Sepsis: a roadmap for future research

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Sepsis is a common and lethal syndrome: although outcomes have improved, mortality remains high. No specific anti-sepsis treatments exist; as such, management of patients relies mainly on early recognition allowing correct therapeutic measures to be started rapidly, including administration of appropriate antibiotics, source control measures when necessary, and resuscitation with intravenous fluids and vasoactive drugs when needed. Although substantial developments have been made in the understanding of the basic pathogenesis of sepsis and the complex interplay of host, pathogen, and environment that affect the incidence and course of the disease, sepsis has stubbornly resisted all efforts to successfully develop and then deploy new and improved treatments. Existing models of clinical research seem increasingly unlikely to produce new therapies that will result in a step change in clinical outcomes. In this Commission, we set out our understanding of the clinical epidemiology and management of sepsis and then ask how the present approaches might be challenged to develop a new roadmap for future research.

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Introduction: facing the challenge of sepsis

Patients with sepsis—a severe infection associated with organ dysfunction[1]—constitute a large proportion of the critically ill population and, although outcomes have improved,[2] mortality remains higher than 25–30%, and even 40–50% when shock is present.[3] No effective specific anti-sepsis treatments exist, therefore management of patients with sepsis relies mainly on early recognition allowing correct therapeutic measures to be started rapidly, including administration of appropriate antibiotics, source control measures when necessary, and resuscitation with intravenous fluids and vasoactive drugs when needed.

Although substantial advances have been made in the understanding of the host response to infectious organisms, progress in the development of new therapeutic drugs has been restricted. Many reasons exist for this limited progress, but one key issue has been the heterogeneity of the patient population. A major hindrance has been the reliance on the so-called systemic inflammatory response syndrome criteria to screen patients for possible sepsis. These criteria (hypothermia or hyperthermia, tachycardia, tachypnoea, and abnormal white blood cell count) are present in most critically ill patients but they are not specific for infection and so have restricted use in differentiating between patients with sepsis and those with other inflammatory disorders. One approach has been to move away from using signs or markers of infection to diagnose sepsis, and instead to focus on the presence or development of organ dysfunction, and to assess whether the organ dysfunction might be due to sepsis.[1] The presence of any organ dysfunction without a clearly identifiable cause should always raise the possibility of sepsis.

Increased awareness and early effective management of patients with sepsis have helped to improve outcomes in the past 15 years, but mortality remains unacceptably high. The critical care management of these patients has improved, although some of the reduction in the mortality is probably due to an increase in the overall number of reported cases of sepsis, with the inclusion of more patients with less severe illness than was reported before. In the USA, this effect is driven partly by the introduction of new diagnostic codes for severe sepsis.[4] The complexities associated with the interpretation of the epidemiological data are discussed in more detail later in this Commission. Nevertheless, the issue of increased sepsis reporting is important to emphasise, not so much to temper our enthusiasm about the decreasing mortality, but mainly to stress the persistent, pressing need for new therapies.[5] In fact, investigators analysing the separate components of sepsis management have difficulty identifying any one factor or factors that might provide an explanation for the apparent improvement in sepsis outcomes. Early, effective antibiotic therapy is essential, but in many places it is confounded by the problem of multidrug-resistant organisms; physicians are faced with the dilemma of trying to restrict the routine use of third-line so-called reserve drugs versus the need to ensure early adequate coverage of potentially multidrug-resistant organisms. Emergency or intensive-care physicians still struggle with the optimum amount of fluid to give,[5] and the approach developed by Rivers and colleagues[6] of early goal-directed therapy based on measurements of central venous oxygen saturation has been challenged by the results of larger, more pragmatic, multicentre studies.[7,8] The significant reduction in mortality reported in a multicentre Spanish study[9] of early sepsis management was partly attributed to the administration of activated protein C (APC), which is no longer available. Tight blood sugar control is another controversial issue,[10] as is corticosteroid administration in severe septic shock.[11] Outcomes of patients with sepsis have certainly improved, but mainly through increased awareness of sepsis, improved general patient management, and improved processes of care, rather than by any specific measure.

The challenge of definitions

An unresolved issue

An important difference exists between a definition that is used to identify an individual who has a specific disorder and a definition that is used for epidemiological

purposes. The former definition might have therapeutic, prognostic, or sociological implications for the patient and will need to be pragmatic and easy to apply. By contrast, an epidemiological definition will often be used for clinical trials or for public health surveillance reasons and will need to be as robust and rigorous as possible. Much of the debate around the definition of sepsis has occurred because the distinction between these two functions has been blurred, perhaps partly because sepsis is essentially an artificial construct and not a specific disease entity.

Building on the early observations of Bone and colleagues,[12] consensus conferences in 1991[13] and in 2001[14] led to the development of a simple notion, in which sepsis was the systemic response to infection, with increasing degrees of severity identified as severe sepsis or septic shock. Terms such as systemic inflammatory response syndrome or multi-organ dysfunction syndrome were suggested as a way of acknowledging that substantial similarities existed between sepsis and other inflammatory disorders not mainly caused by infection. However, these terms are not sensitive or specific enough and have mostly been discarded.[15]

The present consensus definition,[14] which includes a long list of probable signs of sepsis, is inadequate, and a working group of the Society of Critical Care Medicine and European Society of Intensive Care Medicine is developing a further update and revision of the criteria. One approach to this issue has been the introduction of threshold decision making, in which diseases are defined as being present when one or more predefined criteria are breached, an idea that appeared in the 2012 guidelines for the management of sepsis[16] for the first time. However, this approach relies on availability of a clinical or biochemical biomarker that can provide the so-called gold standard criterion for diagnosis. Unfortunately, no satisfactory biomarkers exist that can be used to diagnose sepsis, unlike disorders such as hypertension or diabetes mellitus, which have clear cut, internationally accepted criteria.

