Could Biomarkers Direct Therapy for the Septic Patient?

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Abbreviations: ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; Ang, angiopoietin; CARS, compensatory anti-inflammatory response syndrome; cfu, colony forming units; CLP, cecal ligation and puncture; CRP, C-reactive protein; DAMPs, damage-associated molecular patterns; DIC, disseminated intravascular coagulation; EEG, electroencephalography; GM-CSF,
granulocyte macrophage colony-stimulating factor; HLA-DR, human leukocyte antigen D related; ICAM-1, intercellular adhesion molecule 1; ICU, intensive care unit; IL, interleukin; KIM-1, kidney injury molecule 1; LPS, lipopolysaccharide; MMP-8, matrix metalloproteinase-8; MODS, multiple organ dysfunction syndrome; MRI, magnetic resonance imaging; NAG, N-acetyl-β-(D)-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; NIRS, near-infrared spectroscopy; PAMPs, pathogen-associated molecular patterns; PCT, procalcitonin; PD-1, programmed cell death 1; S1P, sphingosine-1-phosphate; SIRS, systemic inflammatory response syndrome; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule 1; VWF, von Willebrand factor
Abstract

Sepsis is a serious medical condition caused by a severe systemic inflammatory response to a bacterial, fungal, or viral infection that most commonly affects neonates and the elderly. Advances in understanding the pathophysiology of sepsis have resulted in guidelines for care that have helped reduce the risk of dying from sepsis for both children and older adults. Still, over the past three decades, a large number of clinical trials have been undertaken to evaluate pharmacological agents for sepsis. Unfortunately, all of these trials have failed, with the use of some agents even shown to be harmful. One key issue in these trials was the heterogeneity of the patient population that participated. What has emerged is the need to target therapeutic interventions to the specific patient’s underlying pathophysiological processes, rather than looking for a universal therapy that would be effective in a “typical” septic patient who does not exist. This review supports the concept that identification of the right biomarkers that can direct therapy and provide timely feedback on its effectiveness will enable critical care physicians to decrease mortality of patients with sepsis and improve the quality of life of survivors.
Introduction

Sepsis is a serious medical condition caused by a severe systemic inflammatory response to a bacterial, fungal, or viral infection (Martin, 2012; Dellinger et al., 2013). As sepsis worsens, organ dysfunction (severe sepsis) or severe sepsis plus hypotension (septic shock) can develop (Dellinger et al., 2013). Sepsis affects approximately 700,000 people annually in the U.S. alone (Chang et al., 2015). Over 25% of these patients die before leaving the hospital, making sepsis the 9th leading cause of death overall (Heron, 2015). In addition, relative to the total number of cases, sepsis is the most costly medical condition, especially given that over 20% of sepsis survivors are readmitted within 30 days and nearly 30% of these patients have recurrent sepsis (Torio and Andrews, 2013; Chang et al., 2015). The estimated annual health care burden is over $20 billion in the U.S. alone (Torio and Andrews, 2013).

While sepsis affects people of all ages, the most commonly affected populations are neonates, due to an immature immune system and still developing organs, and the elderly, due to chronic and comorbid conditions. In the U.S., sepsis is the 6th and 7th leading cause of death in neonates and infants, respectively, and the 10th leading cause of death in patients age 65 and older (Heron, 2015). Mortality rates for children with septic shock range from 9% to 25% (Hartman et al., 2013; Weiss et al., 2015), and for older adults mortality rates are as high as 45% (Daviaud et al., 2015). Although the absolute number of deaths from sepsis is increasing due to increasing incidence (Martin, 2012; Gaieski et al., 2013), advances in the understanding of the pathophysiology of sepsis have reduced the risk of dying from sepsis for both children (Kissoon et al., 2011; Ruth et al., 2014) and older adults (Stevenson et al., 2014).
The pathophysiology of sepsis is very complex and still poorly understood. Initially, local activation of the innate immune response by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) results in a “cytokine storm” (Wang and Ma, 2008; Gustot, 2011; Laszlo et al., 2015). If the inflammatory response becomes systemic it manifests as the systemic inflammatory response syndrome (SIRS, (Conference, 1992)). Patients who survive the initial hyperinflammatory state may progress to a hypoinflammatory state and develop the compensatory anti-inflammatory response syndrome (CARS, (Bone, 1996; Ward et al., 2008)), which increases the susceptibility to recurrence of the primary infection or a secondary acquired infection. Disruption of the balance between these inflammatory and anti-inflammatory responses in sepsis leads to multiple organ dysfunction syndrome (MODS), which significantly increases mortality (Wang and Ma, 2008; Gustot, 2011; Boomer et al., 2014).

