Toward Resolving the Challenges of Sepsis Diagnosis

SHAWN D. CARRIGAN,1 GEORGE SCOTT,2 and MARYAM TABRIZIAN1*

Sepsis in the United States has an estimated annual healthcare cost of $16.7 billion and leads to 120,000 deaths. Insufficient development in both medical diagnosis and treatment of sepsis has led to continued growth in reported cases of sepsis over the past two decades with little improvement in mortality statistics. Efforts over the last decade to improve diagnosis have unsuccessfully sought to identify a “magic bullet” proteic biomarker that provides high sensitivity and specificity for infectious inflammation. More recently, genetic methods have made tracking regulation of the genes responsible for these biomarkers possible, giving current research new direction in the search to understand how host immune response combats infection. Despite the breadth of research, inadequate treatment as a result of delayed diagnosis continues to affect approximately one fourth of septic patients. In this report we review past and present diagnostic methods for sepsis and their respective limitations, and discuss the requirements for more timely diagnosis as the next step in curtailing sepsis-related mortality. We also present a proposal toward revision of the current diagnostic paradigm to include real-time immune monitoring.

© 2004 American Association for Clinical Chemistry

The clinical definitions of sepsis used in diagnosis extend beyond systemic infection to focus on the symptoms generated by the host response in combating an infection. Despite a broad range of clinical measures, the complexity and heterogeneity of host response to infection, even within distinct populations, frequently lead to delayed diagnosis, which in turn leads to ineffective and belated antimicrobial treatment (1–4). The necessity of improved diagnosis is evident when considering the 18–29% mortality rates reported in broad epidemiologic studies (5, 6). Enhanced diagnostics could foster substantial reductions in the 120,000 annual sepsis-related mortalities and associated $16.7 billion annual healthcare cost in the United States through reduced duration of hospitalization and more efficient pharmaceutical treatment (5, 6).

Over the past decade, sepsis has been diagnosed according to the consensus guidelines established in 1991 as an infection in addition to the symptoms of systemic inflammatory response syndrome (SIRS)3 (7). Amendments to these criteria were initiated by convening an international conference in 2001 in response to a survey revealing that 71% of responding clinicians considered the existing definitions insufficient (8). In addition to the previous criteria, the 2001 conference added several new diagnostic criteria for sepsis. Of particular interest was the inclusion of the biomarkers procalcitonin (PCT) and C-reactive protein (CRP), despite the overall conclusion that it was premature to use biomarkers for sepsis diagnosis (8). The primary recommendation of the panel was the implementation of the Predisposition, insult Infection, Response, and Organ dysfunction (PIRO) staging system to determine optimum treatment for individual patients by stratifying their individual symptoms and risks (Fig. 1) (8, 9).

The focus of the PIRO system on individualized patient treatment acknowledges the concerns of authors who have previously identified the need for individualized diagnostics and treatment (8–10). However, the PIRO outline fails to address the necessity of prompt diagnosis. Given that swift diagnosis is crucial in permitting timely treatment, it is imperative that diagnostic methods be rapid enough to provide treatment guidance in correspondence with the progression state of infection. Because

1 McGill University, Biomedical Engineering Department, Montreal, QC, Canada.
2 MDS Pharma Services, St. Laurent, QC, Canada.
*Address correspondence to this author at: McGill University, Biomedical Engineering Department, 3775 University St., Duff Medical Building, Rm. 316, Montreal, QC, Canada, H3A 2B4.
Received February 2, 2004; accepted May 5, 2004.
Previously published online at DOI: 10.1373/clinchem.2004.032144

3 Nonstandard abbreviations: SIRS, systemic inflammatory response syndrome; PCT, procalcitonin; CRP, C-reactive protein; PIRO, Predisposition, insult Infection, Response, Organ dysfunction; TLR, toll-like receptor; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; IL-1ra, interleukin-1 receptor agonist; LPS, lipopolysaccharide; IL-18BPa, interleukin-18 binding protein a; NF-xB, nuclear factor xB; and ICU, intensive care unit.
of this need for rapid diagnostics, the majority of current research has focused on the search for a “magic bullet” biomarker as a provision for confirmed pathogen presence [for a listing of studied biomarkers, see Ref. (9)]. Despite a wealth of case studies correlating sepsis to specific biomarkers, lack of attention to the timing of patient sampling in relation to the period elapsed since infection onset has generated conflicting results for even the most promising biomarkers. The persistent trend of increasing rates of septic infection, particularly those caused by pathogens resistant to antimicrobial agents, fortifies the need to resolve these conflicts or find alternative methods of rapid sepsis identification and interim patient monitoring.

To help better understand the challenges in fulfilling these goals, we will review current diagnostic methodologies and discuss some of the inherent dilemmas in resolving sepsis diagnosis, concentrating on the impact of delayed diagnosis and initiation of antimicrobial treatment. The focus of the reviewed research on the diagnostic utility of biomarkers pertains to the differentiation of sepsis from SIRS in clinical cases, a considerably more difficult task than is differentiation of severe sepsis or septic shock. In analyzing this research, we refrained from comparing concentrations of biomarkers in survivors and nonsurvivors; although these data may provide useful hindsight, this end-term mortality measure fails to adequately consider the course of individual infections. Finally, we discuss suggestions for potential improvements to the monitoring of patient immune status, recognizing the importance of treatment timeline.

