

# Barriers to the effective treatment of sepsis: antimicrobial agents, sepsis definitions, and host-directed therapies

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Sepsis is a complex clinical syndrome involving both infection and a deleterious host immune response. Antimicrobial agents are key elements of sepsis treatment, yet despite great strides in antimicrobial development in the last decades, sepsis continues to be associated with unacceptably high mortality (~30%). This is the result, on one hand, of the rise of antimicrobial resistant organisms and, on the other hand, of the dearth of effective host-directed immune therapies. A major obstacle to the development of good host-directed therapies is the lack of understanding of the host immune response. The problem is exacerbated by poor nonspecific clinical definitions of disease. Poor definitions have had a profound impact on sepsis research, from epidemiologic studies to the failed clinical trials of host-directed therapies. Therefore, better definitions must be developed to enable advancement in the field.

**Keywords:** sepsis; antimicrobial resistance; host-directed therapies; sepsis definitions; immunology

## Introduction

Sepsis, a complex clinical syndrome defined by the clinical response to a suspected or proven infection, is a leading cause of death worldwide. Mortality (~30%)<sup>1</sup> and incidence (750,000 cases in the United States, 18 million worldwide)<sup>2,3</sup> remain unacceptably high even in industrialized nations where there is widespread availability of antibiotics and advanced supportive care. There are two major aspects to consider in sepsis, the underlying infection and the host immune responses that fail to clear the infection and that paradoxically contribute to morbidity and mortality.

In the 1960s, following a golden age of antibiotic development, many were ready to “close the book on infectious diseases,” in the words of one American official.<sup>4</sup> Ironically, far from an eradication of infectious diseases, between 1940 and 2004 more than 300 new infectious diseases emerged or became recognized, including new strains of drug-resistant organisms (e.g., methicillin- and vancomycin-resistant *Staphylococcus aureus*).<sup>5</sup> In fact, antibiotic-resistant microbes account for 21%

of emerging infectious disease events,<sup>5</sup> although even susceptible microbes are a threat in sepsis. Increased host susceptibility (e.g., owing to an aging population, the widespread use of immunosuppressive therapies, and the HIV/AIDS pandemic) was another potent driver of the emergence of new pathogens. Here, challenges in sepsis therapeutics are discussed. First, we briefly consider the problem of antibiotic selection in the face of blossoming antimicrobial resistance, and second, we explore barriers to the development of host-directed therapies that target the underlying deleterious immune responses.

## Antimicrobial agents are the cornerstone of sepsis treatment

When antibiotics first appeared in the early 1930s, they revolutionized the treatment of sepsis. In the preantimicrobial era, bacterial diseases causing pneumonia and diarrhea were feared owing to high rates of mortality. With the introduction of antibiotics, however, the mortality from these diseases plummeted. In the United States, death due to

infections of all types declined from 265 per 100,000 individuals in 1910 to 12 in 1960. Over the same period, death due to pneumonia and diarrheal disease also declined from 184 and 118 per 100,000 population to 39 and 5, respectively.<sup>6</sup>

### *Antimicrobial efficacy is limited by increasing resistance*

Today, antimicrobial agents remain a key element of sepsis treatment, but appropriate use is hampered by increasing numbers of drug-resistant microbes, as well as by the threat of further resistance developing. Since penicillin was first introduced into general use in 1945, the march of antibiotic resistance has been steady and relentless. Notable events include the emergence of methicillin-resistant *S. aureus* (MRSA) less than 1 year after the introduction of methicillin<sup>7</sup> and its rapid rise to become the first “superbug” in the 1990s. In the early 2000s, MRSA represented up to 70% of organisms isolated from U.S. intensive care units (ICUs).<sup>8</sup> An increase in the incidence of vancomycin-resistant *Enterococcus* spp. (VRE) infections was also significant, representing up to 40% of enterococcus isolates in U.S. ICUs.<sup>8</sup> Recently, the problem of multidrug resistance among Gram-negative microbes has gained prominence, largely due to the withering pipeline of new antimicrobial agents to treat these pathogens.<sup>9</sup> Bacteria such as *Klebsiella pneumoniae* and *Escherichia coli* elaborate plasmid-encoded extended-spectrum  $\beta$ -lactamases (ESBL), while *Pseudomonas aeruginosa* expresses derepressed chromosomal  $\beta$ -lactamases, both of which inactivate most  $\beta$ -lactam antibiotics. The plasmids encoding ESBLs frequently express resistance genes for other antibiotics, such as fluoroquinolones and aminoglycosides. The rapid spread of ESBLs through the 1990s and early 2000s resulted in a number of publicized outbreaks and instigated the increasing use of carbapenem  $\beta$ -lactam antibiotics (a class of last-line antimicrobial agents) to treat these infections.<sup>10,11</sup> This then encouraged the emergence of multidrug-resistant bacteria with plasmid-encoded carbapenemases, notably in *Klebsiella* and *Pseudomonas*, which are active against all  $\beta$ -lactam antibiotics including carbapenems.<sup>12</sup> The only antibiotics now generally regarded as active against these organisms are colistin and the new tetracycline analog tigecycline, both considered suboptimal for use in critically ill patients.<sup>13,14</sup>

Therefore, the threat of antimicrobial resistance and the need for stewardship is real. Balancing obligations for antimicrobial stewardship, however, with that of providing optimal treatment to individual patients, is a major challenge for clinicians.

