REVIEW

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Multilevel omics for the discovery of biomarkers in pediatric sepsis

Xinyu Wang[1]* | Rubo Li[2]* | Suyun Qian[2] | Dan Yu[1]

[1]Laboratory of Dermatology, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, Key Laboratory of Major Diseases in Children, Ministry of Education, National Center for Children's Health, Beijing, China
[2]Department of Pediatric Intensive Care Unit, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China

Correspondence
Suyun Qian, Pediatric Intensive Care Unit, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing 100045, China.
Email: syqian2020@163.com

Dan Yu, Laboratory of Dermatology, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, Key Laboratory of Major Diseases in Children, Ministry of Education, National Center for Children's Health, Beijing 100045, China.
Email: danyu@ccmu.edu.cn

*These authors contributed equally to this work.

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ABSTRACT

Severe sepsis causes organ dysfunction and continues to be the leading reason for pediatric death worldwide. Early recognition of sepsis could substantially promote precision treatment and reduce the risk of pediatric death. The host cellular response to infection during sepsis between adults and pediatrics could be significantly different. A growing body of studies focused on finding markers in pediatric sepsis in recent years using multi-omics approaches. This narrative review summarized the progress in studying pediatric sepsis biomarkers from genome, transcript, protein, and metabolite levels according to the omics technique that has been applied for biomarker screening. It is most likely not a single biomarker could work for precision diagnosis of sepsis, but a panel of markers and probably a combination of markers detected at multi-levels. Importantly, we emphasize the importance of group distinction of infectious agents in sepsis patients for biomarker identification, because the host response to infection of bacteria, virus, or fungus could be substantially different and thus the results of biomarker screening. Further studies on the investigation of sepsis biomarkers that were caused by a specific group of infectious agents should be encouraged in the future, which will better improve the clinical execution of personalized medicine for pediatric sepsis.

KEYWORDS

Biomarkers, Early diagnosis, Pathogen identification, Pediatric, Sepsis

INTRODUCTION

Sepsis is one of the leading causes of death in children and is characterized by a sustained inflammatory response due to a disrupted host immune response caused by infection.[1] Approximately 15%–16% of children admitted to pediatric intensive care units (PICUs) in Asia develop severe sepsis, with a mortality rate of approximately 40%.[1] Besides,

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35%–50% of childhood sepsis deaths occur in previously healthy children, who often start with nonspecific symptoms and rapidly progress to shock and multisystem organ failure.[2] Therefore, early and accurate diagnosis and treatment is the key to reduce the risk of sepsis and improve the prognosis.[3] Specific biomarkers allow timely diagnosis, monitoring, grading of severity, and prediction of clinical outcomes in sepsis.[4] Due to the complex intersection between pro-coagulant, fibrinolysis, pro-inflammatory, and anti-inflammatory mechanisms in sepsis pathology, it can be theorized that the optimal biomarker would play a role in multiple septic pathways.[5] Biomarkers can be used clinically to assist in the diagnosis of sepsis and the development of new therapeutic strategies.[6–14] The high dimension of multi-omics profiling technology has been used to reveal the complexity of sepsis immunity and inflammation. To find reliable biomarkers, different biomarkers related to sepsis can be screened for broad-spectrum changes at four levels: genomics, transcriptomics, proteomics, and metabolomics. Combined multi-omics analysis could better analyze the systemic responses as well as local tissue-specific responses to sepsis.[15] In addition, some other biomarkers, such as cell-free DNA and monocyte activation are emerging as sepsis biomarkers.[16,17] The application of machine learning in studying sepsis biomarkers and the establishment of a model in assessing the risk of death from septic shock could also help to promote the finding of novel biomarkers for sepsis diagnosis and prognosis. Several studies have previously summarized the progress of research on sepsis markers in children.[3,18–25]

In this study, we reviewed the research progress about pediatric sepsis markers from different levels according to the omics tools used for biomarker identification. Since the neonatal immune response against sepsis is different from that of children, sepsis biomarkers screened by using neonatal samples were not included in this review.[26,27] Biomarkers were summarized according to the stage of sepsis progression (sepsis, severe sepsis, septic shock) of the patient samples that have been used in the studies.

