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The pediatric sepsis biomarker risk model: potential implications for sepsis therapy and biology

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Abstract

Sepsis remains a major cause of morbidity and mortality in adult and pediatric intensive care units. Heterogeneity of demographics, comorbidities, biological mechanisms, and severity of illness leads to difficulty in determining which patients are at highest risk of mortality. Determining mortality risk is important for weighing the potential benefits of more aggressive interventions and for deciding whom to enroll in clinical trials. Biomarkers can be used to parse patients into different risk categories and can outperform current methods of patient risk stratification based on physiologic parameters. Here we review the Pediatric Sepsis Biomarker Risk Model that has also been modified and applied to estimate mortality risk in adult patients. We compare the two models and speculate on the biological implications of the biomarkers in patients with sepsis.

Keywords

biomarker; pediatric; PERSEVERE; risk stratification; sepsis

Septic shock is a common cause of morbidity and mortality in adult and pediatric intensive care units [1,2]. During septic shock, pathogen invasion into the bloodstream leads to innate immune cell recognition, activation and massive cytokine release. Bacterial toxins together with cytokines can cause endothelial cell damage leading to microvascular thrombosis and vascular leakage [3]. The end result is systemic hypoperfusion and decreased oxygen delivery, resulting in end organ damage, and ultimately, death. Therapy for sepsis has remained virtually the same through recent times: antibiotics and supportive care. Many physiologic and pharmaceutical interventions have been tested to improve outcomes in septic shock, but almost all have failed to show definitive improvement due, in part, to tremendous heterogeneity with regard to etiologies, patient history, biological responses,

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physiology and degree of illness. The important issue of sepsis heterogeneity was reviewed extensively in a recent publication by Iskander *et al.* [4].

One potential approach to managing septic shock-related heterogeneity is to develop clinically applicable stratification tools [5]. We recently developed and validated a sepsis outcome risk stratification tool for children with septic shock called the pediatric sepsis biomarker risk model (PERSEVERE) [6,7]. Importantly, the derivation and validation procedures included full-term neonates. PERSEVERE assigns a mortality probability based on a panel of biomarkers obtained at the time of clinical presentation. The biomarkers that go into PERSEVERE were selected objectively based on extensive genome-wide expression studies, and subsequent classification and regression tree (CART) analysis [8,9]. PERSEVERE has recently been adapted for adults with septic shock [10]. While PERSEVERE was developed to provide a simple, accurate and objective method for risk stratification, there is a wealth of information contained within the biomarker data that could shed light on the heterogeneous pathophysiology that we have failed to impact with one-size-fits-all therapeutic strategies.

Figures 1 & 2 show the CART-derived decision trees for children and adults, respectively. Interestingly, while some of the biomarkers that predict mortality are the same between adult and pediatric patients, others are not. In pediatric patients, granzyme B (GZMB), heat shock protein 70 kDa 1B (HSPA1B), C-C chemokine ligand 3 (CCL3), IL-8 and matrix metalloproteinase 8 (MMP8) contribute to the predictive capacity of the decision tree. In adult patients, GZMB, HSPA1B, CCL3, IL-8, IL-1 α (IL1A) and C-C chemokine ligand 4 (CCL4) contribute to the predictive capacity of the decision tree. Thus, while GZMB, HSPA1B, CCL3 and IL-8 are common to both models, MMP8 is unique to the pediatric model, whereas CCL4 and IL1A are unique to the adult model. The implication is that the injury pathways most closely associated with mortality may differ between children and adults. Further support for this concept is evident in the different cutoff values for the biomarkers in the pediatric and adult decision trees. In this review, we will describe the seven biomarkers used in the decision trees and the clues they provide to understanding the causes of poor outcomes in sepsis. We will then speculate on the potential biological implications of the decision trees.

Granzyme B

GZMB is primarily involved in cytotoxic T lymphocyte (CTL)-directed target cell apoptosis. It is a serine protease produced primarily by CTLs and NK cells that engage their targets, then exocytose granules containing several proteins including granzymes and perforin. GZMB binds to target cells via the mannose-6-phosphate receptor and is then endocytosed. Once intracellular perforin allows GZMB to escape the vesicle, GZMB then targets caspase proteins to initiate apoptosis [11]. In patients with sepsis, GZMB expression correlates with severity of sepsis and may serve as a marker for CTL activation [12].

