Biomarkers of Sepsis

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Sepsis is a highly heterogeneous clinical disorder currently characterized almost exclusively by the use of physiologic variables. A burgeoning interest in the potential descriptive role of biomarkers in sepsis holds the promise of transforming the diagnosis from a clinical one to a biologic one, and so permitting better evaluation and use of a spectrum of adjuvant therapies. Biomarkers provide information in one of three domains: diagnosis, prognosis, and monitoring of response to treatment. Their primary prognostic utility, however, is not in forecasting outcome, but in identifying patients who are more likely to benefit from (or be harmed by) a particular intervention. A proposed template for staging sepsis in a manner analogous to systems used in oncology provides a framework for evaluating sepsis biomarkers. The model stratifies patients on the basis of predisposition, insult, response, and organ dysfunction, generating the acronym PIRO. This brief review considers the methodologic basis for biomarker development and validation and situates some emerging sepsis biomarkers within the framework of the PIRO model.

Introduction

Sepsis is an enormously complex clinical syndrome that arises from the activation of an innate host response to danger. Historically, the term has been restricted to describe patients in whom a systemic host inflammatory response is evoked by invasive infection [1]. However, newer insights into the biology of innate immunity suggest that in terms of impact on outcomes, the specific insult evoking the response is less important than the nature of the response itself [2].

Consistent with the syndrome's intrinsic biologic complexity, its treatment is multimodal. Resuscitation and restoration of tissue perfusion is the first management priority [3,4••]. Infection is the most common cause of the syndrome, and once resuscitation has been initiated and the immediate cardiorespiratory threat to life has been addressed, the next priority is diagnosis of the site and microbiology of infection. Then clinicians can initiate appropriate anti-infectious measures in the form of broad spectrum antibiotics and source control.

For the majority of patients with uncomplicated sepsis, these measures will suffice to reverse the process, and the patient will recover. In a minority of patients, however, the inciting insult evokes a more fulminant response, characterized by organ dysfunction and necessitating more invasive approaches to diagnosis and support, generally within the confines of an intensive care unit (ICU). Sepsis of sufficient severity to jeopardize normal physiologic organ function is termed "severe sepsis," and if the consequences include cardiovascular dysfunction of sufficient severity to compromise tissue perfusion, the process is known as "septic shock" [5]. Beyond measures to support vital organ function in the ICU, some patients with severe sepsis or septic shock derive clinical benefit from adjuvant therapies targeting discrete pathologic derangements that contribute to the clinical syndrome. Two of these are sufficiently well-characterized and have become valid therapeutic targets. Recombinant human activated protein C (drotrecogin alpha activated) provides exogenous replacement of depleted levels of an important endogenous anticoagulant and has been shown to improve survival rates for sicker patients with severe sepsis or septic shock [6]. Pharmacologic doses of glucocorticoids reverse an acute state of adrenal insufficiency that is relatively common in septic shock and characterized by refractory hypotension and an impaired response to adrenocorticotrophic hormone (ACTH) stimulation [7].

Unfortunately, the optimal management of the septic patient with currently available therapies and the development of new, more effective therapies has been hampered by the imprecision of current descriptive methods and a lack of validated measures to link measurable changes in systemic biology to potentially useful therapeutic interventions. This concept is best appreciated by considering an exception to this generalization. Maintenance of a normal glucose level in the critically ill has been associated with reduced septic morbidity and increased survival [8], and the practice has been widely adopted as a key element in the optimal management of the septic patient. Glucose levels are monitored closely, and exogenous insulin is administered to maintain levels within a normal range. The intervention is only feasible because there is a reliable marker—blood glucose—to guide a specific therapy—insulin.

Other therapies of potential use in sepsis, including activated protein C, antibodies to tumor necrosis factor (TNF), or strategies to neutralize endotoxin, have been evaluated on the basis of the crude physiologic criteria of sepsis syndrome [9] or the systemic inflammatory response syndrome (SIRS) [1], without determining whether the target was present in the patient population or if the dose of the intervention was appropriate to inhibit or replenish the target in question.

