Diagnostic Challenges and Laboratory Considerations for Pediatric Sepsis

Khushbu Patel[1,2*] and Erin McElvania[3]

Background: Sepsis is a leading cause of death for children in the US and worldwide. There is a lack of consensus how sepsis is clinically defined, and sepsis definitions and diagnostic guidelines for the pediatric population have remained unchanged for more than a decade now. Current pediatric definitions are largely based on adult guidelines and expert opinion rather than evidence based on outcomes in the pediatric populations. Without a clear definition of sepsis, it is challenging to evaluate the performance of new laboratory tests on the diagnosis and management of sepsis.

Content: This review provides an overview of common etiologies of sepsis in pediatric populations, challenges in defining and diagnosing pediatric sepsis, and current laboratory tests used to identify and monitor sepsis. Strengths and limitations of emerging diagnostic strategies will also be discussed.

Summary: Currently there is no single biomarker that can accurately diagnose or predict sepsis. Current biomarkers such as C-reactive protein and lactate are neither sensitive nor specific for diagnosing sepsis. New biomarkers and rapid pathogen identification assays are much needed. Procalcitonin, although having some limitations, has emerged as a biomarker with demonstrated utility in management of sepsis in adults. Parallel studies analyzing the utility of procalcitonin in pediatric populations are lagging but have shown potential to affect sepsis care in pediatric populations. Multibiomarker approaches and stepwise algorithms show promise in the management of pediatric sepsis. However, a major hurdle is the lack of validated clinical criteria for classification of pediatric sepsis, which is necessary for the development of well-designed studies that can assess the clinical impact of these emerging biomarkers.

IMPACT STATEMENT

In the past 2 decades, declines in mortality rates owing to pediatric sepsis have staggered, and sepsis remains a major cause of mortality in the pediatric population. Updated guidelines and definitions for the diagnosis and management of pediatric sepsis are much needed to meaningfully assess the impact of new biomarkers and technologies in the field.

[1]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX; [2]Department of Pathology and Laboratory Medicine, Children's Health, Dallas, TX; [3]Department of Pathology, NorthShore University HealthSystem, Evanston, IL.
***Address correspondence to this author at:** UT Southwestern Medical Center, 1935 Medical District Dr., Mailstop B1.06, Dallas, TX 75235. E-mail khushbu.patel@utsouthwestern.edu.
DOI: 10.1373/jalm.2017.025908

[4] **Nonstandard abbreviations:** Sepsis-3, Third International Consensus Definitions for Sepsis and Septic Shock; SIRS, systemic inflammatory response syndrome; IL, interleukin; CRP, C-reactive protein; PCT, procalcitonin; SBI, serious bacterial infection.

According to the CDC 2017 National Vital Statistics Report, sepsis is a leading cause of death in children <9 years of age and a significant cause of morbidity and mortality for children of all age-groups (*1*). Pediatric sepsis results in approximately 80 000 hospitalizations and 7000 deaths per year in the US (*2*). The terms "sepsis," "severe sepsis," and "septic shock" represent a disease continuum with severe sepsis being defined as sepsis with progressive organ dysfunction and septic shock being defined as severe sepsis with persistent cardiovascular dysfunction (Fig. 1). In 2016, the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[4] were introduced. Although the Sepsis-3 task force acknowledged the need for pediatric-specific definitions, these new definitions currently apply only to adult populations (*3*).

Risk of death increases with increasing sepsis severity (*4*). In 1966, the mortality rate for pediatric septic shock was 97% (*5*). This rate was reduced to approximately 9% by the early 1990s and is currently around 8.2% (*6*). Half of all pediatric sepsis occurs in patients with underlying comorbidities, with one-third of all pediatric sepsis occurring in low-birth-weight neonates (*7*). Although there has been a concentrated effort in recent years for early recognition of sepsis with an aim to decrease pediatric sepsis-related mortality, the reduction in the mortality rate has been small. For immunocompromised children, this is likely because of 2 factors. The first is a concurrent increase in the

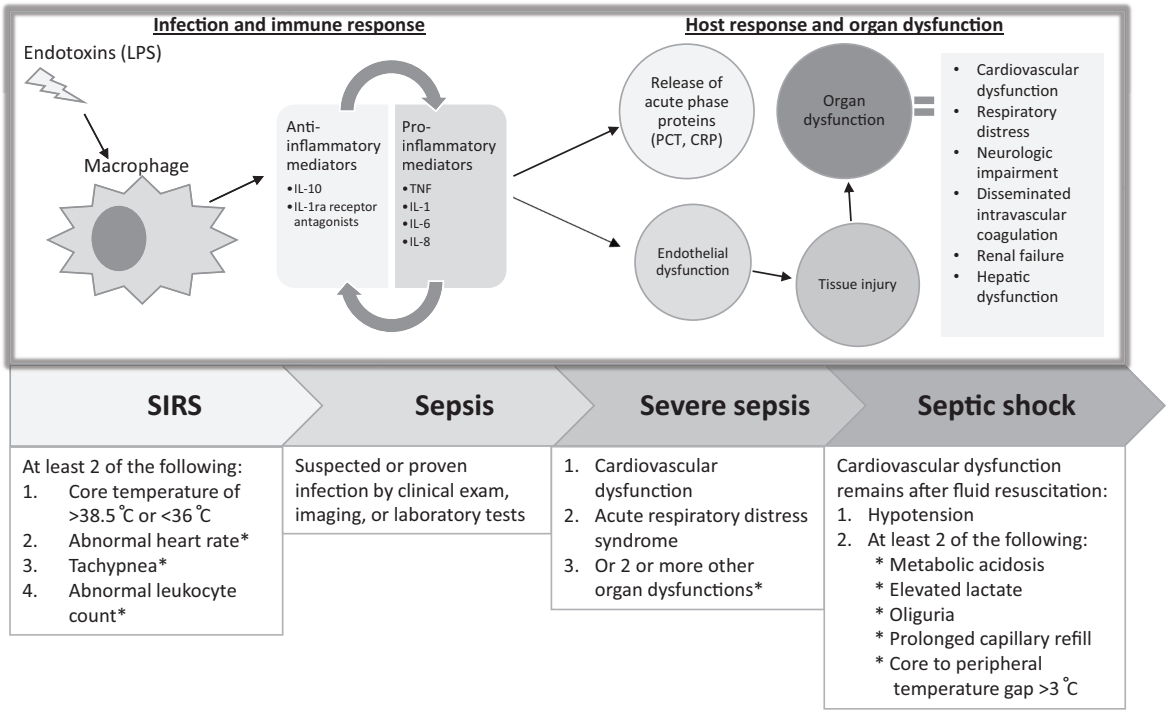

Fig. 1. Schematic depicting sepsis continuum.
Pathogenesis of sepsis is illustrated above, and definitions and criteria for SIRS, sepsis, severe sepsis and septic shock are listed below. *Organ dysfunction and laboratory metrics that require age-specific cutoffs. Specific age-based cutoffs can be found in Mathias et al. (*4*). LPS, lipopolysaccharide; TNF, tumor necrosis factor.