**Understanding Septic Infection**

Septic infection can be caused by pathogenic gram-negative and -positive bacteria, fungi, and yeast. Epidemiologic analysis of hospital records throughout the United States over the period spanning 1979–2000 revealed a mean annual increase in sepsis of 13.7% per year (6). With culture-identified pathogens reported in 51% of diagnoses, a breakdown of infectious causes was as follows:
52.1% gram-positive, 37.5% gram-negative, 4.7% polymicrobial, 4.6% fungal, and 1.0% anaerobic bacteria. Remarkably, gram-positive infections increased at a mean rate of 26.3% per year over the study period. This increase is attributed to increased nosocomial infections from such sources as catheterization and immunosuppressive therapies (11) and is particularly alarming considering that reported rates of methicillin-resistant *Staphylococcus aureus* isolates range from 29% to 45% and demonstrate an increasing trend (12–14). Primary sites are respiratory tract infections (40–44% of cases), followed by genitourinary infections (9–18% of patients), and intraabdominal infections (9–14%) (1, 5).

Infections are fought in the body by both cellular defenses, including monocytes, macrophages, and neutrophils, and humoral defenses incorporating antibodies and the complement pathways. Recognition of pathogens by extracellular CD14 and toll-like receptors 2 and 4 (TLR2 and -4) on the membranes of monocytes and macrophages triggers the release of cytokines to activate cellular defenses [see Refs. (9–11, 15–18) for reviews]. Cellular activation leads to differentiation of T-cells into type 1 helper cells (Th1), which secrete proinflammatory cytokines such as interferon-γ (IFN-γ), interleukin-18 (IL-1β), IL-2, and IL-12, and type 2 helper cells (Th2), which secrete anti-inflammatory cytokines such as IL-4, -10, and -13. The degree to which these cytokines are released is a function of many variables, including infectious circumstance, genetic susceptibility, and coexisting conditions. Trends in recent findings lead to the consensus that sepsis results from an imbalance in the host regulation of proinflammatory SIRS and the compensatory antiinflammatory response syndrome (Fig. 2).

Recent studies have resolved some of the unknowns pertaining to pro- and antiinflammatory cytokine secretion through the measurement of cRNA to monitor gene expression of stimulated leukocytes. In what has been labeled the “macrophage activation program”, common responses to various gram-negative and -positive stimuli include transcription up-regulation of 132 genes and down-regulation of 59 genes from a total of 977 transcription responses (19). Similar results obtained with peripheral blood mononuclear cells identified 206 genes with analogous kinetics for gram-positive and -negative stimuli (20). Both studies reported a predominance of transcription changes related to immune activation, signal transduction, and transcription factors, including up-regulation of pro- and antiinflammatory cytokines. Despite similar cell-line responses to various strains, dissimilar activation mechanisms were found across a limited (n = 3) sample of patient-donated cells (20). Additional surprising results stemmed from the observed active microbial defense against host immune function, with bacteria demonstrating strain-dependent effects on cytokine secretion (19, 20). Although these findings do not resolve our limited understanding of host immune dysregulation during sepsis, they underscore the complexity of host response, particularly when the regulated controls imposed by scientific study are removed. They also emphasize the speculative nature of delineating clear conclusions about the nature of in vivo cytokine secretion and interaction during sepsis.

**Current Diagnostic Methods**

Traditional sepsis diagnostics include culturing techniques and symptom classification systems. A positive culture from blood, urine, cerebrospinal fluid, or bronchial fluid represents the most certain method of diagnosis. Unfortunately, clinical symptoms frequently manifest themselves in the absence of a positive culture; among the studies we reviewed, positive cultures ranged from 8% to 73% in neonatal diagnoses (21–29) and from 8% to 88% in adult diagnoses (30–39). An additional drawback of culture-based diagnosis is the 24- to 48-h assay time. However, culture-based pathogen identification remains an
essential part of diagnosis to verify the efficacy of antimicrobial treatment, whether rendered before or after culturing. Automated culturing systems detect bacteria based on solution pH or the presence of CO₂, with detection times from 11–31 h and false-positive and -negative diagnoses in the 2–3% range (40, 41).

Classification systems are intended to aid clinical diagnosis in the absence of a positive culture. Systems such as Acute Physiology Age and Chronic Health Examination (APACHE), Simplified Acute Physiology Score (SAPS), and SIRS represent existing attempts to stratify patients according to various physiologic indices. Although diagnostic usefulness has been demonstrated by the APACHE II score (42, 43), a majority of studies revealed no diagnostic differentiation of sepsis from SIRS by APACHE II (30, 36, 44–46) or SAPS II (35, 38).

**Biomarker Detection**

Biomarker quantification by immunoassay is limited almost exclusively to commercially available ELISAs. Similar in principle to ELISA, PCT is measured by an immunoluminometric assay (47, 48). Alternatively, reports of flow cytometric measurement of PCT (49) and other biomarkers exist (50), although application is limited. Common among these methods is the need for incubation, which leads to a 2–3 h delay in subsequent diagnosis.

