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Review Article

A NOVEL COMBINATION OF BIOMARKERS TO HERALD THE ONSET OF SEPSIS PRIOR TO THE MANIFESTATION OF SYMPTOMS

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ABSTRACT—Sepsis, which kills over 200,000 patients and costs over $20 billion in the United States alone, presents a constant but preventable challenge in the healthcare system. Among the more challenging problems that it presents is misdiagnosis due to conflation with other inflammatory processes, as its mechanisms are identical to those of other inflammatory states. Unfortunately, current biomarker tests can only assess the severity and mortality risk of each case, whereas no single test exists that can predict sepsis prior to the onset of symptoms for the purpose of pre-emptive care and monitoring. We propose that a single test utilizing three, rather than two, biomarkers that appear most quickly in the blood and are the most specific for sepsis rather than trauma, may improve diagnostic accuracy and lead to lessened patient morbidity and mortality. Such a test would vastly improve patient outcomes and quality of life, prevent complications for sepsis survivors, and prevent hospital readmissions, saving the American healthcare system money. This review summarizes the current use of sepsis biomarkers to prognosticate morbidity and mortality, and rejects the current single-biomarker and even combination biomarker tests as non-specific and inaccurate for current patient needs/pro-inflammatory cytokines, general markers of inflammation, and proteins specific to myeloid cells (and therefore to infection) are discussed. Ultimately, the review suggests a three-biomarker test of procalcitonin (PCT), interleukin-6 (IL-6), and soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) to diagnose sepsis before the onset of symptoms.

KEYWORDS—Hemodynamic monitoring, prevention, pro-inflammatory cytokines, regulation, systemic inflammation

INTRODUCTION

Sepsis, recently redefined as life-threatening organ dysfunction caused by a dysregulated host response to infection (1), is one of the top 10 leading causes of hospital deaths in the United States, with approximately 200,000 deaths occurring per year (2). The average mortality rate among patients with sepsis is as high as 33.2% (1). In patients with septic shock and further complications, the mortality rate is much higher and often exceeds 60% (4, 5). It is estimated that sepsis-related costs in United States hospitals exceeds $20 billion in expenses annually, which is approximately 5% of the country's healthcare budget (1). Although mortality in the United States has decreased with the improved and standardized treatment for sepsis over the last several decades, the overall numbers of cases and hospitalizations have increased significantly within the same time period (6). Current guidelines not only elucidate standards of care for healthcare professionals, but also highlight the importance of cohesive modeling and diagnostic criteria to treat a disease both so common and so deadly (7).

Due to its high mortality, its high rate of organ dysfunction, and its high costs to hospitals, the importance of early detection of sepsis cannot be overstated. Given the fact that patients of increased age and lower socioeconomic status have decreased access to the critical care in a hospital setting, this group of patients is especially vulnerable to higher death rates from sepsis in general. Therefore, fast and precise detection of sepsis by biomarkers may save lives, especially if it can be a point-of-care test (2, 8).

Clinical trials have overwhelmingly shown that early detection is both necessary and beneficial, as early diagnosis initiates more aggressive treatment of sepsis, preferably within the first 6 h after detection; since early trials, standards of state-of-the-art care have been changed, producing better patient outcomes (9). Without early aggressive interventions with crystalloid, fluid replacement, hemodynamic monitoring, vasodilators, or vasoconstrictors as needed in addition to traditional antibiotic treatment, sepsis patients who receive only antibiotic treatment have twice as high a risk as experimental patients of death from a sudden cardiac event (10). Although the diagnostic criteria of sepsis have changed (1), the symptoms of sepsis have not, thus stressing the validity for much better and more accurate diagnostic tools with biomarkers.

A number of biomarkers upregulated in the process of sepsis exist that have proven useful, although not definitive, in predicting sepsis severity and mortality. However, as of yet, there is no single standard biomarker or even a combination of biomarkers that are universally dependable for definitive early diagnosis (1, 11). Although very little exists to predict sepsis prior to the onset of symptoms in patients at risk, newly

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developed and emerging technologies—combined with existing biomarkers that set a lower potential for diagnostic error—may allow clinicians to achieve better diagnostic and predictive accuracy.

