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Biomarkers for pediatric sepsis and septic shock

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Abstract

Sepsis is a clinical syndrome defined by physiologic changes indicative of systemic inflammation, which are likely attributable to documented or suspected infection. Septic shock is the progression of those physiologic changes to the extent that delivery of oxygen and metabolic substrate to tissues is compromised. Biomarkers have the potential to diagnose, monitor, stratify and predict outcome in these syndromes. C-reactive protein is elevated in inflammatory and infectious conditions and has long been used as a biomarker indicating infection. Procalcitonin has more recently been shown to better distinguish infection from inflammation. Newer candidate biomarkers for infection include IL-18 and CD64. Lactate facilitates the diagnosis of septic shock and the monitoring of its progression. Multiple stratification biomarkers based on genome-wide expression profiling are under active investigation and present exciting future possibilities.

Keywords

biomarker; CCL4; CD64; C-reactive protein; IL-8; IL-18; lactate; procalcitonin; septic shock

Sepsis and septic shock are syndromes encountered all too often in pediatric hospital medicine. While septic shock falls squarely within the purview of critical care medicine, many clinicians of various specialties are confronted with the task of diagnosing and initiating appropriate therapy for sepsis before, and hopefully to prevent, its progression to severe sepsis and septic shock. Septic shock represents an extreme along a diagnostic and physiologic continuum whose recognition is usually not subtle, whereas the identification of sepsis is often more difficult.

This diagnostic dilemma derives in large part from the fact that sepsis, like other complex disease states such as cancer, is a syndrome rather than a discreet pathologic entity with a clear, unified, maladaptive process at its core. Expert panels have been convened to establish clinical criteria upon which to diagnose sepsis [1,2], but a cohesive understanding of the underlying pathologic mechanisms is absent.

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Within the current framework, a child is diagnosed with sepsis when he or she develops symptoms of the systemic inflammatory response syndrome (SIRS) that are attributable to documented or suspected infection. There are two components necessary, therefore, for the diagnosis of sepsis: recognition of SIRS and infection. Criteria for both have also been specifically developed for children by expert committees [1].

SIRS is defined clinically by the presence of physiologic signs and one laboratory study that indicates activation of the immune/inflammatory response (Box 1). Infection is defined by laboratory documentation of a pathogen by positive culture, tissue stain or PCR test, or a clinical syndrome associated with a high probability of infection. Severe sepsis occurs when organ system dysfunction accompanies sepsis. Septic shock develops with progression to cardiovascular failure sufficient to result in decreased delivery of oxygen and metabolic substrate, which are inadequate to meet tissue demands [1]. Septic shock may or may not be associated with hypotension.

Box 1

Definition of systemic inflammatory response syndrome

The presence of at least two of the following four criteria, one of which must be abnormal temperature or leukocyte count:

- Core temperature of $>38.5^{\circ}\text{C}$ or $<36^{\circ}\text{C}$
- Tachycardia, defined as a mean heart rate >2 SD above normal for age in the absence of external stimulus, chronic drugs or painful stimuli; or otherwise unexplained persistent elevation over a 0.5–4-h time period OR for children <1 -year old: bradycardia, defined as a mean heart rate <10 th percentile for age in the absence of external vagal stimulus, β -blocker drugs or congenital heart disease; or otherwise unexplained persistent depression over a 0.5-h time period.
- Mean respiratory rate >2 SD above normal for age or mechanical ventilation for an acute process not related to underlying neuromuscular disease or the receipt of general anesthesia
- Leukocyte count elevated or depressed for age (not secondary to chemotherapy-induced leukopenia) or $>10\%$ immature neutrophils

Adapted from [1].