Mortality from sepsis has significantly decreased over the years because of early recognition and resuscitation, as documented in the “Surviving Sepsis Campaign” (Dellinger et al., 2013). Following recommendations from this document, current therapies are mostly supportive care. Initial treatment includes broad-spectrum antibiotics and then fluid resuscitation if necessary. As sepsis severity increases, therapy may require administration of vasopressors, use of mechanical respiratory support, and/or renal replacement therapy (Dellinger et al., 2013). Over the past three decades, there have been over 60 large Phase II and III randomized, controlled clinical trials of single pharmacological agents for sepsis, all of which have failed, with some agents even found to be harmful (Fink and Warren, 2014). Reflecting on these failed
trials, many leaders in the field believe that the heterogeneity of the patient population that participated in these trials is a key issue (Cohen et al., 2015). To address this issue, various critical care societies are currently revising the definition of the sepsis syndrome to refine the inclusion criteria for participants in future sepsis trials. One common theme is surfacing: infection plus organ dysfunction should be considered as inclusion criteria rather than just the current two or more SIRS criteria (Vincent et al., 2013; Cohen et al., 2015; Drewry and Hotchkiss, 2015; Kaukonen et al., 2015). In addition it has been suggested that there is a need to target therapeutic interventions according to the specific patient’s underlying pathophysiological processes, rather than looking for a universal therapy that would be effective in the “typical” patient population with sepsis (Boomer et al., 2014; Christaki and Giamarellos-Bourboulis, 2014).

The complex pathophysiology of sepsis and the ineffectiveness of current targeted therapies suggest that treatments guided by biomarkers could provide a new therapeutic strategy. A biomarker, as defined by an NIH working group, is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (Group, 2001). There are general and overlapping categories of biomarkers, which have been reviewed and discussed in detail elsewhere (Marshall et al., 2009; Kaplan and Wong, 2011; Dupuy et al., 2013; Sandquist and Wong, 2014). Biomarker categories include 1) screening biomarkers to identify patients at increased risk and inform on prophylactic interventions, 2) diagnostic biomarkers to establish the presence or absence of disease and inform treatment decisions, 3) stratification biomarkers to stage severity and outcome of disease and help identify subgroups of
patients who may benefit or be harmed by therapy, 4) monitoring biomarkers to inform on the effectiveness of a therapy, and 5) surrogate biomarkers to inform on disease progression and potential outcomes of therapy. A number of excellent recent reviews have been published on the potential diagnostic and prognostic value of biomarkers in sepsis (Pierrakos and Vincent, 2010; Sandquist and Wong, 2014; Biron et al., 2015; Cohen et al., 2015). Therefore, this review will not focus on the current status of sepsis biomarkers. Rather, the goal of this review is to support the concept that the appropriate biomarkers could be used to direct targeted therapies (Fig. 1). For example, diagnostic biomarkers could direct therapies such as those targeting immune status, endothelial injury, coagulation, and microcirculatory failure; and stratification biomarkers could inform the decision to rapidly employ renal replacement therapy or mechanical ventilation.

**Patient Heterogeneity**

Both the incidence and the outcome of sepsis are greatly influenced by patient heterogeneity including patient-specific factors such as age, comorbid medical conditions, and genetic predispositions. The very young (<1 year) and the elderly (>65 years) account for 76% of all deaths due to sepsis in the U.S. (Heron, 2015). Premature and very-low-birth-weight infants suffer the highest mortality (Heron, 2015), which may be due to increased hemodynamic fragility, low cardiac output, and organ hypoperfusion, as well as a decreased ability to mount an appropriate immune response (Adkins et al., 2004; Aneja and Carcillo, 2011; Shah and Padbury, 2014). Likewise, in elderly patients, debilitated physiological processes, reflected in higher incidences of congestive heart
failure, coronary artery disease, diabetes, hypertension, and chronic obstructive pulmonary disease, underlie a 13-fold higher relative risk for the development of sepsis (Martin et al., 2006) and an increased mortality from sepsis (Boss and Seegmiller, 1981). These physiological differences in sepsis with respect to age and comorbid medical conditions suggest that treatments will need to be based on the specific conditions of the individual patient.

The presence of MODS is an important factor in the treatment of sepsis. While there is substantial variation in the percentages of patients with any specific organ involvement, pulmonary, renal, cardiovascular, hepatic, neurological, and hematological dysfunction may result from primary and secondary causes (Gaieski et al., 2013; Iskander et al., 2013; Stubbs et al., 2013; Ziaja, 2013; Fiusa et al., 2015). In all populations, the mortality rate of sepsis greatly increases as multiple organ systems fail (Gaieski et al., 2013; Iskander et al., 2013; Romanovsky et al., 2013).

Because of the inherent heterogeneity of patients enrolled in clinical trials, a level of heterogeneity in the therapeutic response should be expected. Most important, however, while some patients will benefit from the intervention, others could be harmed (Marshall, 2014). To demonstrate overall efficacy of a treatment, studying larger numbers of patients to increase statistical power is required in trial design, but this will increase costs and may still not uncover efficacy in the general population for the reasons just described. Early-phase sepsis trials should focus on identifying an appropriate study population and use more specific inclusion criteria to limit trials to the target populations suggested by preclinical studies. This approach could reduce heterogeneity and increase the likelihood of demonstrating efficacy.
Biomarkers

Validation of biomarkers requires an extensive amount of research and resources. A limitation of biomarker research in human sepsis is that there are rarely data on preillness levels of a biomarker. Because many septic patients have comorbid chronic inflammatory conditions such as coronary artery disease and diabetes, biomarkers alone are unlikely to accurately inform on the severity of sepsis (Ginde et al., 2014) but may inform on possible outcomes (Kojic et al., 2015). For example, the ideal biomarker could identify those patients at risk for specific organ dysfunction and thereby help direct more targeted therapy. In addition, gene expression patterns could lead to candidate serum protein markers to predict organ-specific injury (Basu et al., 2011). Given the complex nature of sepsis, achieving this goal will likely require monitoring multiple biomarkers through the course of the disease and the course of therapy (Wong et al., 2015a). Biomarkers also depend on the specific pathogen, or often pathogens, causing sepsis. Consequently, there is a need to accurately and quickly distinguish sepsis from noninfectious SIRS. These are all areas where proteomics and genomics (Cao and Robinson, 2014; Cao et al., 2014; Christaki and Giamarellos-Bourboulis, 2014; DeCoux et al., 2015) and rapid identification of positive blood cultures (Bhatti et al., 2014b; Bhatti et al., 2014a) could become very helpful.

