mortality among children in pediatric intensive care units (PICUs). Rapid treatment in the first hours after the diagnosis is important for a positive patient outcome. However, diagnosis of septic shock in the early stage is difficult because of complexity and heterogeneity of the disease. Prognosis of patients with pediatric septic shock is equally challenging and is not well done by current tools. Therefore, developing new diagnostic and prognostic tools that might accelerate diagnosis and improve prognosis is important.

What This Study Adds to the Field

This study suggests that nuclear magnetic resonance spectroscopy-based metabolomics can be considered as a potentially useful approach to aid in the diagnosis and early prognosis of septic shock in the PICU.

shock without appropriate resuscitation and restoration of blood pressure increases mortality risk by 40% (7). Septic shock is a very dynamic process, and the clinical status of a child can deteriorate quickly (8). The first hours after the diagnosis are called the “golden hours” for a patient's survival; therefore, aggressive and goal-directed treatment should be initiated as quickly as possible (9). It is reported that those children in whom septic shock is recognized early and properly treated have a much higher survival rate than children who were diagnosed later (10–12). Thus, developing diagnostic approaches that might accelerate disease recognition is extremely important to improve patient outcomes and decrease mortality.

In this study we investigated whether we could use a metabolomics approach for the diagnosis and prognosis of pediatric septic shock. Metabolomics is defined as “the quantitative measurement of the metabolic response of living systems to pathophysiological stimuli or genetic modification” (13, 14) and is based on analytical platforms such as proton nuclear magnetic resonance spectroscopy (¹H NMR) and/or mass spectrometry (13, 14). It has been described that metabolomics is a very efficient tool for discovering biomarkers of various infectious diseases, such as severe childhood pneumonia (15) or hepatitis C virus infection (16). So far, most of metabolomics studies associated with sepsis have been reported for animal models (17–19), and there have been no published metabolomics studies of pediatric septic shock. In the present study we used ¹H NMR and computational analysis to detect and measure concentrations of different metabolites in pediatric serum samples (see Figure E1 in the online supplement). A single ¹H NMR spectrum might be described as a complex distribution of unique spectral intensities

from individual metabolites, which can be identified and quantified during a process called targeted profiling (20). The metabolites and their concentrations provided a dataset on which multivariate statistical analysis was performed. Techniques such as principal component analysis (PCA) (21, 22), partial least squares discriminant analysis (PLS-DA) (23), and orthogonal partial least squares discriminant analysis (OPLS-DA) (22) were applied to separate metabolic variation in the studied subjects. The results highlighted and separated metabolic changes in septic shock and those associated with increased mortality from metabolites identified in control pediatric serum samples. The present study highlights a promising application for early diagnosis and prognosis of septic shock in the pediatric intensive care unit (PICU) and sets the stage for further evaluation of metabolomics in a clinical setting. Some of the results of this study have previously been reported in the form of an abstract during the American Thoracic Society International Conference in 2012 (24).

METHODS

Data Collection

For information about data collection and demographic and clinical characteristics of the enrolled subjects, *see* Table 1 and the online supplement.

NMR Spectroscopy and Metabolite Concentration Profiling

For the sample preparation protocol, *see* the online supplement. NMR spectra were obtained on a Bruker AVANCE 600 MHz spectrometer (Bruker BioSpin Ltd., Milton, ON, Canada) using a standard Bruker 1D spectroscopy presaturation pulse sequence (noesypr1d) with a mixing time

of 100 ms (20, 25). The concentration of 4,4-dimethyl-4-silapentane-1-sulfonic acid was used as a reference to determine metabolite concentrations during targeted profiling (20) (Chenomx NMR Suite 7.1; Chenomx Inc., Edmonton, AB, Canada). Each concentration was normalized to the total sum of the concentrations, excluding the two highest concentrated metabolites, lactate and glucose, which otherwise would dominate the normalization (20, 26).