Since the dissemination and adoption of these guidelines, the diagnosis of SIRS has become fairly straightforward. When a patient meets those stated criteria, the designation is applied. Distinguishing SIRS from sepsis, however, can often be a murky endeavor owing to the added criterion of infection. Although infection can be suspected immediately in a given clinical scenario, it is often 24–48 h before that suspicion can be confirmed or ruled out definitively by laboratory testing. SIRS can be triggered by a variety of noninfectious conditions including burns, trauma, pancreatitis, autoimmune/ inflammatory disorders, transplant rejection, graft-versus-host disease and many others. The diagnostic dilemma of distinguishing between SIRS and sepsis is further complicated by the fact that these conditions are often similar to infectious processes in their clinical presentation and frequently predispose patients to secondary infections. This distinction between SIRS and sepsis, however, is a very important one from a clinical standpoint as it dictates essential management decisions, such as the initiation, selection and duration of antibiotic therapy.

Beyond diagnosing sepsis, clinicians are also faced with the task of monitoring the patient's response to therapy toward either resolution of illness or its progression to septic shock,

multisystem organ failure and finally death. Here too, clinical management guidelines exist for adults [3] and children [4] that focus primarily on physiologic parameters of well-being such as hemodynamic indices, blood oxygen saturations and signs of end-organ perfusion (e.g., mental status, urine output and peripheral perfusion). While these various indicators provide an overall picture of patient status, they are incomplete and inadequate in providing accurate real-time assessments of the underlying disease progression or resolution. Additionally, they fail to provide prognostic information also crucial to guide patient stratification and therapeutic interventions

For all of these reasons, there is a need to develop biomarkers in the field of sepsis in order to supplement clinical assessments and provide information to bear on diagnostic, monitoring and therapeutic decision making, as well as staging of sepsis.

Overview of biomarkers

A broad definition of biomarkers was put forth in 2001 by a special panel at the NIH [5]. This group described a biomarker broadly as any “characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” In that sense, many of the clinical, physiologic parameters used in assessing hospitalized children could be considered biomarkers. In its day-to-day application, however, the term ‘biomarker’ is most often used to refer to a test performed on some body fluid (e.g., blood, urine and cerebrospinal fluid) that provides clinicians with patient information not readily obtainable otherwise using current diagnostic or monitoring modalities. This latter, narrower concept of biomarkers will be used in this discussion.

Over the years, dozens of putative markers for sepsis and septic shock have been described in the literature. Organizing, understanding and utilizing these markers can be a daunting task and it is helpful to sort them into classes distinguished by their clinical application. Four general types described in recent publications include: diagnostic, monitoring, stratification and surrogate biomarkers [6,7]. Diagnostic biomarkers serve to establish the presence or absence of a disease state or other clinical condition. This class includes the subset of screening biomarkers. Monitoring biomarkers generally consist of molecules or proteins whose levels change dynamically as a disease process evolves or in response to therapeutic interventions, affording the clinician the ability to track the course of disease and assess adequacy of treatment. Stratification biomarkers are useful to sort a population of patients into classes of severity with the intent of applying therapeutic interventions to groups where the most benefit will be realized at the least risk. Finally, surrogate biomarkers are used to predict outcome of a disease process rather than follow its course or to titrate therapy. Surrogate biomarkers serve as proxy end points for severe or rare patient-centered outcomes such as death or significant complications.

The effectiveness of biomarkers is commonly measured in terms of their sensitivity, specificity and by creating receiver operating characteristic (ROC) curves, which allow for the calculation of the AUC. Sensitivity is often defined as the proportion of a population with a disease in whom the test in question gives a positive result. Specificity is the proportion of that population without the disease in whom the test gives a negative result. Biomarkers that are highly sensitive have low false-negative rates and those that are highly specific have low false-positive rates. Optimally, a good biomarker will be both sensitive and specific. It is very rare that a diagnostic biomarker is strictly present or absent. Much more commonly, the presence of a biomarker is measured as a continuous variable and cutoffs are defined along that continuum to establish the presence or absence of disease. The sensitivity and specificity of a biomarker are dependent upon where those cutoffs are placed.