The question of whether these imprecise definitions have clouded our ability to develop new treatments for sepsis is something we will return to later in this Commission.

Research in sepsis: success or frustration?

The past 20 years have been very productive for research in sepsis. By the, albeit crude, measure of PubMed citations for sepsis, during the 10 year period 1975–85 about 6500 papers were published; between 1996 and 2006 that figure was three times greater, nearly 20 000.

Nevertheless, an unavoidable if uncomfortable truth is that despite many hundreds of clinical trials, involving tens of thousands of patients, and costing hundreds of millions of dollars, not one so-called novel drug has come to the market and passed the test of time by being incorporated into routine clinical practice.[17] This result is at best frustrating, although acknowledgment of it by no means undervalues the real improvements that have been made in the management of patients with sepsis. However, we do need to consider what the reasons might be for this absence of successful drugs. We do not intend to discuss the reasons exhaustively, but a brief summary will provide a useful framework for the later parts of this Commission.

Issues with the basic science?

The proliferation of novel drugs for sepsis has been driven by an increased understanding of the pathophysiology of sepsis, which in turn has identified potential new drug targets. Many of these new targets are components of the inflammatory response, cytokines such as tumour necrosis factor (TNF), interleukin 1, interleukin 6, or platelet activating factor, or components of the coagulation cascade or vasoactive molecules, such as nitric oxide. These discoveries have been reviewed in detail elsewhere and are based on reproducible findings from many laboratories[18] and the basic observations are very unlikely to be flawed. Difficulties might have arisen, however, when experiments moved from in-vitro studies to animal models.[19] That simple endotoxin challenge in young healthy mice is at best only a first step has become increasingly clear; more complex and pathologically relevant models such as caecal ligation and puncture in mice, or larger animal haemodynamic models, are probably more informative. Issues such as the timing of the intervention, the dose of both the challenge and the potential therapeutic drug, and even the choice of the infecting agent can all have a profound effect on the relevance of the results.[19–21] Although some drugs might have entered phase 2 clinical trials on the basis of unjustifiable enthusiasm from preclinical data, this issue alone is unlikely to be an adequate explanation for their ineffectiveness.

Ineffective drugs?

That all the novel drugs failed in clinical trials because they were simply inactive, or had too little biological activity to be effective is, at least theoretically, possible. A more plausible explanation, however, relates to pharmacokinetic or pharmacodynamic considerations. Particularly in the early years of sepsis trials, little consideration was given to the appropriate timing of treatment, partly because no biomarkers of sepsis were (or are) available to effectively and accurately diagnose sepsis. For example, trials with anti-TNF monoclonal antibodies were done without regard for whether the patient actually had raised concentrations of TNF at the time when the antibody was given. Only in recent years has the dynamic nature of the disease been fully recognised: the cytokine profile changes with time, the underlying infection can move from being localised to disseminated, the immunological profile can change from being proinflammatory to more immunosup-

pressed.[21,22] To our knowledge, only one phase 2 trial has ever been completed that specifically used pharmacodynamic endpoints to decide whether to proceed to a full phase 3 trial[23] (a trial of thrombomodulin alfa using the same approach is in progress). In that case, a drug that had been developed on the basis of strong basic science and a full portfolio of preclinical models did not show a significant clinical pharmacodynamic effect and was withdrawn from further development. That at least some of the failures of the phase 3 trials resulted from the inactivity of the drugs or the fact that they were given in the wrong dose or at an inappropriate time in the disease process, or both, is certainly possible.

Clinical trial design

The design of clinical trials in sepsis has improved immeasurably in the past 20 years, a topic helpfully reviewed in a journal supplement in 2009.[24] Many of the sepsis studies done between 1980 and 2000 were far too small, gave the investigational drug at variable times after the insult, used endpoints that were imprecise or unrealistic, and ignored pharmacokinetic and pharmacodynamic considerations. Aspects of supportive care we now know to be crucial for survival—eg, the prompt administration of appropriate antibiotics[25]—were absent from these early trial designs, which alone would have led to substantial heterogeneity in the patient population. But perhaps the largest issue was the extent of heterogeneity resulting from the vague and imprecise definitions of sepsis used for clinical trials. Does a young, previously well child with acute meningococcal sepsis really have the same disease as an elderly patient with diabetes and postoperative staphylococcal pneumonia? Moreover, should they both be entered into the same clinical trial of a novel drug targeting a particular point in the pathological process (figure 1)? We[26,27] and others have pointed out that, although sepsis is a useful clinical concept, it covers such a broad range of clinical scenarios that it might not be a sensible way to categorise patients for clinical trial reasons, in which a heterogeneous population will create a low signal to noise ratio.