Statistical Modeling

Normalized concentrations were used for multivariate analysis (SIMCA-P+ 12.0.1; Umetrics, Umeå, Sweden). The PCA model was designed to identify and exclude outliers before PLS-DA models were constructed with class identification (healthy control, systemic inflammatory response syndrome [SIRS]/ICU control, septic shock). To evaluate the PLS-DA model, R2Y and Q2 metrics were calculated using a sevenfold cross-validation method (27). The R2Y metric describes the percentage of variation explained by the model; Q2 shows the predictive ability of the model. The difference between these metrics describes the model's goodness of fit. Next, the OPLS-DA method was applied to models including only two classes: septic shock versus healthy, SIRS/ICU control subjects versus healthy, and septic shock versus SIRS/ICU control subjects within all subjects and specific age groups (infants, toddlers, school age). Additionally, two OPLS-DA models were constructed to reveal mortality factors using (1) 20 septic shock samples (10 nonsurvivors and 10 age- and sex-matched control subjects), and (2) 23 patients with complicated course (10 nonsurvivors and 13 survivors). The OPLS-DA method for age groups and mortality models was based on potentially relevant metabolites selected in two-sample *t* tests with *P* less than 0.2 as a threshold. For each OPLS-DA model, the area under a receiver operator curve (AUROC) was calculated (Metz ROC Software, Chicago, IL) (28). The sensitivity, specificity, and accuracy were determined on the basis of sample class prediction during sevenfold cross-validation (Y-predcv) in SIMCA-P+ software. The results of the ROC analysis were then

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE SUBJECTS ENROLLED IN THE STUDY

	Neonates	Infants	Toddlers	School Age
Age range	1 wk to 1 mo	1 mo to 1 yr	2–5 yr	6–11 yr
Age, yr*	0.1	1 (0.5–1.4)	3.4 (2.4–4.4)	8.3 (7.5–9.4)
No. of subjects	7	47	54	32
Boys/girls, n	4/3	28/19	30/24	16/16
Race, n				
White	1	28	34	17
Black/African American	4	5	2	5
Asian	N/A	2	3	1
Native Hawaiian/Pacific Islander	N/A	N/A	1	N/A
Unknown/unavailable	2	12	14	9
Number of healthy control subjects	0	13	18	9
SIRS/ICU control subjects				
No. of patients	2	13	16	9
PRISM III-APS score*	3.5 (3.25–3.75)	10 (7–11)	8 (4.5–14)	8 (6–10)
PCT, ng/ml*	1.7	2.1 (1.0–2.4)	1.1 (0.1–2.4)	1.1 (0.1–3.85)
Patients with septic shock				
No. of patients	5	21	20	14
Complicated course, n	2	10	7	4
Deaths, n	1	6	2	1
PRISM III-APS score*	24 (12–27)	17 (15–23)	12.5 (8–19.8)	11.5 (10.3–20.8)
PCT, ng/ml*	3.3 (2.5–4.1)	10.3 (2–17.4)	5.7 (2.6–25.8)	10.7 (5.3–21.4)
No. (%) of patients with:				
Gram-positive bacteria	2 (40)	12 (57)	12 (60)	4 (29)
Gram-negative bacteria	1 (20)	8 (38)	6 (26)	6 (43)
Polymicrobial infection	N/A	1 (4)	N/A	N/A
Negative cultures	2 (40)	N/A	2 (10)	3 (21)
Infection site, n				
Blood	3	16	13	7
Lung	N/A	5	5	1
Urine	N/A	N/A	N/A	1
CSF	N/A	N/A	N/A	N/A

Definition of abbreviations: CSF = cerebrospinal fluid; ICU = intensive care unit; N/A = not applicable; PCT = procalcitonin; PRISM III-APS = Pediatric Risk of Mortality III-Acute Physiology Score; SIRS = systemic inflammatory response syndrome.

All children were divided into four age groups according to previously published age-specific categories for sepsis (29).