Inflammatory Biomarkers

Should the goal of therapy for septic patients be to decrease inflammation or boost host immunity? This is an extremely important and difficult question with no
simple answer. Hotchkiss and Sherwood (Hotchkiss and Sherwood, 2015) have published a recent commentary on this issue. However, biomarkers have the potential to help critical care physicians make important clinical decisions regarding immunotherapy (Hotchkiss et al., 2013b).

In general, blood levels of soluble adhesion molecules such as E-, L- and P-selectin, ICAM-1, and VCAM-1 correlate with the presence of sepsis in neonates, children, and adults; however, there are age-related differences in the absolute levels, and it is still unclear whether changes in levels might correlate positively or negatively with outcomes (Zonneveld et al., 2014). C-reactive protein (CRP) is an acute-phase protein released by the liver. It is used as an inflammatory biomarker but cannot distinguish between infection and noninfectious diseases (Kojic et al., 2015). Procalcitonin (PCT) is a propeptide of calcitonin released in the blood in response to bacteria-associated proinflammatory mediators and inflammatory cytokines. Its use as a specific biomarker for sepsis has come under question (Talan, 2015). CRP and PCT may be more general biomarkers of moderate to severe inflammation but could be useful for informing on the response to treatment (Wacker et al., 2013; Rule et al., 2015). However, the levels of PCT and CRP in combination may be able discriminate bacterial sepsis from other causes of SIRS (Han et al., 2015).

Cytokines including tumor necrosis factor α (TNF-α) and interleukin (IL)-1, IL-6, IL-8, IL-27, and others have been evaluated individually and in combination as potential biomarkers for screening and diagnosis (Xiao et al., 2015). Although cytokine levels are relatively easily and rapidly measured with multiplex assays (Mera et al., 2011), the cost–benefit of monitoring them has yet to be established. Serial cytokine
measurements would be required to inform on immune status; however, this would require a thorough understanding of the complex kinetics of synthesis, release, and clearance. Moreover, elderly septic patients present with generally higher levels of inflammatory biomarkers due to comorbid conditions (Ginde et al., 2014), and because pre-sepsis levels would be unknown, interpreting changes (or not) in cytokine levels would be extremely difficulty. These issues may explain, at least in part, why clinical trials targeting cytokines failed to demonstrate efficacy; use of some agents was even harmful (Fink and Warren, 2014).

Septic patients in the immunosuppressive phase could potentially benefit from immune-enhancing therapies, but these patients must first be identified. Potential biomarkers, which have been suggested for this purpose, include decreased monocyte human leukocyte antigen – antigen D related (HLA-DR) expression for monocyte unresponsiveness, increased programmed cell death 1 (PD-1) expression for T cell exhaustion, lymphopenia, and increased percentage of regulatory T cells, among others (reviewed in (Hotchkiss et al., 2013b) and (Boomer et al., 2014)). The use of specific, biomarker-directed immunotherapies such as granulocyte macrophage colony stimulating factor (GM-CSF) to stimulate mature myeloid cell expansion in patients with granulocytopenia (Mathias et al., 2015), and IL-7 to promote lymphocyte expansion in patients with lymphopenia (Unsinger et al., 2010; Hutchins et al., 2014) are examples of new therapeutic strategies with the potential to advance the treatment of the immunosuppressed septic patient (Hotchkiss et al., 2013a; Boomer et al., 2014).

Endothelial Biomarkers
Sepsis is associated with severe injury to vascular endothelial cells. Injury to the endothelium leads to dysregulation of vascular tone, hemostasis, and the permeability barrier. Microvascular leakage is a hallmark of sepsis and results from damage to the endothelial cell barrier lining the microvasculature. Identifying possible targeted therapy to stabilize and even promote repair of the endothelial barrier is a very active area of research (Darwish and Liles, 2013). Syndecan-1 and heparan sulfate are potential biomarkers of damage to the endothelial glycocalyx, the antiadhesive and anticoagulant surface of the endothelial cell (Schmidt et al., 2012) and, if confirmed as such, could be used as diagnostic and monitoring biomarkers to direct and evaluate anticoagulant therapy (Ostrowski et al., 2015; Yini et al., 2015).

A number of signaling pathways regulating vascular permeability appear to be disrupted during sepsis. One such system is the endothelial-specific angiopoietin growth factor (Ang) and its Tie-2 tyrosine kinase receptor (Maisonpierre et al., 1997; Ziegler et al., 2013). Plasma levels of Ang-1, Ang-2, and Tie-2 have been show to predict the onset of acute lung injury (Agrawal et al., 2013), 28-day mortality in septic adults (Fang et al., 2015), and sepsis severity in children (Wang et al., 2014). If verified, levels of Ang-1, Ang-2, and Tie-2 in septic patients may inform on the effectiveness and outcome of therapy affecting the endothelium, such as the use of statins (Ghosh et al., 2015). Another system is the sphingosine-1-phosphate (S1P) signaling pathway, a key regulator of endothelial stability and vascular permeability (Lee et al., 1999; Wang and Dudek, 2009). Serum levels of S1P in septic patients are decreased and inversely associated with disease severity (Winkler et al., 2015). If S1P levels can be verified as a biomarker of microvascular injury, S1P could be used to screen, diagnose, and/or
stratify patients and direct the use of S1P receptor 1 agonists, shown to protect the endothelial glycocalyx in vitro (Zeng et al., 2014) and to heal the renal microcirculation in a murine model of sepsis (Wang et al., 2015b).