Ineffective initial coverage for sepsis patients can be catastrophic, leading to increased mortality.<sup>15–19</sup> One analysis suggested that mortality increases 7.6% for each hour that effective antibiotics are delayed within the first 6 h of sepsis-associated hypotension.<sup>20</sup> Consequently, international guidelines for the treatment of severe sepsis and septic shock emphasize that intravenous antibiotics be administered as soon as possible after diagnosis, preferably within 1 hour.<sup>21</sup> Despite this, the need to avoid resistance development necessitates that antibiotic stewardship be practiced, such that the antibiotics used may not always be appropriate for the (initially unknown) organisms causing disease. Since a microbiological diagnosis takes at least 24 h to obtain,<sup>22</sup> clinicians treat empirically with antibiotics while awaiting the results. Polymerase chain reaction (PCR) and analogous technologies have the potential to shorten diagnosis times but are not yet available in many clinical labs.<sup>23</sup> Also, they are not clearly superior to conventional culture, and are limited by cost, the range of species identified, and the lack of susceptibility information.

Ideally, empiric antibiotic selection should be personalized for each patient. It should be guided by careful consideration of local susceptibility patterns and patient-specific factors that increase the risk of antibiotic-resistant infection such as recent antibiotic use, recent hospitalization, residency in a nursing home, chronic dialysis, immunosuppression, and other factors.<sup>24,25</sup> In practice, however, first responders rarely have the time to consider all potential factors that increase the risk of infection by a resistant microbe. Therefore, the prescription of empiric antibiotics may simply reflect local preferences or guidelines for a particular broad-spectrum agent. In many health jurisdictions, however, the use of antibiotics such as vancomycin and meropenem (with activity against MRSA and ESBL-producing organisms, respectively) are discouraged as part of the initial therapy, due to fears of resistance developing. Consequently, in more than 20% of cases, the initial empiric antibiotics is retrospectively recognized as inadequate.<sup>17,19</sup> Commonly used antimicrobial agents for sepsis and the gaps in

**Table 1. Important gaps in antimicrobial coverage of commonly used antimicrobials in sepsis**

Commonly used antimicrobial types <sup>a</sup>	Specific examples of commonly used antimicrobial agents	Important gaps in antimicrobial coverage
Carbapenems	Imipenem, meropenem, doripenem, ertapenem	Carbapenemase-producing organisms.
$\beta$ -lactams	Piperacillin–tazobactam, cephalosporins	Carbapenemase- and ESBL <sup>b</sup> -producing organisms.
Quinolones	Moxifloxacin, ciprofloxacin, levofloxacin	Quinolone-resistant organisms, which include many of the carbapenemase- and ESBL-producing organisms.
Aminoglycosides	Gentamicin, tobramycin, amikacin	Most Gram-positive organisms, aminoglycoside-resistant organisms, and most of the carbapenemase- and ESBL-producing organisms.
Macrolides	Azithromycin, clarithromycin, erythromycin	Macrolide-resistant organisms including many Gram-positive organisms and most of the carbapenemase- and ESBL-producing organisms.
Anti-Gram-positive	Glycopeptides (including vancomycin, oritavancin, telavancin), linezolid, daptomycin	Vancomycin-resistant <i>Staphylococcus aureus</i> and <i>enterococcus</i> spp. (for vancomycin). Typically used in combination with one of the above classes of antimicrobial agents.

<sup>a</sup>Taken from Ref. 106.

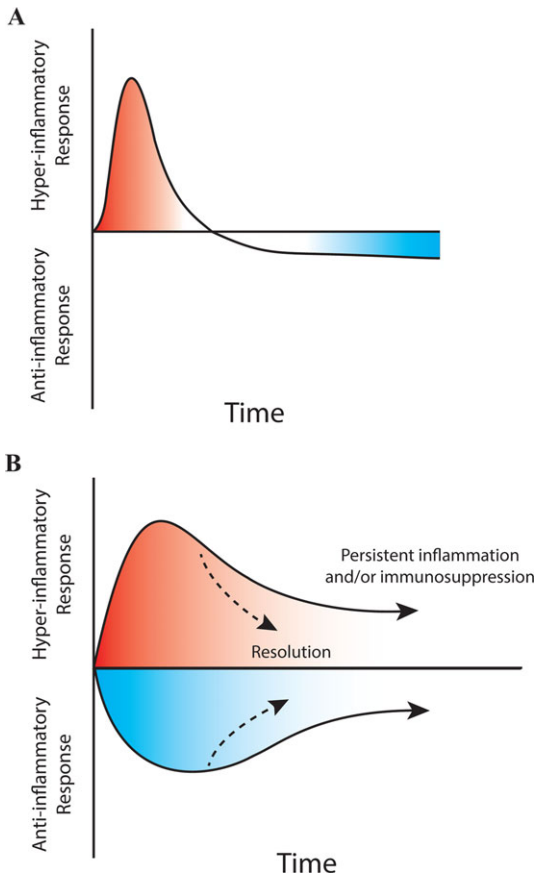
<sup>b</sup>ESBL, extended spectrum  $\beta$ -lactamase.

their coverage are listed in Table 1. While current initiatives are attempting to increase the discovery of new antibiotics, as well as adjunctive strategies that include antiresistance agents, immunomodulators, phages, probiotics, and microbiota, these have yet to demonstrate clinical success.