This relationship can be displayed graphically as a ROC curve and the AUC calculated for each candidate biomarker to facilitate comparison. The higher an AUC, the more accurate the marker, with AUCs near 1 having very good sensitivity and specificity and those near 0.5 having very poor sensitivity and specificity. Because testing conditions and study parameters differ between investigations, it is very difficult to compare ROC curves from one report to the next. The ROC curve with its attendant AUC provides relative not absolute values. Because of this relationship, a detailed discussion of comparative statistics related to these variables will not be undertaken in this article. If desired, the reader is encouraged to seek that specific information from the studies referenced.

Diagnostic biomarkers for sepsis

The diagnostic biomarkers employed in sepsis are primarily restricted to those useful in confirming or eliminating the suspicion of infection, as SIRS is defined by clinical criteria and sepsis is further designated by the presence or suspicion of infection. As stated earlier, this is an important distinction that has immediate bearing on the medical management of the patient. If there is no infection and the child's SIRS is caused by another noninfectious, inflammatory process, antibiotic over-usage can be avoided. If an infection, however, is confirmed or strongly suggested at the outset of illness by biomarker analysis, a more aggressive antibiotic regimen may be selected for the patient or more invasive measures taken to identify and remove the infectious source. Applied in this manner, diagnostic biomarkers might also be considered stratification biomarkers because they allow for selection of a population subset at higher risk for severe disease and thus merit the use of more toxic therapies to prevent poor outcome.

C-reactive protein

One of the earliest discovered biomarkers used to diagnose infection is C-reactive protein (CRP), so named for its ability to precipitate from serum in the presence of pneumococcal cell wall C-polysaccharide. CRP is an acute-phase reactant found in the blood that is produced by hepatocytes in the setting of infection or tissue injury. CRP production is triggered by cytokines (IL-1, IL-6 and TNF- α) and levels increase within 4–6 h of an inflammatory stimulus. Serum CRP concentration doubles approximately every 8 h from that stimulus and peaks at around 36–50 h. It has a short half life of 4–7 h.

Some of the first applications of CRP in pediatrics were related to identifying neonates with sepsis in whom clinical manifestations of severe illness are often nonspecific. CRP was also used in a variety of other clinical scenarios to distinguish infection from inflammation, but it was noted early on that CRP alone lacked the specificity to consistently discriminate between bacterial, viral and noninfectious inflammatory conditions. Owing to its poor specificity, it was often used in combination with other biomarkers as part of a panel of tests to assist clinicians with diagnosis. It has also been used to follow response to therapy once an infectious diagnosis has already been established. The overwhelming majority of recent literature regarding CRP has compared its diagnostic accuracy with that of newer candidate biomarkers, most notably procalcitonin (PCT).

Procalcitonin

Procalcitonin is a precursor for the hormone calcitonin, which is produced in the thyroid to regulate serum calcium concentrations. All tissues in the body have the capacity to produce PCT, but only the thyroid C cells express the appropriate enzymes that cleave the prohormone into mature calcitonin [8]. For some time, it has been recognized that PCT levels are increased in children with sepsis and bacterial infection. Under normal conditions, the thyroid gland is the only tissue that produces PCT and serum levels are very low. When

the body is challenged with infection, however, significant production of PCT by nonthyroidal tissues occurs throughout the body. Although the exact proximal stimuli that mediate PCT secretion are unknown, evidence suggests that early inflammatory signals such as TNF- α , IL-1 β and IL-6 play a role. Elevations in PCT are generally observed before CRP rises and levels peak within a much shorter time frame. Additionally, when the patient responds appropriately to therapy, PCT levels return to normal much quicker than those of CRP.