The catalog of proteic molecules associated with sepsis is extensive and includes cytokines, chemokines, adhesion mediators, soluble receptors, and acute-phase proteins (9, 17). Initial identification of the proteins to monitor generally stems from animal models of sepsis. Although caution is needed in extrapolating the conclusions of animal studies to humans, results from these studies contribute considerable to understanding septic inflammation and host response [for reviews of animal studies, see Refs. (51–53)]. Current proteic biomarker research focuses primarily on PCT and several interleukins in continued pursuit of a diagnostic magic bullet biomarker for infection. The standard method for characterizing diagnostic utility is the ROC curve, which plots the sensitivity (true-positive diagnoses) of the diagnostic marker(s) at a specified cutoff value against the specificity (false-positive diagnoses). Table 1 compares sensitivity and specificity values among the surveyed studies for delineating infectious from noninfectious illness by use of the most commonly reported biomarkers.

Tumor necrosis factor-α (TNFα) is considered the likely initiating factor in the activation of host response and subsequent cytokine release during infection, with concentrations increasing to 24 times (828 ng/L) their preinfection concentrations at 2 h post-lipopolysaccharide (LPS) challenge during in vivo experimental endotoxemia (54). However, the diagnostic utility of TNFα is insufficient for distinguishing infectious inflammation, with ROC analyses indicating poor sensitivity and specificity for differentiating sepsis from lack of infection in adults and neonates (Table 1). Furthermore, published values are generally <100 ng/L during sepsis in studies of both adult (30, 55–58) and neonates (59–63), which is well below peak concentrations found during experimental endotoxemia (54). Difficulties in using TNFα for sepsis diagnosis arise from the central role this cytokine plays in the inflammatory response, its short-term concentration in response to bacterial challenge, its short half-life of 17 min (64), and from excessive concentrations of soluble receptors p55 (sTNF-RI) and p75 (sTNF-RII) during sepsis (55, 58, 60).

Similar to TNFα, IL-6 is implicated in the early host response to bacterial challenge. Increased concentrations correlating to infection have been demonstrated by several studies. Adult values for sepsis are reported in the range of 300–2700 ng/L (30, 65, 66), above the 100 ng/L range for SIRS (30, 66). However, there are reports of insignificant differences between concentrations in

<table>
<thead>
<tr>
<th>Table 1. ROC analysis results for various biomarker-based prediction of sepsis in adult and neonatal cases.*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Marker</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>TNFα</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>IL-6</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>IL-1ra</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>IL-8</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>CRP</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PCT</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

* Values listed are for differentiating infected individuals from uninfected controls rather than from healthy individuals. Sensitivities and specificities listed are minimum, mean (in bold), and maximum percentages.
* NA, not available.
SIRS and sepsis (30, 35) and between septic and nonseptic trauma patients (67). These contradictions are confirmed by the insufficient sensitivity and specificity of ROC analyses for adult diagnosis (Table 1).

Although its usefulness in adult diagnosis has not been tested clinically, IL-6 is considered applicable in neonatal diagnosis. Reports indicate concentrations for septic neonates ranging from 47 to 1617 ng/L (range in healthy neonates, 2–42 ng/L) (21, 59–62, 68, 69). Despite variability in the septic range, ROC analyses indicate acceptable sensitivity (Table 1). Additionally, IL-6 increased in one study 2 days before the diagnosis of neonatal sepsis (70). However, care is required in interpreting measurements in relation to the gestational period and age at sampling. Separate studies showed that increased concentrations at birth decreased substantially in both term and preterm septic neonates during the first 24 h of life (28, 71, 72). An additional study found increased concentrations in septic preterm but not in full-term infants (68).

Another family of proinflammatory cytokines frequently linked with sepsis is the IL-1 family, including IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1ra), which exists in substantial excess of IL-1β during sepsis (55). The diagnostic usefulness of IL-1β is minimal given conflicting reports of both increasing (55) and decreasing (56) concentrations associated with sepsis in adults; similar increases (59) and decreases (73) have been published for neonates. In contrast, concentrations of IL-1ra have been shown to be consistently increased in sepsis patients, with adult concentrations of 2–31 μg/L (55, 74–76) (in healthy adults, concentrations are undetectable) and neonatal concentrations of 6–30 μg/L (60, 70) (concentrations in uninfected neonates, 2–3 μg/L). Furthermore, one study found that IL-1ra in neonatal sepsis increased to 3 and 15 times the concentration in healthy neonates 4 and 2 days, respectively, before clinical diagnosis (70); ROC analysis demonstrated 93% sensitivity and 92% specificity for the day of diagnosis (Table 1). As always, consideration of the inflammatory cause is warranted, because concentrations drastically exceeding the range typical for adult sepsis (mean = 174 μg/L) have been reported for thoracoabdominal aneurysm repair (76). Increased concentrations of sIL-1RII, another molecule that decreases IL-1β activity, have also been reported, although the evidence remains insufficient to draw diagnostic conclusions (76, 77).

