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Emerging infection and sepsis biomarkers: will they change current therapies?

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Abstract

Introduction—Sepsis is a heterogeneous syndrome characterized by both immune hyperactivity and relative immune suppression. Biomarkers have the potential to improve recognition and management of sepsis through three main applications: diagnosis, monitoring response to treatment, and stratifying patients based on prognosis or underlying biological response.

Areas Covered—This review focuses on specific examples of well-studied, evidence-supported biomarkers, and discusses their role in clinical practice with special attention to antibiotic stewardship and cost-effectiveness. Biomarkers were selected based on availability of robust prospective trials and meta-analyses which supported their role as emerging tools to improve the clinical management of sepsis.

Expert Commentary—Great strides have been made in candidate sepsis biomarker discovery and testing, with the biomarkers in this review showing promise. Yet sepsis remains a dynamic illness with a great degree of biological heterogeneity – heterogeneity which may be further resolved by recently discovered gene expression-based endotypes in septic shock.

Keywords

sepsis; immune system; IL-27; procalcitonin; presepsin; nCD64; cfDNA; bioscore; PERSEVERE; endotype

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Declaration of interest

H Wong and his institution hold a patent US patents for the PERSEVERE biomarkers described in the review. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

1. Introduction

Despite a global push to combat sepsis, it continues to kill millions of people worldwide annually [1]. The incidence of sepsis, both in the United States and globally, continues to rise resulting in a significant loss of life and growing financial burden [2, 3]. In the U.S. alone, the annual incidence of severe sepsis ranges from 300 to 1,000 cases per 100,000 people [2, 4], with mortality ranging from 30% to 50% [4], and total yearly healthcare costs estimated at 16.7 to 20 billion dollars [4, 5].

Physicians and medical societies have sought to better define sepsis, reflecting the goal of earlier recognition and reduction of delays in treatment. The 1991 consensus conference focused on sepsis as a combination of systemic inflammatory response syndrome (SIRS) plus a documented or suspected infection [6]. The conference further delineated the differences between sepsis, severe sepsis (evidence of organ dysfunction) and septic shock (arterial hypotension) [6]. Ten years later, these definitions were reexamined and other signs and symptoms of sepsis were added to the consensus statement to help clinicians identify septic patients [7].

More recently, a task force was convened in 2014 to revisit the definition. The recurring problem is the lack of a gold-standard diagnostic test to easily identify patients with sepsis. Sepsis remains a heterogeneous syndrome with vast immunologic changes as opposed to a disease with one key pathophysiologic feature. With this backdrop in mind, the current consensus statement defined sepsis as a “Life-threatening organ dysfunction caused by a dysregulated host response to infection,” and suggested several criteria to identify septic patients [8].

This new, seemingly streamlined, consensus definition urges the clinician to put aside the terms ‘severe sepsis’, and ‘SIRS,’ and instead get to the crux of the matter: diagnosing and appropriately treating this often deadly condition. However this is more easily said than done, as the concept of sepsis can be somewhat nebulous and patient conditions are dynamic. Biomarkers might represent key adjuncts to guide the diagnosis and management of sepsis. Biomarker development in the field of sepsis has largely focused on the presence or absence of infection, and not sepsis per se. By definition, the diagnosis of sepsis requires the presence of an infection, which is often a more tangible or quantifiable target for biomarker discovery than sepsis itself. Thus, for the purpose of this review, the words “sepsis” and “infection” will be used interchangeably.

The pathogenesis of sepsis is complex with patient-to-patient variability. An immunologic cascade is triggered by the presence of a given pathogen: the innate immune system acts first to stem the infection by releasing pro-inflammatory cytokines and small inflammatory molecules, followed by the adaptive immune system which mounts a more specialized attack using antigen-presenting cells to initiate proliferation of a specific clonal population of T-cells and B-cells, and a multitude of cytokines [4, 9–11]. Neutrophils serve to clear pathogens via phagocytosis, but can also cause direct tissue injury, cytotoxicity, and increased vascular permeability [12]. This necessary response to bacterial invasion has also

been proposed to cause direct damage to the host and account for some of the morbidity and mortality seen with sepsis [12, 13].

Given this background of immunologic storming, several studies have assessed the utility of anti-inflammatory agents in sepsis, including tumor necrosis factor antagonists [14, 15], corticosteroids [16], IL-1 receptor antagonists [17], and anti-endotoxin antibodies [18]. None of these yielded significant clinical improvement. In fact, animal models of peritonitis with sepsis showed that TNF- α blockade leads to increased mortality [19]. Further meta-analysis evaluating anti-inflammatory therapies yielded generally negative results except in a small subgroup (10%) of patients [20].

There is growing support for the notion that following the acute state of immunologic hyperactivity, a state of relative immune suppression takes over, leaving the host vulnerable to secondary infections or failed eradication of the primary infection [11, 13, 21, 22]. Once the initial response has ended, counter-inflammatory cytokines seek to restore homeostasis [21, 23]. Additionally, there is significant death of lymphocytes, dendritic cells, gastrointestinal epithelial cells and thymocytes [21–24]. Hotchkiss et al. examined spleens from patients whom had died from sepsis and discovered that a more prolonged disease state led to greater depletion of B-cells and CD4+ T-cells [24]. Similar findings of immunosuppression were found in the lungs of septic patients [25]. Their organs had inhibited cytokine secretion, paucity of CD4+, CD8+, and HLA-DR cells, increased expression of certain inhibitory receptors and ligands, and proliferation of a suppressor cell line [25]. Once the pendulum has swung in this direction, patients are at high risk of infection. It is therefore important to consider what state of the disease process a given patient is in. This concept also helps to explain why previous trials of immunomodulators might have failed. The intricacies of the immune response in sepsis provide the key to better understanding the process and more effectively treating patients.