### Immune status in sepsis

While the infectious origin of sepsis is well accepted, an understanding of the immune status of the host is still evolving. Generally, there is agreement that a strong inflammatory response initiated by the innate recognition of microbial signature molecules by pattern-recognition receptors (PRRs) such as Toll-like receptors (TLRs) is critical to the pathogenesis of early sepsis.<sup>26</sup> In cases of severe sepsis, the inflammation is prolonged and more intense, resulting in greater plasma levels of proinflammatory cytokines, such as TNF- $\alpha$  and IL-1 $\beta$ , accompanied by a heightened physiologic response and resulting in organ dysfunction.<sup>27,28</sup> Multiple organ failure leading to death can then occur as a result of an ensuing cycle of inflammation and coagulation.<sup>26</sup> Previously, it was thought that the threat to organ function posed by this systemic hyperinflammatory response leads to a subsequent

anti-inflammatory (immune-suppressive) response characterized by the reduction of proinflammatory mediators and the upregulation or stabilization of anti-inflammatory mediators.<sup>29</sup> Recently, however, this has been challenged, based on a leukocyte transcriptome analysis of patients with severe trauma and burns as well as a human model of endoxemia. This revealed a common early response to severe injury in which a proinflammatory innate immune response occurs simultaneously with an anti-inflammatory immune response, as well as the suppression of genes involved in adaptive immunity.<sup>30</sup> Postmortem studies of patients who die of sepsis indicate that immune suppression is the dominant pathology at the time of death.<sup>31</sup> Tissue from patient lungs and spleen demonstrate marked lymphocyte apoptosis, upregulation of inhibitory cell receptors (such as PD-1), downregulation of markers of cell activation (including HLA-DR) and decreased production of both pro- and anti-inflammatory cytokines (including TNF- $\alpha$ , interferon- $\gamma$ , and IL-10).<sup>32</sup> Although the exact timing of these events with respect to the course of sepsis remains unclear, this has led Hotchkiss *et al.* to postulate that it is the balance between the proinflammatory and immune-suppressive states

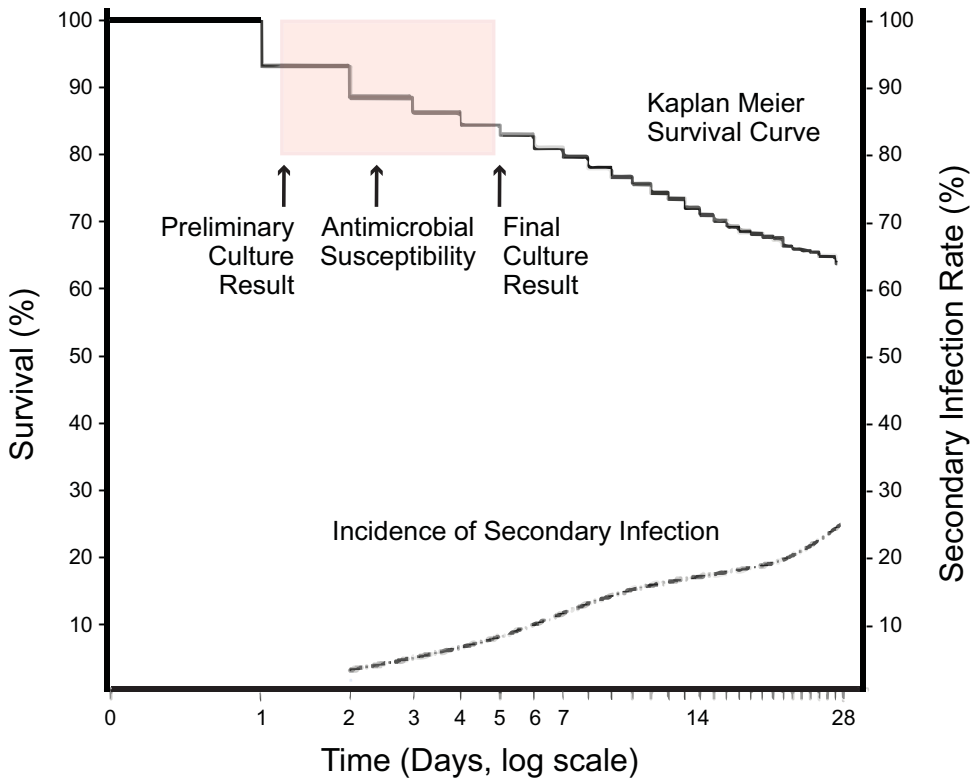


**Figure 1.** Changing paradigms of host immune pathology during sepsis. (A) The old concept suggested that an initial hyperinflammatory state was followed by an immunosuppressive state. (B) The proposed new concept suggests that both a hyperinflammatory response and an immunosuppressive anti-inflammatory response occur simultaneously in early sepsis, although the actual timing and magnitude of these events are not established in detail. Early resolution of these responses is thought to be associated with uncomplicated disease, whereas persistent inflammation and/or immunosuppression are proposed to be associated with worse outcomes (i.e., organ dysfunction or increased susceptibility to secondary infections, respectively). The character, relative magnitude, and dynamics of these responses are an active area of research