KNOWN DATA ON SEPSIS DETECTION BIOMARKERS

The diagnosis of sepsis can be initially very difficult. The sequential organ failure assessment score (SOFA score) can only be used to determine the extent of patient's organ dysfunction. This scoring system addresses an individual's respiratory, cardiovascular, hepatic, renal, coagulation, and neurological systems in the intensive care unit. An increase of two points in a patient's SOFA score, consequent to an infection, allows the classification of organ failure or dysfunction, and signifies an increase in in-hospital mortality of more than 10%—higher than the average mortality rate of patients in the hospital for electrocardiogram (ECG)-diagnosed ST-elevated myocardial infarction (1, 11). Septic shock, a further sequela, is identified by hypotension to the point of requiring aggressive rehydration, hemodynamic monitoring, and vasopressors to keep mean arterial pressure above 65 mmHg and lactate levels greater than 18 ng/mL (1). The formerly accepted diagnostic sequelae of systemic inflammatory response syndrome (SIRS) leading to sepsis and thus to multi-organ dysfunction syndrome (MODS) are no longer considered valid. Previously, this diagnosis was made based on values exceeding arbitrary cutoffs of two of the four diagnostic parameters, temperature, heart rate, white blood cell count, and respiratory rate or P_aCO_2 (1).

Currently, inflammatory biomarkers are typically used in prediction of morbidity and mortality in extant cases of sepsis. High levels of lactic acid, for example, are correlated with higher 90-day mortality rates in patients with septic shock than those with lower levels (12). Although these markers are effective enough in prognosticating the severity of sepsis course and likelihood of patient mortality to be considered as options for standard practice, they have nevertheless not been used to predict sepsis and circumvent its progress before symptoms begin. Given that the process of sepsis has been extensively studied and is well-known, this is an issue that may be relatively easy to address.

Biomarkers related to the symptoms of sepsis rather than the mechanisms of inflammation have also been tested, such as CT-proAVP (C-terminal segment of pro-arginine vasopressin), which aids in regulation of blood pressure; however, these biomarkers have not proven effective in diagnostic testing (13). Central venous pressure is an aggressively monitored vital statistic and predictor of morbidity and mortality in sepsis patients, but possibly due to the complexity of sepsis, these biomarkers are unusable for such a purpose themselves (13, 14). This data only emphasizes the need for effective biomarkers usable in standard testing.

In the typical sepsis exemplified by severe bacteremia and its consequences, the inflammatory response begins when Toll-like receptors (TLRs), particularly TLR4, are stimulated by endotoxins—such as lipopolysaccharide (LPS)—that bacteria produce as virulence factors (15). This process initiates a global, nonspecific innate inflammatory response that produces a cascade of first pro-inflammatory, followed by anti-inflammatory, cytokines; with normal regulation, this cycle of cytokines continues to its resolution (1, 15). Resolution may involve either recovery or death of the patient. Although the process is complex due to this somewhat vicious cycle, the most prominent cytokines in the early phase, which are often taken as an estimate of severity if measured, are three cytokines most often defined as standard: interleukin (IL)-1-β, IL-6, and tumor necrosis factor alpha (TNF-α). These first response molecules are initially upregulated in the early phase of a normal systemic inflammatory response and subsequently followed by rising levels of anti-inflammatory cytokines, most prominently IL-10; IL-10 levels have been found to rise in as little as 2 h after TLR stimulation (15). Another participating anti-inflammatory molecule, MAP kinase phosphatase 1 (MKP-1), is stimulated by TLR-activated pathways (4). Finally, adaptive immunity-associated cytokines IL-2 and IL-4 are also associated with sepsis development and its severity, although the association is less clear than with other pro-inflammatory cytokines (11).