2. Biomarker Review

In 2001 a biomarker definitions working group at the NIH defined a biological marker, or biomarker, as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [26]. They further classified two distinct categories of biomarkers: that which denotes the natural history of a disease, and that which demonstrates the effect of a therapeutic intervention [26, 27]. Building upon these definitions, more recent work has established four broad classes of biomarkers: diagnostic, monitoring, surrogate, and stratification [27–29]. Diagnostic biomarkers serve to either rule in or rule out a specific disease process or clinical state. Monitoring biomarkers allow clinicians to gauge the effect of a certain intervention and thereby adjust said intervention. A surrogate biomarker is “intended to substitute for a clinical endpoint... [And] is only a useful measure if it is accurate, with high sensitivity and specificity for the suspected outcome” [27]. Perhaps more abstract than the others, surrogate biomarkers offer a tangible measure of a given end-point to better predict that end-point or outcome [29].

Stratification biomarkers serve to stage diseases based on prognosis or underlying biological mechanism, thus enabling the concept of *enrichment*: selection of a patient population in which an intervention effect is more likely than in an unselected population. *Prognostic* enrichment refers to the selection of a patient population that is more likely to have a disease-related event, such as mortality. *Predictive* enrichment refers to the selection of a patient population that is more likely to respond to a therapeutic intervention based on an underlying biological mechanism. These concepts of enrichment are fundamental for embracing precision medicine.

Looking more specifically at sepsis, biomarkers serve many roles. As there remains no gold standard diagnostic test for sepsis, this is a crucial function that could be filled by one or several biomarkers. Additionally, they could be used to stratify patients by degree of risk to better decide which patients receive higher-risk therapies [29–31]. They can also be used to gauge response to antibiotics, and help determine appropriate length of treatment, resulting in significant healthcare cost saving. As of yet, there are no adequate surrogate biomarkers for sepsis. The remainder of this review will focus on specific examples of well-studied biomarkers, which, in our opinion, have the most evidence to support their inclusion. Additionally, this review will discuss the role of these biomarkers in clinical practice with special attention to antibiotic stewardship and cost-effectiveness. Table 1 provides a summary of the biomarkers covered in this review.

2.1 Procalcitonin: diagnostic, prognostic, and monitoring applications

Procalcitonin (PCT) has been studied for many years and is currently approved for clinical use. It does not fall under the category of “emerging,” but we include it as an important comparator for newer biomarkers. PCT is the prohormone of calcitonin, produced by thyroid C cells for calcium homeostasis [32]. Under typical conditions, PCT is only produced by the thyroid; however once an infection takes hold, other tissues produce this prohormone [33]. One diagnostic advantage of PCT is that levels rise quickly after the onset of infection, with meaningful increases within four hours, and peak concentration at twelve to forty-eight hours [34, 35]. In clinical practice, PCT has largely replaced C-Reactive Protein (CRP) as the adjunctive biomarker of choice when evaluating presumed septic patients, as PCT has proven to be the superior biomarker [36–40]. A meta-analysis of critically ill adults with systemic inflammation (either from trauma or surgery) found PCT to be a more accurate diagnostic tool than CRP, with an odds ratio for diagnosis of infection of 15.7 for PCT vs. 5.4 for CRP, and a Q* value (the intersection of summary receiver operating characteristics curve with the diagonal line where sensitivity equals specificity) of 0.78 and 0.71 for PCT and CRP respectively [40]. Although it exceeds the diagnostic ability of CRP, concern remains about its ability to consistently distinguish between sepsis and sterile inflammation [40–43]. A different meta-analysis evaluating the ability of PCT to separate sepsis from non-infectious inflammation among critically ill patients showed under-performance of the biomarker, with mean sensitivity and specificity of 71%, and an area under the summary receiver operator characteristic curve (AUC of the ROC curve) of 0.78 [43].

Procalcitonin might be able to serve as a prognostic tool. Several studies have demonstrated a relationship between elevated PCT levels and illness severity including risk of mortality

[33, 44–47]. Boussekey et al. examined admission PCT levels in patients with community acquired pneumonia [45]. They found higher baseline levels in patients who died or went on to acquire septic shock, organ dysfunction, ARDS, or DIC during their ICU stay [45]. In addition, persistently elevated concentrations of PCT have been associated with multi-organ dysfunction and death [46].

Perhaps the best application of PCT is as a monitoring tool. Much like the rapid rise in PCT level with infection, once appropriate antibiotics are instituted, serum PCT levels drop precipitously [34]. Several randomized prospective trials evaluated the use of procalcitonin-guided algorithms for determining antibiotic duration [48–52]. The general study design involves randomizing patients to a standard treatment arm (in which a physician determines when to discontinue antibiotics) or a procalcitonin-guided arm whereby antibiotics are discontinued using a combination of clinical improvement and procalcitonin level or PCT delta from admission. None of the studies report any increase in adverse events or outcomes [48–52]. There was, however, a significant decrease in the mean duration of antibiotics [48–52] and length of ICU stay [48], an increase in the number of antibiotic-free days [52], and although it was a small study, Schroeder et al. report a reduction in the cost of antibiotics of 17.8% [49]. Two recent meta-analyses support these findings [53, 54], as does a 2016 multi-center prospective randomized trial [55].

Yet, one criticism of these meta-analyses is that they incorporated studies using PCT-guided algorithms at different points of antibiotic use – for initiation, discontinuation, or both [56]. Given this degree of heterogeneity, it is difficult to form valid conclusions about PCT and its exact role in guiding antibiotic therapy. Lam et al. individually evaluated each strategy in their 2016 review [56]. When studied as an antibiotic initiation tool, PCT did not decrease antibiotic usage, and in fact in one study, the PCT-guided group actually had higher antibiotic usage and morbidity [57]. When used adjunctively to guide de-escalation of antibiotic therapy it performs better, with the majority of reviewed studies showing shorter duration of antibiotic therapy [56]. The one dissenting study did not find any difference in length of antibiotic use, length of stay, or mortality [58]. However, Lam et al. postulated this was most likely related to the study's stringent antibiotic cessation criteria – requiring patients to have a much lower PCT level for discontinuation of therapy – when compared to other studies [56]. An expert review panel from 2013 proposed using PCT to help guide antibiotic discontinuation, with suggested minimum treatment lengths varying based on degree of illness, immunocompromised status, and bacteremia [59]. Thus, although the data appear weak for PCT-guided institution of antibiotics, the majority of the literature supports PCT-based algorithms for de-escalation of antibiotic therapy.