Apoptosis of immune cells is a key step in sepsis-associated immune suppression and while triggers for apoptosis in immune cells are likely multifactorial [13], GZMB is likely an important participant. Interestingly, in a murine model of sepsis, GZMB transcripts are

upregulated in megakaryocytes. These transcripts are then carried in platelets and have been shown to mediate apoptosis in lymphocytes [14].

Heat shock protein 70 kDa 1B

Molecular chaperones are proteins that facilitate the proper folding of other proteins under normal conditions or times of cellular stress. Perhaps, the most studied intracellular chaperones are those in the HSP70 family, including HSPA1B. HSPA1B participates in multiple cellular functions including protein folding, protein transport and management of cellular oxidant stress [15]. HSPA1B has also been shown to be anti-apoptotic for cells undergoing increased physiologic stress [16].

Intracellular HSPA1B is an anti-inflammatory molecule that helps cells deal with oxidative stress and decreases the cellular response to proinflammatory molecules like TNF- α [17,18]. Extracellular HSPA1B acts as a damage-associated molecular pattern (DAMP) to stimulate a more robust immune response [19,20]. In models of sepsis, aged HSPA1B null mice are more susceptible to sepsis-related mortality, but not young HSPA1B null mice [21]. There has also been interest in increasing cellular HSPA1B levels in patients with sepsis to improve outcomes [22]. In surgical patients, glutamine can be supplemented in the diet and has been shown to increase HSPA1B levels and is also associated with shorter ICU stays [23]. Similarly, increased extracellular levels of HSPA1B are associated with decreased mortality in adults with severe trauma [24]. However, increased extracellular HSPA1B levels correlate with increased mortality in children with sepsis [25].

Interleukin-1 α

IL1A is constitutively expressed in non-hematopoietic cells and upon disruption of the cellular membrane is released into the plasma where it acts as a potent stimulant of inflammation. In this way, it acts as a DAMP, similar to HSPA1B. IL1A can be cleaved into two functional portions, with the C-terminus being a potent stimulator of inflammation and secretion of IL-6 and IL-8, while the N-terminus can translocate to the nucleus to act as transcription factor [26,27]. Mice deficient in the IL-1 receptor are not susceptible to lipopolysaccharide (LPS)-induced sepsis mortality, even at doses lethal to 100% of wild-type controls [28].

Interleukin-8

IL-8 is a member of the C-X-C chemokine family. IL-8 is the major human chemoattractant for neutrophils and is produced by stromal cells and monocytes. Mice deficient in the IL-8 receptor demonstrate neutrophilia but a decrease in tissue extravasated neutrophils, suggesting that IL-8 plays a major role in neutrophils leaving the circulation [29]. A specific role for IL-8 has been suggested by human endothelial cells treated with LPS, where IL-8 contributes to tissue factor release into the circulation [30]. IL-8 has been shown to differentiate between sepsis patients with and without acute respiratory distress syndrome [31]. An elevated level of IL-8 alone is highly predictive of poor outcomes in children with septic shock [32], but not in adults [33].

C-C chemokine ligand 3 & C-C chemokine ligand 4

CCL3 and CCL4 were originally identified as a small molecule purification that led to increased infiltration of inflammatory cells after injection. Later this purification was actually shown to contain two separate molecules, CCL3 (also known as macrophage inflammatory protein-1 α) and CCL4 (also known as MIP-1 β). While these two molecules share a great deal of homology, there are important differences. CCL3 is a member of the C-C chemokine family that is secreted by monocytes and macrophages and can be secreted by T cells. It is primarily chemotactic and activates macrophages to enhance killing, and induces secretion of TNF, IL-6 and IL-1. CCL3 has some selectivity in that it can also be chemotactic for B cells, CD8 T cells and eosinophils [34,35]. Notably, peripheral blood mononuclear cells from burn patients have significantly decreased secretion of CCL3 and in murine models this has been shown to increase mortality [36].

CCL4 (MIP-1 β) is also a member of the C-C chemokine family and is primarily chemotactic for lymphocytes and monocytes [37]. Healthy patients given a single dose of LPS have been shown to have an acute rise in both CCL3 and CCL4 that peaks at around 2 h [38]. Patients with bacterial but not viral meningitis have been shown to have a large increase of CCL4 in the cerebral spinal fluid [39].

Matrix metalloproteinase-8

Matrix metalloproteinases are zinc-dependent protein peptidases that are typically secreted as inactive proenzymes that require extracellular activation. MMP8, also known as neutrophil collagenase, is secreted by neutrophils and has been shown to be activated by bacteria [40]. The best known substrate for MMP8 protease activity is collagen, but it is now well established that chemokines are also MMP8 targets, and MMP8-mediated chemokine processing has important effects on chemokine function [41].