BIOMARKERS SCREENED BY GENOMICS

Genomics aims to study the function of a genome and predict what problems may happen if genetic mutations that could interfere with biological function have been generated.[28] Common methods used in genomics include single nucleotide polymorphism (SNP) analysis, DNA microarray technology, and next-generation sequencing (NGS). SNPs have been widely used to predict genetic risk for disease progression, DNA microarray technology makes large-scale gene expression analysis feasible, and the advent of NGS has made whole-genome sequencing much less time-consuming but expensive.[29–31]

Pediatric sepsis often starts with nonspecific symptoms and rapidly progresses to shock and multisystem organ failure. In addition to pathogenic virulence factors, genetic variation in primary immunodeficiency disease (PID) may underlie fulminant sepsis and could serve as potential sepsis biomarkers.[32] Asgari et al.[32] used whole-exome sequencing and bioinformatics analysis to explore genetic factors contributing to fulminant *Pseudomonas aeruginosa* sepsis in previously healthy children. They identified 12 potentially pathogenic variants, including two new pathogenic variants of known PID genes: *BTK* and *DNMT3B*. Some researchers have conducted a study in a similar cohort (children with bacterial sepsis who were healthy prior to admission).[33] To investigate whether PID gene variants could be used for sepsis prediction, they analyzed the screened gene variants and compared them with 240 PID genes that have been reported to be associated with bacterial infection. They found 41 unique predicted pathogenic PID variants in 20% of the affected children, with the most common variants being *CHD7*, *SAMD9*, *TCF3*, and *BACH3*. However, they did not observe a significant correlation between the PID variants with the current clinical or laboratory characteristics of sepsis patients. Future studies were required to investigate the functional relevance of these variants in depth to determine whether these PID gene variants are associated with susceptibility to bacterial sepsis in children.

In addition to primary immunodeficiency disease-relevant genes, several other genes have also been suggested to be good sepsis biomarker candidates. Plasminogen activator inhibitor-1 (PAI-1) is a major inhibitor of both tissue and urinary plasminogen activator.[34] Hermans et al.[35] demonstrated that the concentration of PAI-1 at admission was associated with the severity and prognosis of sepsis and that PAI-1 concentrations were significantly higher in deceased children than in surviving children. There is a common functional polymorphism in the *PAI-1* gene, which is an insertion (5G)/deletion (4G) polymorphism that occurs in a single base pair at 675 bp upstream of the predicted transcription start site.[36] They also reported that children with meningococcal bacteremia who carried the 4G/4G genotype had higher PAI-1 concentrations than 4G/5G or 5G/5G genotypes and were at increased risk of death. These findings were further confirmed by a meta-analysis conducted by Brouwer et al.,[37] which linked the 4G/4G allele to increased mortality.

Some other biomarkers with potential research value have also been proposed, but still require further validation. Yang et al.[38] performed a meta-analysis of the polymorphism of the serum angiotensin-converting enzyme (CD143) gene in intron 16 insertion/deletion (ins/del) to assess the risk of sepsis associated with the ins/del polymorphism. They found that in comparison to pure del/del, the risk of sepsis significantly increased in pure ins/ins, and heterozygous del/ins, and the two genotypes were

combined (ins/ins + del/ins). Further age group analysis showed that this gene polymorphism was significantly associated with childhood sepsis but not correlated with adult sepsis. The Arg702Trp, Gly908Arg, and the Leu1007fsincC variants of the *NOD2/CARD15* gene were associated with susceptibility to risk factors of sepsis, among which the Leu1007fsincC variant displayed the most significant correlation with increased sepsis-related mortality.[39] Gökay et al.[40] found that the occurrence of the C/C genotype of *MyD88* SNP-938 was significantly higher than the C/A genotype in the children with sepsis, indicating that children with the *MyD88* SNP-938C/C genotype had a greater propensity for sepsis.

As sepsis progresses, the patients may fall into septic shock. Whitmore et al.[41] found that septic shock children with the Toll-like receptors 1 (TLR1) rs5743618 (1805G/T) SNP took longer to recover from PICU, suggesting that this SNP may exacerbate the disease outcome by promoting a hyperinflammatory response during the disease process such as septic shock. In addition, the 449G polymorphism in the *DDAH2* gene was found to correlate with the increased probability of cold shock complications in children of all ages affected by sepsis.[42]

Michalek et al.[43] analyzed two bactericidal permeability-increasing protein (BPI) gene polymorphisms, *BPI* (G545/C) Taq and *BPI* (A645/G) 216, and demonstrated that the *BPI* Taq gene was significantly different between healthy controls and different subgroups of sepsis, including systemic inflammatory response syndrome (SIRS), sepsis, septic shock, multiple organ dysfunction syndrome, in which all non-survivors carried a *BPI* Taq GG genotype, which is highly predictive of gram-negative bacterial infections. However, the *BPI* 216 SNP showed no significant difference between affected patients and healthy controls. Although the role of the *BPI* 216 polymorphism in sepsis and infection is unknown, researchers have demonstrated that children with septic shock/multiple organ distress syndrome carrying the *BPI* 216 AG/GG allele have an increased mortality rate, particularly associated with bacterial infection. The study by Michalek et al.[43] demonstrated for the first time that polymorphisms in *BPI* Taq and *BPI* 216 are prevalent in children with infectious shock and multiple organ distress syndrome, suggesting that this mutation increases mortality in children with sepsis (Table 1).