2.2 Interleukin-27 (IL-27): diagnostic applications

IL-27 is a heterodimeric cytokine composed of the IL-27p28 and Epstein-Barr virus-induced gene 3 (EBI3) subunits [54, 60]. These two subunits originate primarily from antigen presenting cells upon exposure to either an infectious or inflammatory stimulus [60]. IL-27 promotes helper T-cell type I response, and is involved via its signaling on CD4+ T-cells in B-cell class switching, which generates pathogen-specific antibodies [54]. Interestingly, IL-27 also has anti-inflammatory properties, releasing IL-10 through T-cells [61, 62].

A recent genome-wide expression study of pediatric patients with SIRS vs. those with sepsis sought to identify candidate predictor genes for sepsis [63]. Among the class-predictor genes identified, EBI3 had the highest predictive strength to differentiate between these two conditions [63]. The study subsequently measured IL-27 serum protein concentrations in critically ill children to determine its ability to differentiate between sterile inflammation (SIRS) and sepsis. The specificity of IL-27 for sepsis was 90%, with a positive predictive value of 94%, and the AUC for the ROC curve was 0.81, better than the AUC of 0.74 seen with procalcitonin [63]. An ensuing study prospectively examined IL-27 as a diagnostic marker in critically ill children in an intensive care unit [64]. They classified patients as either bacterially infected or not based on clinical data, laboratory tests, and blood cultures. As a result, some patients were considered to have strong enough evidence of bacterial infection despite negative cultures. The ROC curve yielded an AUC of .64 (comparable to the 0.61 for PCT) [64]. In a secondary analysis focused on patients with positive blood cultures, the AUC rose to 0.75, superior to the 0.64 of PCT [64].

Studies of IL-27 among adult populations have not performed quite as well, with the AUC for the IL-27 ROC curve only reaching 0.68 compared with 0.84 for PCT [65]. However, a decision tree for sepsis diagnosis showed improved AUC when IL-27 was incorporated with the PCT-based model [65]. IL-27 did perform better when looking at patients whose source of infection was not the lung, with an AUC approaching 0.8 [65].

Wong et al. postulated that the differing results between adults and children might be due to the more robust immune response of a growing child compared to that of an adult [66]. This notion is supported by recent work demonstrating high levels of dendritic cell-derived IL-27 in infancy with lowest levels in adulthood [67, 68]. Additionally, there was a secondary peak of IL-27 levels at 10–12 years of age [68]. Kraft et al. looked at macrophage expression of IL-27 and found that human cord blood-derived macrophages express genes for both the EBI3 and IL-27p28 subunits in greater quantity than macrophages obtained from adults [69]. They also created a mouse model to further study the age-related production of this biomarker. They evaluated gene expression using RNA from spleens of mice of different ages, discovering that IL-27p28 increased during the first twelve days of life and then remained stable at that level into adulthood, but EBI3 increased over the first three weeks of life and then dropped precipitously as the mice entered adulthood [69]. The authors suggested that the EBI3 subunit was essentially the limiting factor in IL-27 production in adulthood, which was confirmed using flow cytometry of splenocytes from these same mice [69]. IL-27 level and the number of macrophages producing IL-27 were higher in neonatal mice (compared with adults), with both rising until the end of infancy [69].

Thus, IL-27's application may be best suited in the pediatric population. Further studies are currently underway to better ascertain the diagnostic utility of this biomarker.

2.3 Presepsin: diagnostic, prognostic, and monitoring applications

Cluster of differentiation 14 (CD14) is a membrane surface glycoprotein found on monocytes and macrophages that functions as a receptor for lipopolysaccharides (LPS) and LPS-binding proteins [70, 71]. CD14 is generally considered a pro-inflammatory molecule, acting to recognize pathogens, and triggering activation of other cytokines and chemokines

[72]. In response to inflammation, plasma proteases cleave CD14, yielding soluble CD14 fragments, including sCD14-ST, also known as presepsin [70, 71]. In a healthy host, presepsin levels are quite low. Following pathogen stimulation, presepsin can be detected rapidly [73]. In a rabbit model of sepsis, presepsin was found within two hours of cecal ligation and puncture, earlier than PCT, with peak levels at three hours [74]. Additionally, an *in vitro* study looking at the response of human monocytes to LPS showed detectable levels of presepsin at one-hour post-exposure, again with a peak at the three hour mark [73]. The rapid kinetics of presepsin expression following exposure to infection is an ideal characteristic for a candidate diagnostic biomarker for sepsis.

A recent meta-analysis evaluated presepsin's ability to distinguish between systemic inflammation and sepsis using studies published from 2012–2014 [71]. The AUC for the summary ROC curve was 0.89, with a pooled sensitivity of 86% and specificity of 78% [71]. The authors report this data as superior to that of other biomarkers, including PCT [71].

In 2011, Shozushima et al. conducted a prospective cohort study measuring presepsin, PCT, CRP, and IL-6 at six time-points in patients with evidence of at least two SIRS criteria, comparing them with healthy controls [75]. Although the sample size was small, only 41 cases matched with 128 controls, the results were compelling. For distinguishing between infected vs. non-infected, the AUC of presepsin was 0.85, compared with 0.82, 0.65, and 0.67 for CRP, PCT, and IL-6, respectively [75]. Presepsin had an 80.1% sensitivity and 81% specificity for differentiating sepsis from SIRS [75].

A 2014 study by Behnes et al. prospectively studied 116 patients admitted to the ICU with suspected sepsis, measuring biomarker values at days 1, 3, and 8, and following the patients to six months. The AUC to diagnose septic shock at day 1 of ICU treatment was 0.80, comparable to PCT and IL-6 [76]. By day 3, the AUC to diagnose any form of sepsis was 0.84, and at day 8 it remained at 0.82, both of which exceeded the abilities of PCT and IL-6 [76].