MMP8 transcripts and protein activity have been shown to be upregulated in children with sepsis and to correlate with organ failure burden and mortality [42]. Similar observations have been made in adults with sepsis [43]. Genetic ablation or pharmacologic inhibition of MMP8 in adult mice confers a survival advantage following an experimental sepsis challenge. As a mechanism for this protection, it is suggested that MMP8 may be directly activating NF- κ B [42]. MMP8 has also been shown to be important in maintenance of the blood-cerebrospinal fluid barrier in the choroid plexus of the brain. After LPS challenge, MMP8 $^{+/+}$ mice showed leakage of a labeled dye into the cerebrospinal fluid space while MMP $^{-/-}$ mice did not, possibly due to depletion of type I collagen [44].

Potential biological interpretation of the decision trees

The CART-derived decision trees (Figures 1 & 2) partition patients into terminal nodes (TNs) that provide a mortality probability for patients diagnosed with septic shock or severe sepsis. An individual patient occupies a given TN based on sequential biomarker value cutoffs. Some patients may be assigned to a TN based on just two biomarker levels (Figure 1, TN1 and Figure 2, TN12), while others may be assigned based on four biomarker levels (Figure 2, TN2 and 3). Here, we speculate on how the combination of biomarkers levels that

assign a patient as low, intermediate or high risk may inform our understanding of sepsis biology.

Table 1 compares the median values of the stratification biomarkers between children (median age = 3.3 years) and adults (median age = 62 years). With the exception of IL1A, the median values for all of the stratification biomarkers were higher in the pediatric patients. These findings are consistent with pediatric immune cells being more robust in secretion of immune proteins [45]. In a study testing this concept, Barsness and colleagues showed that peritoneal macrophages harvested from pediatric patients secreted more cytokines after *ex vivo* stimulation with LPS and IL-1 β than did peritoneal macrophages harvested from adult patients [46,47]. This observation may be a manifestation of immunosenescence that occurs with aging (see below).

IL1A is the one exception among the stratification biomarkers, where adults with sepsis had a higher level of IL1A when compared with pediatric patients. Given the role of IL1A as a potent DAMP released with cellular damage, it is possible that basal secretion of IL1A is much higher in adult patients as aged, senescent cells have been shown to secrete IL1A [48,49]. Whether increased IL1A directly participates in sepsis pathology, or whether it is simply a marker of patients with a greater mass of senescent cells and thus less able to recover injured tissue, is unknown. However, the absence of an IL1A receptor is protective from sepsis in mice [28], suggesting that IL1A may be directly participating in sepsis pathology. Interestingly, in young adults with a low CCL3 and elevated lactate and HSPA1B, a low level of IL1A is associated with a higher mortality probability (Figure 2, TN7).

The traditional paradigm for understanding sepsis pathology is centered on the innate immune system. In this paradigm, challenge with a pathogen triggers the innate immune system to generate an overwhelming, hyperinflammatory response intended to eradicate the invading pathogen, but subsequently leads to unintended secondary cellular damage to the host. This paradigm does not explain all sepsis pathology. Indeed, focus has moved to the immunosuppression paradigm as a major cause for mortality [50,51]. This paradigm focuses on the adaptive immune system, and its suppression occurring concomitantly with activation of the innate immune system. Suppression of the adaptive immune response during sepsis is complex and multifactorial, but may explain why many patients who die from sepsis do not die in the first 48 h after admission, but rather die in the following days due to secondary infections or failure to control the initial infection [52]. Further, it is now apparent that patients with sepsis cannot be simply compartmentalized into either a 'hyperinflammatory' or 'immune suppression' phenotype. Rather, it appears that both phenotypes can be present concomitantly in a given patient [4].

Portions of both the pediatric and the adult decision trees are consistent with the hyperinflammatory paradigm. In the pediatric decision tree, a combination of increased CCL3, increased IL-8 and increased GZMB concentrations leads to a TN (Figure 1, TN4) with a high mortality probability (0.47), consistent with the hyperinflammatory paradigm. A high IL-8 concentration (Figure 1, TN3) in the setting of low CCL3 and HSPA1B concentrations, or a high MMP8 concentration (Figure 1, TN6), in the setting of high CCL3

and low IL-8 concentrations suggest hyperinflammation is incomplete in some patients, reflected in the fact that these TNs have intermediate mortality probabilities. A combination of low CCL3, low HSPA1B and low IL-8 concentrations suggest minimal inflammation, leading to a TN with a very low probability of mortality (Figure 1, TN2).