BIOMARKERS SCREENED BY TRANSCRIPTOMICS

Transcriptomics is an important methodology for gene function exploration, allowing to elucidate the transcription and regulation patterns in cells at a holistic level.[44,45] Detection biomarker at transcriptional level is very convenient, fast, and cheap by using polymerase chain reaction (PCR). Transcript biomarkers screening will greatly benefit from transcriptome analysis. Single-cell sequencing has also been used for the transcriptional level analysis of sepsis patient samples, which will provide us with a deeper understanding of sepsis pathology and novel clues for sepsis biomarker identification.[44] Moreover, the advantage of using transcript as biomarkers is that in addition to the encoded genes or protein, transcript biomarkers also include non-coding products, such as microRNAs (miRNAs), which are small non-coding RNAs that are post-transcriptionally regulated and represent ideal diagnostic targets being highly conserved.[46–48] Additionally, a growing number of studies gradually emphasized the important role of transcriptome studies about non-coding RNAs in pediatric sepsis.[49,50] Most of the current biomarkers were identified at the transcriptomic level, which is probably due to the low cost and fast detection of biomarkers in the clinic. Xie et al.[50] have identified 160 upregulated and 61 downregulated genes by enrichment analysis of differentially expressed genes (DEGs) in pediatric sepsis compared with healthy controls. Specifically, nine key genes including *ITGαM*, *TLR8*, *IL1β*, *MMP9*, *MPO*, *FPR2*, *ELANE*, *SPI1*, and *C3AR1* were found to be upregulated in sepsis children. These genes play crucial roles in the infiltration and activation of white blood cells. Among them, *ITGαM* was the most significantly upregulated gene. It has been demonstrated that the increased expression of *ITGαM* on polymorphonuclear neutrophils is associated with a decreased survival rate. Additionally, *ITGαM* blocking has been shown to significantly inhibit these processes.[51,52] They also demonstrated that five miRNAs were possible key miRNAs related to sepsis progression, including has-miR-204-5p, has-miR-211-5p, has-miR-590-5p and has-miR-21-5p.

A total of 1941 differentially expressed messenger RNAs (mRNAs) and 225 long non-coding RNAs (lncRNAs) have been identified.[49] By using a weighted gene co-expression network analysis (WGCNA), they found that the turquoise modules displayed a significant association with pediatric sepsis. A total of 15 mRNAs (*MAPK14*, *ITGαM*, *HK3*, *ALOX5*, *CR1*, *HCK*, *NCF4*, *PYGL*, *FLOT1*, *CARD6*, *NLRC4*, *SH3GLB1*, *PGS1*, *RAB31*, and *LTB4R*) and four lncRNAs (GSEC, NONHSAT160878.1, XR_ 926068.1, and RARA-AS1) as hub genes, were found to be upregulated. In addition, the area under the receiver operating characteristic (ROC) curve was found to predict the sepsis diagnosis, which was higher than 0.88 when compared to the one in the healthy group, suggesting that they may have a good diagnostic value for pediatric sepsis.[49] Similarly, by using WGCNA, Zhou et al.[52] demonstrated that the differentially expressed genes in pediatric sepsis were

TABLE 1 Biomarkers that were identified using genomics for the diagnosis, triage, or prognosis of pediatric sepsis

Authors	Sepsis marker	No. of patients	No. of controls	Age	Sample	Sepsis stage	Pathogen
Asgari et al.[32]	12 variants including *BTK* and *DNMT3B*	11	–	6–60 months	Blood or tissue	Severe sepsis	*P. aeruginosa*
Borghesi et al.[33]	41 variants such as *CHD7*, *SAMD9*, *TCF3* and *BACH3*	176	–	>28 days and <17 years	Blood	Sepsis	Bacteria
Hermans et al.[35]	*PAI-1* (4G/4G)	Rotterdam cohort: 37; London cohort: 138	Rotterdam cohort: 137; London cohort: 89	0.5–17.9 years 0.3–17.9 years	Serum	Sepsis	*Meningococcus*
Yang et al.[38]	*CD143* ins/del	53 98 98 246	135 100 287 963	<18 years 1.5–180 months 1–168 months <37 weeks	Blood	Sepsis	–
Tekin et al.[39]	*NOD2/CARD15* (Arg702Trp, Gly908Arg, Leu1007fsincC)	128	128	Mean age: 3–3.4 years	Blood	Sepsis	Bacteria and fungi
Gökay et al.[40]	*MyD88* (938-C/C)	65	65	Mean age: 47.54 months	Blood	Sepsis	–
Whitmore et al.[41]	*TLR-1* SNP 1805G/T	110 (Database GSE26440, GSE26378)	70	≤10 years	Serum	Septic shock	–
Weiss et al.[42]	*DDAH2* SNP 449G/C	56	26	≤18 years	Plasma	Septic shock	–
Michalek et al.[43]	*BPI* Taq (G545>C), *BPI* 216 (A645>G)	345	641	0–19 years	Blood	SIRS, sepsis, severe sepsis, septic shock or MODS	Gram-negative bacteria

Abbreviations: MODS, multiple organ dysfunction syndrome; SIRS, systemic inflammatory response syndrome; SNP, single nucleotide polymorphism.

primarily enriched in processes such as neutrophil degranulation, neutrophil-mediated immunity, fibrin-1-rich particle cavity, cysteine metabolism, export of rRNA-containing ribonucleoprotein complexes from the nucleus, negative regulation of NIK/NF-κB signaling pathway, and T-cell receptor signaling pathway.