The measurement of PCT has been applied to many of the same clinical scenarios as the analysis of CRP levels, but it has generally performed better in distinguishing children with bacterial infections from those without. With specific regard to separating sepsis from SIRS, multiple studies have demonstrated the diagnostic superiority of PCT [9–13]. Three studies are of particular interest. Simon *et al.* measured PCT and CRP levels in 64 children who developed SIRS and compared values between those with *a posteriori* confirmation of infection and those without. Those with confirmed infection (sepsis) had significantly higher PCT values than those without (SIRS only), but CRP levels did not differ between the two groups [13]. The AUC for PCT in that study was 0.71 versus 0.65 for CRP. Arkader *et al.* demonstrated that in children with sepsis, serum PCT concentration was significantly elevated above that of noninfected children with SIRS following cardiopulmonary bypass (AUC: 0.99). In this setting, however, CRP could not distinguish the two states (AUC: 0.54) [9]. In a group of 359 children cared for in a pediatric intensive care unit, Rey *et al.* showed that PCT was superior to CRP in distinguishing six classes of patients: those without SIRS or sepsis; SIRS alone; local infection; sepsis; severe sepsis; and septic shock. PCT levels increased significantly with the severity of illness (AUC: 0.91), but CRP failed to mirror the trend as dramatically (AUC: 0.75) [12]. A minority of studies evaluating both PCT and CRP in sepsis have shown that while PCT is a good diagnostic marker for sepsis, it was not statistically more accurate than CRP [14–16].

While PCT performs quite well as a diagnostic biomarker, it excels when called upon as a monitoring or prognostic bio-marker. Multiple studies in sepsis show that PCT levels fall quickly as appropriate antibiotic therapy is initiated [9,10,12,17,18]. Additionally, both admission and serial PCT levels have been correlated to severity of disease, multisystem organ failure and mortality, indicating that PCT is an excellent indicator of outcome. Hatherill *et al.* studied PCT in 75 children with septic shock and determined that children with higher admission PCT levels ultimately had worse organ failure and lower survival. When serum PCT levels were trended over several days they observed that children whose levels of PCT remained elevated were at much greater risk of severe disease and death than those children whose levels dropped in response to therapy [18]. Han *et al.* showed that serum PCT levels were preferentially elevated in children with bacterial sepsis compared with those with viral or fungal etiologies and, again, those who progressed to persistent multiple organ failure and death [17]. In both of their studies, Casado-Flores *et al.* and Carrol *et al.* showed that PCT was better than CRP as a predictor of severity of disease [10,14].

Serial measurement of PCT levels has also been used as a monitoring biomarker to direct and limit antibiotic usage. The purpose of this application is to reduce both bacterial antibiotic resistance as well as patient-centered side effects such as nephrotoxicity and drug reactions. Multiple adult studies using PCT-guided algorithms have shown substantial reductions of antibiotic exposure without increases in adverse outcomes. Kopterides *et al.* recently reviewed this literature and performed a meta-analysis [19]. A recent pediatric study looking at early onset sepsis in the neonatal period demonstrated similar findings [20].

Clinicians caring for ill children are confronted with several other scenarios where separating SIRS from sepsis is essential. Children who have been subjected to cardiopulmonary bypass for correction of congenital heart defects and those presenting with burns constitute two classes of patients who almost uniformly present with SIRS. In these settings it can be difficult to determine whether a child needs increased therapy for an infectious process or to withhold potentially nephrotoxic antibiotics. PCT analysis has been shown to be helpful here as well. Arkader *et al.* described PCT kinetics after cardiopulmonary bypass and showed that while PCT was elevated it never exceeded the reference values for SIRS and returned to pre-bypass levels by postoperative day 2. CRP, however, was significantly increased and stayed elevated until the third postoperative day [21]. McMaster *et al.* showed similar postoperative PCT kinetics, but also demonstrated that children who developed local infections, suspected sepsis and confirmed sepsis had increasing PCT levels that were statistically significant (AUC: 0.84). CRP in this setting was a poor marker of infection and sepsis (AUC: 0.62) [22].

The pediatric burn literature on PCT is sparse. One small study with multiple methodological weaknesses determined that serial PCT measurements were inferior to serial CRP measurements in detecting sepsis [23]. The criteria for sepsis in that study, however, were poorly defined and the increment in PCT used to determine sepsis fell within the range of their population's baseline. Much of the adult literature supports the use of PCT as a good diagnostic aid for the detection of sepsis in the setting of burns [24].