that might be the main factor driving the pathogenesis of sepsis and determining outcomes.<sup>31</sup> The old and new paradigms are compared in Figure 1. Our own data support the notion that an immune-suppressive state appears early during sepsis. A secondary analysis of mortality data from the Vasopressin versus Norepinephrine Infusion in Patients with Septic Shock (VASST) trial<sup>33</sup> demonstrates increasing rates of secondary

infection starting just 2 days after the onset of septic shock, which were inversely correlated with survival (Fig. 2). One intriguing mechanism of immune suppression in sepsis has been proposed to be endotoxin tolerance, although again the timing of this event in sepsis is uncertain.<sup>31,34,35</sup> Endotoxin tolerance is defined as the loss of responsiveness to endotoxin (bacterial lipopolysaccharide (LPS)) or other bacterial signature molecules that interact with PRRs during subsequent encounters with these bacterial signatures. Phenomena resembling endotoxin tolerance have been shown to affect most immune cells (including neutrophils and monocytes) during sepsis.<sup>31</sup> Indeed, the dynamics of the immune-suppressive state during sepsis represents one of the most interesting areas in sepsis research today. This is due to the consistent and dramatic failures of treatments that have targeted the hyper-inflammatory response (cytokine storm) in sepsis.

In contrast to the relative success of antimicrobial agents and supportive therapies designed to treat various sequelae of sepsis, host-directed therapies for the treatment of sepsis (mostly anti-inflammatory) have been uniformly disappointing. Among the initially promising but ultimately failed therapies tested in phase III human clinical trials are intravenous immunoglobulin,<sup>36</sup> a TLR4 (LPS receptor) antagonist,<sup>37</sup> TNF- $\alpha$  antagonists,<sup>38–40</sup> an IL-1 receptor blocker,<sup>41</sup> recombinant human activated protein C (rhAPC),<sup>42</sup> talactoferrin<sup>43</sup> (a glycoprotein with anti-inflammatory properties), monoclonal antibodies directed against the common lipid A portion of LPS,<sup>44</sup> and others. An examination of the basis for these failures reveals a common pattern. Clinical phase II trials often suggested remarkable benefits in post hoc analyses for selected subgroups of patients. The benefits, however, evaporated in large double-blind, placebo-controlled, randomized clinical trials, or in the case of rhAPC, during postmarketing assessments. This story has repeated itself so frequently that sepsis therapeutics has become known as the graveyard for the pharmaceutical/biotech industry.<sup>45,46</sup> One consequence of the failure of host-directed therapies has been an increasing emphasis on antimicrobial agents. For example, recent studies have examined alternative antibiotic dosing schedules<sup>47,48</sup> or ways that antibiotics can be combined for greater effect.<sup>49–51</sup> However, we would argue that, for sepsis, an increased focus on antibiotics is unlikely to have a



**Figure 2.** Kaplan–Meier survival curve for patients enrolled in the VASST trial (solid line).<sup>33</sup> The dashed line shows the rate of secondary infections (ventilator-associated pneumonia and central venous catheter bloodstream infection) from the Vancouver regional 2010 critical care report. The red box shows a typical timeline for a microbiologic diagnosis.

dramatic effect on outcomes. A basic tenet of infectious diseases, first described by Leslie Webster in the 1920s, is that infection severity is promoted by higher pathogen dosage and virulence, and limited by host resistance mechanisms.<sup>52</sup> Therefore, as host resistance diminishes, even weakly virulent organisms can become opportunistic pathogens. In fact, it seems likely that increasing host susceptibility (e.g., transplant immunosuppression, anti-TNF- $\alpha$  monoclonal antibodies, and malignancy) is substantially responsible for both the increased incidence of sepsis and the persistently poor outcomes reported today, despite decades of advances in both antimicrobial strategies and supportive care technologies.

### Barriers to the development of effective host-directed therapies

The mortality rate associated with severe sepsis has remained stubbornly high despite great advances in supportive care, including limiting secondary injury to the lungs through low tidal volume ventilation,<sup>53</sup>

reducing copious fluid administration and subsequent tissue edema,<sup>54,55</sup> and limiting exposure to benzodiazepine sedatives. These treatment failures in sepsis are caused, to some extent, by increasing antimicrobial resistance leading to ineffective empirical antibiotic selection, as discussed above. However, we suggest that a greater impediment to success in sepsis treatment is the lack of effective host-directed therapies. To promote the development of innovative therapies, however, we need a better understanding of the immune pathology of this syndrome. To achieve this, we need to first address the problem of nonspecific sepsis definitions.

### *The problem with nonspecific definitions of sepsis*

Sepsis definitions have, to date, relied on clinical signs and symptoms, together with a suspicion or identification of infection.<sup>21,56,57</sup> These definitions have inherent limitations. First, each of the clinical signs and symptoms, and indeed infection, can

occur in patients that do not have sepsis. Second, they provide little information about the stage of disease, which is critical with regard to treatment. And third, and perhaps most important, they provide no information about the specific pathogen and host immune factors that are central to disease pathogenesis.