Research priorities for the next 5–10 years

Experimental research will, of course, continue to identify drug targets but models of potential therapeutic interventions have to be clinically relevant and the role of ex-vivo and computer-simulated models should be investigated further. One important target is endothelial cell function, which is particularly important in the pathophysiology of sepsis and was a target of the intervention with APC.[28] Changes in endothelial function result in changes in endothelial permeability, which contributes to organ failure. Therapies targeting endothelial function might, therefore, have favourable effects and represent an active area of continuing research. For example, some vasopressin derivatives have been shown to restrict oedema formation,[29] and administration of interferon β in patients with acute respiratory distress syndrome might restrict lung oedema formation by upregulating CD73.[30]

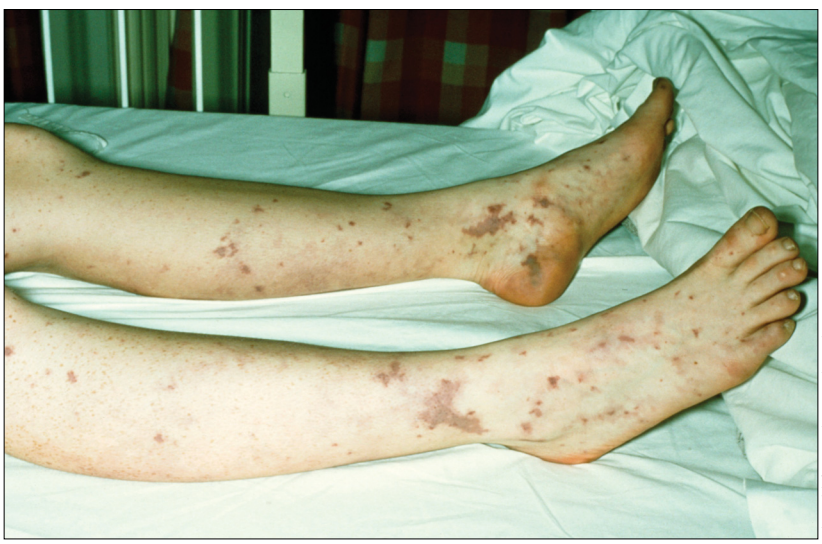

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Figure 1: **Legs of patient showing meningococcal septicaemia**

In addition to continued preclinical investigation, efforts have to be made to improve clinical trial design. This goal might be achieved in several ways.

First, identification of the patient population that might benefit from each intervention should be improved. One suggestion was to use the PIRO (predisposition, infection, response, organ dysfunction) construct, developed as a framework to assemble various elements that could be used to characterise a patient with sepsis.[14] However, which elements to include remains unclear and how PIRO scores could be used to identify patients for inclusion in clinical trials also needs further precision. For example, inclusion of patients with different types of microorganism in the same trial has been criticised, but although some differences in host response to different microorganisms exist, the differences might not be of sufficient importance to affect response to treatments. Similarly, restriction of study inclusion to patients with just one source of infection—eg, severe pneumonia[31]—has not so far been very successful.

Instead, strategies that focus on the type of host response of the patient might allow a so-called personalised medicine approach. That the initial sepsis response is predominantly proinflammatory is well recognised, but this stage is rapidly followed by a longer phase of relative immunosuppression. Administration of an anti-TNF antibody to patients already in a predominantly immunosuppressed phase would not be desirable and could even be harmful. Likewise, administration of the proinflammatory growth factor, granulocyte colony-stimulating factor, to all patients with sepsis was not associated with improved outcomes.[32]

However, how to characterise the type and extent of immune response in the individual patient is unclear. One approach has been to use a gene expression based method to classify patients with sepsis in a way that would potentially inform therapeutic decisions.[33] Many biomarkers have been suggested for this purpose, but none is ideal. Importantly, circulating substances might not represent the cellular response very well and other types of markers might be more relevant. For example, plasma interleukin-6 concentrations were used in an attempt to identify patients who might benefit from an anti-TNF strategy, but this approach was not successful.[34] In view of the complexity of the sepsis response, a single biomarker is unlikely to be suitable for use in all circumstances. Panels of several biomarkers might be more helpful in decision making than the individual components, but further research is needed to define precisely which biomarkers should be included in such panels. The biomarker should also be chosen on the basis of the intervention under trial. As an example, a trial is in progress in which thrombomodulin is given only to patients with coagulopathy (NCT01598831) as the result of an earlier trial that suggested benefits in these patients.[35]

Additionally, study populations could be selected according to individual organ dysfunction, such as the kidney or the lungs, depending on the likely mode of action of the intervention. For example, a trial is in progress (NCT02182440) that is giving alkaline phosphatase to patients with sepsis-related acute kidney injury, with the primary target of an improvement in renal function. Another trial[30] evaluated interferon beta and focused on lung function in patients with acute respiratory distress syndrome, some of whom had sepsis-related respiratory failure.

Second, innovative new trial designs should be investigated instead of or alongside the traditional randomised controlled trial (RCT).[36] One example is the adaptive trial, in which key clinical trial characteristics (eg, dosing amounts, randomisation ratios, and inclusion or exclusion criteria) are modified during the trial by use of new information acquired from early trial data. These new study designs might help speed up the study process, enabling ineffective therapies to be discarded earlier than with traditional trials and therefore allow resources to be concentrated on those interventions most likely to be effective.

Third, study outcome endpoints should be carefully selected according to the intervention being studied. The traditional 28 day mortality endpoint, although still important, is widely seen as inadequate and other endpoints, including longer-term survival rates, quality-of-life markers, organ function variables, or intervention-free durations (eg, mechanical ventilation-free days and renal replacement therapy-free days) should be considered. These endpoints should not only provide evidence of scientific efficacy but also of patient-centred benefit.[37]

The seeming inability to develop an effective sepsis therapy despite many potential drugs and huge advances in our knowledge of the underlying sepsis response has been disappointing and is likely to be related more to clinical trial design and patient selection criteria than to the specific interventions studied. An understanding of the reasons behind these apparent failures will help improve clinical trial development and should represent a key priority for future research.