Furthermore, Yao et al.[53] have identified DEGs and WCGNA in one dataset, and then 41 common genes were screened between DEGs and WGCNA. A four-gene signature consisting of *ANXA3*, *CD177*, *GRAMD1C*, and *TIGD3* allowed to distinguish pediatric sepsis patients from healthy controls, suggesting that this signature could be used as a novel biomarker.

It was found that sepsis could result in neutrophil dysfunction, which in turn promotes the severity of sepsis.[54] Researchers detected a reduced membrane metalloendopeptidase expression level from neutrophils of patients affected by septic shock and found that it may be involved in the development of pediatric sepsis by regulating the neutrophil function.[55,56,60] Toufiq et al.[57] found that *ANXA3* expression (restricted to neutrophils) was significantly increased in the plasma of patients affected by sepsis under *in vitro* conditions and it was statistically associated with poor clinical outcomes.

Moreover, the studies performed by Yao et al.[53] overcame the shortcomings of previous studies, as many samples were screened and their results were validated using six other datasets. Besides, some of the key genes in this study (*ANXA3* and *CD177*) were previously reported to be associated with sepsis, whereas *GRAMD1C* and *TIGD3* were not.[57–60] Karam et al.[61] found that the serum miRNA-146-a expression levels were significantly lower in the sepsis children group compared to controls, which was correlated with the severity of sepsis: sepsis shock group < severe sepsis < sepsis. Additionally, it was found that the miRNA-146-a had a sensitivity of 86.6% and a specificity of 56.6% with an area under the curve value of 0.803 for the diagnosis of

sepsis, suggesting that serum miRNA-146-a could be considered as a potential biomarker for an early diagnosis of sepsis and prediction of its severity.

In addition to the high-throughput screening, some well-known immune factors have also been studied as sepsis biomarkers. Interleukin 27 (IL-27), playing an important role in inflammation modulation in partnership with innate and adaptive immune cells, has been broadly studied as a sepsis biomarker but remains controversial. Epstein-Barr virus-induced gene 3 (*EBI3*), a subunit of the heterodimeric cytokine IL-27, plays a role in regulating T-cell function.[62] Wong et al.[63] found that *EBI3* showed the highest predictive strength for bacterial infection among the 221 diagnostic candidates they screened for sepsis. Because serum IL-27 concentrations are easily measured, serum IL-27 protein concentrations can be used as a diagnostic marker for bacterial infections in critically ill children instead of EBI3 concentrations. However, the study conducted by Eckerle et al.[64] stated that the detection of IL-27 alone may be not specific for the diagnosis of sepsis and needs to be combined with other possible indicators to obtain an accurate sepsis prediction. NOD-like receptor family CARD domain containing 4 (NLRC4), is involved in the regulation of infection in sepsis.[65] Zhu et al.[66] found that the mRNA and protein expression levels of NLRC4 in the blood of children with bacterial sepsis were significantly increased compared with those of the control group.

In the septic shock cohort, Mohammed et al.[67] have identified 53 differentially expressed pediatric septic shock biomarkers, among which 47 were upregulated, while 7 were downregulated, with *DDIT4*, *NDUFV2*, and *TNFRFS10C* being the most highly expressed genes; *AREG*, *CCL20*, and *CFH* the least expressed ones. The aforementioned genes were also implicated in immune response and chemokine-mediated signaling pathways. Five (*CCL3*, *CDC20*, *TNFRSF10C*, *EBI3*, and *TOP2A*) of the 53 identified genes have already been shown to be involved in sepsis.[68–72] In their study, the most common pathogens identified in 181 children with sepsis were *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pyogenes*.

In addition, Wong et al.[73] found that 2482 RNAs were differentially expressed in children with septic shock compared to healthy children, of which 1081 were upregulated and 1401 were downregulated. They also found that many of the downregulated genes in septic shock patients were involved in gene ontologies related to metal or zinc homeostasis. In their study, there were 63 differentially expressed genes in the septic shock non-survivor group compared to the survivor group, of which 36 were upregulated and 27 were downregulated. The levels of two subtypes of metallothionein increase in non-survivors, augmenting the body's oxidative stress response and consequently elevating the mortality rate associated with infectious shock in children.