*Median (interquartile range).

compared with the predictive values of procalcitonin (PCT) levels and to the Pediatric Risk of Mortality III-Acute Physiology Scores (PRISM III-APS) collected for the enrolled patients.

RESULTS

Predictive Models of All Subjects

The PCA model identified five outliers: two healthy control subjects (infant and toddler), one SIRS/ICU control (school age), and two septic shock samples (toddler and school age). The samples were placed outside the 95% confidence interval of the Hotelling's T-squared distribution in the score scatter plot (Figure 1). Outliers might seriously disturb a model (21); therefore, for all subsequent steps of statistical analysis these outliers were excluded. Based on the PCA results showing sample grouping, a supervised PLS-DA analysis was performed to reveal specific metabolic changes in defined groups and improve the separation between specimens. Three PLS components were used to build the model, and the results are presented by three-dimensional score scatter plots (Figure 2). The scores of healthy control subjects are visibly distinguished from SIRS/ICU control subjects and septic shock samples, indicating specific differences in metabolic profiles of the subjects. Patient groups are well clustered, and the R²_Y and Q² metrics are 0.48 and 0.35, respectively. Despite the fact that some of SIRS/ICU control subjects and septic shock specimens

do overlap, which may result from similar biological responses of these cases, the PLS-DA model appears to be highly relevant. In this model there is a visible tendency reflecting separation of patient groups that is in agreement with the morbidity and severity of septic shock. The disease reveals a very specific metabolic response in a child's body that is much stronger than other parameters such as age and sex. When we applied statistical methods to distinguish all studied specimens according to age or sex, the results revealed poor models, whose patterns could not be fitted. Moreover, a direct comparison between age categories within one patient class (healthy, SIRS/ICU control subjects, or septic shock) did not represent any significant separation, indicating that changes in metabolism of the studied individuals were mainly associated with health condition rather than with age or sex.

Additionally, an OPLS-DA method was applied to compare metabolic variance in patient groups consisting of only two classes: septic shock and healthy subjects, SIRS/ICU and healthy control subjects, septic shock and SIRS/ICU control subjects. The score scatter plots for each statistical analysis are presented in Figure E2. Both OPLS-DA models: SIRS/ICU patients versus healthy control subjects (Figure E2A) and septic shock versus healthy control subjects (Figure E2B) show clear separation of groups and are described by high values of R²_Y and Q² parameters that indicate powerful and reliable models. The scores of the OPLS-DA model that contains patients with septic shock and

Figure 1. A score scatter plot of the first two principal component analysis (PCA) principal components (PC1 and PC2). The PCA model summarizes the variation in the data set of septic shock samples (red triangles), systemic inflammatory response syndrome (SIRS)/intensive care unit (ICU) control subjects (green squares), and healthy control subjects (blue dots) and highlights outliers. Five samples (two septic shock samples, one SIRS/ICU control subject, and two healthy control subjects) are placed outside the ellipse that describes the 95% confidence interval of the Hotelling's T-squared distribution.

Figure 2. The 3D partial least squares discriminant analysis (PLS-DA) score scatter plot for patients with septic shock (red), systemic inflammatory response syndrome (SIRS)/intensive care unit (ICU) control subjects (green), and healthy control subjects (blue). Studied groups are well clustered and distinguished along three PLS components. The sphere describes the 95% confidence interval of the Hotelling's T-squared distribution.

SIRS/ICU control subjects (Figure E2C) are not as well distinguished as the scores in the previous plots. However, there is a visible tendency that allows for clustering septic shock specimens and SIRS/ICU control subjects along the first PLS component. The calculated validation metrics and AUROC for each model are summarized in Table 2.