number of indwelling catheters and medical devices used in pediatric patients, which are a nidus for bacterial biofilms that may go on to cause sepsis. Second, an increasing number of immunosuppressive therapies are being used in the pediatric population for long periods, including immunobiologic therapies that suppress cytokine function and cancer chemotherapy agents that alter or deplete the body's immune cells. These immunomodular therapies have been successful in suppressing chronic inflammatory disease symptoms and prolonging the lives of children with cancer, but these agents also put patients at increased risk for the development of sepsis. There is still much work to do, as unrecognized pediatric sepsis is a preventable cause of mortality. In this review, we will discuss the role of laboratory testing in the management of pediatric sepsis, as well as the utility of emerging biomarkers in both the diagnosis and management of sepsis.

PEDIATRIC SEPSIS DEFINITIONS

It is well established that prompt recognition and initiation of broad-spectrum antibiotics dramatically increases the survival rate in all populations (*8*). Initial clinical presentation of sepsis in children, which includes fever, tachycardia, tachypnea, hypotension, and hypothermia, is highly variable, nonspecific, and is often missed. The most striking feature of pediatric sepsis is that children can sustain tachycardia for prolonged periods, which means hypotension may not present until much later in the sepsis continuum compared with adults with sepsis. Therefore, pediatric sepsis definitions are aimed at identifying compensated septic shock. Currently the pediatric community relies on the 2005 recommendations set forth by the International Pediatric Consensus Conference for diagnostic criteria (*9*). Pediatric severe sepsis is defined as ≥2 systemic inflammatory response syndrome (SIRS) criteria, confirmed or suspected invasive infection, and cardiovascular, acute respiratory distress syndrome or ≥2 organ dysfunctions (Fig. 1). These definitions were modified from consensus definitions created for adult patients in 1992 (*10*), with the major difference being age-specific cutoffs for physiologic and organ dysfunction laboratory parameters.

One of the major criticisms of pediatric sepsis definitions is that they are adapted from adult criteria and are based on expert consensus, but they have not been clinically validated in the pediatric population. Furthermore, their impact on clinical outcomes is unknown (*11*). In both adults and children, the SIRS criteria have been criticized for their broader inclusion of mild symptoms and lack of specificity (*12*, *13*). On the other hand, these definitions may also miss patients with infection and single-organ failure with a high mortality risk (*14*, *15*). A subanalysis of a recent epidemiological study, the Sepsis Prevalence, Outcomes, and Therapies (SPROUT) study of approximately 7000 pediatric intensive care unit patients with sepsis found only a 42% consensus between the 2005 definitions and physician diagnosis, demonstrating that the SIRS criteria are not effective in identifying patients with sepsis or those at risk of escalating to septic shock (*16*).

To address some of the limitations of previous sepsis definitions, the Adult Sepsis Definition Taskforce introduced the Sequential Organ Failure Assessment (SOFA) score to the new Sepsis-3 definition. The new adult sepsis definitions have moved away from using SIRS criteria and now redefine sepsis as life-threatening organ dysfunction caused by a dysregulated host response to systemic infection. Further, the new definitions removed the term severe sepsis, recognizing that the >10% mortality rate associated with sepsis makes the condition already severe. The purpose of this addition was to identify patients at high risk for progression to sepsis and to specify sepsis as the presence of life-threatening organ dysfunction with infection as opposed to an uncomplicated infection. The new scoring criteria were developed

and validated using a large cohort of >1 million adult patients (*17*). Although Sepsis-3 definitions were not validated separately for pediatric populations, recent studies have demonstrated the feasibility of developing pediatric SOFA (pSOFA) and Pediatric Logistic Organ Dysfunction-2 (PELOD-2) scores for use in pediatric patients (*18*, *19*).

A fundamental challenge in creating consensus definitions for the pediatric populations is the degree of age stratification required for various organ dysfunction and laboratory parameters. Further studies validating the utility of the pSOFA and PELOD-2 scores in pediatric populations are in motion, and it is hoped they will pave the way for the much-needed update in pediatric consensus definitions (*19*, *20*). Definitions for sepsis have important implications in assessing the impact of novel diagnostic tools on patient outcomes. Well-validated consensus definitions that can accurately diagnose and depict a patient's position on the sepsis continuum with high specificity are instrumental in designing studies for new diagnostics and incorporating new findings into clinical practice. Sepsis is mainly a clinical diagnosis often made before culture results are available. Although laboratory testing is used to support criteria for organ dysfunction, current sepsis guidelines do not incorporate any laboratory biomarkers, apart from lactate, into their criteria. Use of inflammatory biomarkers such as acute phase proteins and cytokines for the diagnosis, management, and prognosis of sepsis has been an active area of research.

BACTERIAL AGENTS OF SEPSIS BY AGE

Bacteria are the most common microorganism causing sepsis in children. Toxins such as endotoxin and lipopolysaccharide found on the outer cell membrane of gram-negative bacteria or secreted exotoxins and enterotoxins result in many symptoms associated with sepsis. Toxins can act as super antigens resulting in a massive activation of the inflammatory system, cause destruction of host cells, and allow dissemination of bacteria to distant body sites. Although the complete pathophysiology of sepsis is still not completely understood, this dysregulation of the immune response can lead to the organ dysfunction seen in severe sepsis and progress to circulatory, cellular, and metabolic dysfunction, which are characteristics of septic shock (Fig. 1). Next, we will discuss the characteristics of sepsis by pediatric age-group, as well as current and future perspectives of laboratory testing for the diagnosis and management of sepsis.