Beyond differentiating between sepsis and inflammation, presepsin is also able to stratify patients. The absolute value of presepsin directly correlated with illness severity [75, 76]. Additionally, higher presepsin levels have been associated with worse outcome [70, 76, 77]. Behnes et al. found a significant difference in presepsin levels between survivors and non-survivors both at thirty days and six months [76]. This relationship persisted when adjusting for possible confounding variables such as APACHE II score, total ICU-days, gender, and creatinine [76]. Another study similarly showed higher initial presepsin levels in patients who did not survive to ICU-discharge or to 28-days [77]. A 2013 study by Ulla et al. also determined that higher initial presepsin levels were associated with increased risk of 60-day in-hospital mortality [70]. They also tested PCT in this regard but found no significant relationship between PCT and survivorship [70].

There is evidence to sub-classify infected patients based on absolute presepsin levels [75, 76], making presepsin a candidate monitoring biomarker. A 2014 multi-center prospective study showed that persistently elevated presepsin levels were seen in patients with higher SOFA and APACHE II scores at day 7 of hospitalization [78]. The other biomarkers tested

(PCT, CRP, IL-6) did not demonstrate this relationship [78]. In addition, patients with higher SOFA and APACHE II scores, stratified into the 'unfavorable' groups, had significantly longer duration of antibiotic usage: 16.5 vs. 10.6 and 14.9 vs. 10.0 respectively [78]. As these two scoring systems are currently recognized scales to gauge illness severity in critically ill adult patients, it is fair to say that sicker patients have persistently elevated presepsin levels. Therefore, presepsin could aid in evaluating the response to therapy and elucidate a patient's overall trajectory. If a patient's presepsin level is rising, it has acutely crossed an absolute value threshold from one degree of illness severity to another, or if levels are persistently elevated, the clinician could use this information to adjust antimicrobial therapy or institute higher-risk therapies. Conversely, although this has not yet been tested, down-trending presepsin levels or those that cross the threshold value from septic to inflamed could compel a clinician to discontinue antibiotics, thereby improving antibiotic stewardship, limiting unnecessary exposure to antimicrobials, and decreasing healthcare expenditure.

The other important factor in determining a biomarker's utility as a diagnostic tool is the timeline for results. Both Shozushima et al. and Shirakawa et al. used a chemiluminescent enzyme immunoassay to rapidly and accurately measure presepsin levels, decreasing result time from the previously reported 4 hours down to just 1.5 [75, 79]. Even faster results have been demonstrated using the PATHFAST presepsin assay, with results available in one hour, and non-inferiority when compared with other assays [80, 81].

2.4 Neutrophil CD64 (nCD64): diagnostic, prognostic, and monitoring applications

Cluster of Differentiation 64 (CD64) is an immunoglobulin Fc- γ receptor I found ubiquitously on monocytes and sparingly on neutrophils [82]. However, neutrophil CD64 (nCD64) expression markedly increases after infection as the innate immune system tries to optimize phagocytosis [82, 83]. Levels rise within two hours of pathogen exposure, and return to their normal low level within a few days after the inciting infectious agent dissipates [83, 84]. Thus, nCD64 could be a promising diagnostic and monitoring tool.

A 2015 meta-analysis using adult studies sought to determine the relationship between nCD64 and the diagnosis of sepsis [85]. The AUC of the summary ROC curve was 0.95 with a Q^* value of 0.89, and pooled specificity and sensitivity of 85% and 76% respectively [85]. A prior meta-analysis by Cid et al. included studies performed on patients of all ages, and found the pooled sensitivity to be 79%, specificity 91%, and AUC 0.94 for nCD64 expression to predict bacterial infection [86]. When adult-only studies were sub-analyzed, the pooled sensitivity and specificity improved [86].

A 2015 prospective study by Dimoula et al. measured nCD64 in adults within 24-hours of admission to an ICU [87]. Patients with sepsis had higher nCD64 expression than their non-septic counterparts, as did septic patients with positive blood cultures when compared to those with negative blood cultures [87]. For identifying patients with sepsis, the AUC was 0.94; the sensitivity and specificity were 89% and 87% respectively with a negative predictive value of 97% [87]. Similar AUC's have recently been reported by Gibot et al. (0.95 to predict sepsis) and Icardi et al. (0.94 to predict bacterial infection) [88, 89].

Icardi et al. looked at the CD64 index in patients with blood cultures drawn, and used a combination of healthy patients and those undergoing uncomplicated surgeries as controls [89]. Using an *a priori* cutoff point, a CD64 index greater than 1.19 had a sensitivity of 94.6% and specificity of 88.7% for predicting infection [89]. Furthermore, a CD64 index less than 1.19 was 100% predictive of a negative blood culture [89].

Regarding prognostic capacity, Dimoula et al. found that higher nCD64 expression was associated with severity of illness [87]. In addition, although the AUC of baseline nCD64 level for prediction of hospital death was only 0.65, there was a statistically significant difference in nCD64 expression between in-hospital survivors and non-survivors [87]. Livaditi et al. similarly found that higher nCD64 expression was associated with worsening degree of sepsis, higher SOFA and APACHE II scores, as well as non-survivorship at 28-days [90].

There is also evidence that nCD64 could be useful in monitoring response to therapy. One study with robust data on organism type and antibiotic resistance reported that 34% of the 130 septic patients in their study received inappropriate antibiotics, in part due to gram-negative bacterial resistance patterns [87]. This subset of patients whom had received inappropriate empirical antibiotic coverage had persistently elevated levels of nCD64, in contrast to patients who had received appropriate antibiotics, where a decrease in nCD64 expression was seen at day 4 of illness [87]. They further quantified this relationship, with multivariable analysis showing that nCD64 expression above a set point of 260 MFI at day 3 was independently associated with inappropriate antibiotic usage, with a significant adjusted odds ratio of 10 [87]. Finally, in a small group of patients who were initially not infected but developed sepsis during their ICU admission, a considerable increase in nCD64 expression was seen between two sample collection points [87]. nCD64 expression could, therefore, be useful when determining the reason for a patient's acutely changing or conversely stagnant clinical course.