Ultimately, the pathology of septic shock comes from complete dysregulation of pro- and anti-inflammatory cycles of cytokines and other regulatory molecules. Uncontrolled production in the liver of pro-inflammatory cytokines, without sufficient rallying of the body's natural defenses against such a process, leads to an equally uncontrolled, nonspecific, systemic inflammatory process that the body is often incapable of countering; therein, within this out-of-control process, lies the danger of septic shock. This is also the reason that, while DNA and other markers of pathogens can be detected in the laboratory, this method is not viable as a clinical diagnostic tool; the mechanisms are too unpredictable and the required time too long (16).

These are not novel and solely dangerous processes. In effect, sepsis and septic shock are simply ''too much of a good thing''; indeed, the same cytokines, chemokines, and inflammatory cells that are damaging and deadly in sepsis protect us as they eliminate infections in normal circumstances. While systems and tests exist that can identify some markers of these processes, development of more accurate systems/tests that use a current combination of known biomarkers to predict the onset of sepsis can improve healthcare costs and patient outcomes.

PRO-INFLAMMATORY CYTOKINES

The production of multiple cytokines correlates with the severity and mortality of sepsis cases, and indicates that the process of uncontrolled inflammation specific to sepsis has begun. Although appearance of MCP-1 (monocyte chemotactic protein) and IL-8 correlates with an increased 28-day mortality, high 24-h concentrations of IL-6, IL-8, and G-CSF (granulocyte colony-stimulating factor) are associated with increased organ dysfunction. Out of these four cytokines, only MCP-1 is independently associated with early prognosis of sepsis outcome (17). However, it must be noted that correlations/associations do not translate to causation or to usefulness in the pre-symptom diagnosis of sepsis.

The three cytokines usually associated with the early phase of inflammation (IL-1β, TNF-α, and IL-6) are themselves also good predictors of sepsis-induced 28-day mortality. The major problem with early-phase cytokines as independent predictors of sepsis outcome and development is the fact that sepsis is biphasic, namely early pro-inflammatory processes are followed by late-occurring anti-inflammatory processes The two contradictory phases then begin to alternate with pro- and anti-inflammatory phases as the patient's condition fluctuates and overall condition worsens, leading to clinically defined organ dysfunction and later failure (18). In fact, in the later stages of sepsis, the ''confused'' body, with its organs overwhelmed by too many contradictory stimuli, becomes hypo-responsive to and then overwhelmed by massive amounts of bacteria-produced endotoxins such as LPS. This late stage is in complete opposition to an earlier stage: hyper-responsiveness to the cytokine storm of sepsis (19). In that early stage, markers such as the Soluble Triggering Receptor Expression on Myeloid Cells (sTREM-1, to be presented later) that correlate positively with other prognostic markers and tests will correlate negatively with those same molecules. All of these complex interactions are further complicated by the varied progression of sepsis, the pathologies of which may cause very different levels of organ dysfunction (19).

The measurements of cytokines are useful for the diagnosis and prediction of sepsis in patients at risk but with certain limits. Specifically, IL-6 has recently been shown as a potential diagnostic marker for sepsis. In fact, Roche Diagnostics Operations has patented an immunoassay based on antibody complex formation and detection in the solid phase for IL-6. Based on this assay, IL-6 levels may be used as a potential diagnostic marker for potential sepsis; an IL-6 level 10-fold more or higher than the baseline value is considered a fairly early indication of sepsis (20). Roche indeed calculated the baseline IL-6 levels, for the purpose of clearly distinguishing pre-sepsis from trauma or other non-bacterial hematological factors during a trauma procedure, from the patient's blood during said surgery (20).

IL-6 levels rise before C-reactive proteins, which are commonly tested in cases of suspected sepsis and other inflammatory processes. Measuring IL-6 and C-reactive proteins offers the possibility for detecting sepsis before the early-phase cytokine storm can initiate the innate inflammatory process that damages organs and tissues. A recently reported clinical study showed good predictive values for IL-6 before symptoms began, but the sample size was small, with only 3/20 patients diagnosed prior to developing sepsis (4). Nevertheless, this cytokine retains a potential as a valuable marker to be evaluated in future studies, as it rises early in concordance with increasing concentrations of endotoxin and, thus, sepsis severity; studies of IL-6 and endotoxinemia have shown strong associations between the biomarker and the condition in tests of patients with abdominal sepsis (21).