Biomarkers of sepsis could transform this syndrome defined by crude, nonspecific physiologic criteria into one or more diseases that can be diagnosed and monitored as biochemical derangements that are potentially responsive to manipulation. This short review will summarize the current state of the art and outline the challenges in achieving that transformation.

Biomarkers: Taxonomy and Methodologic Principles

A biomarker can be defined as a biochemical or cellular measure of a biologic state or process. This definition differentiates biomarkers from physiologic measures in that physiologic measures (eg, temperature or cardiac index) reflect events in vivo, whereas biomarkers can be determined ex vivo and reflect a state of biologic affairs at a discrete point in time. As the term is commonly used, it implies a measurement that is distinct from routinely performed laboratory investigations, although this distinction is more a reflection of an exaggerated sense of anticipation than of clinical utility. Thus a Gram's stain or quantitative culture of bronchoalveolar lavage fluid can be appropriately considered biomarkers of infection, whose performance characteristics are amenable to study in the same way as a biochemical parameter such as procalcitonin or C-reactive protein.

From a pragmatic perspective, the utility of a medical biomarker arises through its capacity to provide the clinician with information beyond that which is available from readily obtained clinical and biochemical parameters. In other words, biomarkers have value to the extent that they refine the description of a disease state and inform further clinical decisions. This process of refinement and information can occur in one or more of three domains: diagnosis, prognosis, and prediction of treatment response.

A marker may provide information to definitively establish a diagnosis (eg, documentation of trisomy 21 to diagnose Down's syndrome, or bacteremia with *Pseudomonas* to diagnose invasive bacterial infection). Also, a marker could help increase or decrease the probability of a diagnosis, perhaps directing the clinician to perform further tests (eg, fecal occult blood as a marker for colonoscopy to look for cancer, or *Pseudomonas* bacteremia as a marker to suggest the need for further studies to identify a focus of infection amenable to source control measures).

A marker may also serve to provide information about prognosis, in general by situating a patient within a subpopulation whose outcome is either better or worse than that of the entire population. This information may be but is not often of value in informing a decision to alter therapy. For example, among women with breast cancer, preoperative levels of CA 15.3 or carcinoembryonic antigen (CEA) provide the best measure of tumor burden and hence of prognosis, whereas specific tumor markers such as estrogen receptor status or HER-2 provide information on potential response to estrogen receptor blockade and herceptin or anthracycline-containing chemotherapy, respectively [10,11].

Finally, a marker may provide information on a patient's response to therapy and so permit titration of therapy to optimize efficacy. Such markers play a key role in the management of the critically ill patient whose insulin dose is titrated to serum glucose levels or whose serum lactate level is monitored as an index of tissue perfusion.

In general terms, an ideal biomarker for a process such as sepsis should maximize four key characteristics. First, it should show biologic plausibility, being credibly and specifically linked to the discrete process it purports to measure. Implicit in this concept is the expectation that the biomarker adds information by delineating a subpopulation of patients with a clinical condition. For example, endotoxemia is a measure of the presence in the blood of products from gram-negative bacteria, and perhaps by inference, of infection with viable gram-negative organisms [12]. Similarly, as the metabolic byproduct of anaerobic metabolism, lactate is a plausible marker of tissue ischemia. Neither is a marker of sepsis per se, but rather of a distinct facet of sepsis that may be present to a variable degree in patients meeting nonspecific criteria for sepsis. As a result, each marker adds specific information about an aspect of the disease that is potentially amenable to therapy. Biologic plausibility is not a prerequisite for a useful marker; however, the utility of a marker is enhanced when the clinician can link the test directly to the pathologic state that is being measured.