A brief look at how sepsis definitions have evolved over time is instructive. Sepsis syndromes were described in the earliest writings of ancient Greek, Roman, and Chinese authors.<sup>58</sup> The word sepsis derives from the Greek verb form *sepo*, meaning “I rot.”<sup>59</sup> It was not until 1989 that Bone *et al.* proposed specific physiologic criteria as a screen for patients with “sepsis syndrome” for a clinical trial of methylprednisolone (Table 2).<sup>60</sup> These criteria formed the basis for a consensus definition established in 1992 by the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM).<sup>56</sup> According to this definition, sepsis is suspected infection in addition to the presence of a “systemic inflammatory response syndrome” (SIRS), which for simplicity was defined by only four variables: temperature, respiratory rate, heart rate, and white blood cell count. In addition, severe sepsis was defined as sepsis with organ failure; septic shock was severe sepsis with refractory hypotension (Table 2). These definitions were widely adopted and then used in virtually all subsequent epidemiologic studies and clinical trials of sepsis. By the late 1990s, however, there was increasing recognition that the definitions were vague and that they did not accurately reflect clinical experiences with the disease.<sup>57</sup> SIRS was criticized for being nonspecific. It is present in diseases leading to sterile inflammation such as trauma, burns, or pancreatitis.<sup>61</sup> In one study of more than 200 patients presenting to the emergency department with severe sepsis (based on the 1992 ACCP/SCCM definition), roughly 20% who were treated as such ultimately did not have a defined infection.<sup>62</sup> Moreover, there was no difference in white blood cell count or temperature (two SIRS parameters) among patients with confirmed infection compared to those without infection.<sup>62</sup> Conversely, it was evident that some patients with clinical sepsis did not have SIRS. Moreover, the presence of SIRS itself was subject to interpretation. A more liberal interpretation of the SIRS-related definition of sepsis, for example, in cases where two SIRS criteria

are present within a 24-h period (rather than simultaneously), significantly increased the numbers of patients deemed to have sepsis.<sup>63</sup> Hence, in 2001, the ACCP/SCCM consensus definitions were updated at an international sepsis definitions conference involving the ACCP and the SCCM, as well as the European Society of Intensive Care Medicine (ESICM), the American Thoracic Society (ATS), and the Surgical Infection Society (SIS).<sup>57</sup> Sepsis was still defined as suspected infection along with a host inflammatory response. However, instead of a strict requirement for the presence of SIRS, a host inflammatory response is considered possible when any of greater than 20 signs or symptoms is present (Table 2). This definition was reiterated in the 2012 Surviving Sepsis Guidelines.<sup>21</sup> While the new definition is more faithful to the reality of clinical diagnosis at the bedside, it is even less specific than its predecessor. As a result, the 1992 definitions continue to dominate the scientific literature and the problem of poor nonspecific definitions persist.

An inadvertent consequence of having poor definitions for sepsis is the avoidance of the term sepsis in important clinical studies. For example, there are good studies looking at sepsis due to specific pathogens in well-defined patient populations in which the words SIRS and sepsis do not appear. A recent study of MRSA bacteremia in hemodialysis patients is one such instance.<sup>64</sup> Hemodialysis is an important host factor that increases susceptibility to invasive MRSA. An estimated 23.4% of MRSA bacteremia occurs in hemodialysis patients.<sup>64</sup> Using population-based data from nine American cities gathered between 2005 and 2011, Nguyen *et al.* concluded that the incidence of MRSA infection (mostly bacteremia) had decreased annually by 7.3%. Neither the word SIRS nor sepsis appeared in this paper. Another important omission is that sepsis is excluded as a cause of death and morbidity in the Global Burden of Diseases (GBD) project, the most comprehensive and important effort to assess the epidemiology of various diseases around the world.<sup>65</sup> Among the 235 diseases and injuries in more than 180 countries assessed in the most recent iteration of the GBD project in 2010,<sup>66</sup> sepsis (occurring in most adults) was not included as a disease. In contrast, maternal sepsis and neonatal sepsis are included as diseases. This likely reflects the fact that new mothers and neonates are relatively homogenous populations, being of