Recently, a temporal version of PERSEVERE has been developed that considers how the biomarkers change from day 1 to day 3 of septic shock [53]. Consistent with the hyperinflammatory paradigm, persistent increases of IL-8 and HSPA1B over the initial 3 days of septic shock are associated with poor outcomes. Conversely, subjects with initially high CCL3 or IL-8 concentrations, who subsequently show decreased concentrations of these two biomarkers, have a lower probability of a poor outcome.

In the adult tree, a combination of increased CCL3 and increased IL-8 leads to the TN (Figure 2, TN12) with the highest mortality probability (0.75). A high CCL3 and a high concentration of GZMB, even with a low IL-8, also portends a high mortality probability (Figure 2, TN11). Conversely, when CCL3 is high but both IL-8 and GZMB concentrations are low, the probability of mortality is very low (Figure 2, TN10). These combinations of biomarker levels are consistent with the paradigm of a hyperinflammatory response leading to unintended organ injury and increased risk of death in adults with sepsis.

Both the pediatric and adult decision trees support the immune suppression paradigm as well. In the pediatric tree, TN7 includes infants with high CCL3 and high IL-8 concentrations, but a low GZMB concentration. This TN has the highest mortality probability (0.63) in the pediatric decision tree. Conversely, in older children this exact combination of biomarkers was associated with a very low mortality probability (TN8). Thus, the combination of young age and a low GZMB level seems to be a particularly high-risk situation, and may reflect low NK and cytotoxic T-cell function that can be seen in neonatal patients [54].

In the adult decision tree, a combination of low CCL3, low HSPA1B, high lactate and low CCL4 leads to a TN2 with a mortality probability of 0.70. Conversely, this same combination of biomarker levels but with a higher CCL4 concentration leads to a TN3 with a mortality probability of 0.1. One could speculate that patients that occupy TN2 have immune suppression, given the importance of CCL3 and CCL4 for immune cell function. Similar phenomena may be operative in TN4, TN7 and TN9 of the adult tree, which are dependent on chronic disease status and age, respectively. Many of the chronic diseases of adulthood are associated with immune suppression, and age is a major factor in the concept of immunosenescence.

Conclusion

While biomarker-based stratification tools are a promising modality for predicting patients who are at high risk for poor outcomes, the study of biomarkers may also provide new insight into sepsis pathology. In particular, understanding how the various combinations of biomarkers are associated with different mortality risk in both adults and children may help us to understand the intricacies of the immune response to pathogens invading the

bloodstream, and subsequently help to identify personalized treatment approaches. The answer to the question of whether patients with sepsis die from hyperinflammation or immune suppression is probably that both are true, but is patient and context specific. The heterogeneous label of sepsis includes both states of the immune system and biomarker-based stratification may help determine where on the spectrum each patient lies.

Expert commentary

The primary driving factor for the development of PERSEVERE is to more effectively stratify patients with sepsis. PERSEVERE has several potential clinical applications. PERSEVERE-based stratification can serve as a benchmark for quality improvement efforts focused on sepsis outcomes. By comparing actual mortality with the PERSEVERE-derived mortality probability, caregivers can evaluate objectively if their care processes are improving upon the predicted outcome [7]. PERSEVERE can also be used for stratified analyses of clinical trials and treatment outcomes. For example, we recently demonstrated that increased fluid administration and a positive fluid balance are associated with poor outcomes in patients with a low PERSEVERE mortality probability, but not in patients with intermediate or high mortality probabilities [55].

The current assay platform to measure the PERSEVERE biomarkers does not meet the time-sensitive needs of critically ill patients with severe sepsis or septic shock. However, the necessary technology is readily available to develop a rapid assay platform to reliably measure the PERSEVERE biomarkers within a short time frame. With the pending development of a rapid assay platform that can generate biomarker data in less than 2 h, PERSEVERE also has the potential to enhance patient selection for interventional clinical trials. For example, PERSEVERE could allow for the exclusion of patients at the extremes of low and high mortality probability, who are unlikely to benefit from an experimental therapy, while simultaneously identifying patients with an intermediate mortality probability who may have a higher chance of benefitting from an experimental therapy. An analogous approach was employed previously in a clinical trial involving an anti-TNF- α antibody, in which study subjects were risk stratified based on a pre-specified serum IL-6 concentration [56].