Predictive Models of Age Groups

It has been reported that clinical parameters used to define SIRS, organ dysfunction, and sepsis are strongly affected by the age of pediatric patients (29). We tested this relationship by designing OPLS-DA models for specific age groups: infants, toddlers, and school age (Table 1). Neonates were not considered because of

TABLE 2. SUMMARY OF THE QUALITY OF THE RESULTS OF ORTHOGONAL PARTIAL LEAST SQUARES DISCRIMINANT ANALYSIS MODELS FOR ALL SERUM SAMPLES OF DIAGNOSTIC GROUPS AND SPECIFIC AGE GROUPS

OPLS-DA Models	Samples	Quality Results OPLS-DA						
		R2Y	Q2	P Value	Sensitivity : Specificity	PPV:NPV	ACC	AUROC
SIRS/ICU control subjects vs. healthy control subjects	All	0.74	0.60	1.56×10^{-13}	$0.90 \pm 0.10 : 0.95 \pm 0.07$	0.95:0.90	0.92	0.95
	Infants	0.72	0.62	2.2×10^{-5}	$0.92 \pm 0.14 : 0.92 \pm 0.16$	0.92:0.92	0.92	0.97
	Toddlers	0.87	0.69	7.2×10^{-7}	$0.94 \pm 0.12 : 0.88 \pm 0.15$	0.88:0.94	0.91	0.98
	School age	0.41	0.29	0.087	$0.75 \pm 0.30 : 0.78 \pm 0.27$	0.75:0.78	0.77	0.85
Septic shock vs. healthy control subjects	All	0.83	0.68	4.82×10^{-20}	$0.90 \pm 0.08 : 0.97 \pm 0.05$	0.98:0.86	0.93	0.98
	Infants	0.92	0.78	2.15×10^{-7}	$0.95 \pm 0.09 : 1.0$	1.0:0.92	0.97	1.0
	Toddlers	0.92	0.75	1.5×10^{-7}	$0.90 \pm 0.14 : 1.0$	1.0:0.90	0.94	0.99
	School age	0.65	0.61	1.3×10^{-4}	$0.92 \pm 0.14 : 0.89 \pm 0.2$	0.92:0.89	0.91	0.98
Septic shock vs. SIRS/ICU control subjects	All	0.46	0.28	4.5×10^{-6}	$0.78 \pm 0.11 : 0.72 \pm 0.14$	0.80:0.68	0.75	0.82
	Infants	0.50	0.41	2.9×10^{-4}	$0.91 \pm 0.13 : 0.62 \pm 0.26$	0.79:0.80	0.79	0.88
	Toddlers	0.53	0.30	0.026	$0.63 \pm 0.22 : 0.69 \pm 0.23$	0.71:0.61	0.66	0.82
	School age	0.63	0.52	0.0013	$0.92 \pm 0.14 : 0.88 \pm 0.23$	0.92:0.88	0.91	0.94

Definition of abbreviations: ACC = accuracy; AUROC = area under a receiver operator curve; ICU = intensive care unit; NPV = negative predictive value; OPLS-DA = orthogonal partial least squares discriminant analysis; PPV = positive predictive value; SIRS = systemic inflammatory response syndrome.

R2Y metric describes the percentage of variation explained by the model; Q2 metric describes the predictive ability of the model. The difference between R2Y and Q2 indicates the model's goodness of fit. Sensitivity, specificity, PPV, NPV, and ACC were calculated based on the predictive values of Y-variables obtained in a sevenfold cross-validation step during OPLS-DA model construction. Sensitivity and specificity are reported with 95% confidence intervals. The AUROC statistic provides additional interpretation of discriminatory power of the models.