The global epidemiology of sepsis

Sepsis is both one of the best known yet most poorly understood medical disorders. First recognised by Hippocrates, sepsis can be described as the state in which a host mounts an inflammatory response to an invading pathogen with poor results. The lay definition states that sepsis is a life-threatening disorder that arises when the body's response to an infection injures its own tissues and organs. Sepsis leads to shock, multiple organ failure, and death, especially if not recognised early and treated promptly.[38]

Although this definition seems clear, it also emphasises how difficult the global epidemiology of sepsis is to measure and understand. For example, how are infections counted? When is the body's response to an infection injurious? How can such injury be measured, and when is it due to the body's response to infection? Finally, how does the provision of treatment affect the clinical manifestations, and therefore diagnostic features, of sepsis?

General approaches used to measure sepsis

Unlike troponin for acute coronary syndrome or a radiograph for fracture, sepsis does not have only one diagnostic test and instead is a clinically defined syndrome[39] subject to revision[40] and debates about inter-rater reliability. The prodrome of sepsis can be non-specific or brief and the short-term mortality high, leading to missed cases in point-prevalence studies, the simplest study design for sepsis epidemiology. Three general approaches to measurement of sepsis exist, each with strengths and limitations: clinically defined sepsis in prospective clinical registries, administrative coding of sepsis in hospital discharge databases, and causes of death from vital statistics records.

The first approach relies on a combination of infection and organ dysfunction to count cases of clinically defined sepsis in prospective databases of patients, typically treated in intensive care units (ICUs). Infection can be defined by use of specific criteria,[41] clinician discretion, or expert adjudication. Organ dysfunction is based on organ-specific definitions or composite scores, such as the Sequential Organ Failure Assessment (SOFA) score.[42] Organ failure scores are used most often in patients in ICUs, with definitions of organ dysfunction scores relying partly on provision of ICU-based supportive interventions, such as mechanical ventilation and vasopressors. As such, access to ICU beds and receipt of

life-support technology affect whether a patient is counted. Furthermore, registries cannot capture all ICUs, and therefore the total number of ICU-treated sepsis cases might not be known at the population level.

The second approach measures sepsis incidence in hospital discharge databases, which count all admissions for an entire region or country and assign diagnoses by International Classification of Diseases (ICD)-9 or ICU-10 codes. Such databases allow for calculation of sepsis incidence at the population level and include cases treated on hospital wards. One method of using ICD codes mimics the clinical definition of sepsis, and counts cases in which patients are both infected and have organ dysfunction.[43] However, organ dysfunction might be unrelated to the infection if ICD codes are listed at discharge without information about the sequence of events. Another method uses septicaemia codes plus organ dysfunction and is based on an older conceptual model that equated septicaemia with sepsis.[44] However, since most clinical series report negative cultures in about 40% of patients with severe sepsis,[45] many cases will be missed. Generally, these two approaches have excellent positive predictive value but moderate sensitivity compared with the clinical gold standard. Finally, for the past decade the US ICD-9-CM coding scheme has included codes for septic shock and severe sepsis, which could improve concordance with the clinical definition.

Irrespective of strategy, codes are generated by medical records abstractors and are only as accurate as chart documentation, and probably poorer than prospective screening by clinical data collectors. Medical records abstractors sometimes interpret records and apply administrative codes differently by country. At one end of the range, countries such as the USA have a very complete capture of hospital discharges because hospitals are paid via a scheme that reimburses for care. Increasing pressure on hospitals to report sepsis for reimbursement purposes might increase measured incidence. At the other end of the range, many countries, especially middle-income or low-income countries, have no mechanism to track the number of hospital discharges accurately, let alone with assignment of ICD codes.

Another limitation of hospital discharge databases is that cases are restricted to those treated in hospital. When hospital access is restricted, cases will be underestimated.[46] Therefore, the third potential approach to measure sepsis burden is to examine death records. These capture all deaths, irrespective of location. Unfortunately, cause of death can be difficult to ascertain and might not be medically certified in high-mortality areas without vital statistics infrastructure, relying on verbal autopsy.[47] The end result of death certification, irrespective of method, is a set of ICD-10-coded deaths reported nationally to WHO. This method therefore faces the same challenges as ICD coding of hospital discharge databases. An added difficulty is that most death records assign infectious deaths to the underlying infection, such as pneumonia. Finally, even if death records were perfectly accurate, incidence can only be back-calculated from mortality through inferences about the case-fatality rate, which is not precisely known.

The three different approaches to sepsis measurement have had restricted cross-validation. For example, a study[43] using ICD-9 codes to examine the incidence of sepsis in the USA compared the number of patients with sepsis identified by ICD-9 coding with prospective clinical screening in wards and ICUs in five hospitals. Very close agreement existed between the two methods for the number of cases of sepsis per 100 hospital admissions and per 100 ICU admissions, and good agreement existed for clinical characteristics and hospital mortality.[43] However, different ICD coding schemes necessarily yield different estimates—for example, a scheme requiring septicaemia cannot yield the same count as another that merely requires the patient be infected.