The remaining proinflammatory cytokine biomarker commonly associated with sepsis is IL-18, which induces IFN-γ and amplifies Th1 differentiation. The higher concentrations of IL-18 observed in gram-positive compared with gram-negative infections (42, 78) have been corroborated by in vitro stimulation; whole blood stimulation with gram-negative LPS indicated a slight increase after 24 h, whereas gram-positive S. aureus stimulation induced a significant increase after 4 h, peaking at 24 h (42). The usefulness of this marker has been reported in several studies, including differentiation of sepsis from severe injury (42); values ranged from 588 to 1121 ng/L for sepsis, compared with 64–188 ng/L for healthy or uninfected control individuals (42, 79–81). The diagnostic value of this marker may be impacted by the late increase in IL-18 during sepsis, with peak concentrations occurring 3–10 days after injury in one report (42). Potential diagnostic improvements may stem from simultaneous measurement of IL-18-binding protein isoform a (IL-18BPa), which binds the majority of circulating IL-18 to block activity (80, 81). In one study, IL-18BPa was higher in septic than in healthy individuals (22 vs 2 μg/L (80). No information has been reported pertaining to IL-18 or IL-18BPa in neonatal sepsis.

In addition to the proinflammatory endothelial and phagocytotic activators, antiinflammatory cytokines also increase during sepsis. A major focus of current research is IL-10, which strongly inhibits the inflammatory cytokines TNFα, IL-1, IL-6, IL-12, and IL-18 in addition to suppressing transcription of the genes responsible for their production by inhibiting translocation of nuclear factor-κB (NF-κB) (82). LPS challenge in vivo induced a rapid increase, with peak concentration occurring 3–8 h postchallenge (54, 77). In reported studies, IL-10 concentrations were increased (22–383 ng/L) in septic adults, whereas it was undetectable in all control and healthy individuals, with the exception in one study (55, 58, 74, 77, 83). However, IL-10 concentrations are reported to increase within 2 h of blunt trauma, in correlation with injury severity (84), and in uninfected surgical patients (83), indicating a tendency to participate in the general compensatory antiinflammatory response syndrome. With regard to neonatal sepsis, limited findings indicated increased concentrations relative to healthy neonates (113 vs 36 ng/L) (28).

Among the other proteic markers correlating with sepsis, the chemoattractant IL-8 was higher in adult studies (30, 85), although the main focus of IL-8 diagnosis has been in neonatal studies. IL-8 concentrations in septice neonates were 94–4335 ng/L compared with 2–42 ng/L for healthy neonates (27, 59–62, 69). Although one study identified no diagnostic utility (23), the majority of studies found consistently increased concentrations of IL-8; ROC analysis for differentiating sepsis from uninfeclcted control individuals gave a sensitivity of 92% and specificity of 70% (Table 1). However, conflicting reports exist pertaining to the impact of gestation period on the correlation of increased concentrations in septic neonates (59, 68).

CRP, an acute-phase protein involved in coagulation, is an additional biomarker widely used in sepsis diagnosis. Acting as an opsonin for gram-positive bacteria to aid in their phagocytosis, CRP increases late during the onset of sepsis. Opinions on the diagnostic usefulness of CRP vary, with reports claiming both high and low value in adult diagnosis. Reported concentrations in septic patients range from 12 to 159 mg/L (30, 35, 38, 46, 86–88), demonstrating significant overlap with the 13–119 mg/L range in SIRS patients (30, 35, 38, 46, 87) and giving ex-
pectedly low sensitivity and specificity based on ROC curve analysis (Table 1). Studies involving neonates revealed similar diagnostic ambiguity, although ROC analyses demonstrated substantially higher mean specificity. The lower cutoff range stipulated in neonatal studies is indicative of the lower production of CRP in septic and healthy neonates, as is clearly reflected in values of 1–77 mg/L for septic and 0.1–6 mg/L for healthy neonates (21, 27, 28, 59, 60, 62, 68, 72, 89, 90). Although the diagnostic usefulness is superior in neonates than in adults, application of CRP for diagnostic purposes is best left until 24 h after birth. Several studies demonstrated that CRP increases over the initial day of life, with correspondingly increasing diagnostic sensitivity at 24 and 48 h, based on ROC curve analyses (23, 63, 72).

In contrast to the above biomarkers, the role of PCT in the host immune response is still incompletely understood [for reviews of PCT, see Refs. (91–93)]. Its implication in the cytokine cascade stems from demonstrated increases of TNFα before increases in PCT (54, 94) and a documented correlation between the concentrations of these biomarkers (r = 0.86) (35). Furthermore, a study involving in vivo LPS stimulation showed an exponential increase over the period 2–6 h after injection, with a plateau from 8 to 24 h (54). As the most common biomarker analyzed for correlation with sepsis, diagnostic use of PCT for sepsis and the immunoassay used have been patented by B.R.A.H.M.S. Diagnostica (www.brahms.de); this may contribute to the focusing on PCT in preference to other biomarkers. Measurement of PCT as a biomarker is favorable given its half-life of 22–29 h (95) and its prolonged increase during sepsis (54). Additionally, gram-positive and gram-negative cause increases in PCT concentrations that are not significantly different (37, 38, 78, 88). However, the definitive evidence of the role of PCT in immune response has been offset by contradictory reports of increased concentrations attributable to various inflammatory insults, including hepatic dysfunction (38), trauma (increases within 3 h of trauma) (43, 96), anti-T-cell therapy (57, 94), burns, heat stroke, and fungal infection (36) [for the exclusion criteria, see Ref. (92)]. Conversely, leukopenic patients have significantly lower concentrations of PCT during sepsis, with concentrations failing to exceed 2 μg/L even during septic shock (97). Although PCT evidently correlates with infection severity (Fig. 3), increasing evidence indicates that PCT fails to provide a definitive diagnosis. Several authors found no diagnostic usefulness for PCT in differentiating sepsis from trauma or SIRS (37, 43, 44, 46, 88, 96). The broad ranges of sensitivity and specificity values reflect these contradictory findings (Table 1).