While the diagnostic test characteristics of nCD64 indicate clinical utility, a limiting factor for clinical application is the method of measurement. Determining nCD64 expression requires flow cytometry, which necessitates specific equipment and trained personnel. Included in the Icardi et al. article is a brief cost analysis. The cost to run each CD64 analysis was roughly \$10 per test [89]. They reasoned that if antibiotics were discontinued once the CD64 index was below the cutoff for infection, they would have saved 98 days of broad-spectrum antibiotics which equates to \$32,000, or a 20% reduction in the cost of antimicrobials used by the patients enrolled in this study [89]. Not only could this biomarker result in significant healthcare cost saving, but it could also decrease antibiotic resistance by limiting exposure to broad-spectrum antibiotics.

2.5 Interleukin-8: prognostic applications

Interleukin-8 (IL-8) is chemokine produced by macrophages for the purpose of mobilizing and activating other pro-inflammatory cells, primarily neutrophils [23]. It was implicated as a candidate stratification biomarker by Wong et al. in 2007 [91]. They conducted microarray analyses in children with septic shock, using blood samples obtained within 24-hours of admission, to look at differences in regulation of gene probes between survivors and non-

survivors [91]. Among sixty-three differentially regulated gene probes, IL-8 mRNA expression was higher in non-survivors [91]. This finding was further validated by the detection of higher serum levels of IL-8 protein (via ELISA) in patients who died [91]. Members of that same research group then conducted an independent validation study to determine the prognostic significance of IL-8 [92]. Using a specified cutoff, serum IL-8 levels higher than 220 pg/ml had a sensitivity of 78%, specificity of 64% and positive predictive value of only 25% for predicting mortality [92]. The negative predictive value for serum concentrations less than 220 pg/ml was 95% [92]. A *post hoc* analysis also showed that patients with IL-8 concentrations less than 220 pg/ml had significantly lower 28-day mortality [92]. IL-8 could therefore serve to stratify patients, especially in the setting of employing experimental or higher risk therapies.

Kraft et al. examined IL-8 levels in pediatric burn patients, placing patients either into the low IL-8 group (serum concentration less than 234 pg/ml) or the high IL-8 group (greater than 234 pg/ml) [93]. The high IL-8 group had a significantly higher (ten-fold) mortality rate compared to the low group during the first sixty days post-injury [93]. Within the high level group, higher concentrations of IL-8 correlated with incidence of sepsis and multi-organ failure. Interestingly in the low group, higher IL-8 levels correlated with burn size and likelihood of multi-organ failure but not incidence of sepsis [93].

In adults with sepsis, IL-8 concentrations greater than 220 pg/ml were significantly associated with all-cause 28-day mortality, however the negative predictive value of mortality of an IL-8 concentration less than 220 pg/ml was only 74% [94]. Livaditi et al. looked at the ability of several biomarkers to predict mortality and illness severity and only found significant results for IL-8 and CD64 [90]. Although the study size was small (forty-seven adult ICU patients and twelve healthy volunteers), increasing IL-8 levels correlated with worsening degree of sepsis, as well as 28-day mortality [90]. The AUC of the ROC curve to predict severe sepsis or septic shock was 0.94, the AUC for 28-day mortality was 0.73, with a statistically significant odds ratio of mortality of 1.26 [90]. At this point, IL-8 as a prognostic tool warrants further investigation in both the pediatric and adult realms.

2.6 Cell Free DNA (cfDNA): diagnostic and prognostic applications

Cell free DNA (cfDNA) is comprised of short-lived fragments of DNA produced secondary to cellular necrosis and apoptosis [95, 96]. cfDNA is present in healthy people, at minimal levels, as clearance of apoptotic cells is readily accomplished by phagocytes [96]. In the setting of sepsis, there is rapid cellular turnover and increased apoptosis with impaired clearance of dying cells, leading to accumulation of cfDNA [97]. A murine model of sepsis showed increased levels of cfDNA at six hours post-cecal ligation and puncture, with peak values seen at the twenty-four hour mark [98].

Most of the work on cfDNA has focused on its use as a prognostic biomarker with just a few studies examining its diagnostic potential. One prospective study looking at febrile adults yielded an AUC of 0.99 for cfDNA to diagnose infection, along with a sensitivity of 95%, specificity of 97% and a positive predictive value of 99% [99]. A similar AUC was found when diagnosing sepsis in this same population, with an AUC of 0.95 [99]. These remarkable test characteristics require prospective validation.

A 2011 prospective cohort study using samples obtained one to four days following a positive blood culture revealed that the maximum cfDNA value was significantly higher in non-survivors as compared with survivors, with the AUC for the ROC curve for mortality measuring 0.81 [100]. Dwivedi et al. performed a retrospective observational study using plasma samples from eighty patients with severe sepsis to assess the prognostic strength of cfDNA [101]. The mean cfDNA level was significantly higher in non-survivors than in both survivors and healthy volunteers [101]. They determined the AUC for the ROC curve to predict ICU mortality was 0.97, which far exceeded any other variables assessed including APACHE II (AUC of 0.64) and IL-6 (AUC of 0.56) [101]. Interestingly, a difference in cfDNA levels persisted over the course of twenty-eight days: cfDNA levels started higher and persisted in non-survivors as compared to survivors whose levels started lower and remained that way [101]. There are several other studies similarly demonstrating the ability of cfDNA to estimate the risk of mortality [102, 103]. With this data in mind, cfDNA could be considered as a stratification tool to identify patients at highest risk of mortality.

Another recent study looked at the prognostic value of cfDNA in adult patients with at least one positive blood culture for *S. aureus* [96]. Samples were taken at days three and five after the initial blood culture, with the primary endpoints being mortality at various time points [96]. Patients requiring ICU admission had higher cfDNA levels than those able to avoid the ICU [96]. Higher cfDNA levels at both days three and five were associated with mortality [96]. Finally, Rhodes et al. found the AUC for the ROC curve for baseline cfDNA to predict ICU mortality was 0.84, however this was a smaller sample size [102].