the small number of available samples ($n = 7$). The results are presented in Table 2, and the score scatter plots are shown in Figure 3. It is noted that the models obtained for SIRS/ICU control subjects versus healthy, septic shock versus healthy, and septic shock versus SIRS/ICU control subjects are quite similar between infants and toddlers. Validation metrics R2Y and Q2 are also comparable within these two age categories. The score scatter plots (Figure 3) show very clear separation between SIRS/ICU patients and healthy control subjects and between septic shock specimens and healthy control subjects for infants and toddlers. Moreover, these models are described by high values of R2Y, Q2, and ROC parameters (Table 2), confirming their great strengths. The score scatter plots of patients with septic shock and SIRS/ICU control subjects for infants and toddlers are not so well distinguished, and some of the samples are overlapping; however, these OPLS-DA models are still more powerful and reliable (higher values of R2Y and Q2 metrics) than the model constructed for all subjects. Interestingly, the school-age samples represent much different metabolic behavior than younger children. Unlike infants and toddlers, the scores plot of the OPLS-DA model for SIRS/ICU patients versus healthy control subjects sample data are converged almost in the same region, and validation parameters calculated for this model have low values: R2Y = 0.42 and Q2 = 0.28. Nonetheless, the OPLS-DA models containing patients with septic shock (septic shock specimens vs. healthy control subjects and septic shock specimens versus SIRS/ICU control subjects) demonstrate good separation and are described by reasonable values of R2Y and Q2 metrics.

To describe specific biopatterns within the OPLS-DA age group models, OPLS regression coefficients were calculated, and only metabolites with significant changes in concentration ($P < 0.05$) were considered (Table 3). As a result, the numbers of the most meaningful metabolites for separation between SIRS/ICU patients and healthy control subjects include the following: 20 metabolites for infants, 10 for toddlers, and 7 for school-age children. The metabolic patterns found in the OPLS-DA model consisting of patients with septic shock and healthy control subjects show 10 significant metabolites within infants, 12 within toddlers, and 14 for school-age patients. Seventeen OPLS regression coefficients (metabolites) appeared to be the most important

for differentiating individuals with septic shock from SIRS/ICU patients for infants, whereas for toddlers and school-age children the numbers are 4 and 15, respectively.

Mortality Models

In the first mortality model (10 septic shock nonsurvivors and 10 age- and sex-matched septic shock survivors), we detected 11 significant metabolites. The OPLS-DA score scatter plot shows good separation between survivors and nonsurvivors along the first PLS component (Figure E3A). Although one sample from nonsurvivors is situated close to the survivor group, none of the studied samples were predicted incorrectly (Figure E3B). The calculated R2Y and Q2 metrics were, respectively, 0.63 and 0.47. The second mortality model was based on the serum samples collected from 23 patients with septic shock with a complicated course (i.e., two or more organ failures ≥ 7 d after onset of septic shock [10 nonsurvivors and 13 survivors]). In total, 18 metabolites were selected as potentially important variables. The OPLS-DA modeling method led to clear separation between the two groups of patients with high validation metrics, including R2Y = 0.93 and Q2 = 0.82. As seen in the score scatter plot, nonsurvivors are very well distinguished from patients who survived (Figure E4A), and none of the nonsurviving patients was predicted as a surviving patient in this analysis (Figure E4B). The calculated AUROC values were 0.91 for the first mortality model and 1.0 for the second model (Table 4).

Comparison with Conventional Predictors for Septic Shock and Its Outcome in the PICU

In addition to investigating the use of metabolomics for septic shock diagnosis and its outcome prediction in the PICU, we compared the results of ROC analysis for metabolomics data and PCT and PRISM III-APS data for our models: septic shock and SIRS/ICU control patients and age- and sex-matched survivors and nonsurvivors (see Table 5). The AUROC values obtained for septic shock diagnosis are quite similar for all predictors. However, comparing the results calculated for the mortality model using a metabolomics approach seems to be much more

Figure 3. The orthogonal partial least squares discriminant analysis score scatter plots for models consisting of two classes: (A) Systemic inflammatory response syndrome (SIRS)/intensive care unit (ICU) control subjects versus healthy control subjects, (B) patients with septic shock versus healthy control subjects, (C) patients with septic shock versus SIRS/ICU control subjects within three different age groups: infants, toddlers, and school-age children. Patients with septic shock are marked in red, SIRS/ICU control subjects in green, and healthy control subjects in blue.

favorable than the PCT concentration and the PRISM III-APS score in the prognostic evaluation of mortality for patients with septic shock in the PICU.