Published use of PCT as a diagnostic tool in neonatal sepsis remains limited compared with its use in adults. Results demonstrated a postnatal surge of endogenous production, peaking at 24 h of life (72, 95), rendering comparison of the limited published studies difficult because of differences in the ages of the neonates studied.

Increased concentrations have been noted in both early- and late-onset cases, with values ranging from 12 to 78 μg/L (0.8–2.5 μg/L for healthy neonates) (72, 89, 90). ROC analysis indicated sensitivity equivalent to that of adult diagnosis and superior specificity, although this may result from studies including neonates suffering primarily from bacterial insults (Table 1). However, differentiation between infectious and noninfectious inflammation showed dependence on patient age during sampling, with conflicting reports of peak sensitivity and specificity over the initial 48 h of life (23, 24). As with contradictory findings in adult inflammation, increased PCT has been reported in uninfected neonates suffering from respiratory distress syndrome, indicating the potential for increases in response to noninfectious inflammation (98).

The preceding discussion incorporated the biomarkers most commonly associated with sepsis to date and is not intended to provide an exhaustive review. Several other biomarkers, including soluble cytokine receptors, cell surface receptors, endothelial activation factors, complement proteins, and transcription mediators, have shown strong correlation to sepsis in limited reports. For example, some studies have demonstrated the enhanced usefulness of markers such as NF-κB and C3a when compared directly with the above-mentioned biomarkers (55, 66). Another potential candidate for future research is high-mobility group-box protein 1 (HMGB-1), which activates NF-κB (99). Regardless of the marker, it is evident that consideration of the root inflammatory cause, infectious recurrence, previous treatment, and patient age are all critical factors in assessing the usefulness of biomarkers for differentiating sepsis. Nevertheless, objective biomarker quantification and monitoring should eventually assist in providing prompt diagnosis and treatment, thereby preventing regression from sepsis to severe sepsis, shock, and mortality.
DNA FINGERPRINTING

Current genetic studies indicate that detection of specific DNA sequences common to all bacteria is another possible means of diagnosis. When 7 gram-positive and 13 gram-negative strains were studied, the 16S rRNA gene was detected in all (100). A similar study found that the mecA gene was highly specific for the detection of methicillin-resistant strains of staphylococci (101). Finally, the presence of 18S rRNA indicated the presence of fungal infections caused by Candida albicans (102). These studies used PCR detection of their respective sequences and reported 100% detection efficiency for their specific targets in a 5–6 h time frame. Although limited, these studies demonstrated the potential for a highly specific diagnostic method, and consistent findings could lead to replacement of culturing with testing for genes found in pathogens.

GENETIC SUSCEPTIBILITY TO INFECTION

Genetic monitoring of sepsis also extends to the patient, focusing on genetic susceptibility to infection rather than the presence of pathogen RNA. Preliminary studies on the impact of gender on susceptibility have produced conflicting results. Stimulation of peripheral blood mononuclear cells with LPS shows twofold increases of TNFα and IL-6 in males relative to females (103). Gender differences are also associated with higher concentrations of IL-6 and PCT and increased sepsis and organ dysfunction related to severe trauma (104). The apparent enhanced immunity conferred by female gender may stem from immune stimulation provided by the female sex hormones or from the lack of immunosuppressive androgenic hormones (105). Whatever advantages may exist, in epidemiologic studies female sex benefits have been minimal. Reported cases of sepsis in males were 48.1–49.6%, which is in excess of female cases when adjusted for population (5, 6). However, the rates of sepsis-related mortalities in these studies are contradictory, with indications of marginally higher mortality rates in men (29.3% vs 27.9%) (5) and of no significant difference (22.0% vs 21.8%) (6).

Although gender-based immunity remains uncertain, advanced probing of genetic susceptibilities demonstrated numerous polymorphisms related to immune deficiency. The research published to date has focused on the alleles responsible for TNFα, IL-1/IL-1ra, and IL-10 production, with additional research identifying links to pathogen processing through the alleles for TLR4 and CD14 [for reviews, see Refs. (106–108)]. Correlation of the frequencies of different polymorphisms and haplotype combinations with sepsis has identified several genetic traits that may impart susceptibility to deficient immune response (Table 2).