One important consideration is the feasibility of measuring cfDNA. Most of the mentioned studies used either a DNA assay kit and a fluorometer to quantify cfDNA levels [96, 100], a spectrophotometer [101], or PCR amplification [102], which are time-consuming techniques. A 2015 paper by Yang et al. described a rapid point-of-care test – a microfluidic device that concentrates fluorescent-labeled DNA with a direct current electrical field to measure the fluorescence intensity, which is directly proportional to the cfDNA concentration [104]. Further research must be done to validate measurements, but in initial testing, the cfDNA quantification was significantly different between healthy volunteered samples and non-survivors of severe sepsis [104]. This test results in just five minutes and is very low cost [104].

3. Combination Biomarker Sets

Individual biomarkers can stand alone, but when combined, they often have superior value. The following combination biomarker sets have shown particular potential.

3.1 An 11-Gene Sepsis MetaScore with diagnostic and monitoring applications

In a 2015 study, Sweeney et al. evaluated mRNA expression as a means of distinguishing sepsis from sterile inflammation [105]. Using a large number of time-matched samples drawn from publically available datasets, they sought to differentiate between septic/infected patients and those with SIRS or trauma using their multicohort gene expression analysis framework [105]. A set of 11 genes was identified that best discriminated between sepsis and sterile inflammation to create a Sepsis MetaScore (SMA), which yielded a mean ROC

AUC of 0.87 [105]. This set was then validated using an additional eight data sets, again resulting in similar mean AUC's [105].

The authors then analyzed how the SMA changed with time. As patients progressed toward infection, the SMA significantly increased [105]. Conversely, the SMA decreased from days two to five as patients received appropriate antimicrobial treatment [105]. Thus, this gene set has the potential to monitor treatment response with the supposition that if infected patients were not being adequately treated with antibiotics their SMA would not improve.

Recently, Sweeney et al. again leveraged multi-cohort analysis of publically available datasets to derive a 7-gene classifier differentiating between viral and bacterial infection [106]. This 7-gene classifier was then combined with the SMA to develop an integrated antibiotic decision model (IADM). In a pooled analysis of 1,057 samples representing twenty cohorts, the IADM had a sensitivity of 94%, a specificity of 60%, and a negative likelihood ratio of 0.1 for bacterial infection [106]. This indicates that the IADM is particularly effective at identifying patients with an extremely low likelihood of having bacterial infection thereby avoiding unnecessary antibiotic prescription.

3.2 Pediatric Sepsis Biomarker Risk Model (PERSEVERE): prognostic applications

Wong et al. used genome-wide expression profiling to identify candidate prognostic biomarkers for pediatric septic shock [91, 107–109]. Among the 117 gene probes with predictive strength in microarray-based studies [29, 33], twelve candidate biomarkers were chosen for the PERSEVERE model based on biological plausibility and the ability to measure the gene products (proteins) in the serum compartment [30]. They collected samples from children less than 11 years of age admitted to the PICU who were diagnosed with septic shock [30]. They derived a decision tree using classification and regression tree (CART) methodology. This showed that a combination of five of the twelve biomarkers achieved the most accurate results (C-C chemokine ligand 3 or CCL3, heat shock protein 70 kDa 1B or HSPA1B, IL-8, neutrophil elastase 2 or ELA2, and lipocalin 2 or LCN2) [30]. The CART analysis yielded two groups: low risk and high risk. Within the low risk group mortality was 1.2% vs. 42.9% in the high-risk group [30]. After testing this in a validation cohort, the CART analysis was then updated using all subjects from both cohorts. At this point, three biomarkers appeared to be the driving force for stratification: CCL3, HSPA1B and IL-8, with ELA2 and LCN2 being replaced by granzyme B (GZMB) and matrix metalloproteinase 8 (MMP8) in the CART analysis [30]. In the new low-risk group, 28-day mortality was just 1.3% vs. 31.9% in the high-risk group [30]. A total of 119 patients were stratified into the high-risk arm with 81 patients surviving – leaving 81 false positives [30]. Of these false positives, 30% had persistent multi-organ failure after seven days compared with only 9% of the living low-risk subjects [30]. These false positive subjects also had significantly longer PICU length of stay, and significantly fewer PICU-free days [30]. Overall, the PERSEVERE model had a 93% sensitivity, 74% specificity, 99% negative predictive value, with an AUC of the ROC curve to predict mortality of 0.88 [30]. Thus, the PERSEVERE model predicted a combination of morbidity and mortality for these patients. These same PERSEVERE biomarkers were used to develop an analogous model for adults with sepsis [110, 111].

Wong et al. further explored the PERSEVERE model (including the biomarkers CCL3, IL-8, HSPA1B, GZMB and MMP8) by using samples from two different points during patients' first three days of sepsis with the primary end-point being a complicated course, defined as at least two organ systems failing at seven days or death within 28-days [112]. The sensitivity and specificity were 91% and 70% respectively, with a PPV of 47%, NPV of 96% and AUC of the ROC curve of 0.84 [112]. To further validate the temporal PERSEVERE model (tPERSEVERE), they performed a prospective study, again using samples from day 1 and day 3 of pediatric patients with sepsis [113]. The AUC for predicting a complicated course was 0.67, which prompted a redesign of the classification tree using baseline PERSEVERE mortality risk as the first level of decision, and relying heavily on IL-8, CCL3 and MMP8 levels [113]. This new model delivered a sensitivity of 88%, specificity of 64%, PPV 49%, NPV 93%, and AUC for predicting a complicated course of 0.84 [113]. Interestingly, IL-8 was a key decision point in the model. In the patients with higher risk of mortality IL-8 levels started high and remained elevated at day 3 [113]. In patients stratified to the lower risk group based on the initial decision point of low PERSEVERE-based mortality risk, patients whose IL-8 levels were elevated on day 1 had a 44.4% probability of a complicated course, compared to patients with low initial IL-8 levels whose likelihood was only 6.3% [113]. In addition, patients with higher CCL3 at day 3 were less likely to have a complicated course [113]. The authors postulated that the findings with IL-8 and CCL3 harken back to the notion of sepsis being a dynamic process with both excessive inflammation and a state of relative immune suppression, suggesting that tPERSEVERE could potentially serve as a monitoring tool [113].