DISCUSSION

This study describes a novel application of metabolomics for the diagnostic and prognostic evaluation of pediatric septic shock. The diagnosis of sepsis and septic shock remains a clinical challenge in pediatric critical care, especially because of the disease's complexity and heterogeneity in this population. Additionally, there is no other factor more crucial in the recognition of septic shock than time. However, making a firm diagnosis early is critical, since early recognition of septic shock and rapid medical intervention may significantly improve patients' outcomes and increase their chance for survival (9, 30). Consequently, a global health initiative has been created that aims to develop a multifaceted medical approach and aims to identify effective biomarkers to aid in the early diagnosis and treatment of septic shock and to improve the quality of clinical care for children with sepsis syndrome (7, 31). It seems obvious that early identification of factors that influence the outcome in pediatric septic shock may improve early diagnosis and treatment of children who are at the highest risk of death. Therefore, research and

actions that open the door to quick recognition of pediatric septic shock in the PICU is an important goal and a goal of this study.

In this work we were able to separate patients with septic shock from noninfected PICU patients (with SIRS) and healthy children using serum samples and an NMR-based metabolomics approach. Both septic shock serum samples and SIRS/ICU control serum samples were collected within 24 h of admission to the PICU, allowing for early diagnostic and prognostic evaluation of the disease. Metabolomics has previously been suggested as a potential technique for early diagnosis of sepsis (17–19), but our research appears to be the first study that uses serum metabolomics to evaluate septic shock in a pediatric population. It is certainly the largest pediatric metabolomics study to date. In addition, there is currently no other study in the literature that describes analysis of metabolic profiles within the three patient groups: septic shock, SIRS/ICU control subjects, and healthy pediatric control subjects. We present models that consist of only two groups of patients (SIRS/ICU patients and healthy control subjects, patients with septic shock and healthy control subjects, or patients with septic shock and SIRS/ICU control subjects), and these groupings resulted in differences in serum metabolite concentrations depending

TABLE 3. THE LIST OF POTENTIALLY IMPORTANT METABOLITES BASED ON THE ORTHOGONAL PARTIAL LEAST SQUARES DISCRIMINANT ANALYSIS REGRESSION COEFFICIENTS ($P < 0.05$) FOR MODELS

	SIRS vs. Healthy			Septic vs. Healthy			Septic vs. SIRS		
	Infants	Toddlers	School Age	Infants	Toddlers	School Age	Infants	Toddlers	School Age
2-Aminobutyrate	↓		↓	↓	↓				
2-Hydroxybutyrate				↑	↑	↑	↑		↑
2-Hydroxyisobutyrate	↑								
2-Hydroxyisovalerate	↑			↑	↑	↑			
2-Methylglutarate					↑				
2-Oxoisocaproate	↑			↑		↑			↑
3-Hydroxybutyrate		↑			↑				
3-Hydroxyisovalerate	↑								
Acetate		↓		↓					
Acetone		↑	↑		↑				
Adipate				↓			↓		
Alanine			↓				↓		↑
Arginine	↓					↑		↑	↑
Asparagine									↑
Betaine					↑				
Carnitine						↑			
Citrate	↓	↓	↓			↓		↑	
Creatine						↑			↑
Creatine phosphate	↑					↑			↑
Creatinine	↑			↑		↑			↑
Ethanol			↓						↑
Glucose				↑	↑		↑	↑	
Glutamate							↓		
Glutamine	↓		↓		↓				
Glycerol	↓						↑		↑
Glycine	↑						↓		
Histidine						↑			↑
Hypoxanthine							↓		
Isobutyrate		↑			↑				
Isoleucine							↓		
Lactate	↑	↑		↑	↑	↑	↓		↑
Methanol	↓	↓	↓						↑
Methionine							↓		
Myo-Inositol	↑					↑			
O-Acetylcarnitine						↑			
Ornithine							↓		
Phenylalanine	↑	↑			↑	↑			↑
Pyroglutamate	↑								
Pyruvate	↑						↓		
Serine							↓		
Suberate	↑						↓		
Taurine		↑					↓	↓	↑
Threonine				↓			↓		
Tyrosine	↓								
Urea		↓							

Definition of abbreviations: ICU = intensive care unit; SIRS = systemic inflammatory response syndrome.