Two polymorphisms in the TNF locus, TNFα-308 and TNFβ-252, correlate with immune dysfunction. Greater secretion of TNFα in trauma response has been linked to homozygous TNF1 genotypes of the TNFα-308 polymorphism, although no correlation was found with the development of severe sepsis (109). In contrast, trauma patients homozygous for either the TNFβ-252 TNFB1 or the TNFB2 allele were shown to have a significantly increased potential to develop sepsis; odds ratios for the B1/B1 and B2/B2 genotypes were 13 and 11, respectively (109). Homozygous TNFB patients also had increased production of TNFα, IL-6, and IL-8 on endotoxin challenge. Increased sepsis-related mortality risk has been linked to both homozygous TNFB1 and TNFB2, with conflicting results as to which genotype imparts greater susceptibility (109,110). Interestingly, the haplotype TNF1:TNFB2 appears to impart genetic resistance, as demonstrated by a negative correlation with sepsis (109).

Genes for proinflammatory IL-1α, IL-1β, and their receptor competitor IL-1ra have also been analyzed for correlation with sepsis. None of the genotypes for the IL-1α polymorphism IL-1A or the TaqI and AvaI polymorphisms on the IL-1β gene showed correlation with sepsis in infected patients (110, 111). The frequency of the IL-1ra RN2 polymorphism, which is associated with increased IL-1ra secretion, has been noted in two separate studies to correlate with sepsis occurrence, both as homozygous RN2 and as heterozygous RN1/RN2 (110, 111). Despite the lack of sepsis correlation with specific IL-1α or IL-1β alleles, genotype-associated mortality indicates increased

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Gene polymorphism</th>
<th>Effect</th>
<th>Referenced studies</th>
</tr>
</thead>
</table>
| **Table 2. Polymorphisms correlated with sepsis susceptibility or mortality.**

<table>
<thead>
<tr>
<th>Gene polymorphism</th>
<th>Effect</th>
<th>Referenced studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP 308 G/A (TNF1/TNF2)</td>
<td>TNF1/TNF1 genotype correlates with higher TNFα secretion in trauma</td>
<td>(109)</td>
</tr>
<tr>
<td>SNP 252 G/A (TNFB1/TNF2)</td>
<td>Homozygous genotypes correlate with sepsis and increased inflammation on challenge</td>
<td>(109, 110)</td>
</tr>
<tr>
<td>VNTR intron 6 (A1–A7)</td>
<td>Increased mortality in A2/A2 genotypes</td>
<td>(111)</td>
</tr>
<tr>
<td>VNTR intron 2 (RN1–RN5)</td>
<td>RN1/RN2 and RN2/RN2 genotypes correlate with sepsis</td>
<td>(110, 111)</td>
</tr>
<tr>
<td>SNP 592 C/A</td>
<td>A/A and A/C genotypes have low in vitro IL-10 and correlate with mortality</td>
<td>(112)</td>
</tr>
</tbody>
</table>

*a Polymorphisms indicate type, position on the gene, alteration for single-nucleotide polymorphisms, and common nomenclature where applicable. Noted effects may not be applicable to all cultural populations.

*b SNP, single-nucleotide polymorphism; VNTR, variable number tandem repeat.
risk for IL-1A A2/A2 homozygotes, AxAI B1/B2 heterozygotes, and B2/B2 homozygotes (111). Divergent conclusions relating to the mortality risk of the IL-1ra RN2/RN2 genotype have been reported. However, a limited number of patients (n = 8) with the homozygous TNFβ2 and IL-1ra RN2 haplotype demonstrated 100% mortality (110).

The gene for the antiinflammatory protein IL-10 have three identified polymorphisms, 592C/A, 819 C/T, and 1082G/A. Analysis of these polymorphisms in healthy individuals, in patients in the intensive care unit (ICU), and in septic patients indicated no correlation between the polymorphisms and the occurrence of sepsis (112). However, presence of the 592A allele was associated with the lowest LPS-stimulated IL-10 release in vitro, whereas the homozygous C/C genotype produced the highest secretion. In addition, the 592 A/A and A/C genotypes were associated with survival, whereas the C/C homozygous genotype was associated with mortality. No correlations were reported between the other polymorphisms and in vitro IL-10 secretion, likelihood of infection, or risk of mortality.

Polymorphisms also correlated with increased concentrations of sCD14 (homozygous CD14 −159T), impaired TLR4 pathogen processing (TLR4 −896G), and reduced IL-6 secretion (IL6 −174C) (106). The relative knowledge concerning these polymorphisms is still quite limited for diagnostic application. Caution is warranted in interpreting the results of these initial genetic studies because certain polymorphisms and haplotypes that may appear to be statistically indicative of genetic susceptibility are not necessarily prevalent enough in the general population to permit such associations. Furthermore, conclusions drawn from studies comparing septic patients with healthy individuals may fail to be corroborated by future studies comparing septic patients with aseptic ICU patients. In addition to the noted statistical limitations, the caveat found to date with this type of analysis arises from discontinuity across populations. Studies examining the TNFa −308 polymorphism demonstrated conflicting results among French, German, and Taiwanese populations (107), and studies examining IL-1ra RN similarly find conflicting results among German, Chinese, and Spanish populations (108). Although not confirmed genetically, similar epidemiologic findings in the United States indicate that non-whites have approximately twice the risk for septic complications as do whites (6). This finding requires further investigation to differentiate between anthropologic and social impacts. However, as this field of research develops, with additional studies lending credence to the impacts of certain polymorphisms and haplotypes, it is possible that future patients will be screened for genetic susceptibilities to predict their likelihood of developing systemic infection.