Procalcitonin has also been studied in populations of children with abnormal immune systems and has generally been found to facilitate the diagnosis of sepsis. In immunosuppressed children after liver transplantation, Coelho *et al.* showed that PCT could be used to differentiate SIRS associated with graft rejection from sepsis [25]. In children with cancer who present with febrile neutropenia, PCT and CRP are both helpful in diagnosing bacterial infections, but results are mixed as to whether PCT is better than CRP [26–29]. In a group of children who had received bone marrow transplants and were suspected of having sepsis, Sauer *et al.* determined that PCT performed better as a marker of illness severity and mortality than further aiding the diagnosis [28]. This may have been because the patient population had already been selected for those at high risk for bacterial infection.

Many physicians care for children in developing countries where the rates of serious bacterial infection and sepsis are much higher. Even in settings such as these, PCT has been able to accurately distinguish true bacterial infection from those with no identifiable infection in children presenting with symptoms of significant illness. Carrol *et al.* recently reported in an investigation using a five biomarker panel that PCT and CRP were both effective at diagnosing infected children, with AUCs of 0.86 and 0.81, respectively [30]. Furthermore, they identified PCT as the best marker for predicting outcome.

There is much more controversy surrounding the use of PCT in the diagnosis of sepsis in both term and premature infants. Several investigators advocate PCT as a good diagnostic marker of sepsis, with some demonstrating better performance than CRP and others mere equivalence [16,31–36]. Some groups, however, emphasize lack of specificity of PCT in this very young age group [37,38]. A significant reason for this has its roots in perinatal PCT kinetics where multiple researchers have demonstrated a normal, physiological surge in serum PCT levels that peaks approximately 24 h of life and returns to normal on about the third day of life [31,33]. To address this phenomenon, Turner *et al.* developed a nomogram for neonatal PCT levels [39].

CD64

CD64 is a neutrophil cell surface marker also known as Fc γ R1. It is the first of three receptors on the neutrophil whose function is to bind the Fc portion of IgG (hence γ) antibodies that facilitate bacterial opsonization and phagocytosis. CD64 is constitutively expressed on neutrophils, albeit at low levels, until the immune system encounters an infectious agent whereupon surface expression of CD64 is highly upregulated. The level of CD64 expression is measured by flow cytometric analysis of blood samples. In pediatrics, CD64 has been investigated primarily in the setting of neonatology where it has been used to identify premature and term neonates with sepsis [16,40,41]. In most of these studies, CD64 has appeared to perform moderately well, often in combination with other markers or hematologic parameters, but it is unclear what this molecule adds to the field of more established biomarkers. Few of these neonatal studies compared CD64 head-to-head with CRP or PCT and those that did had conflicting results as to whether or not CD64 was significantly better. In a small group of older children, Groselj-Grenc *et al.* showed that CD64 was able to distinguish between sepsis and SIRS better than CRP and PCT, especially when combined with the measurement of lipopolysaccharide-binding protein. In this study, however, only PCT was able to provide prognostic information [16]. In another setting, where CD64 was used to identify bacterial infections in a pediatric emergency department, Rudensky *et al.* demonstrated that CD64 was elevated in children with documented infections, but CD64 was unable to distinguish between viral and bacterial infections [42]. This group also found that CRP was more sensitive than CD64 and that PCT was more specific. Cid *et al.* recently conducted a meta-analysis of the literature on CD64 and calculated mean pooled results for various performance characteristics [43]. For the pediatric studies, they found for CD64 a mean sensitivity of 71% and a mean specificity of 87%. Ultimately, they concluded that CD64 across all subgroups appeared to be a good marker of infection, but noted importantly that the methodological quality of the few available studies was poor.

IL-18

IL-18 is a cytokine produced by activated macrophages that participates in the induction of cell-mediated immunity. Very little has been written about IL-18 as a diagnostic biomarker. Several years ago, various investigators reported that IL-18 levels were increased in the serum of adult patients with sepsis. Two very small, recent pediatric studies are contradictory. Kingsmore *et al.*, using a high-throughput proteomic immunoassay, reported that IL-18 and seven other serum markers were preferentially elevated in preterm infants who developed symptoms of infection [44]. Bender *et al.* determined in their population of neonates that IL-18 had no diagnostic ability [45]. Much more study is needed to clarify the utility of this biomarker in the diagnosis of sepsis.