Thus, each approach to counting sepsis cases is unique, and extrapolation across approaches is hazardous. Estimates depend on access to care plus clinician judgment, documentation, and diagnostic testing to establish the presence of infection and extent of organ injury. One final factor that affects the measured burden of sepsis is that sepsis is linked to the end of life. For example, an elderly patient who develops pneumonia might die of severe sepsis, but be recorded as having died of pneumonia or old age. Although, arguably, many diagnoses can be missed in elderly or frail patients, perhaps the comment from prominent sepsis researcher John Marshall is worth bearing in mind, "What was the old name for severe sepsis? Natural causes".[48]

Regional and global estimates of sepsis incidence and case-fatality

Using ICD-9 codes for infection and organ dysfunction, one study[43] based on hospital discharge records from 1995 estimated an incidence of 300 cases of severe sepsis per 100 000 US inhabitants that year, resulting in a national estimate of 751 000 total cases. About half these cases occurred outside the ICU. Hospital mortality was 28% overall and higher in ICU cases. More recently, a study used administrative data from 20% of US hospitals and four methods[43,44,49,50] to estimate sepsis incidence from 2004 to 2009.[51] The absolute incidence varied by more than three times (300–1031 cases per 100 000 population per year) and the case-fatality rate varied by two times (14·7% to 29·9%). Irrespective of approach, sepsis incidence in the US hospitals increased and mortality decreased with time. By contrast, a study[52] using a Swedish database of all hospital discharges that assessed three sepsis coding approaches[43,44,53] showed that only 8·3% of patients identified as having severe sepsis by any approach met the criteria for all of them. Therefore, not only did the incidence range from 130 to 350 per 100 000, but the cohorts were also almost entirely separate.

Clinically identified sepsis has mostly been restricted to patients in the ICU, and as such true population incidence estimates do not exist. Nonetheless, case-fatality rates seem to be decreasing with time in both clinical and administrative series at a rate of 3–5% per year for the past 20 years.[54] Despite this trend, sepsis remains a prominent cause of overall mortality, contributing to a third to a half of all hospital deaths.[55]

Thus, many studies—at least from high-income countries—report the occurrence of rising incidence and falling case fatality. In each instance, investigators struggle with identifying whether the diminishing case-fatality rate is due to advances in care or a diluting effect through increased detection of patients that are not as sick as those who would have previously been detected. Both factors are probably contributing. Improvements in care are suggested by randomised trials published in 2014 that reported lower control group mortality[7,8] compared with previous trials.[6,56,57] These findings are supported by an observational study[2] of severe sepsis cases in an ICU registry from Australia and New Zealand that reported a relative risk reduction in mortality of 47·5% over 13 years, with absolute mortality reaching 18·4% in 2012. Notably, the inclusion of less severely ill cases (than previously reported) in studies of sepsis epidemiology does not imply the inclusion of inappropriate cases. Rather, clinicians and investigators might be correctly identifying these additional sepsis cases because of a genuine rise in incidence or because they are cases that would previously have been missed because of poor detection strategies. In some countries, such as the USA, an increasing pressure to report sepsis for reimbursement purposes might be a factor.[4] Some investigators have reported a trend of rising sepsis incidence with decreasing incidence of infection or bacteraemia,[4,58,59] suggesting increased coding of sepsis instead of infection, or possibly even in the absence of infection. By contrast, a recent analysis[60] of US administrative data over 7 years showed that the number of hospital admissions with a claim for sepsis and the number with a claim for infection both increased. In view of the uncertain clinical details of these recent additional cases of sepsis, they might have a lower overall risk of death than cases that were typically reported previously, but the risk is not zero. Although the case-fatality rate might be dropping, the total number of deaths after recorded sepsis is rising, because the rise in sepsis incidence is relatively higher than the drop in case fatality. Thus, with time, the burden of sepsis is either rising or being more fully exposed than it was previously.

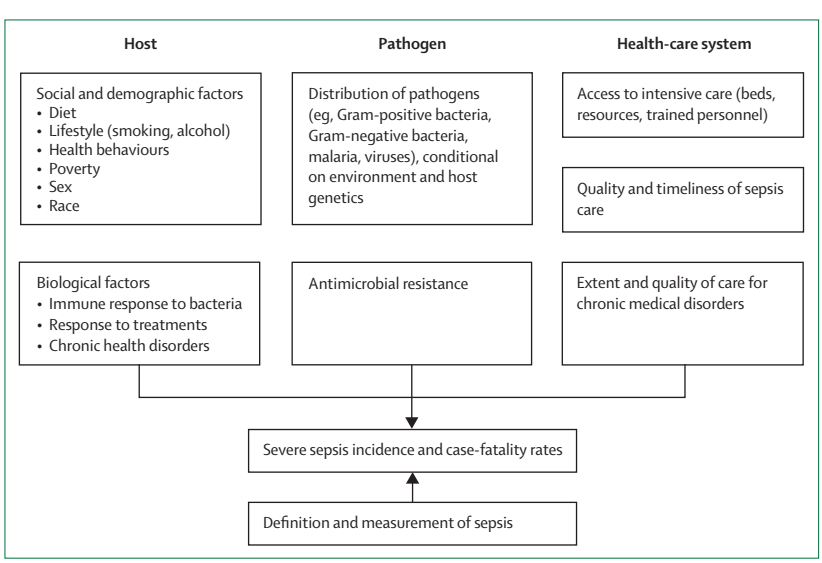


***Figure 2*: Factors determining the incidence and case-fatality rate of severe sepsis**
Adapted from Octavia Peck-Palmer, with permission.

The first estimate of yearly worldwide incidence of sepsis was calculated as 15–19 million.[61] This crude estimate was generated by multiplication of the incidence from US studies estimated from hospital discharge data that used ICD-9 codes[43,44] by world population estimates. As such, the estimate assumed that all countries have similar distributions of risk factors for sepsis, age, sex, and health-care resources, such that sepsis incidence is constant. To provide a more refined estimate, a systematic review[62] identified 33 studies providing population-level data of the incidence of hospital-treated sepsis, all from high-income countries. Applying those data globally, Fleischmann and colleagues[62] estimated the yearly incidence as up to 31 million cases of sepsis and 24 million cases of severe sepsis, with about 6 million fatalities.