Neonates have poorly developed regulatory immune systems and are more susceptible to all manner of infections, some leading to sepsis, than are adults (22). Birth is another traumatic process that carries a unique risk of sepsis for a neonate, in particular disease caused by Group B *streptococci*. This thereby presents an excellent opportunity to test biomarkers as contributions to sepsis prediction. Studies of freshly collected umbilical cord blood demonstrated a rise in pro-inflammatory cytokines that subsequently led to sepsis diagnosed within 2 h after birth (23). Again, IL-6 proved to be a better marker of sepsis as compared to IL-8 (23). In neonates with possible sepsis diagnosis, IL-6 and sTREM-1 have proven accurate in differentiating non-infected infants from infected, although not in differentiating suspected from diagnosed sepsis in retrospect (24). Infants with poorly developed regulatory immune mechanisms are more susceptible to sepsis. Nonetheless, the independent ability of IL-6 to predict sepsis remains relevant (25).

PROCALCITONIN AND C-REACTIVE PROTEIN

Procacitonin (PCT) is a peptide precursor of calcitonin, which is involved in calcium homeostasis. It is produced by the parafollicular cells (C cells) of the thyroid gland and by the neuroendocrine cells in the lungs and intestines in response to hypercalcemia, such as the calcium efflux generated by sepsis-induced tissue damage and necrosis (26, 27). PCT testing is often combined with CRP in order to make a putative diagnosis of sepsis. In addition, CRP serves as a generalized marker of inflammation and may be present as a result of such disparate factors as trauma or surgery (both put patients at risk for infection and possibly sepsis) or pre-existing acute or chronic infection (11, 28). PCT may also rise as a result of other stimuli, but it is more specific for sepsis risk than CRP (29). As with another standard diagnostic marker, erythrocyte sedimentation rate (ESR), CRP is not specific for any particular bacterial infection and does not increase the specificity of diagnostic tests when added to PCT testing. PCT levels are increased in greater magnitude by bacterial infections than either CRP or ESR, possibly because the liver, pancreas, and colon are sites of PCT synthesis during bacterial infection. Thus, PCT proves useful in differentiating pancreatic necrosis caused by bacteria from generalized pancreatitis, for example (30).

PCT shows a marked increase within 2–4 h of the initiation of an inflammatory response (half-life 22–26 h), which allows for easy testing (31). It is, however, not without its drawbacks. The test itself is expensive compared with CRP testing, and while PCT increases are more specific for bacterial infections than CRP or ESR, false-positive results may be observed in such cases as acute respiratory distress syndrome, chemical pneumonitis, severe falciparum malaria, and others (32). It is important to note that trauma and subsequent tissue damage may, and often do, induce sepsis via release of inflammatory compounds and subsequent perpetuation of the immune response. However, high values for biomarkers other than PCT are more indicative of classical bacterial sepsis than trauma (32). This is at least partially because PCT rises higher in correlation with the severity of an infection, whereas CRP quickly reaches a plateau no matter the source or comparative severity of inflammation (33).

The fact remains that PCT is not only a better marker of bacterial infection and sepsis than others, but that it also has some specificity for the type of bacterial infection causing the symptoms. In patients whose PCT scores exceeded the cutoff for suspicion of sepsis, the most common bacteria isolated from their

blood were *Escherichia coli* including extended-spectrum beta-lactamase types, *Klebsiella pneumoniae* and varieties of *Staphylococcus*. Higher PCT levels correlated with Gram-negative rods, which release LPS and thus directly trigger symptoms of sepsis (34). In addition, antibiotic-resistant bacteria appeared to induce higher levels of PCT than either Gram-positive *cocci* or Gram-negative rods, and increased levels were also associated with increased bacteremia and sepsis symptoms (34).

However, PCT alone may not be an accurate predictor of even sepsis morbidity and mortality, as non-significant differences exist between PCT levels in survivors and victims of septic shock (35). It is therefore important to combine PCT with at least one other biomarker to avoid false assumptions of survival or death in patients whose ongoing sepsis is as yet undetermined in terms of morbidity and mortality.