Lactate

Another important biomarker that has specific relevance to distinguishing sepsis from septic shock and predicting the prognosis of the latter is the serum lactate level. For decades, serum lactate has been recognized and utilized as an indicator of tissue hypoxia, which has immediate relevance to the fundamental pathophysiologic difference between sepsis and septic shock. As stated previously in this article, shock is defined as the insufficient provision of oxygen and metabolic substrate to meet tissue demand. Cells rely on glucose and oxygen to produce ATP, which is the 'energy currency' of the cell that fuels all of its life-sustaining processes. Glucose is transported from the blood into the cytoplasm and there is converted to pyruvate via the glycolytic pathway, which yields only a very small amount of ATP. In the presence of sufficient oxygen, that pyruvate is transported into the mitochondria where it is incorporated into the Krebs cycle to produce significant quantities of ATP. This process is known as aerobic respiration. If, however, there is insufficient

oxygen present in the mitochondria for the Krebs cycle to function, pyruvate accumulates in the cytoplasm and depletes the cell of mediators necessary for the continuation of glycolysis. To regenerate those mediators and continue glycolysis for what small amount of ATP it generates, pyruvate is converted to lactate that is then released into the bloodstream. This is known as anaerobic metabolism. Additionally, there are many clinical conditions, such as toxin ingestion or inborn errors of metabolism, which cause lactate production independent of tissue hypoxia.

Under normal physiologic conditions, a small amount of lactate is produced, but almost all healthy tissues, and especially the liver, have the ability to convert lactate to pyruvate for use in cellular metabolism. This recycling of lactate to pyruvate itself is an energy and oxygen intensive process. Serum lactate levels rise when lactate production outstrips the body's ability to metabolize it or when there is a decrement in that metabolic capacity, which is often seen in sepsis-associated multisystem organ failure.

The overwhelming majority of research with serum lactate has been conducted in adults. The relevant pediatric investigations have been reviewed in detail with excellent discussions of the relevant physiology [46–50]. To summarize, it was first noted that serum lactate was increased in patients with sepsis and that this cohort was sicker and had increased mortality. Early on it was also observed that patients whose lactate levels decreased with therapy had better outcomes while those whose elevated levels persisted fared worse [51]. In these regards, the lactate level was used as a diagnostic, monitoring and prognostic biomarker. With the advent of this research, however, many other investigators demonstrated that lactate levels were not specific to tissue hypoxia, showing that lactate could be increased by adrenergic stimuli and lung injury independent of cellular hypoxia [46,52,53]. This challenged the fundamental premise that lactate distinguishes very well between states of adequate perfusion and poor oxygen delivery. Despite these findings, serum lactate provides valuable information about a patient's physiologic status when considered in the context of other clinical signs and symptoms. To that end, the reduction of serum lactate is still advocated as a target for therapeutic interventions [54,55].

Even though we have discussed individual biomarkers in isolation except as compared with each other, a potentially more robust approach to the diagnosis of sepsis may be the combination of separate biomarkers into one panel so that multiple indicators of infection combine to improve specificity and sensitivity of the whole assay. Although little research has been conducted in pediatric sepsis using multiple markers in concert, the concept has been demonstrated by Kofoed *et al.* in adults [56]. In their study of patients presenting with SIRS suspected of having an infectious etiology, they used multiplex immunoassay measuring six markers to identify those with true bacterial infection. The AUC for the six marker panel was significantly greater than the AUCs of each individual constituent.

Stratification biomarkers for sepsis

As mentioned previously, clinical sepsis is a heterogeneous syndrome rather than a discreet pathologic entity. As such, it has been proposed that the development of more effective stratification strategies is a major challenge in the field as a means of better informing individual patient care and clinical research [57]. Accordingly, biomarker-based stratification strategies for sepsis are currently an active area of investigation.