Researchers have also taken advantage of microarray datasets for septic shock biomarker analysis, from which they found that 32 DEGs were associated with pediatric septic shock characterized by a fatal outcome, with *OLFM4* as the most significantly upregulated gene and *MME* as the most significantly downregulated one.[74]

A few studies specifically focused on the screening of lncRNAs and miRNAs as diagnostic biomarkers in pediatric sepsis. For example, Wu et al.[75] revealed that the lncRNAs THAP9-AS1 and TSPOAP1-AS1 may help in the differentiation of septic shock samples from control samples. Zhao et al.[76] identified five genes in the GSE94717 dataset, including *SOCS3*, *KBTBD6*, *FBXL5*, *FEM1C*, and *WSB1*, which were validated using the GSE95233 dataset. In addition, Gong et al.[77] found that *LRG1*, *ELANE*, *TP53*, *LCK*, *TBX21*, *ZAP70*, *CD247*, *ITK*, and *FYN* genes had a diagnostic value for sepsis in three datasets. Although these studies identified potential markers for the diagnosis of sepsis, they were evaluated only in a small size of samples (Table 2).

BIOMARKERS SCREENED BY PROTEOMICS

Proteomics is a biotechnological research technique that was developed in the post-genomic era. It is more complex than genomics and transcriptomes as it involves the study of modifications of specific proteins, and it can be helpful for the identification of differentially expressed proteins in sepsis and contribute to a better understanding of pathophysiological processes.[78] Proteins are the final step products of gene expression, and their function determines the host response to an infectious invasive pathogen and are the most direct markers reflecting the progression of sepsis.

Due to the high cost of experimental tests, only a few researchers used proteomics for pediatric sepsis biomarker screening. Luo et al.[79] have performed a quantitative identification of plasma proteome to analyze the age-related proteomic profile of sepsis in children. They found that developmental age may affect the levels and biological functions of haptoglobin and thrombin-reactive protein 1 (THBS1). Compared with patients with a good prognosis of sepsis, haptoglobin levels were elevated in the poor prognosis infant group and decreased in the toddler group. In addition, they found that THBS1 was only a candidate biomarker for poor prognosis in the infant group of sepsis. Proteins differentially expressed between the septic and non-infected SIRS patients have also been studied.[80] In this study, the authors identified 111 differentially expressed proteins between two groups and found that the protein

TABLE 2 Biomarkers that were identified using transcriptomes for the diagnosis, triage, or prognosis of pediatric sepsis

Authors	Sepsis marker	No. of patients	No. of controls	Age	Sample	Sepsis stage	Pathogen
Zhang et al.[49]	15 mRNAs (*MAPK14*, *ITGAM*, *HK3*, *ALOX5*, *CR1*, *HCK*, *NCF4*, *PYGL*, *FLOT1*, *CARD6*, *NLRC4*, *SH3GLB1*, *PGS1*, *RAB31*, *LTB4R*); 4 lncRNAs (GSEC, NONHSAT160878.1, XR_926068.1, RARA-AS1)	GSE13904: 209	18	≤10 years	Blood	Sepsis	–
Xie et al.[50]	9 key genes *(ITGAM*, *TLR8*, *IL1β*, *MMP9*, *MPO*, *FPR2*, *ElANE*, *SPI1*, *C3AR1*) and 5 possible miRNAs	GSE25504: 27	35	0–111 days	Blood	Sepsis	–
		GSE26378: 82	21	≤10 years			
		GSE26440: 78	32	≤10 years			
Yao et al.[53]	*ANXA3*, *CD177*, *GRAMD1C*, *TIGD3*	GSE119217: 122	12	56 days–18 years	Blood	Sepsis	–
Karam et al.[61]	miRNA-146-a	55	60	2 months–12 years	Serum	Sepsis	–
Wong et al.[63]	*EBI3*, IL-27	60	21	≤10 years	Blood	Sepsis	Bacteria
Zhu et al.[66]	*NLRC4*	42	40	1–6 years	Serum	Sepsis	Bacteria
Mohammed et al.[67]	43 genes, top three: *DDIT4*, *NDUFV2*, *TNFRFS10C* (up); *AREG*, *CCL20*, *CFH* (down)	181	–	Mean age: 3.69 years	Blood	Septic shock	Top 3 pathogens: *S. pneumoniae*, *S. aureus*, *S. pyogenes*
Wong et al.[73]	IL-8, Two MT subtypes	42	15	<10 years	Blood	Septic shock	–
Wang et al.[74]	32 genes, top 10: *OLFM4*, *CEACAM8*, *C15orf48*, *ELANE*, *PRTN3*, *DDIT4*, *PRG2*, *CD24*, *RRM2*, *CEP55* (up); top 10: *MME*, *FGL2*, *HCAR3*, *LOC100134822*, *CXCR2*, *RGS2*, *FCGR3B*, *CPVL*, *FFAR2*, *HTATSF1P2* (down)	6 non-survivors (GSE9692)	24 survivors	<10 years	Blood	Septic shock	–
		12 non-survivors (GSE26378)	70 survivors	≤10 years			
		17 non-survivors (GSE26440)	81 survivors	≤10 years			
		9 non-survivors (GSE4607)	33 survivors	<10 years			
Wu et al.[75]	lncRNAs THAP9-AS1, lncRNAs TSPOAP1-AS1	GSE13904: 106	18	≤10 years	Blood	Septic shock	–
		GSE4607: 69	15	<10 years			