One notable finding from this review was the absence of population-based data on sepsis incidence from low-income and middle-income countries (LMICs). Of course, many diseases from these countries do not have epidemiological data, which is partly the impetus for the Global Burden of Disease project.[63,64] However, that project relies mainly on death records, with their inherent limitations. Thus, maternal and neonatal sepsis are measured, as are separate infections, but not sepsis per se. Some investigators have tried to use Global Burden of Disease categories to estimate the burden of acute illness,[65,66] including sepsis, but these estimates have not been validated against existing population-based estimates.

A conceptual model of host, pathogen, and system factors

Beyond issues related to measurement, the incidence of sepsis depends on host, pathogen, and system factors (figure 2). Although these factors are articulated as separate entities in figure 2, their complex interplay is crucial to consider.

For example, chronic obstructive pulmonary disease (COPD) exemplifies a chronic health disorder that predisposes patients to increased risk of severe sepsis. The risk arises because of an increased risk of lower respiratory tract infection and, if infection is present, an increased risk of organ dysfunction, such as acute respiratory failure, because of decreased physiological

reserve. Thus, COPD affects infection and organ dysfunction, the core components of sepsis. Furthermore, although a host biological risk factor, COPD is exacerbated by health behaviours such as smoking, and might limit exercise and promote other unhealthy behaviours. COPD also affects respiratory tract colonisation and microbiome, changing the incidence and pathogens underlying sepsis. Finally, the availability of health-care resources will contribute to the total size and distribution of severity of COPD in a given community. Thus, COPD, although a biological host factor, interacts with other host factors and even cascades into pathogen and system factors.

Chronic health and behaviour factors

Many other chronic diseases, such as cancer,[67] cirrhosis,[68] and AIDS[69] are associated with an increased risk of sepsis or organ dysfunction. For some comorbidities, such as diabetes mellitus, the relation is more complicated than that of most chronic diseases, with a higher risk of infection but a lower risk of sepsis-related acute respiratory failure.[70]

Social behaviours are also associated with sepsis, with an increased incidence and worse outcomes in people who misuse alcohol[71] and an increased risk of death from pneumococcal pneumonia in smokers[72]—despite sparse data on the effects of smoking in sepsis.[73] Physical activity modulates the inflammatory response[74,75] and in experimental models of sepsis is associated with reduced mortality.[76,77] In a large US community-based cohort,[78] inadequate exercise increased the adjusted risk of dying with a sepsis diagnosis by 2·13 times.

Sex

Sex differences in sepsis incidence have been reported, with an age-specific incidence lower in women than in men.[43,44] Among patients with sepsis, men have a higher adjusted risk of sepsis than women in many studies,[43,79] although the finding is not uniform.[44] Although lifestyle factors might be unmeasured confounders of these associations, sex hormones have many potential effects on different organs,[80,81] including cardiovascular and immune-regulatory functions. Male hormones lead to earlier production and higher concentrations of cytokines after the induction of endotoxaemia and suppress cell-mediated immune responses,[82] whereas oestradiol (a female hormone) enhances the responsiveness of mononuclear cells after exposure to lipopolysaccharide, protecting women early in the disease course.[83] In experimental studies, female hormones were associated with attenuated myocardial dysfunction[84] and improvement in functional capillary density.[85] Sex might interact further with genetic factors, as exemplified in studies reporting a higher risk of sepsis in men with polymorphisms of lipopolysaccharide-binding protein[86] and TNFβ,[87] compared with women with the same polymorphisms.

Host genetic susceptibility

Many studies have examined questions related to genetic determinants in sepsis. Differences in the genotype modulate inflammatory response and affect outcomes by creating differences in recognition of bacterial and viral pathogens and the host response to their presence. However, genetic studies of sepsis face fundamental challenges to conduct and interpretation. First, an adequate phenotype definition is crucial, and the present clinical definitions of sepsis and severity[39] lead to phenotypic heterogeneity. Second, associations between sepsis and a given polymorphism are easily masked by upstream or downstream events of the implicated pathway. Common to any observational study, a polymorphism might be associated with a given phenotype without any causality relation. Third, sepsis does not fit the genetic case-control study design because of challenges in the definition of a control group that could develop sepsis if exposed to the putative genetic risk factor. Other issues include the inadequate sample size of these studies and the complex interactions of genetics with pathogens and comorbidities, leading to different phenotypes.

One of the most studied family of polymorphisms is related to CD14, a component of the innate immune system that brings about the recognition and binding of lipopolysaccharide to toll-like receptor 4 (TLR4). Studies of the relation between the CD14-159C/T polymorphism and sepsis have yielded conflicting results. A 2013 meta-analysis[88] of 17 studies suggested no association between this polymorphism and sepsis, although it did report a trend to an association in the Asian population, a significant association with septic shock, and a borderline significant association with death. Another meta-analysis[89] of 11 studies showed the interleukin-10 1082A/G polymorphism to be associated with sepsis in Asian people but not white people. Similar findings were reported in a meta-analysis[90] of 25 studies that showed an association between TNFα polymorphism TNF2 (adenine at position –308, G/A or A/A) and sepsis but not death. Asian people with this polymorphism had a higher risk of sepsis and risk of death compared with people of other ethnic origin.