IL-8

We recently reported that IL-8 can serve as a robust predictor of outcome in children with septic shock who are receiving standard care [58]. The foundation for this approach was a study involving genome-wide expression profiling in pediatric septic shock [59]. This study

involved microarray analyses using whole blood-derived RNA from samples obtained within 24 h of admission to the pediatric intensive care unit with septic shock. IL-8 was identified as one of 34 genes that were increased in nonsurvivors relative to survivors, based on 28-day mortality.

Subsequently, we measured serum IL-8 protein levels in this same cohort of patients (n = 42) and constructed a ROC curve for 28-day mortality with an AUC of 0.857. From this ROC curve we derived a serum IL-8 level of greater than 220 pg/ml, having 75% sensitivity and specificity for predicting 28-day mortality. We next applied this cutoff value of 220 pg/ml to a validation cohort of patients (n = 139) and found a negative predictive value for mortality of 95%, and a negative likelihood ratio for mortality of 0.3. These data suggested that a serum IL-8 level of 220 pg/ml or less, measured within 24 h of admission to the pediatric intensive care unit, may have the ability to predict survival in pediatric septic shock with 95% probability.

We next tested the ability of serum IL-8 levels to serve as a stratification biomarker in a second validation cohort completely independent of our own database. Specifically, we applied the cutoff value of 220 pg/ml to an existing cohort of children with septic shock previously enrolled in a Phase III trial of activated protein C [60]. The application of the cutoff level to this independent validation cohort (n = 193) yielded a similarly high negative predictive value and low negative likelihood ratio for 28-day mortality.

Based on these data, we have proposed that serum IL-8 measurements, conducted within the first 24 h of admission to the pediatric intensive care unit, can be used to stratify children with septic shock having a low risk of mortality with standard care. This stratification strategy would allow for the exclusion of patients from interventional clinical trials carrying more than minimal risk, and thus improve the risk-to-benefit ratio of a given experimental therapy. A similar approach has been proposed for chemokine CCL4 (MIP-1 β), but the negative predictive value of CCL4 has not yet been validated in an independent cohort of patients [61]. Interestingly, we have preliminarily tested the ability of IL-8 to serve as a stratification biomarker for adults with septic shock. This initial study indicates that IL-8 is not a robust stratification biomarker in adults [62], thus illustrating the need to conduct biomarker-based studies in both pediatric- and adult-specific patient populations.

A multibiomarker-based risk model for pediatric septic shock stratification

The IL-8- and CCL4-based stratification strategies described earlier are clinically appealing because of their relative simplicity and feasibility. However, both stratification biomarkers have insufficient positive predictive values, sensitivities and specificities to develop a comprehensive pediatric septic shock stratification tool meeting a wide variety of clinical and research needs [58,61]. Since the biological response during septic shock is exceedingly complex, it is possible that a multibiomarker stratification strategy can more comprehensively meet these needs. Herein, we describe an ongoing protocol to derive the pediatric sepsis biomarker risk model (Pediatric Sepsis Biomarker Risk Model [PERSEVERE]).

The basis for PERSEVERE is a list of 15 candidate outcome biomarkers (Table 1). The 15 candidate outcome biomarkers have been selected using a genome-wide expression database of nearly 100 children with septic shock and representing the first 24 h of admission to the pediatric intensive care unit [63]. The candidate outcome biomarker selection process involved a three-stage process. In stage one, we generated a list of candidate outcome biomarkers by standard statistical approaches targeted at identification of genes differentially regulated between survivors and nonsurvivors of pediatric septic shock. In stage 2, we generated a second list of candidate outcome biomarkers using class prediction

modeling (i.e., 'survivor' and 'nonsurvivor' classes) to identify the top 5% class predictor genes. In stage three, we conducted Venn analysis of the aforementioned two gene lists. The final 15 candidate outcome genes were selected from the intersection of the Venn diagram based on biological plausibility and the ability to reliably measure the genes' protein product in the serum [7].