Second, a useful biomarker should demonstrate appropriate sensitivity and specificity for the process it measures (Fig. 1). Sensitivity refers to the capacity of a marker to detect a disease in patients in whom the disease is truly present. Conversely, specificity is the marker's capacity to rule out the disease in those patients in whom the disease is truly absent. In practice, a tradeoff exists between sensitivity and specificity: as sensitivity is increased, specificity is lost. A receiver operating characteristics (ROC) curve reflects this relationship. Perhaps more relevant to the clinician are the positive and negative predictive values of the test. The positive predictive value represents the percentage of people with a positive test who truly have the disease, whereas the negative predictive value is the percentage of people with a negative test who truly do not have the disease. The positive and negative predictive values are influenced by the prevalence of the disease in a population: if the prevalence is low, the negative predic-

tive value of the test will be high, even if the reliability of the test is low.

Third, a useful biomarker should be readily, reliably, and reproducibly measured. For an acutely life-threatening condition such as sepsis, the test must yield results rapidly. Also, inter- and intra-assay variability must be low, and the cost should be reasonable for a test that finds wide clinical use. Evaluation of the accuracy of a test can be further confounded by differences in the measured levels versus the measured bioactivity of a marker. For example, in a large clinical trial of a monoclonal antibody to TNF, analysis of a subgroup of patients showed that the antibody reduced circulating levels of immunoreactive TNF, but did not reduce TNF bioactivity [13]. Did the agent bind but not neutralize TNF, or did it neutralize TNF but not another substance in the samples that produced spurious evidence of bioactivity?

While a bioassay may show biologic activity, it may not reflect the specific activity of the substance it purports to measure. For example, the limulus amebocyte lysate (LAL) assay, which is widely used to detect endotoxin, can be activated by cell wall products from fungi and can be artifactually negative due to the presence in plasma of circulating inhibitors of the coagulation response whose activation denotes the presence of endotoxin [14,15]. Finally, the binding of a marker to proteins such as albumin can result in variability in assay results [16].

The final and perhaps most important requirement for a useful marker is that it be directly linked to a therapeutic decision. A large number of measures have been proposed as useful biomarkers of sepsis on the basis of their being differentially altered in patients who meet clinical criteria for sepsis [17•]. In the absence of further information that guides the clinician to institute an intervention or to undertake a further diagnostic test, the mere confirmation of risk does not aid in patient management, and such a marker is unlikely to find widespread use. Two recent reports underline this fundamental challenge facing developers of biomarkers for use in the critically ill. Borgel et al. [18] from France measured circulating levels of a protein known as growth arrest-specific protein-6 (Gas6) in a cohort of critically ill patients with organ dysfunction of either infectious or noninfectious cause. They reported that median Gas6 levels were highest in patients with severe sepsis, and that levels correlated with severity of illness and the degree of organ dysfunction. In a separate study, Lee et al. [19] performed serial assays of plasma gelsolin levels in 31 critically ill surgical patients, and found that levels lower than 61 mg/L were associated with longer ICU stay, longer ventilator dependence, and higher hospital mortality. Similar associations have been reported for scores of putative biomarkers of sepsis [17•]. But unless that knowledge of the level of a marker can prompt the clinician to do something that might change patient outcome (eg, neutralize Gas6 or administer gelsolin), none will find a role in clinical practice.

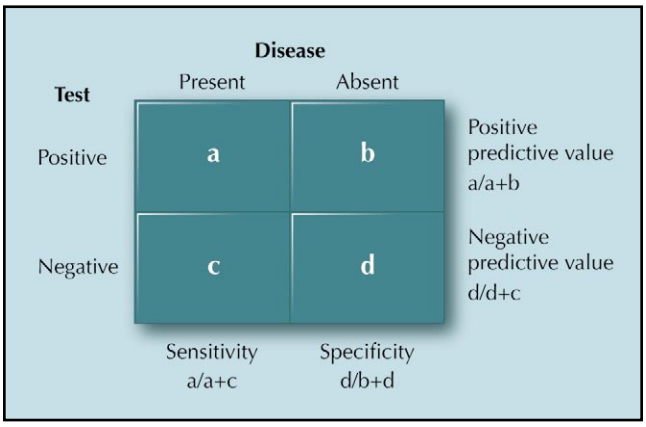

Figure 1. The concepts of sensitivity and specificity relate a diagnostic test to the presence or absence of a disease, as measured by an independent gold standard. Positive and negative predictive values, on the other hand, measure the prevalence of a disease in patients with a positive or negative test.