PERSEVERE, therefore, could be used to select out low-risk patients and find the high-risk patients who would be more suitable candidates for higher-risk experimental therapies or interventional research studies [30, 113]. This is the concept of prognostic enrichment introduced above. Recently, PERSEVERE-II was developed to broaden application across different multi-organ failure phenotypes of pediatric shock including thrombocytopenia associated multiple organ failure (TAMOF) [114]. In a trial simulation, PERSEVERE-II-based prognostic enrichment substantially decreased the number of patients needed for an interventional trial of plasmapheresis in children with TAMOF [114]. Prospective studies are required to further test the ability of PERSEVERE to serve as a prognostic enrichment tool.

3.3 Gene Expression-Based Endotypes of Septic Shock: prognostic and predictive applications

Endotypes are subclasses of diseases based on biological processes. Recently, Wong et al. reported two septic shock endotypes (endotypes A and B) based on the expression patterns of 100 classifier genes. [115]. Endotype A subjects had higher rates of all cause 28-day mortality and complicated course compared to endotype B subjects [115]. By logistic regression, allocation to endotype A was independently associated with increased risk of mortality and complicated course, after accounting for age, co-morbidity, and illness severity [115].

The gene signature for endotype assignment reflects adaptive immunity and the glucocorticoid receptor signaling pathway, both of which are repressed in endotype A

subjects relative to endotype B subjects [115–118]. This suggests that the endotyping strategy could potentially predict treatment response to either immune-enhancing therapies or adjunctive corticosteroids. This is the concept of predictive enrichment introduced above. Indeed, in a *post hoc* analysis, controlling for illness severity and age, corticosteroids were independently associated with increased risk of mortality in endotype A subjects, but not endotype B subjects [115]. Recently, PERSEVERE-based prognostic enrichment was combined with predictive enrichment via endotype allocation as a means to identify children with septic shock who might benefit from adjunctive corticosteroids [119]. This *post hoc* analysis demonstrated that corticosteroid prescription was associated with a more than 10-fold risk reduction for poor outcome among endotype B subjects with a high PERSEVERE-based mortality risk [119]. While this finding requires prospective validation, it illustrates how biomarkers can potentially enable precision medicine in patients with sepsis via prognostic and predictive enrichment.

3.4 Bioscore: diagnostic applications

Gibot et al. examined the combined utility of PCT, sTREM-1 and nCD64 to diagnose sepsis in critically ill patients at the time of ICU admission [88]. Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) is a pro-inflammatory immunoglobulin inciting production of cytokines, chemokines, and reactive oxygen species, and increasing phagocytosis and degranulation of neutrophils [120]. sTREM-1 lacks evidence to support its utility as a standalone biomarker, however it may perform better in a combination biomarker set.

Gibot et al. drew blood within twelve hours of admission and again on day two [88]. Individually, all three biomarkers were found in higher concentration in patients with sepsis, with the AUC for the ROC curve to diagnose sepsis reaching 0.95, 0.91, and 0.73 for nCD64, PCT, and sTREM-1 respectively [88]. Further multiple logistic regression analysis showed that all three were independent predictors of sepsis [88].

They then created a bioscore. Patients received one point for each biomarker that exceeded its predetermined infectious cut-off for infection. A bioscore of 0 equated with infection rates of 3.8%, and a score of 3 equated with a 100% infection rate [88]. The bioscore diagnosed infection with impressive accuracy, yielding an AUC of 0.97 [88]. They then validated the bioscore in an independent cohort, again yielding an AUC of 0.95 [88].

This study provides a clinician-friendly scoring system with strong positive predictive value aimed at better determining which patients are infected. The biggest hurdle with implementing this specific bioscore is the laboratory-based work to determine the biomarker concentrations. At this point PCT can be run at most hospitals with turnaround time within hours. However, sTREM-1 is measured using an ELISA assay and nCD64 can only be quantified using a flow cytometer [88]. With three different laboratory modalities required, obtaining the raw data to determine the bioscore could be challenging. A bioscore has a role, however, especially if the included biomarkers can be quantified in a similar manner, saving costs and time.

4. Conclusion

Sepsis remains a worldwide problem, causing significant morbidity and mortality, and accounting for a considerable portion of healthcare expenditure. As sepsis is a heterogeneous process with individual variability, the diagnosis and treatment of patients remains a challenge. Biomarkers and combinations of biomarkers offer a way to better diagnose, monitor, and stratify patients to tailor the approach to sepsis. At this point, the most salient obstacles for ubiquitous biomarker usage are the feasibility and cost of measuring samples. Work is underway to create rapid point-of-care tests that are both user-friendly and low cost. With the advent of these new methods and likely determining which biomarkers fit best together to yield the robust information, recognition and management of sepsis will only improve.

5. Expert Commentary

Great strides have been made in candidate sepsis biomarker discovery and testing, with the above described biomarkers showing promise. However, sepsis is a dynamic illness with a great degree of biological heterogeneity. The recent reporting of gene expression-based endotypes of children with septic shock provides an opportunity to further resolve this heterogeneity [115]. Of note, Davenport and colleagues recently reported analogous gene expression-based endotypes among adults with sepsis [121]. It appears that the best diagnostic or prognostic approach is to combine multiple biomarkers to resolve sepsis-related heterogeneity.

6. Five-Year View

The goal of biomarker discovery is to produce tangible clinical change. It seems we are on the brink of readily creating such change using PCT to guide antibiotic cessation. The evidence is convincing that PCT-guided algorithms shorten duration of antibiotic usage [48–52]. This translates into healthcare cost savings and improved antibiotic stewardship thereby lessening the burden of antibiotic resistance. As of yet, this practice has not been universally adopted, but with growing substantiation, clinicians will likely incorporate it into their evidence-based clinical practice.