Pediatric sepsis

The most common comorbidities associated with pediatric sepsis (>1 year of age) are neuromuscular, hematologic, immunologic, and neoplastic in nature. Respiratory and primary bacteremia are the most common sites of infection for both immunocompetent and immunocompromised children (*21*, *22*). In otherwise healthy children, sepsis is commonly caused by the gram-positive organisms *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. Common gram-negative agents of sepsis are *Neisseria meningitidis*, *Escherichia coli*, and *Salmonella* spp. Tick-borne pathogens such as *Ehrlichia* and *Rickettsia* spp. can also mimic sepsis-like symptoms. In the past, *S. pneumoniae* and *Hemophilus influenzae* were prominent causes of pediatric sepsis in the US. Vaccination has decreased the amount of invasive disease caused by *S. pneumoniae* by 75% and *H. influenzae* type b to just a few cases per year for children <5 years of age (*7*).

Immunocompromised pediatric patients are at much greater risk for developing sepsis than otherwise healthy children. In addition to the sepsis-causing pathogens above, immunocompromised children are also at risk of developing sepsis from additional pathogens based on the presence of indwelling hardware and the nature of their compromised immune systems. Patients with indwelling catheters are at risk for sepsis caused by skin

flora such as coagulase-negative staphylococci and *Candida* spp. Neutropenic patients with mucositis can develop sepsis owing to gram-negative enterics, *Pseudomonas aeruginosa*, and viridans group streptococci. Patients with functional or anatomic asplenia, such as sickle cell, are at greater risk for developing sepsis from encapsulated organisms such as *S. pneumoniae*, *H. influenzae* type b, *N. meningitidis*, and *Salmonella* spp. Finally, pediatric patients with HIV are at higher risk of developing sepsis from *S. pneumoniae*, *S. aureus*, *P. aeruginosa*, and *H. influenzae* type b (*7*).

Neonatal and infant sepsis

The prevalence of severe sepsis is highest during the neonatal and infant period, with nearly half of all pediatric cases occurring in children <1 year of age (*21*, *23*, *24*). Two-thirds of those who develop severe sepsis in this age-group are children classified as low birth weight (born weighing <2500 g) or very low birth weight (born weighing <1500 g). The most common comorbidities in this group are neuromuscular, cardiovascular, and respiratory diseases, with the respiratory tract and primary bacteremia identified as the most common site of infection for these children (*7*). Among premature and low-birth-weight children, the most common pathogens causing severe sepsis are coagulase-negative *Staphylococcus* spp., *S. aureus*, *Candida* spp., and, less frequently, *P. aeruginosa* and enteric gram-negative bacteria (*7*, *21*, *25*). These organisms reflect the high number of indwelling catheters, surgical procedures, comorbidities, and immunocompromised states of these children compared with the general population. Viral sepsis can clinically mimic bacterial sepsis in the neonatal age-group, with the most common pathogens consisting of herpes simplex virus, enterovirus, respiratory syncytial virus, and influenza virus (*7*). For otherwise healthy children, the most common microbial pathogens causing sepsis in the first 7 days of life are *Streptococcus agalactiae* (group B streptococci) and *E. coli*, which are acquired from the mother during birth (*7*, *26*). Late-onset neonatal sepsis, acquired between 7 and 28 days of life, is most likely to be caused by *S. agalactiae.* In this age range, 70% of sepsis is caused by gram-positive organisms and 30% is caused by gram-negative organisms such as enterics and *P. aeruginosa*.

LABORATORY TESTING FOR PEDIATRIC SEPSIS

There is no single laboratory test that can accurately diagnose sepsis or assess the severity of sepsis. An ideal laboratory marker would be one that can distinguish bacterial, viral, and noninfectious sources of inflammation, allowing for prompt initiating of broad-spectrum antibiotic therapy for bacterial sepsis.

Diagnostic markers used for detecting sepsis exploit the current knowledge of pathogenesis and mechanisms of sepsis (Fig. 1). Sepsis development begins after immune recognition of an invading organism, which stimulates the release of both proinflammatory and antiinflammatory cytokines. Several studies have shown that changes in the levels of cytokines are observed before those of acute phase proteins. Commonly studied cytokines include interleukin (IL)-6, IL-10, IL-1β, and tumor necrosis factor-α (*27–29*). However, the performance of these biomarkers is variable and has not been proven to be superior to clinically available testing of C-reactive protein (CRP) or procalcitonin (PCT). Furthermore, lack of automation and the cost to run cytokine profiles have limited its use in routine clinical practice. CRP, a positive acute phase reactant, is the best-known biomarker for sepsis and often is used along with blood culture to benchmark performance of contemporary biomarkers (*30*, *31*). A major limitation of CRP is that it lacks specificity for bacterial infections and sensitivity to detect sepsis in its early stages. Blood lactate is currently used to determine sepsis severity and is an indicator of organ dysfunction and metabolic abnormality in patients with septic

shock. Additionally, serial measurements are useful in monitoring treatment; however lactate concentrations do not increase till much later in the sepsis continuum.

Blood culture

Blood culture is considered the gold standard for diagnosis; however, it has limitations, including low sensitivity of pathogen detection and prolonged turnaround time. Although most sepsis is caused by bacteria, a bacterial pathogen is isolated from blood in only one-third of sepsis cases, and a causative bacterial agent is isolated from any body site in fewer than half of all sepsis cases (*3*). For this reason, positive blood culture is not required to meet the sepsis criteria for either adults or children, but it remains the gold standard for diagnosing sepsis (*32*). Despite the difficulty, isolation of a bacterial pathogen is extremely useful for tailoring antimicrobial therapy and determining duration of treatment; therefore, optimal blood culture collection should be followed whenever possible to increase the chances of isolating the causative agent of sepsis.