It was once suggested, regarding one of the best studied biomarkers of sepsis, "It is a great answer, but what is the question?" Defining the question that markers might answer is truly the unsolved challenge.

Biomarkers of Sepsis: What is the Question?

The utility of a biomarker of sepsis comes from its ability to inform a diagnostic or therapeutic decision—to identify a subgroup of patients who are more likely to benefit from a given intervention and to maximize clinical benefit of that therapy. The treatment of sepsis includes initial resuscitation and restoration of tissue oxygenation, the eradication of infection, support of impaired organ function, and adjuvant measures to modulate the systemic inflammatory response. Resuscitation is typically guided by physiologic parameters including heart rate, blood pressure, central venous pressure, and urine output, although the lactate level provides evidence of tissue ischemia [4••], and improved lactate clearance is associated with a better prognosis [20]. Gram's stain and culture provide the most commonly used biomarkers of infection, whereas physiologic support in the ICU is generally initiated and titrated on the basis of simple laboratory markers of impaired function such as arterial blood gases, serum creatinine, or circulating platelet count. Biomarkers offer the greatest promise in the development, evaluation, selection, and titration of adjuvant therapies.

Acknowledging the challenging heterogenic population of patients with sepsis, a recent consensus conference recognized the need for more sophisticated systems of describing and staging sepsis. By analogy to the tumor, nodes, metastasis (TNM) system widely used in oncology, the conference suggested that it may be possible to stratify patients by differences in four different domains of the process: the predisposing factors present at the time of onset of illness, the nature of the insult, the extent

Table 1. The PIRO Model

Domain	Current Clinical Measures	Potential Biomarkers
Predisposition	Gender, age, premorbid conditions, cultural and religious beliefs, personal preferences.	SNPs in discrete components of the innate immune system.
Insult	Microbial cultures, Gram's stain, radiologic detection of infection or injured/ischemic tissue.	Assay of microbial products (LPS, mannan), detection of bacterial RNA or resistance genes, procalcitonin.
Response	Temperature, white blood cell count, hemodynamic parameters.	Acute phase reactants (eg, CRP), IL-6, cellular markers of altered function (eg, HLA-DR), detection of target of therapy (eg, TNF, PAF, IL-1).
Organ dysfunction	Quantitative organ dysfunction scales (MODS, SOFA, LODS).	Biochemical or cellular measures of altered function—apoptosis, response to stimuli.

CRP—C-reactive protein; IL—interleukin; LODS—logistic organ dysfunction score; LPS—lipopolysaccharide; MODS—multiple organ dysfunction score; PAF—platelet-activating factor; PIRO—predisposition, insult, response, organ dysfunction; SNPs—single nucleotide polymorphisms; SOFA—sequential organ failure assessment; TNF—tumor necrosis factor. *Data adapted from* Levy et al. [5].

and nature of the host response evoked, and the resultant degree of organ dysfunction [5]. Together, these create the acronym PIRO (Table 1). As with the TNM system, the PIRO system is designed to stratify patients not only on the basis of their risk for adverse outcome (ie, their prognosis), but even more importantly by their potential to respond to specific therapies. Additionally, the model provides a useful template for considering emerging biomarkers of sepsis.