TREATMENTS
Spurred by greater understanding of how the response to infection functions, researchers have repeatedly tried to intervene pharmaceutically to arrest inflammation in the host immune response to infection in addition to standard antimicrobial treatment regimens. The successive failures of more than 70 clinical trials aiming to control inflammation biomediators is reflected in the previously noted epidemiologic increase in sepsis over the past two decades (6). During this period, only one novel sepsis drug was approved by regulatory authorities (113). Interventions focusing on suppression of the inflammatory response, including administration of TNFα antibodies, soluble TNF receptor, granulocyte-colony-stimulating factor, and IL-1ra, have failed to provide substantial reductions in mortality (113). Activated protein C, which inhibits inflammation by hampering monocyte secretion of TNFα, IL-1, and IL-6 and by reducing adhesion between neutrophils and the endothelium, has received recent approval for cases of severe sepsis, based on the results of the PROWESS study (114). Treatment with activated protein C was correlated with reduced mortality, particularly for cases of severe sepsis and organ dysfunction. Another suppressive therapy, the efficacy of which is currently undergoing clinical evaluation, involves low-dose administration of the corticosteroid methylprednisolone, which suppresses inflammation by decreasing the concentrations of TNFα and IL-6 and decreasing NF-κB activity in leukocytes (114). In contrast to these antiinflammatory therapies, treatment with the proinflammatory cytokine IFN-γ has been found to restore HLA-DR and TNFα production in septic patients (15). However, further clinical trials are needed to better ascertain the effectiveness of this therapy (114).

The principal limiting factor in pharmaceutical immunomodulating treatment is the lack of transferability from animal studies to human studies (113, 114). Certainly, part of this failure stems from the inability to control when therapeutic intervention is initiated relative to commencement of the inflammatory response, as would be the case with experimental animal models. Further compounding the issue of timing is the complexity and redundancy of host response, a response that is not yet understood in its entirety (113). Finally, the differential cytokine responses among patients presenting with similar infections and symptoms suggests that any biomediator-directed therapy will likely be ineffective for an unknown proportion of patients (111), particularly when incongruous genetic susceptibilities among different populations are considered.

A separate factor in the hindrance of biomediator-related therapies may be the outcome measures used to measure efficacy. Mortality at day 28, a common measure used to rate the success of a given treatment, provides little information about the progression of infection or its response to treatment. As discussed by Marshall (113), the impact of any treatment, when given at an unknown time point during the progression of the illness, will provide only crude understanding of the treatment efficacy when the measured variables are of such a crude nature. A
definite need exists for refined measures of both the infection and the status of immune activity within the host.

Delayed Diagnosis
The necessity for enhanced measures for diagnosing infection and monitoring immune status becomes clearer when the rate of inappropriate antimicrobial treatment (23–30%) is considered (1–4). Autopsies have confirmed that failure to diagnose and provide antimicrobial intervention is the most common avoidable error in sepsis-related mortality (15). Studies analyzing the effect of delayed diagnosis use different definitions for “adequacy of treatment”, although all emphasize that efficacy of the antimicrobial against the identified pathogen and timeliness of administration are critical. For studies requiring that treatment begin within 48 h of pathogen isolation, inappropriate treatment is reported in 27–29% of cases (3, 4). Narrowing the treatment timeline to 24 h, a separate study found inappropriate treatment in 23% of patients, with 77% of these cases resulting from administration of an ineffective antimicrobial and the remainder attributable to delayed administration (1). Unfortunately, these studies are consistent in reporting increased infection-related mortality rates in inappropriately treated patients (12–28% adequate vs 30–39% inadequate) (1–4). Furthermore, no patient subgroup, including those suffering from septic shock, failed to benefit from adequate treatment. Finally, the above-mentioned studies are consistent in identifying ineffective treatment as an independent predictor of mortality when isolated from other health-related complications.

The consequences of delayed diagnosis become more ominous when considering their effects on pathogen management. Among culture-identified pathogens, 59% of isolated infections are nosocomial, which show higher correlation with inadequate treatment (2). Further complicating treatment is the emergence of resistant bacterial strains such as methicillin-resistant S. aureus, vancomycin-resistant enterococci, and vancomycin-resistant S. aureus in hospital settings (4). Administration of ineffective antimicrobials serves to increase the cost of treatment through additional antimicrobial therapy and lengthened hospitalization. Inadequate treatment also increases the risk of side effects and future patient resistance to treatment with a given antimicrobial. Cumulatively, the effects of delayed diagnosis and treatment ultimately increase a patient’s risk of infection-related mortality.