Although many factors go into an optimal blood culture collection, the most important by far is submitting an adequate volume of blood for culture. Bacteremia patients generally have <5 cfu of bacteria per milliliter of blood (*33*). Two studies by Kellogg et al. found that low-level bacteremia, <1 cfu/mL, was responsible for 71% and 75% of pediatric deaths owing to sepsis (*34*, *35*). With so few bacteria per milliliter of blood, we can see how easily bacteremia can be missed if a small volume is sent for culture. In adults, it is recommended that 20 to 30 mL of blood be drawn from 2 peripheral venipunctures to achieve adequate volume for culture. Blood from each site should be cultured under both aerobic and anaerobic conditions, and ideally blood will be drawn before antibiotics are administered. Obtaining blood through a catheter is not recommended because it is more likely to be contaminated than a venipuncture. It is worth mentioning that to document catheter-associated bacteremia, a catheter tip can be submitted along with a venipuncture. It has been well documented in adults and children that there is an increased yield of blood pathogen detection for each additional milliliter of blood that is submitted for culture (*36–38*). Obtaining an acceptable volume of blood from pediatric patients can be a challenge because before reaching maturity, or generally around 80 pounds of body weight, pediatric patients have lower total blood volumes compared with adults. Although the literature agrees that the volume is the single most important factor in pathogen recovery, there is no consensus on the amount of blood that should optimally be submitted from pediatric patients. Both age- and weight-based recommendations for pediatric blood culture volume have been developed (*39–42*). The most recent guidelines from the Infectious Diseases Society of America recommend weight-based criteria to determine the volume of blood that can be safely drawn from pediatric patients (*43*), with reduced volumes for patients weighing <80 pounds. When <10 mL of blood is cultured, blood should be placed in a single aerobic bottle rather than split between an aerobic and anaerobic bottle set. Studies to determine the maximum amount of blood that can be safely drawn from pediatric patients within a 24-h period were performed on healthy research participants; therefore, they may not accurately reflect the amount of blood that can be collected from an ill patient. Also, the blood volume must cover all laboratory testing, not just blood culture, and the amount of testing can be high for critically ill patients.

Rapid diagnostic testing for blood culture

Recognition of sepsis and prompt treatment are essential for favorable patient outcomes. Guidelines recommend treatment be started within 1 h of suspicion of sepsis. Empiric therapy is chosen based on the patient's medical history, immunologic state, and organisms commonly known to

Table 1. BRAHMS PCT assays.

Instrument platform	Assay principle	Analytical measurement range, ng/mL	Limit of detection, ng/mL	Total imprecision (% CV) near 0.5 ng/mL
Brahms Kryptor	Homogenous immunoassay using time-resolved amplified cryptate emission	0.02–5000	0.02	≤6%
ADVIA Centaur	One-step chemiluminescent sandwich immunoassay	0.02–75	<0.02	<8%
Architect	Chemiluminescent microparticle immunoassay	0.02–100		2.10%
Roche Elecsys e411	Electrochemiluminescent immunoassay	0.02–100	≤0.02	4.20%
Roche e600	Electrochemiluminescent immunoassay	0.02–100	≤0.02	2.60%
Diasorin Liason	Two-step chemiluminescent immunoassay	0.02–100	0.02	<10%
BioMeriux VIDAS	One-step sandwich immunoassay	0.05–200	0.03	7.86%

Analytical metrics are based on manufacturer-provided information (*49*).

cause sepsis in the patient's age-group. In recent years, molecular multiplex assays for rapid identification of bacteria and yeast from positive blood culture broth have become widely adopted. These assays require minimal hands-on time and are technically simple to perform, are random access, and have a run time of 1 to 2.5 h (*44*). In addition to rapidly identifying the most common blood culture pathogens, several of these assays detect key antimicrobial resistance in 1 to 2.5 h, with 1 assay able to provide full phenotypic antimicrobial susceptibility results in 7 h (*45*). In conjunction with antimicrobial stewardship programs, these assays are used to place patients with sepsis on optimal antimicrobial treatment more quickly, with susceptibility results available 24 to 72 h earlier than using traditional methods (*46*). This has been shown to reduce hospital length of stay and decrease overall hospital costs, therefore improving patient outcomes for critically ill patients with sepsis (*47*, *48*). These rapid assays all require positive blood culture broth, so they cannot meet the 1-h cutoff for starting antibiotic treatment in suspected septic patients. Therefore, there is a need to rely on host serum markers of infection.

PCT

In 2016, the Food and Drug Administration expanded the use of PCT in the management of antibiotic treatment for lower respiratory tract infections and sepsis. Prior approval in 2008 was granted for use of PCT in assessment of mortality risk in septic patients. The Brahms PCT assay is now cleared by the Food and Drug Administration on all major manufacturer platforms (Table 1), making it readily available to most hospital laboratories looking to offer PCT with a rapid turnaround time (*49*). So far, no guidance for pediatric use has been issued; however, many studies examining the performance of PCT for similar indications in pediatric populations are emerging.

PCT is a 116-amino acid precursor peptide of calcitonin produced by the CALC-1 gene on chromosome 11. Under normal physiological conditions, PCT is produced only by the C cells of the thyroid gland, and circulating concentrations are low (<0.05 ng/mL). During a proinflammatory response, CALC-1 induction in parenchymal cells results in a rapid increase in circulating PCT (*50*). Serum PCT >0.5 ng/mL is indicative of systemic

Adult PCT cutpoints, ng/mL	
Healthy individuals	<0.05
Local infections	<0.5
Systemic infection/sepsis	≥0.5 to <2
Severe sepsis	≥2 to <10
Septic shock	≥10

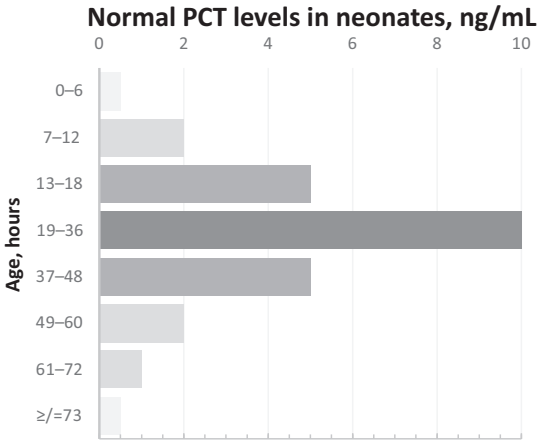

Fig. 2. Manufacturer-provided interpretative ranges for procalcitonin.
Manufacturer-provided interpretative ranges for procalcitonin (*45*) are listed in the table above, and normal ranges of procalcitonin in newborns based on age in hours are depicted below in the bar graph.