Genetic polymorphisms also interact with pathogens by affecting the evolutionary pressure of infectious diseases. Examples are the protective mechanism of haemoglobin alterations against malaria[91] and changes in chemokine receptor 5, leading to protection against HIV.[92] Another example consists of two non-synonymous *TLR4* polymorphisms that bring about changes in the ligand-binding site of TLR4, Asp299Gly and Thr399Ile, which are mainly reported in cosegregation in white people. A different *TLR4* haplotype, the Asp299Gly-derived allele, was identified in African people.[93] In a study[94] of the geographic distribution of these haplotypes in 15 populations from three continents, African people had a high prevalence of the Asp299Gly allele, whereas Indo-European people had both Asp299Gly and Thr399Ile alleles. On the basis of these polymorphisms, the

investigators separated the population into three groups: African, west-Eurasian (Europe), and east-Eurasian (including America). This pattern of distribution might be attributable to the spread of modern human beings from Africa, with the differential frequency of *TLR4* haplotypes being driven by evolutionary pressures after migration.

These polymorphisms result in different phenotypes, with the African Asp299Gly allele enhancing the inflammatory response, compared with the Asp299Gly and Thr399Ile haplotype of white people, while also protecting against malaria. Thus, the disappearance of the Asp299Gly allele in white people might have resulted from negative selection of individuals with strong TNFα response and, consequently, high risk of death. However, in Africa, malaria has contributed to evolutionary pressure, and as such the protection conferred by the Asp299Gly allele to malaria might have surpassed the harmful effects of excessive TNFα production. This case shows a mechanism by which specific pathogens modulate the innate immune system throughout human evolution.

Gene–environment interactions

In face of social and economic determinants of the risk of acquiring sepsis or dying from it, the relevance of genetic background is difficult to assess. Mayr and colleagues[95] reported a higher age-matched and sex-matched incidence of sepsis in black patients in the USA, related to higher risks of admission to hospital with infection and development of acute organ dysfunction once infection occurs. The findings on crude analysis persisted despite adjustment for health behaviours, chronic diseases, health-care access, and measures of socioeconomic status.[95] Although other social and environmental factors—but not quality of sepsis-care[96]—might have accounted for these findings, the alternative explanation focuses on genetics-based racial differences in the biological response to infection. Other studies[97] have suggested that differences in genetics could explain racial disparities in health. For example, a genotype of Mal, a TLR-adaptor protein, is more common in African people than west-Eurasian people and is associated with a decreased proinflammatory cytokine response to bacterial stimuli.[98] Similarly, a genotype of caspase 12, relatively common in Africa (around 20% of the population) but rare elsewhere, has been linked to sepsis susceptibility via host hyporesponsiveness to bacteria.[99,100] Identification of the relative contribution of genetic and environmental factors will need large observational datasets with careful analyses of reliably measured clinical, sociodemographic, and genetic factors.

Pathogen factors

To further complicate epidemiological considerations, variation in pathogens and their characteristics interact with the host to produce variations in sepsis incidence and mortality. About 60%[45] to 70%[101] of patients with sepsis have blood cultures positive for any microorganism; in the more recent study, infections due to Gram-negative bacteria outnumbered those caused by Gram-positive bacteria.[101] Gram-negative infections were associated with an increased risk of mortality in several studies.[102] More recently, a study[103] published in 2014, reported that site of infection is associated with mortality when controlling for organism, with the risk of death highest for intra-abdominal infections and lowest for urinary tract infections.

Researchers are investigating the cellular basis for such observations, as noted in the discussion about genetic risk factors. Additionally, the clinical implications of pathogen–pathogen interactions have been shown in two case-control studies[104] from Kenya about the relation between malaria, bacteraemia, and sickle-cell trait. One study[104] showed that sickle-cell trait conferred protection against both malaria and bacteraemia when the malaria incidence was high. However, the hypothesis of host genetics protecting against both infections was disproved in a natural experiment when the risk of malaria fell substantially with home use of bednets and was accompanied by a decrease in bacteraemia and an attenuated association with sickle-cell trait. Although this result is tantalising, researchers have not yet further explored the effects of a reduction in the effect of malaria in sub-Saharan Africa on bacteraemia and sepsis. Such studies are challenged by the same limitations of sepsis diagnosis already discussed and the challenges of modelling malaria burden, even when diagnosed with a simple blood test.[105]

Health-care system factors

The diagnosis of severe sepsis implies that resources are available to provide patients with initial resuscitation followed by organ support. The decision to admit a patient with sepsis to an ICU depends on availability of ICU beds and non-medical factors. The variable supply of resources leads to a higher ICU-treated incidence of sepsis and higher case-fatality rate in countries with fewer ICU beds.[106] In high-income countries, the supply of ICU beds tracks per-person health-care spending and the number of hospital beds. The USA is an exception, spending more on critical care as a proportion of gross national income and devoting a higher proportion of hospital beds to ICUs than other high-income countries.[106] Perhaps surprisingly, even within the USA, access to extracorporeal support and high-volume mechanical ventilation—both important during a pandemic of respiratory illness—varies regionally.[107] However, extension of these observations on the supply of ICU beds to 36 World Bank-defined low-income countries[108] is impossible in the absence of national data on ICU bed supply, with data available only for Uganda[109] and Nepal.[110] Although ICUs are burgeoning in middle- income countries, these countries still do not have the comprehensive data needed for population-based studies of sepsis.