Abbreviation: mRNAs, messenger RNAs; lncRNAs, long non-coding RNAs; miRNAs, microRNAs; IL, interleukin; MT, metallothionein.

mostly associated with the network was a signal transducer and activator of transcription-3 (STAT3), which interacted with 16 proteins. Besides, four out of the five proteins correlated with STAT3 phosphorylation and activation were increased in the sepsis patient cohort.

Moreover, Pilar-Orive et al.[81] performed another proteomic-based analysis using mass spectrometry (MS) and showed that 44 proteins were differentially expressed between children with sepsis and healthy controls. From these, six proteins were selected for the validation of the data, and high specificity and sensitivity values were reported for soluble interleukin-2 alpha chain receptor (sCD25), serum amyloid-A1 (SAA-1), and leucine-rich alpha-2 glycoprotein (LRG1) with an area under the ROC curve > 0.9.

IL-27 was a sepsis biomarker that can be detected at both transcript and protein levels. Wong et al.[63] performed a clinical cohort study and found significantly higher

TABLE 3 Biomarkers that were identified using proteomics for the diagnosis, triage, or prognosis of pediatric sepsis

Authors	Sepsis marker	No. of patients	No. of controls	Age	Sample	Sepsis stage	Pathogen
Wong et al.[63]	IL-27	231	61	<10 years	Serum	Sepsis	Positive bacteria
Luo et al.[79]	Haptoglobin, THBS1, SAA1/2	90	20	Infants (7–12 months); Toddlers (13–36 months)	Plasma	Sepsis	–
Shubin et al.[80]	STAT3	35	28	1 month–18 years	Serum	Sepsis	–
Pilar-Orive et al.[81]	sCD25, SAA-1, LRG1	40	24	1 month–16 years	Serum	Sepsis	Top three pathogens: *Meningococcus*, *S. pyogenes*, *S. pneumoniae*

Abbreviations: IL-27, interleukin-27; LRG1, leucine-rich alpha-2 glycoprotein; SAA, serum amyloid A; sCD25, soluble interleukin-2 alpha chain receptor; STAT3, signal transducer and activator of transcription 3; THBS1, thrombospondin 1.

IL-27 concentrations in critically ill children with sepsis and significantly lower IL-27 concentrations in patients with SIRS demonstrating that IL-27 predicted infection with a specificity and a positive predictive value of > 90% in critically ill patients. The overall predictive ability of IL-27 was generally better than that of procalcitonin (PCT). The combination of IL-27 and PCT could further improve the overall predictive ability compared with the two biomarkers alone[63] (Table 3).

BIOMARKERS SCREENED BY METABOLOMICS

Metabolomics provides a qualitative and quantitative analysis of all metabolites produced by cells during a specific physiological period, and a comprehensive and systematic study of metabolites in patients to monitor disease dynamics.[82] Metabolite biomarkers can be divided into targeted metabolomics and untargeted metabolomics according to the research purpose. The study of untargeted metabolomics is a global molecular profiling technology, in which nuclear magnetic resonance and gas chromatography-tandem MS are used to analyze all metabolites in biological samples as comprehensively as possible.[83] Targeted metabolomics is the precise qualitative and quantitative analysis of specific target metabolites, mainly studying metabolite groups with specific structural and functional types, such as fatty acids, phospholipids, purine pyrimidines, or amino acids. As a specific model, metabolomics can be used to quantitatively analyze specific metabolite groups according to the research results of non-target metabolomics to make up for the shortcomings of non-target metabolomics.[84]

Mickiewicz et al.[85] investigated specific biological substances that could distinguish sepsis from SIRS in children of different age groups by untargeted metabolomics. The metabolites such as 2-hydroxybutyrate, 2-hydroxyisovalerate, lactate, glucose, ketone bodies, creatine, creatinine, and phenylalanine, can be used as biomarkers for the diagnosis of sepsis shock and its mortality in PICU. These changes in metabolites which were involved in the processes of fatty acid decomposition enhancement, ketoacidosis and amino acid metabolism disorders, hepatic glycogen catabolism, and the destruction of glycerol phospholipid and sulfur metabolism, have been found to contribute to pediatric sepsis. Furthermore, Mickiewicz et al.[86,87] also studied the metabolomics of septic children aged 2–17 years and 1–23 months via targeted metabolomics and found seven metabolites (dimethylamine, mannose, 3-methyl-2-oxovalerate, 3-hydroxyisovalerate, alanine, O-Acetylcholine, and acetate) could distinguish the acute and critical severity of the disease in children with severe sepsis. However, there is no pathogen information on sepsis patients in these two studies.