infection and possible sepsis (Fig. 2). Elevation of PCT usually occurs within 2 to 4 h after onset of inflammation, peaking at approximately 24 to 36 h. PCT has a serum half-life of 25 to 30 h, and concentrations fall rapidly upon resolution of inflammation. The magnitude and duration of PCT levels correlate with disease severity (*51*) and are generally low during viral infections compared with bacterial and fungal infections (*52*, *53*). Because of these characteristics and kinetics, PCT has been proposed to be a prognostic and diagnostic marker that can also be used for monitoring therapeutic response in sepsis. It is important to note the cutoffs listed in Fig. 2 are manufacturer-suggested ranges for orientation purposes and that optimal cutoffs are dependent on the characteristics of the patient population and need to be optimized for the desired clinical use. Additionally, these cutoffs may vary among different assay platforms (*49*). A single PCT result should always be interpreted in the context of the patient's clinical picture, as it can be increased after severe trauma and major surgery, as well as in patients with respiratory distress syndrome, hemodynamic failure, and acute kidney injury (*54*). PCT elevations are also seen in patients with non–small cell lung cancer and medullary thyroid carcinoma (*55*). Furthermore, PCT cannot be used to indicate etiology of infection or to tailor antibiotic therapy; thus, microbiologic data are still essential for management of sepsis.

The most critical consideration for laboratories implementing PCT assays in a pediatric setting is the need for age-specific reference intervals separated by hours of age. This is especially crucial for interpretation of PCT results within the first 72 h of life. In a study of 83 healthy newborns <48 h of age, Chiesa et al. reported that PCT values change hourly, with peak concentrations occurring at 24 h of life, and normalize after 48 to 72 h (*56*, *57*). The mechanism and cellular origin for increase in PCT during the immediate postnatal period is unknown but likely reflects a physiological mechanism rather than a stimulus resulting from infection or pathogen exposure.

Multiple studies assessing the use of PCT as a prognostic predictor in pediatric patients have shown higher PCT levels are associated with sepsis severity and increased risk of death (*58–60*). However, the use for PCT of most interest in pediatric emergency departments is its accuracy in ruling out infections that do not need prompt and aggressive treatment. Several studies have examined the role of PCT in distinguishing viral from bacterial sources of inflammation in pediatric patients. A metaanalysis of 8 studies including 616 pediatric patients showed that PCT can differentiate bacterial and viral meningitis with 96% sensitivity and

89% specificity (*61*). Another metaanalysis of 7260 children assessed the ability of PCT to detect both a broad spectrum of serious bacterial infections (SBIs) from bacterial meningitis to urinary tract infections and a subgroup of the most severe SBIs—invasive bacterial infections. This subgroup included only bacterial meningitis, sepsis, and bacteremia. This metaanalysis found that PCT sensitivity and specificity for detecting SBI (55% and 85%, respectively) was much lower compared with that for IBI (82% and 86%, respectively) using a threshold of 0.5 ng/mL. The negative predictive value of PCT was approximately 99% for invasive bacterial infection and ranged from 79.5% to 96.7% for SBI (*62*). Collectively, PCT seems to slightly outperform CRP in detecting bacterial infections.

The recent Food and Drug Administration clearance of the PCT assay to guide antibiotic use has tremendous implications for antimicrobial stewardship programs. However, outcome studies in pediatric populations for this use are limited. The largest multicenter randomized controlled study in neonates, the NeoPIns trial, evaluated the use of PCT-guided decision-making in reducing total antibiotic exposure without adverse outcomes (*63*). Although the study reported shorter antibiotic courses in the PCT arm, it was underpowered to determine its impact on reinfection or death. Furthermore, this trial included centers largely based in the European Union, where antibiotic-prescribing practices are different. In fact, 1 criticism of the NeoPIns trial is that the treatment courses in the control arm were unnecessarily long compared with standard treatment courses. It has been argued that neonatal intensive care units that have effective antibiotic stewardship programs may not achieve the same results (*64*). Similarly, in adults, several European trials have reported decreased usage of antibiotics with PCT-guided treatment of lower respiratory tract infections. In contrast, a recent study of 14 US hospitals that included 1656 adult patients showed that PCT-guided decision-making did not result in less use of antibiotics (*65*). More research measuring the impact of PCT on antibiotic usage in both adult and pediatric populations is needed.

One challenge implementing PCT diagnostic cutoffs and PCT-guided therapeutic decision-making into clinical practice based on published outcome studies is the use of different assay platforms and, in some cases, the lack of information on how PCT testing was performed. Differences in study design such as inclusion criteria and outcome measures make it difficult to compare findings from different studies. With the widespread availability of PCT assays in the US, we anticipate more studies assessing the role of PCT for its various applications to be further elucidated. Laboratories wanting to successfully implement PCT testing need to determine their own interpretative criteria and will likely require a coordinated effort involving clinical laboratories, infectious disease physicians, and pharmacists.

FUTURE PERSPECTIVES

Because of the heterogeneity of sepsis as a disease state and the populations it affects, there will unlikely be a single marker, "one size fits all" approach to diagnosis and management. To capture the disease heterogeneity, multibiomarker panels and step-by-step algorithms will likely be the future of sepsis diagnosis and management (*66*). In a recent multicenter study of 11 emergency departments in the European Union, Gomez et al. validated a diagnostic algorithm that included assessment of clinical features, age, PCT, CRP, and absolute neutrophil count (*67*). The goal of this algorithm was to determine whether febrile infants <90 days of age can be treated as outpatients without lumbar puncture or antibiotics. This step-by-step approach had a sensitivity and negative predictive value of 92% and 99.3%, respectively.

Multiple "-omics" approaches have also been applied for discovery of novel prognostic and diagnostic markers (*4*, *29*). Wong et al. developed a

multiplex mRNA quantification platform of 100 genes that could accurately classify patients with septic shock and potentially identify patients who will benefit from corticosteroid therapies (*68*). A few studies have analyzed serum and urine metabolites of septic patients (*69*–*72*). In sepsis-associated inflammation, glucose consumption through mitochondrial oxidative phosphorylation is shifted to the production of lactate and pentose phosphate pathway, leading to decreased ribitol, ribonic acid, and 2,3,4-trihydroxybutyric acid, as well as increased glucose, lactate, and ketones. A combination of metabolic profiling and inflammatory mediator profiling has been attempted to identify pediatric patients with or without sepsis (*73*).