Markers of predisposition

Genetic factors are potent determinants of the probability of survival following infection [21]. This influence is believed to arise through a high degree of variability in individuals' innate immune response to infection, a consequence of a high prevalence of polymorphisms in the genes encoding the mediators of this response [22]. The most common of these are single nucleotide polymorphisms (SNPs), resulting from substitution of a single base pair in the gene. If the SNP occurs in an exon (the region of the gene that codes for protein), the amino acid sequence of the protein—and hence its function—will likely change. For example, a SNP in the gene for Toll-like receptor (TLR) 4, the cell surface protein that is responsible for the recognition of endotoxin, results in a single amino acid change in the extracellular domain of the receptor and renders affected mice hyporesponsive to endotoxin [23]. Mutations in *Tlr4* are found in humans and exert variable effects on infectious risk, protecting from Legionnaire's disease [24] but increasing the risk of sepsis following burn injury [25].

One of the better studied SNPs relevant to sepsis is a G->A substitution at the -308 position in the promoter region of the gene for tumor necrosis factor (TNF)-α. The A variant is found in approximately 20% of the general population but at significantly higher rates in patients with sepsis, in whom it is associated with higher circulating TNF levels and a higher mortality risk [26,27]. In patients with rheumatoid arthritis, data are emerging to suggest that patients with this SNP [28] or others involving the TNF or HLA-DR loci [29] have a better response to anti-TNF therapies. Whether these might also delineate sepsis patients more likely to benefit from anti-TNF therapy is unknown.

SNPs and other genetic alterations are attractive candidate biomarkers for the stratification of patients with sepsis. Their interpretation and use, however, pose significant challenges. Reports of associations are highly dependent on study design and conduct [30], often resulting in contradictory conclusions regarding the association of specific markers with adverse outcome. Multiple polymorphisms are often found in a single gene or in several genes that are in linkage disequilibrium, which may mean researchers will need to evaluate gene families or clades rather than individual SNPs [31•]. Since fast, reliable technology to identify genetic variation is improving rapidly [32], such determinations may be feasible for decision-making in sepsis in the near future.

Markers of insult

Although the diagnosis of infection requires either culture or Gram's stain demonstrating a pathogen invading normally sterile tissues, conventional bacteriologic methods have limitations in the management of sepsis. The time required for an organism to grow in culture and to be identified by standard techniques is often of the order of several days, long after decisions about anti-infective therapy must be made. Moreover, cultures may be falsely positive in patients whose mucosal surfaces are colonized with potential pathogens or falsely negative in patients receiving antibiotics. Alternate approaches to establish the presence of invasive infection and the identity and antibiotic susceptibility patterns of the infecting organism(s) are desirable.

One of the more promising and certainly best-studied biomarkers of invasive infection is the calcitonin precursor procalcitonin (PCT). An association between elevated levels of PCT and active infection was first made more than a dozen years ago [33], and several observational

studies have confirmed the association [34•], though elevated levels of PCT may be seen in certain noninfectious disorders. More importantly, recent interventional studies have suggested that the use of PCT as a diagnostic biomarker can safely reduce the use of empiric antibiotics in patients with suspected community-acquired respiratory tract infections [35] and shorten the course of therapy for patients with pneumonia [36••].

C-reactive protein, an acute phase reactant synthesized by the liver, is widely used in Europe as a diagnostic biomarker of invasive infection. Its performance is promising, though it consistently appears to be less sensitive and less specific than PCT in establishing a diagnosis of infection [34•].

A cleaved receptor from myeloid cells, soluble triggering receptor expressed on myeloid cells (sTREM)-1, has also shown promise as a rapid marker of invasive infection. Levels of sTREM-1 in bronchoalveolar lavage fluid are elevated in patients with pneumonia [37], and levels rise progressively in patients who develop ventilator-associated pneumonia [38].

Bacterial products can also be identified, both as a signature of invasive infection and as a therapeutic target in their own right. Lipopolysaccharide from the cell wall of gram-negative bacteria can be detected rapidly (in half an hour) using a point-of-care chemiluminescence-based assay [12]. Endotoxemia is much more common than culture-proven infection, though its absence increases confidence in the conclusion that infection is absent. Perhaps the greater promise of an endotoxin assay lies in identifying appropriate patients to be treated with endotoxin-neutralizing strategies and in following their response to intervention to optimize the dose and duration of treatment.