During sepsis, injured endothelial cells release microparticles containing proteins and lipids that can augment thrombosis, inflammation, and microvascular injury. Understanding the role of microparticles in the progression of sepsis and organ injury is also a very active area of research. Souza and colleagues (Souza et al., 2015) have published an excellent recent review. As a potential biomarker, microparticles may help stratify patients and inform on the potential outcomes of therapy targeting the endothelium.

**Coagulation Biomarkers**

Sepsis with its associated inflammation induces a state of dysregulated hemostasis. Up to 30–50% of septic patients have clinical overt disseminated intravascular coagulation (DIC), which leads to dissemination of fibrin-rich microvascular thromboses and contributes to MODS (Levi and van der Poll, 2013). Many specific monotherapeutic agents that interfere with dysregulated hemostasis in septic patients have been tried without success in large randomized, controlled trials. These agents include heparin, antithrombin III, recombinant tissue pathway inhibitors, recombinant activated protein C, and recombinant soluble thrombomodulin (Warren et al., 2001; Abraham et al., 2003; Nadel et al., 2007; Saito et al., 2007; Jaimes et al., 2009; Ranieri et al., 2012). These trials, however, enrolled heterogeneous septic patients and did not use coagulation biomarkers as titratable therapeutic endpoints. On reflection, the failure
of these trials suggests that, as described earlier, study design and patient selection rather than the specific agents may be at fault (Cohen et al., 2015). Currently, clinicians rely on point-of-care global measurement of the hemostatic pathway including prothrombin time, activated partial thromboplastin time, the fibrin degradation product D-dimer, and platelet count to provide blood product support for hemostasis (Wada et al., 2013). For future clinical trials, in addition to the point-of-care assays, measuring, replacing, and normalizing the activities of heparin, antithrombin III, tissue factor, activated protein C, and/or thrombomodulin in septic patients with a defined severe coagulopathy perhaps might lead to better outcomes.

Sepsis-induced thrombotic microangiopathies driven by either von Willebrand factor (VWF)/platelets and/or complement pathways will lead to dissemination of VWF/platelet-rich microvascular thromboses and MODS (Nguyen et al., 2014; Charchaflieh et al., 2015). Measuring VWF, the presence of ultralarge VWF multimers, and ADAMTS-13 (VWF-cleaving protease) and directing therapies to normalize their activities have been shown to improve outcome in children with sepsis-induced thrombocytopenia-associated multiple organ failure (Nguyen et al., 2008; Fortenberry et al., 2012). Low levels of ADAMTS-13, high VWF activity, and the presence of ultralarge VWF multimers have been associated with worse outcomes in septic adults (Martin et al., 2007; Bockmeyer et al., 2008). Sepsis-induced complement pathway dysregulation leading to DIC and thrombotic microangiopathies has been demonstrated in human clinical studies and animal models (Charchaflieh et al., 2015; Zhao et al., 2015). Eculizumab, a monoclonal antibody against complement C5, has been shown to improve outcome in severe *Escherichia coli*-induced hemolytic uremic syndrome, which
leads to thrombotic microangiopathy and MODS (Lapeyraque et al., 2011; Noris et al., 2012). Research is needed to prove whether other types of infection-induced thrombotic microangiopathy and MODS can be ameliorated by measuring and normalizing complement activities.

Physiological Biomarkers

Biomarkers are generally assumed to mean small molecules. However, physiological biomarkers are the most rapidly determined indices of organ dysfunction and can often be measured at the bedside. The complex pathophysiology of sepsis can have a major impact on the translation of experimental therapies to clinical practice. This is one of the key reasons that animal models of sepsis have had, thus far, a limited impact on therapy in humans (Dyson and Singer, 2009; Marshall, 2014; Efron et al., 2015). Changes in cardiac output, organ perfusion, drug delivery to therapeutic target, and clearance of the drug are dynamic and often unpredictable in septic patients. Physiological biomarkers could be of great help in interpreting successes, failures, and undesirable outcomes in sepsis clinical trials.

The application of transthoracic echocardiography with speckle-tracking technology to detect subclinical myocardial dysfunction may have value in directing cardioprotective strategies, even if the ejection fraction remains normal (Shahul et al., 2015). This type of monitoring may also help in establishing prognosis in septic shock patients because, despite a normal ejection fraction, severe right ventricular wall longitudinal strain dysfunction is associated with a high rate of mortality in patients with severe sepsis and septic shock (Orde et al., 2014). There is a pressing need for
biomarkers to screen for sepsis-induced cardiomyopathy. Extracellular histones may emerge as such a biomarker (Alhamdi et al., 2015; Kalbitz et al., 2015).