As we look forward, the role of precision medicine in sepsis will hopefully be better elucidated. The discovery of distinct endotypes points towards a new path – developing the tools to ascertain specific immunologic states thereby better targeting therapy. Building upon advances from oncology and virology, recent work has focused on the co-inhibitory cell surface molecules programmed cell death receptor-1 (PD-1), and its ligand, PD-L1, to determine what, if any, role they play in sepsis. PD-1 class molecules are found on T-cells, B-cells, and antigen presenting cells including monocytes and macrophages [122]. PD-1/PD-L1 coupling results in inhibitory T-cell signaling, apoptosis, down-regulation of T-cell activation and functional unresponsiveness [25, 123]. These receptors have been implicated in T-cell exhaustion: unrelenting antigen exposure causes loss of T-cell function [25]. Several studies have demonstrated that these molecules are up-regulated in sepsis, making it a candidate biomarker and a candidate therapeutic target [124–126]. In animal

studies, inhibition of PD-1 or PD-L1, either through genetic modification or introduction of an antibody, resulted in improved survival and decreased lymphocyte apoptosis [127–130]. Similar findings have been seen with *in vitro* analysis of human cells [126]. Chang et al. found a much higher percentage of lymphocyte apoptosis in septic patients compared with healthy controls [126]. When septic patients' cells were treated with anti-PD-1 or anti-PD-L1 antibodies, lymphocyte apoptosis was significantly reduced [126].

Clinical and translational studies involving PD-1 are relatively scant, as this pathway in sepsis is quite novel. Although the sample sizes were small, two studies found that increased percentage of PD-1 class molecules were seen in non-survivors [124, 126]. Additionally, higher expression of PD-L1 and PD-L2 on monocytes at day 3–5 of illness was associated with the development of secondary nosocomial infections [125]. The AUC of monocyte PD-L1 percentage to predict 28-day mortality was 0.73; and higher levels of this ligand were seen in patients with septic shock when compared to those who did not develop shock [124]. This early work suggests PD-1 class molecules could help with prognostics, and offers a novel treatment adjunct. Further studies must be undertaken, but clarifying patients' immunologic states and developing targeted therapies are the next steps to bolster precision medicine in sepsis.

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Reference annotations

* Of interest

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Key Issues

- Sepsis is a heterogeneous syndrome with patient-to-patient variability characterized by both immune hyperactivity and relative immune suppression.
- Biological markers, or biomarkers, have the potential to improve the diagnosis of sepsis, facilitate prognostication about morbidity and mortality, evaluate treatment response, and guide antimicrobial cessation.
- Procalcitonin (PCT), the prohormone of calcitonin produced widely during infection, is currently the biomarker of choice to aid in the diagnosis of sepsis although some studies show under-performance in differentiating between sepsis and sterile inflammation. PCT shows promise as a prognostic tool and as a monitoring device, guiding the discontinuation of antibiotics, thereby decreasing duration of therapy, length of stay, and healthcare expenditures.
- Interleukin-27 (IL-27) is a heterodimeric cytokine composed of two subunits, which originate from antigen presenting cells with diagnostic potential, especially in the pediatric population.
- Presepsin is a soluble fragment cleaved from cluster of differentiation 14 (CD14), a membrane surface glycoprotein on monocytes and macrophages. Recent studies demonstrate superior diagnostic ability when compared with procalcitonin, as well as a possible role both in stratification of patients and monitoring response to therapy.
- Neutrophil CD64 (nCD64) is an immunoglobulin receptor up regulated on neutrophil surfaces following pathogen exposure with strong diagnostic, prognostic, and therapeutic monitoring potential.
- Interleukin-8 (IL-8) is a chemokine produced by macrophages being studied as a tool to prognosticate about illness severity and mortality.
- Cell Free DNA (cfDNA) are fragments of DNA produced during cellular necrosis and apoptosis, which may aid in both diagnosis and prognosis of sepsis.
- Several combination biomarker sets have significant prognostic potential (PERSEVERE, gene expression-based endotype classification), diagnostic utility (11-gene sepsis MetaScore, Bioscore), and ability to monitor response to therapy (11-gene set sepsis MetaScore).

Table 1

Biomarker Composition and Potential Roles

Biomarker	Description	Circulating Protein	Gene-Based	Diagnostic	Prognostic	Monitoring
Procalcitonin (PCT)	Prohormone of calcitonin, produced widely during infection [32, 33]	Yes		Yes	Yes	Yes
Interleukin-27 (IL-27)	Heterodimeric cytokine composed of two subunits originating from antigen presenting cells [54, 60]	Yes		Yes		
sCD14-ST (Presepsin)	Soluble fragment of CD14, a membrane surface glycoprotein on monocytes and macrophages, that is a receptor of LPS and LPS-binding proteins [70, 71]	Yes			Yes	Yes
Neutrophil CD64 (nCD64)	Immunoglobulin Fc-γ receptor I found on neutrophils after pathogen exposure [82, 83]	Yes		Yes	Yes	Yes
Interleukin-8 (IL-8)	Chemokine produced by macrophages [23]	Yes			Yes	
Cell Free DNA (cfDNA)	DNA fragments produced by cellular necrosis and apoptosis [95, 96]	Yes		Yes	Yes	
11-Genes MetaScore	11-gene set based on mRNA expression during sepsis [105]		Yes	Yes		Yes
Pediatric Sepsis Biomarker Risk Model (PERSEVERE)	Model based on five biomarkers (CCL3, IL-8, HSPA1B, GZMB, MMP8) discovered using genome-wide expression profiling [30, 112, 113]	Yes			Yes	
Gene Based-Expression Endotype Classification	Denotes degree of variability in gene expression of genes coding for the adaptive		Yes		Yes	

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Biomarker	Description	Circulating Protein	Gene-Based	Diagnostic	Prognostic	Monitoring
	immune system and glucocorticoid signaling [115]					
Bioscore	A score from 0–3 based on levels of PCT, sTREM-1, nCD64 [88]	Yes		Yes		