**Table 2. Sepsis definitions over time**

“Sepsis syndrome” as defined by Bone <i>et al.</i> in 1989 <sup>60</sup>	ACCP/SCCM consensus definitions in 1992 <sup>56</sup>	International consensus definitions in 2001 <sup>57</sup>
<p>Clinical evidence of infection</p> <p>Rectal temperature &gt;101°C or &lt;96 °C</p> <p>Heart rate &gt;90 beats per minute</p> <p>Respiratory rate &gt;20 breaths per minute</p> <p>At least one of the following manifestations of organ dysfunction:</p> <ul style="list-style-type: none"> <li>• Alteration of mental status</li> <li>• Hypoxemia</li> <li>• Elevated plasma lactate level</li> <li>• Oliguria</li> </ul>	<p><b>Sepsis:</b> Clinical evidence of infection along with a systemic response to infection, manifested by two or more of the following due to infection:</p> <ul style="list-style-type: none"> <li>• Temperature &gt;38 °C or &lt;36 °C</li> <li>• Heart rate &gt;90 beats per minute</li> <li>• Respiratory rate &gt;20 breaths per minute or PaCO<sub>2</sub> &lt;32 mmHg</li> <li>• White blood cell count &gt;12,000/cu mm, &lt;4,000/cu, or &gt;10% immature (band) forms.</li> </ul> <p><b>Severe sepsis:</b> Sepsis associated with organ dysfunction, hypoperfusion, or hypotension. Hypoperfusion and perfusion abnormalities may include, but are not limited to:</p> <ul style="list-style-type: none"> <li>• Temperature &gt;38 °C or &lt;36 °C</li> <li>• Lactic acidosis</li> <li>• Oliguria</li> <li>• Alteration of mental status</li> </ul> <p><b>Septic shock:</b> Sepsis induced with hypotension despite adequate fluid resuscitation.</p>	<p>Clinical evidence of infection and some of the following:</p> <p>General variables</p> <ul style="list-style-type: none"> <li>• Fever or hypothermia</li> <li>• Heart rate &gt;90 beats per minute</li> <li>• Elevated respiratory rate</li> <li>• Alteration of mental status</li> <li>• Significant edema or positive fluid balance</li> <li>• Hyperglycemia in the absence of diabetes</li> </ul> <p>Inflammatory variables</p> <ul style="list-style-type: none"> <li>• Leukocytosis, leukopenia, or normal WBC count with &gt;10% immature forms</li> <li>• Elevated plasma</li> <li>• C-reactive protein</li> <li>• Procalcitonin</li> </ul> <p>Hemodynamic variables</p> <ul style="list-style-type: none"> <li>• Arterial hypotension</li> <li>• Mixed venous oxygen saturation &gt;70%</li> <li>• Elevated cardiac index</li> </ul> <p>Organ dysfunction variables</p> <ul style="list-style-type: none"> <li>• Arterial hypoxemia</li> <li>• Acute oliguria or creatinine increase</li> <li>• Coagulation abnormalities</li> <li>• Ileus</li> <li>• Thrombocytopenia</li> <li>• Hyperbilirubinemia</li> </ul> <p>Tissue perfusion variables</p> <ul style="list-style-type: none"> <li>• Hyperlactatemia</li> <li>• Decreased capillary refill or mottling</li> </ul>

NOTE: ACCP, American College of Chest Physicians; SCCM, Society of Critical Care Medicine.

similar age and immune status. In these hosts, septic infections occur during defined time periods of susceptibility to infections, avoiding the problems inherent in generally defining sepsis. Moreover, the pathogens responsible for sepsis in new mothers and neonates are relatively predictable, namely, group A *Streptococcus* in maternal sepsis<sup>67</sup> and one of only a handful of common bacterial pathogens, such as group B *Streptococcus* and *E. coli*, in neonatal sepsis.<sup>68</sup>

***Inconsistent definitions and poor understanding of the disease reflect poor results in clinical trials***

How have nonspecific epidemiology and poor definitions affected the outcomes of clinical trials of host-directed therapies? These factors have limited our ability to understand how the host immune status relates to disease progression. The clinical phenotypes corresponding to the 1992 ACCP/SCCM definitions do not meaningfully represent the

underlying complex host response. In this regard, since sepsis is regarded as a syndrome associated with a pathological immune response, it is worth mentioning that the innate immune response involves more than 2000 separate genes, encapsulating the potential for considerable complexity and heterogeneity. One consequence of our incomplete understanding has been the pursuit of therapies targeting the most obvious biochemical changes associated with disease. However, the most prominent host responses during sepsis (e.g., proinflammatory cytokines such as TNF- $\alpha$ )<sup>69</sup> may not be the best targets for drug therapy. Cytokines such as TNF- $\alpha$  and IL-1 are not only associated with pathology, but also have known, important, protective roles against infection. Moreover, treatments may be potentially effective in specific subsets of patients (as originally suggested for drotrecogin alfa),<sup>70</sup> and thus nonspecific definitions may lead to testing in the wrong populations. This would be like treating all patients with a clinical diagnosis of sepsis with the same antimicrobial agent and not considering the results of pathogen identification and antimicrobial susceptibility testing. Under these conditions, antimicrobial agents would be unlikely to demonstrate the degree of effectiveness currently observed. Nevertheless, this is the current state of affairs for most host-directed therapies. Just as antimicrobial efficacy is critically dependent on which pathogens are involved, it is likely that treatments targeting the host depend on specific host responses. These responses might be unique for genetically distinct hosts in response to different types of pathogens. Indeed, genome-wide associations have revealed complex genetic associations involving a substantial number of genes that predispose to sepsis.<sup>71</sup>