There is also a need for minimally invasive techniques that permit direct physiological measurements of microvascular flow and organ perfusion in critically ill patients. One of the most routine measurements made in septic patients is blood lactate concentration. Lactate production is a protective response to allow cellular energy production to continue when tissue oxygen supply is inadequate for aerobic metabolism (Suetrong and Walley, 2015). Serum lactate levels remain a strong, independent predictor of lung injury (Mikkelsen et al., 2013). Elevated lactate levels are highly associated with in-hospital mortality and can be used to stratify patient risk (Casserly et al., 2015). Use of lactate as a biomarker to direct management of the microcirculation during the later stages of sepsis can be complicated by changes in lactate clearance (Chertoff et al., 2015). Better understanding of the relationships between lactate levels and actual status of the microcirculation remains a critical need (De Backer et al., 2013).

Organ-specific microcirculation can be measured in animal models of experimental sepsis and used to evaluate the therapeutic potential of new approaches to improve organ perfusion (Dear et al., 2005; Wu et al., 2007; Holthoff et al., 2012; Fink et al., 2013; Taccone et al., 2014). As therapeutic strategies emerge to target microvascular dysfunction, physiological biomarkers measureable at the bedside could assist in directing therapy and confirming efficacy (or revealing harmfulness) (Matejovic et al., 2015). Cine phase-contrast MRI and contrast-enhanced ultrasound can be performed on critically ill septic patients to monitor blood flow in the kidney (Schneider et al., 2013). For example, cine phase-contrast MRI revealed that renal blood flow was
consistently reduced as a fraction of cardiac output in septic patients with established acute kidney injury (Prowle et al., 2012). Although these minimally invasive techniques for monitoring organ perfusion are still in the validation phase, their use could ultimately guide the choice of therapy and evaluate effectiveness. For example, changes in renal perfusion paired with renal injury biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), N-acetyl-β-(D)-glucosaminidase (NAG), and matrix metalloproteinase-8 (MMP-8) (Liangos et al., 2007; Basu et al., 2011; Wang et al., 2015a) could direct the implementation of renal replacement therapies (hemofiltration and/or dialysis) that could improve outcomes in some patients; however, this approach is not without risk (Elseviers et al., 2010; Sun et al., 2014; Prowle and Davenport, 2015; Wald et al., 2015).

Orthogonal polarization spectral imaging and side-stream dark-field imaging techniques can be used at the bedside to obtain real-time measurements of the sublingual microcirculation in humans with sepsis (De Backer et al., 2012). Even though changes in the sublingual microcirculation cannot predict the status of other organ-specific microvascular beds, there is an excellent correlation between decreases in the proportion of perfused small sublingual vessels in early sepsis and decreased survival rates (De Backer et al., 2013; De Backer et al., 2014). These techniques could be used as diagnostic and monitoring physiological biomarkers to direct therapy targeting the vasculature, as suggested in animal models of sepsis (Holthoff et al., 2013; Wang et al., 2015b).

Sepsis-associated encephalopathy with cognitive impairment is a common complication of sepsis that places a substantial burden on survivors of sepsis, their
families, and their caregivers (Iwashyna et al., 2010). While the pathophysiology is still poorly understood, neuroinflammatory and ischemic process are likely contributors to alterations in the blood–brain barrier, release of neurotoxic mediators, and microglial activation (Adam et al., 2013). Changes in standard electroencephalogram (EEG) are associated with increased mortality and delirium in septic ICU patients (Azabou et al., 2015). Near-infrared spectroscopy (NIRS) is being evaluated in high-risk septic patients as a method to monitor brain tissue oxygenation to predict the development of acute neurological dysfunction (Wood et al., (in press)). Interest is growing in identifying biomarkers for sepsis-induced neurological injury (Bersani et al., 2015), especially because animal models are revealing long-term brain alterations that affect learning and memory and persist into adulthood (Comim et al., 2015; Gao et al., 2015). Targeting neurotoxic inflammatory pathways (Gao et al., 2015) may emerge as an approach that could be guided by specific small-molecule and physiological biomarkers.

Accumulating data suggest that during sepsis intracellular redox processes result in increased production of superoxide, hydrogen peroxide, and peroxynitrite, especially in mitochondria (Mayeux and Macmillan-Crow, 2012; Duran-Bedolla et al., 2014). While there are studies to suggest that mitochondrial injury occurs in septic patients (Jeger et al., 2013), the notion that mitochondrial injury is a cause of organ dysfunction is controversial (Fink, 2015). Nonetheless, if there were rapidly measurable mitochondrial injury biomarkers, they could be used to direct mitochondria-targeted antioxidants, such as ubiquinone (MitoQ), vitamin E (MitoVit E), and piperidine nitroxide (MitoTEMPO), which have been shown in animal models to be protective against sepsis-induced
cardiomyopathy (Supavekin et al., 2003; Zang et al., 2012; Yao et al., 2015), renal injury (Patil et al., 2014), and microcirculatory failure (Patil et al., 2014; Sims et al., 2014).