SOLUBLE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS-1 (STREM-1) AND OTHER MYELOID MARKERS

Myeloid markers serve as specific indicators of myeloid leukocyte deployment in response to bacterial infection, and they are often effective in predicting sepsis severity for this reason; for example, neutrophil gelatinase-associated lipocalin can independently predict sepsis morbidity and mortality (36). TREM-1, similar in its ability to mark this mechanism, is a member of the immunoglobulin superfamily and is expressed on the outer surface of neutrophils and monocytes, both cells of the myeloid lineage that are instrumental in perpetuating the early immune response. Like PCT, levels of its soluble form in the blood can be measured by enzyme-linked immunosorbent assay (ELISA) (5). When combined with PCT and CD64 (another cell surface marker for polymorphonuclear immune cells) the incidence of correct sepsis diagnosis rises markedly to above 90% diagnostic success rate when at least two biomarkers of three are elevated above a selected bioscore cutoff (37). CD64 has a higher specificity alone than does sTREM-1—approximately 95% versus 73%. However, its measurement requires flow cytometry and thus it is not feasible for hospitals where quick and inexpensive diagnosis is required (37).

The measurement of sTREM-1 requires the same machinery for analysis as the measurement of PCT, and so this biomarker shows more potential for widespread diagnostic use than CD64, despite their differences in the diagnostic accuracy. Some studies report 79% accuracy for sTREM-1 in differentiating survivors from non-survivors of sepsis (4). In addition, sTREM-1 has proven useful alongside IL-6 in diagnosing neonatal sepsis before symptoms become severe (24). Both molecules are significantly elevated in neonates with suspected or diagnosed sepsis as compared to those uninfected with bacteria; although IL-6 alone still has a higher rate of correct diagnosis (as confirmed by a smaller P value) than sTREM-1 alone, the combination shows very significant differences between patients even suspected of sepsis and those at little risk for it (24).

In addition, sTREM-1 may prove useful in a diagnostic sense with regards to the risk of sepsis severity in a patient not yet expressing symptoms; low levels of TREM-1 in patients, combined with high levels of other cytokines and biomarkers, indicate late severe sepsis due to the hyporesponsive state exemplified in late sepsis (19). In this sense, low values as combined with the most viable biomarkers mentioned above may provide information about the aggressiveness of treatment necessary to save a patient's life well in advance of the most dangerous period. In essence, it helps differentiate ''simple'' sepsis (not insignificant in risk with upward of 20% mortality) from septic shock (over 60% mortality) (3, 19).

sCD14, a soluble monocyte/macrophage surface marker cleaved from the CD14 receptor complex after LPS binding (and thus known as presepsin), presents itself as a possible biomarker. It can be measured via immunoassay and appears to possess better diagnostic capacity for sepsis than PCT (38). High levels correlate to increased sepsis mortality rates even six months out from patient recovery, and its low false-negative rate promotes patient safety (38). However, data regarding its usage in an emergency setting are controversial, and clinical trials have concluded that it is not as useful as PCT in emergent situations (39). While diagnosis of sepsis prior to symptoms does not itself qualify as emergent, the unproven presepsin appears less reliable than sTREM and other biomarkers (39).

WHAT WE KNOW: A NOVEL SET OF BIOMARKERS PROPOSED FOR THE EARLY DETECTION OF SEPSIS PRIOR TO SYMPTOMS

The combination of three sentinel biomarkers, IL-6, PCT, and sTREM-1, is uncommon, but pairs within the three have been attempted before to predict and/or prognosticate sepsis: sTREM-1 and PCT or sTREM-1 and IL-6 (3, 24, 37), while PCT is commonly used in a combination test itself. Recently, IL-6 and PCT together have been shown to predict patient outcome in non-classical cases of shock, that is, cardiogenic, indicating that these biomarkers may prove useful in sepsis of multiple types of etiologies when specific antibodies to bacterial endotoxin cannot be detected (40). Serum values for all three together serve as a highly accurate prognostic indicator of patient outcome. The rise of all three in separate tests has not yet been used as an indicator for a tripartite test, but each is highly correlated with worsened outcome for patients diagnosed with sepsis (4). More common is the combination of two tests together, or two out of three biomarkers (IL-6, PCT, and sTREM-1) in combination with other biomarkers; a combination of at least two stands out in terms of accuracy and ability to quickly diagnose (4, 24, 27).