Wildman et al.[24] reviewed the above studies at the metabolic level in children with sepsis and found that three metabolites (3-hydroxybutyrate, glucose, and acetone) overlapped in these findings, suggesting that these three metabolites may be promising biomarkers targets for further clinical validation.

Grauslys et al.[88] found that in bacterial and viral infection samples, the level of metabolites including isoleucine, urea, creatinine, 2-hydroxyisovalerate, tyrosine, valine, creatine, phosphate, and histidine showed significant changes between the sepsis and control group. Li et al.[89] screened for differentially expressed metabolites of different pathogenic infections in childhood sepsis. The predominant pathogens in the study cohort were *Pseudomonas aeruginosa*, *Candida albicans*, and *Staphylococcus aureus*. They identified nine metabolites including 1-oleoyl-L-alpha-lysophosphatidic acid, cholic acid, hypoxanthine, indoxyl sulfate, isovalerylglycine, histidine, PC (P-16:0/18:1), PI (16:0/18:3) and pregnenolone

TABLE 4 Biomarkers that were identified using metabolomics for the diagnosis, triage, or prognosis of pediatric sepsis

Authors	Sepsis marker	No. of patients	No. of controls	Age	Sample	Sepsis stage	Pathogen
Mickiewicz et al.[85]	2-hydroxybutyrate, 2-hydroxyisovalerate, lactate, glucose, 2-oxoisocaproate, creatine, creatine phosphate, creatinine, histidine, phenylalanine, arginine	60	SIRS: 40; Healthy: 40	3 age groups (Infants: 1 month–1 year, Toddlers: 2–5 years, School age: 6–11 years)	Serum	Septic shock	Gram-positive, Gram-negative bacteria, Polymicrobial infection
Mickiewicz et al.[86]	Mannose, propylene glycol, dimethylamine, 2-hydroxyisovalerate, 3-methyl-2-oxovalerate, 2-oxoisocaproate, 2-hydroxybutyrate, 3-hydroxyisovalerate, choline, alanine, dimethyl sulfone, O-acetylcholine, A2M, taurine, SAA, TRAIL, acetate	PICU: 94 Emergency: 81	63	2–17 years	Serum, plasma	Sepsis	–
Mickiewicz et al.[87]	Dimethylamine, mannose, 3-methyl-2-oxovalerate, 3-hydroxyisovalerate, alanine, O-acetylcholine, acetate	PICU: 46 Emergency: 58	19	1–23 months	Serum, plasma	Sepsis	–
Grauslys et al.[88]	Isoleucine, urea, creatinine, 2-hydroxyisovalerate, tyrosine, valine, creatine, phosphate, histidine	Bacterial infection: 25; Viral infection: 30	58	0–16 years	Plasma	Sepsis	Bacterial, viral infection
Li et al.[89]	1-oleoyl-L-alpha-lysophosphatidic acid, cholic acid, hypoxanthine, indoxyl sulfate, isovalerylglycine, histidine, PC (P-16:0/18:1), PI (16:0/18:3), pregnenolone sulfate	84	59	15 days–13 years	Plasma	Sepsis	Top 3 pathogens: *P. aeruginosa*, *C. albicans*, *S. aureus*

Abbreviations: A2M, α-macroglobulin; PC, phosphatidylcholine; PI, phosphatidylinositol; PICU, pediatric intensive care unit; SAA, serum amyloid A; SIRS, systemic inflammatory response syndrome; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

sulfate. Among them, serum cholic acid, isovalerylglycine, and histidine levels were increased in sepsis survivors of *Staphylococcus aureus* infection. When sepsis survivors were compared with the non-survivor group, they found that two carnitine esters (palmitoylcarnitine and acetylcarnitine) and amino acids (glutamate, tyrosine, tryptophan, methionine and proline) as well as acetylneuraminate and N2, N2-dimethylguanosine displayed elevated levels in the non-survivors. These changes in fatty acids indicated that oxidative stress may be involved in the pathogenesis of sepsis, at least in the severe subgroup[89] (Table 4).

DISCUSSION

The representative biomarkers used in the clinical diagnosis include C-reactive protein (CRP), PCT, and lactate. Although CRP and PCT are the most used markers, which can be rapidly detected by using blood samples at low cost, they have limited sensitivity and specificity for sepsis diagnosis. Infections of certain pathogens like viruses and other non-infection factors, such as surgery and immunotherapy could also influence CRP and PCT levels.[90,91] Besides, PCT performance varies depending on the age of the patient population.[63] A meta-analysis has been performed to study the accuracy of PCT in diagnosing sepsis in neonates and children and found that there were very limited studies in children for the derivation of PCT cut-off values due to the large age span.[92]

Biomarkers screened by genomics reflect the influence of altered genetic information on the onset and progression of sepsis. For example, mutations in genes *CHD7* and *SAMD9* will increase the susceptibility to sepsis in children; mutations in *PAI-1* 4G/4G and *BPI* Taq could be used for prognosis markers of sepsis. However, the limitation is that these genomic biomarkers still require further validation in clinical cohort studies, afterwards the validated biomarkers can be detected by using conventional PCR, which requires less time and cost than whole genome sequencing.