**Combinations of Biomarkers**

The heterogeneity of septic patients suggests that multiple biomarkers may be required to effectively guide therapy (Gibot et al., 2012; Liu et al., 2014; Sandquist and Wong, 2014). Wong and collaborators have begun to use panels of biomarkers to stratify pediatric septic shock patients to predict risk (Wong et al., 2014) and possibly inform on therapeutic decisions (Wong et al., 2015a; Wong et al., 2015b). For example, the PERSEVERE study used a panel consisting of C-C chemokine ligand 3, IL-8, heat shock protein 70 kDa 1B, granzyme B, and MMP-8 to stratify pediatric sepsis patients based on mortality probability (Wong et al., 2014). A panel of candidate biomarker genes has also been used to guide the use of adjuvant corticosteroids (Wong et al., 2015b). Due to the complexity of sepsis and the diversity of patient populations, it should be expected that combinations of biomarkers (small molecule and physiological) will be required to best inform on interventional therapies (Fig. 1).

**Animal Models**

Animal models of sepsis are the subject of a number of excellent reviews that discuss advantages and disadvantages of the various species studied and the various methods used to induce sepsis (Zanotti-Cavazzoni and Goldfarb, 2009; Ward, 2012; Fink, 2013; Efron et al., 2015). It is clear that no animal model of sepsis can fully replicate the complexities and range of sepsis in humans. However, animal models are
essential for preclinical testing of new therapies to help suggest dose, potential toxicities, and efficacy. Animal models are also necessary to identify potential biomarkers and test new monitoring modalities. From a basic science point of view, in vivo models of sepsis provide a means to investigate underlying mechanisms of organ injury that are not possible in very sick septic patients.

Murine and rat models of sepsis are the most frequently used because of their relatively low cost and their public acceptance as laboratory subjects. However, just how relevant murine models of inflammation are to inflammatory diseases is an area of intense debate (Seok et al., 2013; Osuchowski et al., 2014; Efron et al., 2015). Larger species, such as pigs, sheep, dogs, and subhuman primates, are also used in sepsis research, and some of these species better replicate more of the “typical” cardiovascular defects observed in humans with sepsis than do small animals (Zanotti-Cavazzoni and Goldfarb, 2009; Taylor et al., 2012). Not surprisingly, however, large-animal models are rarely used in survival studies. This is an important consideration because increased mortality is a key measure of efficacy in sepsis clinical trials.

Because sepsis is defined as a systemic inflammatory response due to an infection (Dellinger et al., 2013), the most clinically relevant models of sepsis should incorporate both a systemic inflammatory response and an active infection. Models of sepsis that utilize endotoxemia (lipopolysaccharide [LPS] from the outer membrane of gram-negative bacteria) in rodents and larger species are less clinically relevant because these models rely solely on rapid activation of the innate immune system and do not replicate the consequences of active microbial defense and subsequent immune suppression observed in humans with sepsis (Stearns-Kurosawa et al., 2011).
In rodents, the cytokine profile and magnitude of cytokine release are very different between endotoxemia models and active-infection models, such as polymicrobial peritonitis induced by cecal ligation and puncture (CLP). LPS produces a very rapid but transient increase in inflammatory cytokines to levels that can be orders of magnitude higher than with CLP (Kawai et al., 1999; Remick et al., 2000; Miyaji et al., 2003), where the magnitude and kinetics of cytokine release more closely mirror what occurs in human sepsis (Rittirsch et al., 2007). Still, there are advantages to studying endotoxemia. Endotoxemia models are attractive from an experimental point of view because they are highly reproducible and do produce similar effects on the vasculature (although perhaps through different mechanisms (Asano et al., 2015) and similar mortality rates (Remick et al., 2000). A limitation of endotoxemia models is that the systemic inflammatory effects are mediated through interactions with toll-like receptor-4 (TLR4) (Kawai et al., 1999; Palsson-McDermott and O’Neill, 2004), whereas this is not the case in polymicrobial sepsis (Dear et al., 2006). These are mechanistically important differences between endotoxin and polymicrobial models of sepsis that can impact the translation of experimental therapies to clinical practice (Rittirsch et al., 2007).

Consequently, the CLP model and other models of active infection are generally considered to be clinically relevant models of sepsis, especially when they incorporate antibiotic and fluid therapy to more closely mimic the standard care of septic patients (Miyaji et al., 2003; Rittirsch et al., 2007; Dyson and Singer, 2009; Rittirsch et al., 2009). However, the CLP model exhibits high inherent variability because it depends on the resident bacterial flora of the cecal contents, which can vary from animal to animal, and on the consistency of a technician’s surgical skills.
Depending on the dose, intravenous infusion of live \textit{E. coli} in sheep can produce mild hyperdynamic (increased cardiac output) sepsis or severe hypotensive sepsis (Ramchandra et al., 2009; Ishikawa et al., 2011) to mimic the range of sepsis severity and degree of cardiovascular dysfunction observed in humans. Similarly, in adult pigs, intravenous administration of live bacteria can produce hypotensive sepsis associated with pulmonary hypertension and a transient increase in systemic vascular resistance (Garcia-Septien et al., 2010); in addition, when acute kidney injury develops, a dissociation is produced between systemic hemodynamic changes and changes in the renal circulation (Benes et al., 2011). In baboons, intravenous challenge with live \textit{E. coli} can be titrated to produce mild sepsis or severe sepsis with refractory hypotension and coagulopathies (Taylor et al., 2012). An advantage of live-bacteria infusion models is that gram-positive bacteria can also be studied (Soerensen et al., 2012), as well as controlled combinations of bacteria. Another advantage of these intravenous live culture models over the CLP model is that sepsis can be induced without subjecting the animal to a surgical procedure or general anesthesia.