A standard point-of-care test using these three biomarkers has not yet been developed, yet all three methods (for IL-6, PCT, and sTREM-1) are fairly inexpensive and easy to learn, and moreover do not require specialized equipment save for the machine necessary to read an ELISA. Eventually, these tests may prove useful or even essential for effective diagnosis of sepsis. Furthermore, prevention of severe sepsis may save billions of dollars for the US healthcare budget. The elimination of CRP as an attempted test for sepsis diagnosis may also help to defray the cost of three tests (1).

The combination of IL-6, PCT, and sTREM-1 into a diagnostic biomarker seems to complement the deficiencies that individual markers possess. The CRP and PCT combination, a

standard set of diagnostic tests, is markedly inferior as compared to the combination of sTREM-1 and IL-6. PCT's low specificity for severe and potentially antibiotic-resistant bacterial infections may be used in combination with these two biomarkers (24, 26, 30). The elimination of CRP might itself increase sensitivity and specificity of the three tests, as removing the uncertainty caused by its maximum ''ceiling'' of magnitude may help clinicians rule out other sources of inflammation, such as chronic disease and trauma without sepsis sequelae (33).

Why are these three biomarkers more specific for sepsis than any other biomarkers? All three of these biomarkers are specific for very different aspects of the inflammatory process leading to sepsis. A combination of the three biomarkers has not yet been developed, but each biomarker has been used in combination tests before to increase their diagnostic and prognostic accuracy. Together in a single test, the three biomarkers would narrow down the cause or risk of inflammatory pathology specifically to sepsis, usually bacterial sepsis. PCT is a general marker of inflammation and is released under conditions of hypercalcemia, but is at its highest levels in bacterial sepsis due to its release as a reaction to tissue damage. sTREM-1 is released by myeloid cells and is therefore specific to the chemotactic process involved in fighting bacterial infections (29). IL-6, meanwhile, rises to levels in the blood indicative of pathogenesis within hours. While it is a marker of inflammation like PCT, IL-6 has already been noted as more specific for sepsis diagnosis than other pro-inflammatory cytokines, as it is specifically produced by the actions of MAP kinase, NF-κB, and other inflammatory pathways further along in the signaling cascade (23). These three in combination (IL-6, PCT, and sTREM-1) would serve to confirm that circulating levels of inflammatory molecules indicate not only a high risk of sepsis, but also that the anti-microbial chemotactic process has already begun, regardless of whether the patient has begun exhibiting symptoms. We acknowledge the complexity of sepsis and recognize that due to its uncertainty and unpredictability, completely accurate early diagnosis may be difficult if even possible, but defining the early-phase markers correlating with all cases of subsequent sepsis symptoms is possible and would prove clinically beneficial.

The three biomarkers may not be usable as a method of discovering the specific bacteria responsible for a patient's sepsis. However, a positive three-biomarker test within hours of bacterial stimulation that a bacterial infection is taking place may circumvent the need to either wait until bacterial culture returns or nonspecifically treat with broad-spectrum antibiotics under the assumption that a bacterial infection is present, thereby increasing the risk of antibiotic-resistant pathogens (28). Should this one-step test show an increased sepsis risk in a patient brought in for febrile pneumonia or in recovery from surgery, aggressive supportive care could be initiated before symptoms begin, providing the patient's own immune system with the tools necessary to fight off the infection and prevent progression into full sepsis or even septic shock—that is, rehydration and vasopressors to keep blood pressure steady and cells hydrated.