The story of drotrecogin alfa (rhAPC) is particularly instructive. rhAPC is a recombinant form of human activated protein C that has antithrombotic and immune-modulatory effects. It was, for a decade, considered the only success to have arisen out of the dozens of trials of host-directed therapies for sepsis. In 2001, both American and European regulators approved the marketing of rhAPC based on the pivotal Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) trial.<sup>70</sup> PROWESS was a large double-blind, placebo-controlled, randomized trial in more than 1600 patients with severe sepsis that was stopped early due to the significant benefits

observed with treatment. Treatment with rhAPC resulted in a 6% absolute reduction in mortality compared to placebo (25% vs. 31%). However, concerns regarding the conduct of the trial (Eli Lilly, the maker of the drug, had changed the enrollment criteria for patients halfway through) and post hoc analyses showing that the benefits accrued mostly in patients at high risk of death meant that drug approval was limited only to high-risk adult patients (e.g., those with septic shock). Moreover, approval was contingent on the assurance that future clinical trials would be conducted to better define the populations expected to benefit. In the following decade, multiple studies failed to reproduce the benefits seen in PROWESS. Trials among patients at low risk of death and in children were stopped early due to futility.<sup>72,73</sup> Finally, in 2011, when no benefit could be shown for even the most severely ill adult patients with septic shock,<sup>74,75</sup> Eli Lilly voluntarily withdrew the drug from the market.<sup>42</sup> Many explanations have been proposed to explain the seemingly contradictory results. Some intensivists still maintain that rhAPC worked for some of their patients. One plausible explanation is that rhAPC does benefit a select group of patients, but that these patients could not be selected for based on the clinical criteria assessed for inclusion in the trials.

The themes of excessive optimism after a single trial that later cannot be reproduced and difficulties in defining the patient groups most likely to benefit are recurring in clinical trials of host-directed sepsis therapies.<sup>42</sup> The failures of trials involving anti-endotoxin antibodies are another example. Nabacumab (HA-1A) was a human monoclonal antibody specific for endotoxin/lipid A that gained approval for marketing in several European jurisdictions after a single positive trial<sup>76</sup> showed benefit in patients with Gram-negative sepsis.<sup>77,78</sup> Not only did later trials fail to confirm the benefit for patients with Gram-negative sepsis,<sup>79</sup> but one trial was actually stopped early due to an increase in mortality among patients with Gram-positive sepsis.<sup>80</sup> The authors of this trial concluded that the difficulty was in reliably identifying those patients who might benefit from antiendotoxin immunotherapy.<sup>80</sup> The last of the antiendotoxin antibody studies was published in 2000. The therapy was a murine monoclonal antibody (E5). Phase II studies had shown a trend toward benefit in patients with Gram-negative



sepsis.<sup>81,82</sup> Despite efforts to carefully enroll only those patients with probable or documented Gram-negative sepsis, the study was stopped early due to lack of benefit.<sup>83</sup> Again, it was suggested that E5 or other antisepsis strategies might work in particular subgroups. Finally, there is the story of interleukin-1 receptor antagonist (IL-1Ra), which is a protein inhibitor of the proinflammatory cytokine IL-1. In the initial phase II trial, this therapy resulted in a dramatic reduction in mortality (from 44% to 16%).<sup>84</sup> A follow-up phase III trial showed a trend toward benefit among a subgroup of patients with more severe disease.<sup>85</sup> However, when another phase III trial was attempted, no benefit was found.<sup>41</sup> The authors concluded that any activity of the IL-1Ra in sepsis would be difficult to detect in a heterogeneous patient population and further development would rely on identifying a better defined target group. Despite these examples, however, the lack of alternative usable definitions means that the same 1992 ACCP/SCCM definitions for sepsis, or a variation thereof, are still being used in clinical studies today.

### *Moving forward*

Future breakthroughs in sepsis treatment would appear to depend on our success at tackling the problem of a deleterious host response. As discussed above, this problem is exacerbated by the lack of uniform definitions that are suitable for use in clinical studies. In fact, some experts question whether it is ethical to enroll patients in sepsis studies based on the 1992 definitions in light of the dramatic failure of previous studies.<sup>86</sup> During the 2001 International Sepsis Definitions Conference, a new classification system for sepsis developed expressly for the purposes of clinical research was proposed. This was called the PIRO system and is based on the elaboration of four factors: predisposition, infection, response, and organ dysfunction. PIRO was introduced only as a rudimentary template for future investigation rather than as a firm set of definitions. A modified representation of the PIRO concept for sepsis definitions is shown in Table 3.

Unfortunately, a decade after the PIRO concept was proposed, there have been few studies that have advanced the ideals of the concept. Instead, most studies have applied the concept to the development of scoring tools resembling the APACHE (Acute Physiology and Chronic Health Evaluation) or SAPS (Simplified Acute Physiology

Score) scores.<sup>87–92</sup> Again, the challenge for PIRO has been the heterogeneity of disease and how to categorize disease within its framework.

### *Putting the PIRO concept to use for the development of host-directed therapies*

There is an urgent need to formalize PIRO-based definitions for the study of host response and the development of host-directed therapies. The 1992 ACCP/SCCM definitions for sepsis already incorporated parameters representing host response (i.e., SIRS), and organ failure (in the definitions for severe sepsis and septic shock). With regard to infection, individual groups of pathogens, such as Gram-negative bacteria, Gram-positive bacteria, and fungi appear to be associated with different mechanisms of sepsis pathogenesis.<sup>93,94</sup> However, delayed or absent microbiologic results at the time of sepsis diagnosis limits classification of patients by this factor in clinical trials of early sepsis. With the advent of molecular diagnostics for specific microbes, this may change in the near future.<sup>23</sup> Similarly, systems biology–level analyses<sup>95</sup> of host immune responses, using genomics, transcriptomics, and proteomic signatures, would likely help to better define patient subsets and enable new biomarkers to be defined. Finally, if the goal is a greater understanding of the host response, an important next step is to define categories for predisposition.