PERSEVERE will be derived in a formal validation cohort of 220 children with septic shock using the 15 candidate outcome biomarkers and statistical modeling based on principal component analysis and multivariable logistic regression. The goal of the final model will be to predict outcome and illness severity of individual pediatric patients with septic shock. The ability of the model to achieve this goal will then be prospectively validated in a separate cohort of 200 patients. If PERSEVERE comes to fruition, we expect that it will provide an unprecedented decision and stratification tool for the care of individual children with septic shock and for the conduct of interventional clinical trials.

Expert commentary & five-year view

To date, there has been no single biomarker discovered that offers clinicians caring for sick children the absolute diagnostic ability to distinguish sepsis from other inflammatory disorders or to monitor and predict its progression or response to treatment. Likewise, markers for septic shock are inadequate and limited in their utility. It is unlikely that any single biomarker will be able to predict with complete certainty the presence or absence of a disease or of a specific outcome. All biomarkers must be used in their appropriate clinical context as adjuncts to the decision-making process. That being said, however, the use of serum PCT levels appears to be a significant improvement over CRP that has enjoyed broad historical usage. PCT has been shown, to our satisfaction, to improve the ability of clinicians in diagnosing, monitoring and predicting outcome in both sepsis and septic shock. From the literature reviewed here and from our experience, we believe that the serum lactate level can be helpful in identifying occult shock and in monitoring response to therapy, but again this level must be considered as part of the whole clinical context. Other candidate biomarkers require much more investigation before they can be instituted in general pediatric clinical practice. These include novel and interesting markers such as CD64, IL-18, CD163, high-mobility group protein B1, urokinase-type plasminogen activator, soluble triggering receptor expressed on myeloid cells and macrophage migration inhibitory factor. Unable to address all of these, we have touched on the best studied in this article. It is possible that one of these or other molecules will gain broad acceptance and application in the future as research progresses.

Ultimately, owing to the complexity of the immune response and the genetic diversity of the human population, it is extremely unlikely that any one biomarker will ever be able to adequately identify and stratify all pediatric patients with sepsis and septic shock. Furthermore, specific subpopulations with altered immunity, such as those with immunodeficiency or transplant-related immunosuppression, may have completely different biomarker profiles. We believe the solution to these problems lies in rapidly progressing technologies that allow the simultaneous quantification of hundreds and thousands of biological mediators from which biomarker panels will be derived. High-throughput modalities of investigation in genomics, proteomics, lipidomics and metabolomics will continue to identify not only isolated candidate biomarkers, but also characterize patterns of expression and host response using multiple markers simultaneously (i.e., biomarker discovery). This pattern recognition will not only facilitate diagnosis and stratification, but will also help elucidate the underlying mechanisms of sepsis syndrome.

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Table 1

Candidate biomarker gene list for derivation of Pediatric Sepsis Biomarker Risk Model (PERSEVERE).

Gene symbol	Description
<i>CCL3</i>	C–C chemokine ligand 3 (MIP-1 α)
<i>CCL4</i>	C–C chemokine ligand 4 (MIP-1 β)
<i>ELA2</i>	Neutrophil elastase 1
<i>FGL2</i>	Fibrinogen-like 2; acute phase protein similar to fibrinogen
<i>GZMB</i>	Granzyme B
<i>HSPA1B</i>	Heat-shock protein 70 kDa 1B
<i>IL1A</i>	IL-1 α
<i>IL8</i>	IL-8
<i>LCN2</i>	Lipocalin 2 (NGAL)
<i>LTF</i>	Lactotransferrin
<i>MMP8</i>	Matrix metalloproteinase 8
<i>ORM1</i>	Orosomucoid 1; acute-phase protein with unknown function
<i>RETN</i>	Resistin
<i>SULF2</i>	Sulfatase 2; extracellular modulator of heparan sulfate proteoglycans
<i>THBS</i>	Thrombospondin 1