Similar to CRP and PCT, transcript biomarkers were also detected at the mRNA level by using peripheral blood. These markers can be used to diagnose sepsis or reflect the severity and prognosis of sepsis, such as miRNA-146-a, which is both a potential diagnostic marker and a predictor for sepsis severity. A subset of markers have been studied in clinical cohorts to validate the sensitivity and specificity of these markers for the diagnosis or prognosis of sepsis, such as miRNA-146-a, *CDC20*, *LCN2*, IL-27, among which IL-27 was shown to be superior to PCT in predicting infection in the study cohort of severe sepsis, and its combination with PCT was shown to have a greater overall ability to predict infection in patients than each biomarker alone.[63] The current limitation is that most of these biomarkers still require more clinical validation. Then, the validated markers can be easily measured by using Real-Time PCR in the laboratory.

Enzyme-linked immunosorbent assay (ELISA) and MS assays allow to detection of protein markers. Protein biomarkers such as haptoglobin, THB1, and SAA-1 have been studied in clinical cohorts. IL-27 is a relatively clear biomarker capable of combining the transcriptome and proteome. The current limitation of these biomarkers to be applied to clinics is that there are no pediatric studies comparing the effect of these markers in the diagnosis of sepsis with the clinical biomarkers, CRP and PCT, which require further studies. Finding more biomarkers like IL-27, which can be consistently detected at different levels is an effective way to improve the sensitivity and specificity of diagnostic markers for sepsis. At present, the method for the detection of protein biomarkers, such as ELISA is relatively mature with low-cost. However, in the development of new biomarkers, new antibodies may need to be designed and generated, which requires more research and development.

There are fewer studies of sepsis metabolite markers in comparison with the other three species of biomarker. Lactate, a current clinical sepsis marker, is mainly associated with increased energy requirement in infectious and inflammatory states, leading to lactic acidosis in patients with septic shock.[93,94] However, there are multiple other physiologic and pathologic factors that can also influence lactate levels.[95] Besides, the levels of some metabolites are inherently variable depending on the age of children. In this review, the levels of certain metabolites were significantly elevated in the severe sepsis cohort independent of age, such as 2-hydroxybutyrate and 2-hydroxyisovalerate, suggesting that these metabolites have great potential to become diagnostic markers of sepsis. The limitation is that most current metabolic biomarkers have been screened by using the patient samples with severe sepsis. The metabolite biomarkers in the differentiation of sepsis and SIRS still require further investigation. Besides, the detection of metabolites with special structures may require more research investment before the application in clinical diagnosis.

Many of the human studies in this review had small sample sizes and were exploratory though, they have provided some clues of new biomarkers, which are worthy to be further evaluated by using a large number of patient samples in the future. Another limitation of these studies is that most of the pathogens they discussed are bacteria, which is likely due to the fact that the virus is not easy to detect in the blood. However, the host inflammatory responses to different groups of pathogens, such as bacteria or viruses, are usually different. Therefore, we emphasized the importance of infectious agents grouping in the screening of sepsis biomarkers, which may provide clues to help the researchers in the field for precision identification of pediatric sepsis markers, and better improve the clinical execution of personalized medicine for pediatric sepsis.

With the development of state-of-the-art techniques, the research on biomarker screening by using multi-level omics showed increasing growth. In hospitals, such as PICUs, continuous monitoring and treatment of pediatric patients have generated tons of dynamic data. The integration and analysis of massive data has become a new research direction for scientists to continuously explore the mechanisms of disease progression and biomarker selection. Artificial intelligence and deep learning have great advantages in data processing. Taking advantage of these new technologies, it will not only improve data utilization but also optimize the results of multi-omics, which will promote the identification of sepsis biomarkers. However, AI and machine learning still require more training and practice in the future to generate data more precisely.

CONCLUSION

There is no identified single biomarker with sufficient sensitivity and specificity to serve as the gold standard for the diagnosis of sepsis to date. With the increasing application of state-of-the-art techniques to the discovery of diagnosis markers, more and more new biomarkers in pediatric sepsis have been identified. However, most of these biomarkers have not been extensively validated and their diagnostic utility still requires further evaluation by prospective clinical trials. It is most likely not a single biomarker but a panel of genes may be promising for the diagnosis of sepsis. Taken together with the pathogen identification, panels of biomarkers will better improve the early diagnosis, treatment, and prognosis of sepsis for precision medicine. Therefore, further studies to find a combination of panel markers for pediatric sepsis caused by a certain group of infectious agents are needed in the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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