The holy grail of blood culture pathogen identification is the ability to detect pathogens directly from patient blood. For traditional blood culture, blood collected from a patient is injected into broth media and incubated in an automated instrument to allow for biological replication until the bacteria or yeast grows to a concentration that can be detected by the instrument, a process taking from 9 h for patients with high numbers of organism in their bloodstream to 3 to 5 days for slowly growing organisms. Direct detection of bacteria and yeast from blood has been accomplished with the T2Bacteria Panel and T2Candida Panel (*74*, *75*). The T2Bacteria Panel detects 5 of the 6 bacteria known as ESKAPE organisms (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *P. aeruginosa*, and *E. coli*) in a matter of hours after blood culture collection. The ESKAPE pathogens are the leading cause of nosocomial infections and often contain mechanisms of antimicrobial resistance. Unfortunately, most bacteria causing pediatric sepsis are not represented on the panel. The T2Bacteria and T2Candida panels require an additional blood volume of 3 to 4 mL for testing, and because of the limited scope of the microbial pathogens detected, the T2 assays do not replace traditional bacterial culture. Another company, Karius, performs next-generation sequencing on plasma for detection of microbial cell-free DNA (*76*). Their assay can identify a broad spectrum of >1000 bacteria, fungi, DNA viruses, and parasites directly from blood, and the assay can identify multiple organisms from a single blood specimen in the case of polymicrobial bacteremia. Like the T2 assays, Karius requires an additional blood volume of 700 µL of plasma beyond culture. All testing is centralized at their California laboratory, so the turnaround time for results is not ideal for detection of routine bacterial pathogens. For this reason, this technology cannot replace traditional bacterial blood culture currently. Benefits of direct detection of bloodstream pathogens are the speed at which results can be available, if testing is available in-house, because of the elimination of the incubation period before blood culture broth signaling positive. Additionally, because these new molecular assays are not dependent on viable bacterial growth, they are useful for detection of pathogens that do not grow in culture. Furthermore, these techniques can also be used to detect pathogens after antimicrobial therapy has been initiated. The major downside of all molecular testing for bacteria is lack of an isolate for antimicrobial susceptibility testing to guide therapy. More research on this topic is in progress, and we look forward to a day when blood culture pathogens can be detected and identified in a timeframe like chemistry analytes such as lactate and PCT.

CONCLUSION

Sepsis is a multifactorial disease, and better guidelines and diagnostics are needed for early recognition of this condition in the pediatric population. New biomarkers such as PCT show promise as a test that can help identify patients at increased risk of developing sepsis and can also aid in monitoring response to treatment. However, further studies measuring the impact of PCT on improved management and outcomes

are needed. Multibiomarker panels, combining host inflammatory and metabolic parameters, in conjunction with pathogen identification are a favorable approach, as they have potential to stratify patients by etiology of sepsis, as well as type and severity of organ dysfunction, and to identify tailored therapeutics. However, these approaches are still in their infancy. Further, the lack of a consensus definition of the disease will remain a major hurdle in the field; consensus is needed to create standardized criteria for study design across different study sites and for implementation of new findings into clinical practice. It is estimated that an interventional trial that would detect a 5% reduction in mortality would require >2000 participants and >58 pediatric intensive care units (*22*), indicating that large-scale studies are needed to truly demonstrate the clinical impact of these emerging technologies, whether it be reduction in mortality, hospital stay, and/or antibiotic use.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

REFERENCES

1. Heron M. Deaths: leading causes for 2015. National vital statistics report, Vol. 66, No. 5. Washington (DC): US Department of Health and Human Services; 2017.
2. Hartman ME, Linde-Zwirble WT, Angus DC, Watson RS. Trends in the epidemiology of pediatric severe sepsis. Pediatr Crit Care Med 2013;14:686–93.
3. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315:801.
4. Mathias B, Mira J, Larson SD. Pediatric sepsis. Curr Opin Pediatr 2016;28:380–7.
5. DuPont HL, Spink WW. Infections due to gram-negative organisms: an analysis of 860 patients with bacteremia at the University of Minnesota Medical Center, 1958–1966. Medicine (Baltimore) 1969;48:307–32.
6. Balamuth F, Weiss SL, Neuman MI, Scott H, Brady PW, Paul R, et al. Pediatric severe sepsis in U.S. children's hospitals. Pediatr Crit Care Med 2014;15:798–805.
7. Long SS, Prober CG, Fischer M, editors. Principles and practice of pediatric infectious diseases. 5th Ed. St. Louis (MO): Elsevier; 2017.
8. Han YY, Carcillo JA, Dragotta MA, Bills DM, Watson RS, Westerman ME, et al. Early reversal of pediatric-neonatal septic shock by community physicians is associated with improved outcome. Pediatrics 2003;112:793–9.
9. Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric Sepsis. International Pediatric Sepsis Consensus Conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med 2005;6:2–8.
10. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992;20:864–74.
11. Schlapbach LJ, Kissoon N. Defining pediatric sepsis. JAMA Pediatr 2018;172:312.
12. Schlapbach LJ, Javouhey E, Jansen NJG. Paediatric sepsis: old wine in new bottles? Intensive Care Med 2017;43:1686–9.
13. Churpek MM, Zadravecz FJ, Winslow C, Howell MD, Edelson DP. Incidence and prognostic value of the systemic inflammatory response syndrome and organ dysfunctions in ward patients. Am J Respir Crit Care Med 2015;192:958–64.
14. Kaukonen K-M, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. N Engl J Med 2015;372:1629–38.
15. Scott HF, Deakyne SJ, Woods JM, Bajaj L. The prevalence and diagnostic utility of systemic inflammatory response syndrome vital signs in a pediatric emergency department. Macy ML, editor. Acad Emerg Med 2015;22:381–9.
16. Weiss SL, Fitzgerald JC, Maffei FA, Kane JM, Rodriguez-Nunez A, Hsing DD, et al. Discordant identification of pediatric severe sepsis by research and clinical definitions in the SPROUT international point prevalence study for the SPROUT Study Investigators and Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network. Crit Care 2015;19:325.
17. Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al. Assessment of clinical criteria for sepsis. JAMA 2016;315:762.