Autologous fecal peritonitis is a variation of the CLP model in which the cecal contents are collected from an animal and administered at a later time. This model has been used in sheep (He et al., 2012; Taccone et al., 2014) and pigs (Benes et al., 2011; Correa et al., 2012; Wepler et al., 2013) to study sepsis-induced cardiovascular injury and evaluate resuscitation protocols. A variation in which the fecal matter is derived from multiple animals can also be used in mice too young or too small for the CLP procedure (Wynn et al., 2007; Wynn et al., 2008). This model offers an additional advantage in that cecal contents can be harvested from multiple animals, pooled,
partially purified, stored at –80°C, and then administered to groups of animals (Starr et al., 2014). The “dose” of fecal contents can be normalized to bacterial colony–forming units (cfu)/body weight prior to administration, which should reduce interexperimental variability.

As discussed earlier, sepsis is not identical in neonates, infants, and adults, and it should not be assumed that results from adult animal models of sepsis are relevant for translation to neonates or even infants with sepsis. Most preclinical studies are performed in adult “middle-aged” animals, yet there are clear differences in the immunological responses (Wynn et al., 2007; Wynn et al., 2008; Turnbull et al., 2009), degree of organ injury (Miyaji et al., 2003; Starr et al., 2015), and mortality (Turnbull et al., 2009; Starr et al., 2014) between very young, adult, and old mice made septic by fecal peritonitis. CLP can be performed in young (weaning age) rat pups as a model of infant sepsis. These animals develop rapid hemodynamic changes and microvascular dysfunction that are not the same as in adult rats (Seely et al., 2011; Sims et al., 2014). Neonatal, 3-day-old piglets subjected to CLP show deterioration of cardiac output, pulmonary hypertension, and shock (Goto et al., 2010), consistent with cold shock (low cardiac output with high systemic vascular resistance) frequently observed in newborns and infants with severe sepsis (Wheeler et al., 2011).

Confounding comorbid or predisposing conditions of most septic patients limit the translation of findings from most animal models. However, researchers are now beginning to study the pathophysiology of sepsis in the presence of comorbid conditions, such as chronic kidney disease (Doi et al., 2008; Leelahavanichkul et al., 2011), type 1 (Osuchowski et al., 2010; Filgueiras et al., 2012) and type 2 (Jacob et al., 2008).
diabetes, and atherosclerosis (Wepler et al., 2013). Such studies are especially important because they could uncover more targeted therapy for comorbid conditions. They could also help explain why a therapy fails in septic patients with preexisting conditions (Doi et al., 2008; Leelahavanichkul et al., 2011; Filgueiras et al., 2012) or why survivors of sepsis may have increased susceptibility to subsequent organ injury (Portella et al., 2013).

Murine models of sepsis are also being used to investigate the development and potential causes of cognitive impairments following sepsis like those observed in human survivors of sepsis (Iwashyna et al., 2010). For example, mice surviving severe sepsis induced by CLP show time-dependent learning and memory impairments (Chavan et al., 2012; Ji et al., 2015) associated with dendritic degeneration (Chavan et al., 2012) and oxidative stress in the prefrontal cortex and hippocampus (Ji et al., 2015).

Preclinical research has several important roles to play in the development of novel therapeutics. However, animal models have limitations that weaken the prediction of clinical efficacy. Still, animal models are necessary for the discovery of plausible biological targets and the demonstration that these targets can be manipulated to improve outcomes, at least in the experimental animal. Preclinical testing seeks to identify targeted therapy that may be effective. Equally important, however, is the use of preclinical testing to suggest ineffective or harmful therapies. For example, administration of TNF-α-neutralizing antibody, while protective in most gram-negative preclinical models, is actually detrimental in gram-positive bacteria models and some other types of pathogens, perhaps due to inhibition of innate antimicrobial defenses (Lorente and Marshall, 2005). Preclinical studies testing efficacy against sepsis-induced
multiorgan failure are more challenging (Andonegui et al., 2009; Kapoor et al., 2010; Coldewey et al., 2013) but are necessary to support translational research and clinical trials.

**Personalized Medicine**

Septic patients still experience unacceptably high morbidity and mortality, even with optimal goal-directed supportive therapy that meets approved guidelines. It is important to consider that the likelihood of survivors of severe sepsis to be readmitted to an inpatient health care facility is higher than matched nonsepsis survivors (Prescott et al., 2014). This is most likely related to the significant long-term physical and mental disabilities that can affect survivors. For example, estimates are that sepsis-associated alterations in mental status and cognitive deficits occur in up to 87% of septic patients (Iwashyna et al., 2012). While decreasing mortality is and should be the goal of therapy, it is becoming increasingly clear that patients who initially survive sepsis experience functional deficits that can diminish quality of life and even increase long-term mortality. Consequently, biomarkers that also direct therapies that decrease the morbidity of survivors would have a major impact on the overall health care burden of sepsis (Fig. 2).

Biomarkers and rapid noninvasive monitoring could offer better and more rapid stratification of patients to identify those who might benefit (or be harmed) by more targeted therapies. The advent of high-throughput technologies and access to advanced bioinformatics have made possible the concept of personalized medicine to direct therapy in septic patients. A personalized medicine approach may have the best chance of identifying specific strategies for such a diverse patient population. A systems biology
approach could complement the use of biomarkers to stratify patients, aid in prognosis, and help identify subsets of patients who may benefit or be harmed by therapy (Tsalik et al., 2015). However, the costs are high, and it is too early to know whether knowledge of the blood metabolome, proteome, and transcriptome could really direct therapy.