SIRS/ICU control subjects vs. healthy, patients with septic shock vs. healthy, patients with septic shock vs. SIRS/ICU control subjects within infants, toddlers, and school-age children. Arrows indicate significant change in the concentration (upward arrow = increased; downward arrow = decreased); empty cells indicate no significant change.

on the age of the studied subjects. Infants and toddlers demonstrated quite similar metabolic response to septic shock, whereas OPLS-DA models for school-age children showed different results than those obtained from the younger patient groups. Interestingly, it has recently been published in an age-specific transcriptomics study of children with septic shock that the school-age children had a much larger number of uniquely regulated gene sets relative to age-matched control subjects than infants and toddlers (32). Therefore, it should not be a surprise that using a metabolomic profiling approach followed a very similar pattern as that revealed by a whole blood transcriptomic response approach during pediatric septic shock.

Some metabolite concentrations changed markedly in specific groups, significantly influencing the separation between healthy

patients and patients with septic shock. Three compounds (2-hydroxybutyrate, 2-hydroxyisovalerate, and lactate) show elevated levels in the model consisting of patients with septic shock versus healthy control subjects regardless of differences in the age (Table 3). These metabolites are mainly associated with increasing demands for energy during infections and inflammatory conditions, and their high concentrations indicate enhanced fat breakdown resulting in a tendency toward ketoacidosis and lactic acidosis in critically ill patients with septic shock (33–35). However, more characteristic biopatterns might be described within specific age categories. In the infant and toddler groups, significantly higher levels of glucose were detected in patients with septic shock compared with both of the control groups: healthy and SIRS/ICU control subjects (Table 3). It is well known that uncontrolled and expanding

TABLE 4. SUMMARY OF THE QUALITY OF THE RESULTS FOR THE MORTALITY ORTHOGONAL PARTIAL LEAST SQUARES DISCRIMINANT ANALYSIS MODELS

OPLS-DA Mortality Models	Quality Results of the Models			
	R2Y	Q2	P Value	AUROC
20 Septic shock specimens (10 nonsurvivors and 10 age- and sex-matched survivors)	0.63	0.47	0.0044	0.91
23 Septic shock specimens with complicated course (10 nonsurvivors and 13 survivors)	0.93	0.82	0.00043	1.0

Definition of abbreviations: AUROC = area under a receiver operator curve; OPLS-DA = orthogonal partial least squares discriminant analysis.

inflammatory responses in sepsis cause hyperglycemia; thus, detection of elevated serum glucose was expected. Additionally, for school-age children there were many other metabolites for which the concentration increased markedly in patients with septic shock versus healthy or SIRS/ICU control subjects (Table 3). The elevated levels of 2-oxoisocaproate, creatine, creatine phosphate, creatinine, histidine, and phenylalanine are mainly associated with an enhanced muscular protein turnover, amino acid oxidation, decreased energy supply, and organ failure during septic shock (36–40). An increased concentration of arginine in the patients with septic shock might be related to cytokine production. It is known that the inflammatory process is modulated by nitric oxide (NO), which is formed from arginine (41), and extracellular arginine availability is crucial for NO synthesis (42); thus, there is a very close relationship between arginine concentration and NO formation (43). Taken together, the metabolites described above may be considered as biomarkers for an early diagnosis of septic shock in PICU patients.