18. Matics TJ, Sanchez-Pinto LN. Adaptation and Validation of a Pediatric Sequential Organ Failure Assessment Score and Evaluation of the Sepsis-3 Definitions in Critically Ill Children. JAMA Pediatr 2017;171:e172352.
19. Leclerc F, Duhamel A, Deken V, Grandbastien B, Leteurtre S, Groupe Francophone de Réanimation et Urgences Pédiatriques (GFRUP). Can the Pediatric Logistic Organ Dysfunction-2 score on day 1 be used in clinical criteria for sepsis in children? Pediatr Crit Care Med 2017;18:758–63.
20. Schlapbach LJ. Time for Sepsis-3 in children? Pediatr Crit Care Med 2017;18:805–6.
21. Watson RS, Carcillo JA, Linde-Zwirble WT, Clermont G, Lidicker J, Angus DC. The epidemiology of severe sepsis in children in the United States. Am J Respir Crit Care Med 2003;167:695–701.
22. Weiss SL, Fitzgerald JC, Pappachan J, Wheeler D, Jaramillo-Bustamante JC, Salloo A, et al. Global epidemiology of pediatric severe sepsis: the sepsis prevalence, outcomes, and therapies study. Am J Respir Crit Care Med 2015;191:1147–57.
23. Randolph AG, McCulloh RJ. Pediatric sepsis: important considerations for diagnosing and managing severe infections in infants, children, and adolescents. Virulence 2014;5:179–89.
24. Wheeler D, Wong HR, Zingarelli B. Children are not small adults! Open Inflamm J 2011;4:4–15.
25. Cohen-Wolkowiez M, Moran C, Benjamin DK, Cotten CM, Clark RH, Benjamin DK, et al. Early and late onset sepsis in late preterm infants. Pediatr Infect Dis J 2009;28: 1052–6.
26. Cortese F, Scicchitano P, Gesualdo M, Filaninno A, De Giorgi E, Schettini F, et al. Early and late infections in newborns: where do we stand? A review. Pediatr Neonatol 2016;57:265–73.
27. Chaudhry H, Zhou J, Zhong Y, Ali MM, McGuire F, Nagarkatti PS, et al. Role of cytokines as a double-edged sword in sepsis. In Vivo 2013;27:669–84.
28. Schulte W, Bernhagen J, Bucala R. Cytokines in sepsis: potent immunoregulators and potential therapeutic targets—an updated view. Mediators Inflamm 2013;2013:165974.
29. Delanghe JR, Speeckaert MM. Translational research and biomarkers in neonatal sepsis. Clin Chim Acta 2015;451: 46–64.
30. Lauc G, Essafi A, Huffman JE, Hayward C, Knežević A, Kattla JJ, et al. Genomics meets glycomics—The first GWAS study of human N-glycome identifies HNF1α as a master regulator of plasma protein fucosylation. Gibson G, editor. PLoS Genet 2010:6:e1001256.
31. Hofer N, Zacharias E, Müller W, Resch B. An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks. Neonatology 2012;102:25–36.
32. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003;348:1546–54.
33. Schelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM, Ascher DP. Volume of blood required to detect common neonatal pathogens. J Pediatr 1996;129:275–8.
34. Kellogg JA, Manzella JP, Bankert DA. Frequency of low-level bacteremia in children from birth to fifteen years of age. J Clin Microbiol 2000;38:2181–5.
35. Kellogg JA, Ferrentino FL, Goodstein MH, Liss J, Shapiro SL, Bankert DA. Frequency of low level bacteremia in infants from birth to two months of age. Pediatr Infect Dis J 1997;16:381–5.
36. Isaacman DJ, Karasic RB, Reynolds EA, Kost SI. Effect of number of blood cultures and volume of blood on detection of bacteremia in children. J Pediatr 1996;128: 190–5.
37. Mermel LA, Maki DG. Detection of bacteremia in adults: consequences of culturing an inadequate volume of blood. Ann Intern Med 1993;119:270–2.
38. Berkley JA, Lowe BS, Mwangi I, Williams T, Bauni E, Mwarumba S, et al. Bacteremia among children admitted to a rural hospital in Kenya. N Engl J Med 2005;352: 39–47.
39. M47-A principles and procedures for blood cultures: approved guideline. Wayne (PA): Clinical and Laboratory Standards Institute; 2007.
40. Baron EJ, Thomson RB. Specimen collection, transport, and processing: bacteriology. Man Clin Microbiol 2011; 228–71.
41. Baron EJ, Weinstein MP, Dunne WM, Yagupsky P, Welch DF. Cumitech 1C blood cultures IV. Baron EJ, editor. Washington (DC): ASM Press; 2005.
42. Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson RB, et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). Clin Infect Dis 2013;57: e22–121.
43. Miller JM, Binnicker MJ, Campbell S, Carroll KC, Chapin KC, Gilligan PH, et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. Clin Infect Dis 2018;67:e1–94.
44. Mestas J, Polanco CM, Felsenstein S, Dien Bard J. Performance of the Verigene gram-positive blood culture assay for direct detection of Gram-positive organisms and resistance markers in a pediatric hospital. J Clin Microbiol 2014;52:283–7.
45. Brazelton de Cárdenas JN, Su Y, Rodriguez A, Hewitt C, Tang L, Garner CD, et al. Evaluation of rapid phenotypic identification and antimicrobial susceptibility testing in a pediatric oncology center. Diagn Microbiol Infect Dis 2017;89:52–7.
46. Pence MA, McElvania TeKippe E, Burnham CA. Diagnostic assays for identification of microorganisms and antimicrobial resistance determinants directly from positive blood culture broth. Clin Lab Med 2013;33: 651–84.
47. Felsenstein S, Bender JM, Sposto R, Gentry M, Takemoto