**Perspectives**

In the near future, biomarkers could direct the therapy of septic patients in order to achieve an optimal outcome. Cohen and colleagues (Cohen et al., 2015) suggested that the ideal profile of a sepsis biomarker should have these characteristics: fast kinetics, high sensitivity and specificity, fully automated technology, short turnaround time, availability as point-of-care tests, and low cost. Many clinicians already use biomarkers to suggest an active infection (CRP, PCT), estimate the severity of under-resuscitated septic shock (mixed venous blood oxygen saturation, lactate), and estimate the severity of organ injury due to sepsis (liver enzymes, troponin, creatinine, ratio of partial pressure of oxygen to fractional inspired oxygen, platelet count, activated partial thromboplastin time, fibrinogen and D-dimer). Physiological biomarker monitoring including echocardiography, pulmonary artery catheter, Doppler ultrasound, and/or continuous pulse-contour cardiac analysis is already being used to direct sepsis resuscitation and is recommended in the “Surviving Sepsis Campaign” document (Dellinger et al., 2013).

After more than three decades of numerous failed large sepsis clinical trials, sepsis is currently being redefined, which will most likely include “MODS due to non-resolving inflammatory response to infection.” Future clinical trials of sepsis therapies
will most likely target an individual patient’s underlying pathophysiological mechanism rather than a general patient population with sepsis. Some of the current proposed mechanisms for sepsis-induced MODS include pathological immune activation, immunoparalysis, mitochondrial dysfunction, disseminated microvascular thromboses, and others (Carcillo et al., 2011). The genetic makeup of the host and pathological organism, sex and age of the host, environmental factors, and specific interventions from different hospitals will all influence the underlying pathophysiological mechanism of the individual septic patient (Rautanen et al., 2015; Srinivasan et al., 2015). In this context, future real-time measurements of biomarkers could identify the pathological mechanism and direct therapeutic strategies in septic patients, including immune modulation for pathological immune activation, boosting the immune response for immunoparalysis, optimizing/rescuing mitochondrial function, reducing oxidative stress, and/or normalizing dysregulated hemostasis. Eventually, more advanced physiological monitoring, such as assessment of the microcirculation of specific organs, will give instantaneous feedback of intervention where titration of therapeutic agents could be optimized.
Authorship Contributions

Participated in research design: NA.

Conducted experiments: NA.

Contributed new reagents or analytic tools: NA.

Performed data analysis: NA.

Wrote or contributed to the writing of the manuscript: Sims, Nguyen, Mayeux.
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Footnotes

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**Figure Legends**

**Fig. 1.** Conceptual framework for the use of discussed biomarkers in combination to direct the development, validation and use of targeted therapies.

**Fig. 2.** Age, genetics, environmental factors, and comorbidities contribute to the diversity of septic patients, with the young and the elderly populations being the most susceptible to sepsis. As the systemic inflammatory response syndrome (SIRS) and sepsis progress to severe sepsis with multiple organ dysfunction syndrome (MODS), biomarkers could be used to guide targeted therapy that would halt the progression of sepsis to increase survival and decrease morbidity.
**Inflammatory Biomarkers**
- E-, L-, P-selectin
- ICAM-1, VCAM-1
- CRP, PCT
- TNF-α
- IL-1, IL-6, IL-8, IL-27
- HLA-DR
- PD-1
- Lymphopenia
  - Increased percentage of T regulatory cells

**Endothelial Biomarkers**
- Syndecan-1
- Heparan Sulfate
- Ang-1, Ang-2
- Tie-2
- S1P
- Microparticles

**Coagulation Biomarkers**
- Prothrombin Time
- Activated Partial Thromboplastin Time
- D-dimer
- Platelet Count
- VWF
- Ultralarge VWF Multimers
- Fibrinogen
- ADAMTS-13

**Organ-Directed and Physiological Biomarkers**

**Brain:**
- Changes in EEG
- Cognitive Impairment
- Perfusion (NIRS)

**Heart:**
- Myocardial Dysfunction
  - (Echocardiography)
- Troponin

**Kidney:**
- Perfusion (Ultrasound, MRI)
- Creatinine Clearance
- NGAL, KIM-1, NAG,

**Liver:**
- ALT, AST

**Lung:**
- Ratio of partial pressure of O$_2$ to fractional inspired O$_2$

**Microcirculation:**
- Blood Lactate
- Sublingual Perfusion
- Mixed venous O$_2$ saturation

**Tissue Injury:**
- MMP-8, Extracellular Histones

**Targeted Therapies**
- Immunomodulation
- Endothelium
- Microvasculature
- Coagulation
- Organ-specific

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Figure 1

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Definitions

**Systemic Inflammatory Response Syndrome (SIRS):**
Two or more of the following:
- fever or hypothermia
- tachycardia
- tachypnea
- leukocytosis or leukopenia

**Sepsis**: SIRS with a known infection

**Severe Sepsis**: Sepsis with 1 organ dysfunction or multiple organ dysfunction syndrome (MODS)

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**Figure 2**

- **Young (< 1 yr)**: Immature Immune System and Organ Development
- **Old (> 65 yrs)**: Weakened Immune System and Comorbidities