What are the important categories defining predisposition? There is a substantial amount of demographic information on certain types of hosts, for which natural clinical distinctions already exist. One such category is the elderly, aged >65 years. This category of patients is distinct epidemiologically, socially, clinically, and immunologically. While the elderly aged >65 years represent only 12% of the U.S. population, they make up 65% of sepsis cases.<sup>96</sup> Overall, they are at a greater risk of severe sepsis and septic shock.<sup>2</sup> This has been attributed to factors such as increased incidence of comorbid illness, institutionalization, frailty, and malnutrition.<sup>97</sup> Immunologically, there are age-related declines in both cell-mediated and humoral immune function.<sup>98</sup> Another well-defined category is HIV/AIDS patients. Categorization of these patients may be particularly important for studies conducted in some developing countries with a high burden of this disease. These patients have a specific, reasonably well-understood immune deficiency and

**Table 3.** The PIRO system for sepsis definitions

Domain	Current	Future
<b>Predisposition</b>	<ul style="list-style-type: none"> <li>• Premorbid illness</li> <li>• Age</li> <li>• Gender</li> </ul>	Genetic polymorphisms in components of the inflammatory and/or immune response
<b>Infection</b>	<ul style="list-style-type: none"> <li>• Culture and sensitivity of infecting pathogens</li> <li>• Detection of disease amenable to source control</li> </ul>	Definition of microbial burden (e.g., bacterial rRNA analysis)
<b>Response</b>	<ul style="list-style-type: none"> <li>• SIRS</li> <li>• Other signs of sepsis</li> <li>• Shock</li> <li>• C-reactive protein</li> </ul>	Defining markers (e.g., differential gene expression) of activated inflammation or impaired host responsiveness
<b>Organ</b>	<ul style="list-style-type: none"> <li>• Organ dysfunction as number of failing organs or composite score</li> </ul>	Dynamic measures of cellular response to insult; correlations with biomarkers.

Modified from the 2001 International Sepsis Definitions Conference.<sup>57</sup>  
SIRS, systemic inflammatory immune response syndrome.

can experience sepsis due to a well-defined group of opportunistic pathogens. In fact, the use of steroids in the treatment of sepsis due to *Pneumocystis jirovecii* pneumonia among HIV-infected patients is one of the few instances in which treatment with an immune modulator is of proven benefit.<sup>99</sup> Other possible categories of predisposition are cancer and diabetes. Patients with cancer are at a 10-fold increased risk of sepsis compared to those without cancer.<sup>100</sup> These patients tend to be immunocompromised, although specific defects may be different between patients, depending on the type and stage of malignancy, as well as treatment. Patients with diabetes are also at increased risk for the development of sepsis.<sup>101</sup> Diabetes leads to hyperglycemia and is associated with neutrophil defects.<sup>102,103</sup> Hyperglycemia, with or without an antecedent diagnosis of diabetes, has been consistently associated with worse outcomes.<sup>104,105</sup> Therefore, there are a number of categories of predisposition that make logical candidates for inclusion in new PIRO-based sepsis definitions.

## Conclusions

Sepsis is a leading cause of mortality and morbidity. Currently, antibiotics are the mainstay of treatment. Appropriate antibiotic selection, however, is limited by increasing antimicrobial resistance, the obligation to prevent further resistance through the practice of antimicrobial stewardship, and the lack of

microbiologic information during the critical early stages of sepsis. Overcoming the barriers to optimal antibiotic use will rely on the discovery of new biomarkers of host status and advances in microbiologic diagnostics. These developments will take time.

On the other hand, research and treatment involving host-directed therapies can be advanced immediately through the development of improved sepsis definitions. Poor nonspecific definitions for disease hinder the conduct of good laboratory and clinical studies. They undermine the importance of the disease when the word *sepsis* is excluded from major epidemiologic studies. Previous attempts at developing effective host-directed therapies have been plagued by repeated failures owing to our limited understanding of the immune etiology of disease. A close examination of a number of clinical trials of host-directed therapies for sepsis show that poor definitions likely led to the application of potentially effective treatments in the wrong populations. Therefore, there is a great need for mechanism-based biomarkers and a more profound understanding of the heterogeneous disease states that fall under the collective name sepsis. Hence, a crucial first step is to address the problem of sepsis definitions. The PIRO concept for sepsis definitions was introduced more than 10 years ago, but its potential has not been realized. In order for PIRO to gain

traction, the sepsis community needs to formalize a set of definitions that are well suited to the needs of researchers. We propose starting with a set of definitions focused on the host, given the potential for host-directed therapies to improve outcomes, with the elaboration of specific categories of predisposition. It is imperative that we develop molecular diagnostics that reflect, report on, and characterize the development of sepsis under each of these categories. Developing both early and mid-stage disease biomarkers for sepsis should become a major priority in society, as it will be critical for the success of future host-directed therapies.

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### Conflicts of interest

The authors declare no conflicts of interest.

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