Need for Real-Time Diagnostics
Our review of sepsis-diagnosis-related literature revealed the continued quest for the magic bullet biomarker. The logic supporting the desire to identify such a marker contains a fundamental error in that it fails to allow for the redundancy, complexity, and heterogeneity of host response to various pathogens and infectious or inflammatory circumstances. Although the possibility of finding such a specific marker should not be precluded, the continual pursuit of such a marker prevents us, as scientific and medical communities, from explaining study-to-study differences in existing biomarkers and from using these markers in diagnosis because of their imperfect specificities.

We propose that current diagnostic methods could be complemented by including real-time monitoring of protein biomarkers during the course of treatment. Existing and novel methods alike require a “wait and see” approach in their diagnostic methods because of their incubation cycles. Although traditional ELISAs (2–3 h), immuno-­luminometric PCT assays (3 h), and DNA detection by PCR (5–6 h) provide more rapid results than culture testing (24–48 h), these methods are incapable of monitoring the exponential changes in biomarkers occurring in both sepsis and experimental in vivo endotoxemia (42, 54, 77, 115). Shown in Fig. 4 is a hypothetical simplified host response to pathogen challenge, indicating the variability of biomarker concentrations at different time points during the progression of infection. Because these changes occur on a time scale of hours, the necessity of measuring biomarker concentrations at regular intervals becomes evident. Furthermore, this idealized response fails to incorporate the impact of the development and coexistence of other complications. To consider these potentialities, immune monitoring should ideally require panels of markers to be checked at regular intervals rather than basing diagnosis on a single value, with the selection of appropriate biomarkers guided by the associated gene regulation demonstrated in recent studies (19, 20, 78). Although ROC analyses have combined the use of multiple biomarkers in studies of adults (66, 85, 86) and neonates (22, 25, 26, 29, 63, 68) to little avail, this differs considerably from what is necessary in terms of on-demand real-time analysis of a patient’s immune status.
An essential requirement of any real-time monitoring system is patient proximity, with samples being analyzed in an ICU setting rather than in a centralized hospital laboratory. Existing real-time protein detection technologies capable of being adapted to suit the needs of clinical monitoring include surface plasmon resonance (116, 117) and quartz crystal microgravimetry with dissipation (118–120). These technologies, optical and acoustic mass sensors, respectively, detect the presence of biomarker proteins through the immobilization of specific antibodies to a biointerface. Real-time binding of the biomarkers to their respective antibodies is exhibited as a mass addition to the biointerface. At present, the capability of these methods would require accepting detection limits on the order of μg/L, limiting the range of currently identified sepsis biomarkers to cytokines whose physiologic concentrations fall within this range. However, the increased application of these technologies in recent years for immune interaction studies, such as antibody affinity determinations and epitope mapping, has encouraged continued improvement in the detection limits of such techniques, consequently improving their usefulness for sepsis biomarker applications.

In addition to these methods, B.R.A.H.M.S. has recently released a more rapid version of their PCT assay, which requires 32 min, representing a substantial step toward providing clinicians with on-demand analysis (47). Extending this novel chemiluminescence assay to other biomarkers could provide a method of analysis that is comparable to surface plasmon resonance and quartz crystal microgravimetry in assay time with the benefit of an order of magnitude improvement in detection limit. A final technology worth noting as a real-time alternative to biomarker detection is a recently developed method for bacterial detection in platelet concentrates, with a reported assay time of 3 min (121). Further testing may reveal that this type of testing is useful in a clinical setting.

Outlined in Fig. 5 are some of the considerations necessary for the proposed real-time immune status monitoring. Although the technology necessary to meet the proposed immune monitoring protocol is unavailable at present, modification of existing technologies could allow the proposed diagnostics to be developed rapidly. The financial burden placed on the healthcare system by sepsis, in conjunction with the desire to provide improved medical treatment, provides sufficient motivation for the development of such technologies.

**Conclusions**

Despite extensive medical advances over the past two decades, the reported number of cases of sepsis continues to increase at an alarming annual rate because of limited progress in treatment and diagnostic methods. Of particular concern is the increase in nosocomial infections caused by bacteria resistant to antimicrobial agents. Diagnosis of these infections, including pathogen isolation, remains challenging given that approximately one half of sepsis cases are culture-negative. Delay in the diagnostic process as a result of inability to identify the cause of the inflammation leads to inadequate treatment in one fourth of cases, with a significant impact on mortality risk in those patients. Over the past decade, research on improving diagnostic timelines has focused on identifying highly specific biomarkers of infectious inflammation. Among the markers associated with sepsis, contradictory results have been reported in all cases regarding the ability of...
individual biomarkers to differentiate sepsis. In an effort to improve diagnostic efficiency and ultimate survivability, we propose that real-time protein detection technologies be used to monitor a panel of sepsis-related biomarkers. Through improved measures of a patient’s immune status, and greater understanding of the host immune response provided by such measures, the confounding factors in diagnosis and treatment can be limited to allow prompt and effective responses.

We thank the Natural Sciences and Engineering Research Council of Canada for funding this work, and K.L. Douglas for comments on the manuscript.

References

31. Blairon L, Wittebole X, Laterre PF. Lipopolysaccharide-binding protein serum levels in patients with severe sepsis due to