We also evaluated whether an NMR-based metabolomics approach could be applied to define metabolic variation between septic shock survivors and nonsurvivors. According to the International Pediatric Sepsis Consensus Conference panel discussion (29), mortality is the most important clinical outcome in sepsis. Clearly, mortality should not be considered as the only end point and thus should be studied together with other factors or scores examining patient survival. There are several scoring systems for estimating pediatric organ dysfunction and children's mortality, for example the Pediatric Logistic Organ Dysfunction (PELOD) score (44, 45), the Multiple Organ System Failure (MOSF) score (46), or the Pediatric Risk of Mortality (PRISM) score (47). The PRISM score, a physiology-based measurement, is the most common currently available system used for mortality prediction in the PICU. Recently, the PRISM score was upgraded to PRISM III-APS (PRISM III-Acute

Physiology Score), including new treatment protocols and therapeutic interventions. It provides a measure of physiologic instability that has been validated against mortality (48, 49). Despite all efforts to improve a sepsis mortality scoring system, determining reliable mortality risk is still challenging and a very difficult process early in the clinical setting. In our study, the constructed metabolomics models show an excellent separation between survivors and nonsurvivors and a very good distinction between the observed and expected outcomes.

It should be noted that this study is just an initial step in pediatric mortality risk assessment using a metabolomics approach, as it refers to data collected from only a small number of patients. Although one can criticize this study as being relatively small, it is the largest pediatric metabolomics study we have found to date. Pediatric studies of 140 patients are generally thought of as reasonably sized studies, as pediatric studies generally do not include as many patients as adult studies do. Nonetheless, the profiling method applied in this study should be validated by a larger cohort of PICU patients in future research. It should be further noted that other methods of metabolomics analysis can be used, such as gas chromatography mass spectroscopy or liquid chromatography mass spectroscopy, which may yield additional features or metabolites that could enhance the separation between the groups presented in this NMR-based study.

In conclusion, this study presents metabolomics as a promising method for diagnostic and early prognostic evaluation of patients with septic shock in the PICU. Our findings show that septic shock leads to significant disruption in biochemical homeostasis that strongly contributes to changes in body metabolites. Moreover, our data strongly suggested that metabolic profiling be used as an additional methodology for the early diagnosis and prognosis of septic shock in the PICU. However, it is suggested that this application should be further evaluated using a larger cohort of critical ill patients. Nonetheless, metabolomics should be considered as a very promising application in the development of better strategies for septic shock diagnosis and prognosis in PICU. Because of the noninvasive nature of the analysis, we recommend that metabolomic profiling be further studied as a technique for early diagnosis of septic shock and prediction of outcome in PICU.

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TABLE 5. COMPARISON OF SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE, NEGATIVE PREDICTIVE VALUE, ACCURACY, AND AREA UNDER A RECEIVER OPERATOR CURVE RESULTS FOR MODELS

Model	Data	Sensitivity : Specificity	PPV:NPV	ACC	AUROC
Septic shock vs. SIRS/ICU control subjects	Metabolomics	0.78 ± 0.11 : 0.72 ± 0.14	0.80:0.68	0.75	0.82
	PCT	0.56 ± 0.16 : 0.94 ± 0.08	0.91:0.66	0.74	0.80
	PRISM III-APS	0.83 ± 0.10 : 0.56 ± 0.16	0.74:0.69	0.72	0.80
Nonsurvivors vs. survivors (age- and sex-matched samples)	Metabolomics	0.80 ± 0.25 : 0.90 ± 0.19	0.89:0.82	0.85	0.91
	PCT	0.29 ± 0.33 : 0.57 ± 0.37	0.40:0.44	0.43	0.51
	PRISM III-APS	0.70 ± 0.28 : 0.80 ± 0.25	0.78:0.73	0.75	0.85

Definition of abbreviations: ACC = accuracy; AUROC = area under a receiver operator curve; ICU = intensive care unit; NPV = negative predictive value; PCT = procalcitonin; PPV = positive predictive value; PRISM III-APS = Pediatric Risk of Mortality III-Acute Physiology Score; SIRS = systemic inflammatory response syndrome. Patients with septic shock vs. SIRS/ICU control subjects and nonsurvivors vs. survivors (age- and sex-matched samples) based on metabolomics, PCT and PRISM III-APS data.

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