PERSEVERE also has the potential to further inform the field with regard to sepsis pathology. As discussed here, the biology of the PERSEVERE biomarkers may be providing additional information beyond a mortality probability. As we learn more about the biology of the PERSEVERE biomarkers and refine the decision trees with additional studies, we are hopeful that PERSEVERE will advance our understanding of sepsis and thus lead to more effective therapeutic regimens. In addition, PERSEVERE has the potential to incorporate other sepsis-related protein biomarkers and metabolomics data as a means of improving predictive capacity and further informing our knowledge of sepsis pathobiology [57–59].

Five-year view

An important next step is the development of a rapid assay platform that can generate biomarker data in a time frame sufficient to guide treatment decisions appropriate to the underlying biology and risk. Such a platform could be used to inform enrollment procedures

for interventional clinical trials in sepsis (companion diagnostics), and could be used to inform decision-making for individual patients with sepsis. Evolution of tools like PERSEVERE that have the capacity for being refined to include new markers that describe the biological processes involved in the pathophysiology of sepsis will help to untangle the complexity and ultimately lead to identifiable therapeutic targets at the individual patient level.

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Key issues

- Sepsis is a heterogeneous disease in terms of population, causes, comorbidities and immune state.
- Biomarker-based stratification tools can be used to decide which patients are at highest risk for death or complicated outcome.
- Biomarkers provide a snap shot of sepsis biology based on selected biomarkers.
- Future sepsis interventional clinical trials could include biomarker-based data to select patients most likely to benefit from a given experimental intervention.
- Using biomarkers to profile sepsis biology for individual patients may ultimately guide personalized therapy.

Figure 1. Decision tree for stratification of children with sepsis

The classification is based on six biomarker based decision rules and one age-based decision rule. The biomarkers used for analysis are as follows: C-C chemokine ligand 3, heat shock protein 70 kDa 1B, IL-8, granzyme B, and matrix metalloproteinase-8. Each node provides the total number of patients in the node, the cut off value for the biomarker or age, and the number of survivors and deaths with the respective rate. All concentrations are in pg/ml. Terminal nodes are considered low risk (TN2, TN5, and TN8), intermediate risk (TN1, TN3, and TN6) or high risk (TN4 and TN7).

CCL3: C-C chemokine ligand 3; GZMB: Granzyme B; HSPA1B: Heat shock protein 70 kDa 1B; MMP8: Matrix metalloproteinase-8; TN: Terminal node.

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Figure 2. Decision tree for stratification of adults with sepsis

The classification is based on nine biomarker based, one age based, and one chronic disease based decision rules. The biomarkers used for analysis are as follows: C-C chemokine ligand 3, heat shock protein 70 kDa 1B, IL-8, granzyme B, C-C chemokine ligand 4, and IL-1 α . Each node provides the total number of patients in the node, the cut off value for the biomarker or age, and the number of survivors and deaths with the respective rate. All concentrations are in pg/ml. Terminal nodes are considered low risk (TN1, TN6, TN8, and TN10), intermediate risk (TN3, TN7, TN9) or high risk (TN2, TN5, TN11 and TN12). CCL3: C-C chemokine ligand 3; GZMB: Granzyme B; HSPA1B: Heat shock protein 70 kDa 1B; IL1A: Interleukin-1 α ; MMP8: Matrix metalloproteinase-8; TN: Terminal node. Reproduced with permission from [10].

Table 1

Comparison of PERSEVERE biomarker concentrations in children and adults.

Biomarker	Children (n = 537)	Adults (n = 881)	p-value
GZMB	14 (5–48)	5 (3–11)	<0.001
HSP70	708,930 (311,1559–1,437,250)	244,947 (125,192–461,690)	<0.001
IL1A	1.0 (0.0–2.0)	2.2 (0.9–4.5)	<0.001
IL-8	281 (80–1189)	168 (77–471)	<0.001
CCL3	97 (58–176)	47 (29–70)	<0.001
CCL4	171 (82–399)	61 (39–113)	<0.001
MMP8	34,079 (11,961–105,239)	15,902 (5291–44,105)	<0.001

All values are provided as medians (pg/ml) with interquartile ranges.

CCL3: C-C chemokine ligand 3; CCL4: C-C chemokine ligand 4; GZMB: Granzyme B; HSP70: Heat shock protein 70; IL1A: Interleukin-1 α ; IL-8: Interleukin-8; MMP8: Matrix metalloproteinase 8.