The system/approach described herein would likely benefit patients and the healthcare systems economically, contribute to better patient outcomes, and improve patient safety. For instance, should they live through the initial crisis, survivors of severe sepsis are far more likely to require hospital readmissions for such sequelae as kidney disease and cardiovascular disease than nonsepsis patients, comprising 77% of all hospital readmissions in a survey of over 43,000 sepsis patients (41). Readmission was 26% within 30 days, and 48% within 180 days, with 25% of patients requiring multiple readmissions. The cost was estimated at $1 billion per year. Many of these patients were beneficiaries of Medicare and Medicaid, and early treatment of sepsis will alleviate strain on the patients' health, on the federal government, and on hospital budgets nationwide, let alone the cost to individual patients (41). Given that cardiovascular conditions are already a leading cause of death in this country, and that kidney disease is notoriously nonspecific in origin, early identification of patients in the initial stages of sepsis may directly or indirectly lead to better patient outcomes.

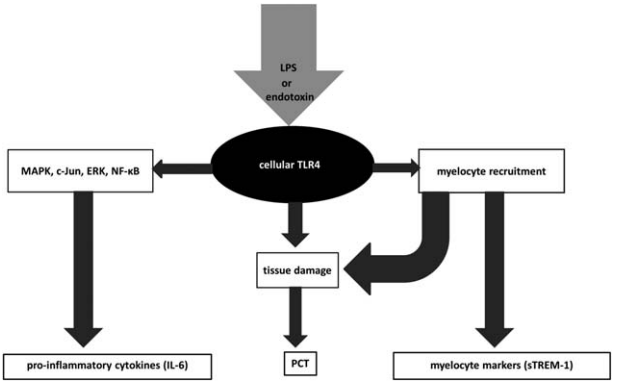

Fig. 1. **The generalized process of sepsis that leads to overexpression of all three proposed biomarkers.** c-Jun indicates JNK pathway DNA-binding protein; ERK, inflammatory pathway within MAPK; LPS, lipopolysaccharide; MAPK, MAP kinase pathway; NF-kB, cytokine pre-transcription complex; TLR, Toll-like receptor; PCT, procalcitonin.

Different patient groups also tend to present different biomarker levels as a hallmark of sepsis, and this must be kept in mind when designing a biomarker test; for example, older adults exhibit higher levels of pro-inflammatory biomarkers within 72 h after sepsis onset, but not later (42). Newborns also show a pattern of pro-inflammatory agents different in concentration and time scale from adults (43). Thus, timing is of the utmost importance, harkening back to the regular testing proposed in other biomarker systems (19). Like other biomarker tests for various diseases, different parameters of normal for different age groups, sexes, and/or other factors yet unexplored may be a requirement.

CONCLUSIONS

We propose that the combination of IL-6, sTREM-1, and PCT as biomarkers for a diagnosis of early-phase sepsis may be very promising, as each biomarker represents a different aspect of sepsis. IL-6 shows the onset of inflammation mediated by LPS stimulation of TLR-4 and subsequent pro-inflammatory signaling cascades. PCT, in response to the hypercalcemia produced by tissue damage, serves as a signal that damage undetectable by clinicians has already begun. Finally, sTREM-1, as a marker of

myeloid cell deployment in response to bacterial stimulation, further narrows the diagnosis to bacterial sepsis (Fig. 1). Clinical evaluation of these three biomarkers should provide valuable information about their diagnostic prowess, and may be easily performed with an enzyme-linked immunosorbent assay (ELISA) that comprises all three biomarkers. We have made plans to perform research to this end, using samples of blood from sepsis patients as well as healthy controls.

Although each of these markers has been tested as a specific biomarker of sepsis, they have never been tested together. A point-of-care test in the early stages of sepsis is clinically paramount. The potential benefits to patients, hospitals, and the healthcare system in general, given the high likelihood of patient death and draining of resources should sepsis progress to multi-organ dysfunction undiagnosed, merit the application of this three-pronged novel diagnostic biomarker system approach to clarify the risk of sepsis. Direct application of these three biomarkers through prompt, point-of-care testing may produce significant benefits for patients, healthcare systems, governments, and insurers.

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