C, Bard JD. Impact of a rapid blood culture assay for gram-positive identification and detection of resistance markers in a pediatric hospital. Arch Pathol Lab Med 2016;140:267–75.
48. Messacar K, Hurst AL, Child J, Campbell K, Palmer C, Hamilton S, et al. Clinical impact and provider acceptability of real-time antimicrobial stewardship decision support for rapid diagnostics in children with positive blood culture results. J Pediatric Infect Dis Soc 2017;6:267–74.
49. B·R·A·H·M·S PCT Assays - B·R·A·H·M·S PCT (Procalcitonin). https://www.procalcitonin.com/pct-assays/ (Accessed May 2018).
50. Linscheid P, Seboek D, Nylen ES, Langer I, Schlatter M, Becker KL, et al. In vitro and in vivo calcitonin I gene expression in parenchymal cells: a novel product of human adipose tissue. Endocrinology 2003;144: 5578–84.
51. Müller B, Harbarth S, Stolz D, Bingisser R, Mueller C, Leuppi J, et al. Diagnostic and prognostic accuracy of clinical and laboratory parameters in community-acquired pneumonia. BMC Infect Dis 2007;7:10.
52. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 1993;341: 515–8.
53. Müller B, White JC, Nylén ES, Snider RH, Becker KL, Habener JF. Ubiquitous expression of the calcitonin-I gene in multiple tissues in response to sepsis. J Clin Endocrinol Metab 2001;86:396–404.
54. Lautz AJ, Dziorny AC, Denson AR, O'Connor KA, Chilutti MR, Ross RK, et al. Value of procalcitonin measurement for early evidence of severe bacterial infections in the pediatric intensive care unit. J Pediatr 2016;179: 74–81.e2.
55. Trimboli P, Seregni E, Treglia G, Alevizaki M, Giovanella L. Procalcitonin for detecting medullary thyroid carcinoma: a systematic review. Endocr Relat Cancer 2015;22: R157–64.
56. Chiesa C, Panero A, Rossi N, Stegagno M, De Giusti M, Osborn JF, et al. Reliability of procalcitonin concentrations for the diagnosis of sepsis in critically ill neonates. Clin Infect Dis 1998;26: 664–72.
57. Stocker M, Hop WCJ, van Rossum AMC. Neonatal Procalcitonin Intervention Study (NeoPInS): Effect of procalcitonin-guided decision making on duration of antibiotic therapy in suspected neonatal early-onset sepsis: a multi-centre randomized superiority and non-inferiority intervention study. BMC Pediatr 2010;10:89.
58. Rey C, Los Arcos M, Concha A, Medina A, Prieto S, Martinez P, et al. Procalcitonin and C-reactive protein as markers of systemic inflammatory response syndrome severity in critically ill children. Intensive Care Med 2007; 33:477–84.
59. Han YY, Doughty LA, Kofos D, Sasser H, Carcillo JA. Procalcitonin is persistently increased among children with poor outcome from bacterial sepsis. Pediatr Crit Care Med 2003;4:21–5.
60. Hatherill M, Tibby SM, Turner C, Ratnavel N, Murdoch IA. Procalcitonin and cytokine levels: relationship to organ failure and mortality in pediatric septic shock. Crit Care Med 2000;28:2591–4.
61. Henry BM, Roy J, Ramakrishnan PK, Vikse J, Tomaszewski KA, Walocha JA. Procalcitonin as a serum biomarker for differentiation of bacterial meningitis from viral meningitis in children. Clin Pediatr (Phila) 2016;55: 749–64.
62. Trippella G, Galli L, De Martino M, Lisi C, Chiappini E. Procalcitonin performance in detecting serious and invasive bacterial infections in children with fever without apparent source: a systematic review and meta-analysis. Expert Rev Anti Infect Ther 2017;15:1041–57.
63. Stocker M, van Herk W, El Helou S, Dutta S, Fontana MS, Schuerman FABA, et al. Procalcitonin-guided decision making for duration of antibiotic therapy in neonates with suspected early-onset sepsis: a multicentre, randomised controlled trial (NeoPIns). Lancet 2017;390: 871–81.
64. Hooven TA. Does a procalcitonin-guided approach to term and late-preterm neonates with suspected early-onset sepsis safely decrease unnecessary antibiotic exposure? Acta Paediatr 2018;107:359–60.
65. Huang DT, Yealy DM, Filbin MR, Brown AM, Chang C-CH, Doi Y, et al. Procalcitonin-guided use of antibiotics for lower respiratory tract infection. N Engl J Med 2018;379: 236–49.
66. Colón-Franco JM, Woodworth A. Current and novel biomarkers for diagnosis and monitoring of sepsis. MLO Med Lab Obs 2014;46:8–9.
67. Gomez B, Mintegi S, Bressan S, Da Dalt L, Gervaix A, Lacroix L, et al. Validation of the "step-by-step" approach in the management of young febrile infants. Pediatrics 2016;138:e20154381.
68. Wong HR, Cvijanovich NZ, Anas N, Allen GL, Thomas NJ, Bigham MT, et al. Developing a clinically feasible personalized medicine approach to pediatric septic shock. Am J Respir Crit Care Med 2015;191:309–15.
69. Mickiewicz B, Vogel HJ, Wong HR, Winston BW. Metabolomics as a novel approach for early diagnosis of pediatric septic shock and its mortality. Am J Respir Crit Care Med 2013;187:967–76.
70. Fanos V, Caboni P, Corsello G, Stronati M, Gazzolo D, Noto A, et al. Urinary 1H-NMR and GC-MS metabolomics predicts early and late onset neonatal sepsis. Early Hum Dev 2014;90:S78–83.
71. Dessì A, Corsello G, Stronati M, Gazzolo D, Caboni P, Carboni R, et al. New diagnostic possibilities in systemic neonatal infections: metabolomics. Early Hum Dev 2014; 90:S19–21.
72. Schmerler D, Neugebauer S, Ludewig K, Bremer-Streck S, Brunkhorst FM, Kiehntopf M. Targeted metabolomics for discrimination of systemic inflammatory disorders in critically ill patients. J Lipid Res 2012;53:1369–75.
73. Mickiewicz B, Tam P, Jenne CN, Leger C, Wong J, Winston

BW, et al. Integration of metabolic and inflammatory mediator profiles as a potential prognostic approach for septic shock in the intensive care unit. Crit Care 2015; 19:11.

74. Hamula CL, Hughes K, Fisher BT, Zaoutis TE, Singh IR, Velegraki A. T2Candida provides rapid and accurate species identification in pediatric cases of candidemia. Am J Clin Pathol 2016;145:858–61.

75. De Angelis G, Posteraro B, De Carolis E, Menchinelli G, Franceschi F, Tumbarello M, et al. T2Bacteria magnetic resonance assay for the rapid detection of ESKAPEc pathogens directly in whole blood. J Antimicrob Chemother 2018;73 Suppl 4:iv20–iv26.

76. Abril MK, Barnett AS, Wegermann K, Fountain E, Strand A, Heyman BM, et al. Diagnosis of *Capnocytophaga canimorsus* sepsis by whole-genome next-generation sequencing. Open Forum Infect Dis 2016;3:ofw144.