Other markers of infection are in the early stages of clinical evaluation. It has been suggested, for example, that abnormalities in wave-form analysis during the performance of an activated partial thromboplastin time provide useful information on the presence of infection [39].

Markers of host response

Variability in genetic predisposition, severity of the inciting insult, and time to and adequacy of early management suggests that the host response will be similarly variable. For treatments that target that response, objective and quantitative measures of response can aid in decisions to start or discontinue therapy and in the titration of optimal dose.

The cytokine interleukin (IL)-6 is one of the best-studied nonspecific markers of host response [40]. IL-6 levels rise precipitously in sepsis, and increased IL-6 levels identify a patient at both increased risk of death and possibly increased benefit from anti-TNF therapy [41]. Circulating levels of IL-6 tend to be more stable over time than levels of TNF.

An altered cortisol response to stimulation with ACTH, characterized by increased basal levels of cortisol but impaired secretion on stimulation, identifies patients at increased risk of ICU mortality [42]. More importantly, it also appears to delineate a group of patients with refractory septic shock who will benefit from treatment with pharmacologic doses of corticosteroids [7]. Similarly serum glucose levels identify patients who will benefit from therapy with exogenous insulin and facilitate dose titration [8,43].

Reduced expression of HLA-DR on the surface of monocytes also identifies an at-risk patient population and could delineate a population of patients who might benefit from treatment with interferon-γ [44].

Markers of organ dysfunction

Organ dysfunction in critically ill patients is commonly quantified through the use of one or more clinical scales [45–47], in no small part because the biochemical and cellular processes responsible for organ dysfunction are poorly understood. Biochemical measures such as the ketone body ratio [48] or measures of cellular process such as programmed cell death or apoptosis [49] may eventually find a role as biologic measures of organ dysfunction, although their current role is minimal.

Conclusions

The identification and validation of biomarkers reflecting discrete facets of a complex disease process such as sepsis can transform this disorder, which is primarily a concept defined by suggestive but nonspecific physiologic parameters, into one or more diseases that can be treated using multimodal therapeutic approaches. However, biomarker development for sepsis is a field in its infancy, hampered by an underdeveloped methodologic framework for biomarker evaluation and a lack of candidate therapies that could validate biomarkers by demonstrating their impact on clinical outcome.

This short review has sought to emphasize that the clinical utility of a biomarker depends on more than its differential expression in patients having sepsis defined by physiologic criteria or its prediction of subsequent adverse outcomes. Pure prognostication has less of a role to play in acute disorders such as sepsis. While an early diagnosis of Huntington's disease or familial polyposis may enable an individual to use antenatal screening or undergo a prophylactic colectomy, early diagnosis of impending complications or death in the critically ill patient provides fewer therapeutic options. Demonstration that a biomarker could identify an infectious complication such as a hospital-acquired pneumonia or anastomotic leak while patients were still asymptomatic might permit pre-emptive therapy, but this strategy has not been tested. Rather, the role of biomarkers is to parse a heterogeneous population of patients, identifying candidates for specific therapy and enabling the optimal initiation, titration, and discontinuation of that therapy. The primary role of a biomarker is to predict response to therapy rather than ultimate prognosis.

The PIRO template described above provides a framework for evaluating putative biomarkers of sepsis. It will be appreciated that the categories are somewhat arbitrary and overlap considerably. For example, assay of TNF may provide a measure of the response to an acute insult, but it also reflects the presence of an insult when the therapy being evaluated seeks to neutralize TNF. Despite these points, it does establish a taxonomic structure that may assist in biomarker development and validation.

Sepsis is common, lethal, and eminently treatable. Initiatives such as the Surviving Sepsis Campaign suggest that preventive measures can have a major impact on the epidemiology of sepsis. The development of new therapies has proceeded much more slowly because of a persisting inability to identify optimal patient populations who might respond to treatments targeting a very broad and even conflicting range of biologic processes. A more rational and explicit incorporation of biomarkers into future research designs may well aid in addressing this challenge.

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