Convenience sampling of high-income and middle-income countries suggests that overall ICU mortality decreases as national income rises, an effect attributable

to health-care resources.[3] This effect probably applies to patients with sepsis as well. A timely example is the present Ebola outbreak in west Africa, in which mortality is estimated at 70%.[111] By contrast, estimated mortality was far lower, 20–25%, during an Marburg virus outbreak in Marburg, Germany, and the former Yugoslavia in 1967, when resources to provide more intense supportive care were presumably more available than in contemporary west Africa.[112] A related issue is variable health-care worker adherence to recommended treatments, although any effect on mortality seems to be dominated by underlying severity of illness.[113]

Extrapolation of high-income country estimates of sepsis incidence to LMICs will almost certainly underestimate the number of cases in these countries because of their higher prevalence of underlying risk factors for sepsis, higher probability of infection and organ dysfunction, and higher case-fatality rates. In sub-Saharan Africa for example, HIV infection is more prevalent than in the rest of the world and is an important risk factor for sepsis; poor basic hygiene and primary care for HIV increase the probability of sepsis; and high rates of antibiotic resistance[114] and an insufficient supply of essential drugs, equipment, and personnel[115,116] conspire to worsen clinical outcomes. Related evidence from health-care-acquired infection showing substantially higher rates of ventilator-associated pneumonia, central line-associated bloodstream infection, and urinary tract infection in LMICs than in high-income countries support this argument.[117] Although some counterbalancing factors might contribute to lowering the incidence of sepsis in LMICs, they are outweighed by the many factors in these countries that contribute to a high incidence of sepsis. For example, because old age is an important risk factor for sepsis, high-income countries with longer life expectancy will have higher age-related sepsis incidence than LMICs. By contrast, sepsis in LMICsis predominantly a disease of young and middle-aged patients with few comorbidities.

High-income countries provide more medical care than do LMICs, such as chemotherapy for cancer, intensive care for trauma, and organ transplantation, which creates immunosuppressed populations at high risk of sepsis. More broadly, any country that invests in the care of chronic medical disorders will probably have to sustain a large population of individuals with increased risk of sepsis. As countries transition from low-income to middle-income status, the relative importance of these factors will change.

The burden of sepsis survival

Increasing data point to the large burdens faced by survivors of critical illness,[118] including long-term physical and neurocognitive impairments, and their caregivers.[119] Although early studies of critical illness survivors were subject to the fallacy of *post hoc, ergo propter hoc* (the conclusion that morbidity recorded after critical illness is caused by critical illness),[120] more recent investigations have accounted for the precritical illness trajectories of patients. Such studies have established that admission to a hospital with sepsis, irrespective of admission to the ward or the ICU, is associated with new functional disabilities, cognitive decline, increased health-care use, and some (but not all) geriatric disorders.[121] The relation between sepsis and chronic illness is probably bidirectional, with sepsis survival leading to new or accelerated chronic disease, which might in turn predispose to recurrent sepsis. Persistent inflammation in survivors of sepsis could be the mechanism underlying this trajectory.[122]

Diagnostic and therapeutic considerations

Diagnostic microbiology

Diagnostic microbiology stands at the epicentre of the tests for sepsis in patients. Nowadays, microbiological studies for the detection of bacteria or fungi in blood, body fluids, or relevant tissues continue to rely for the most part on conventional culture-based systems, which remain the gold standard. Blood cultures are positive in 30–40% of patients with severe sepsis and septic shock.[123] In most instances bloodstream infections are intermittent and the circulating microbial loads are low, typically between one and ten colony-forming units per mL.[124–126] As a result, large volumes of blood are needed to increase the sensitivity of blood culture systems, which is an issue in paediatric patients. Once blood cultures are positive, conventional techniques (Gram stain and phenotypic tests) are used for the identification of the microorganism and susceptibility testing (figure 3). Automated blood

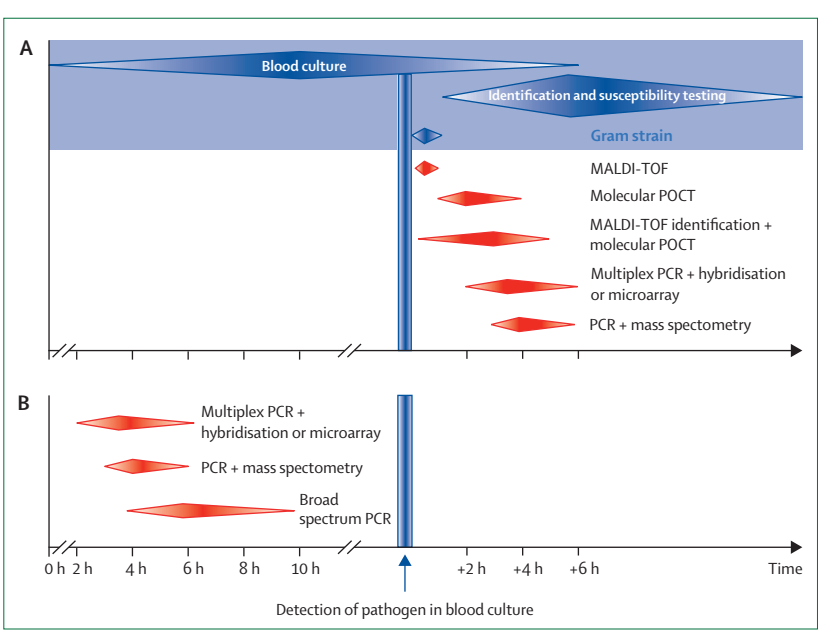


***Figure 3*: Time scale for the diagnostic investigation of bloodstream infections**
Conventional (blue) and new (red) technologies available for the diagnostic investigation of bloodstream pathogens (mainly bacteria and yeasts), starting with positive blood cultures (A) or with whole blood (B). The blue vertical line depicts a time at which blood cultures become positive. Diamonds represent theoretical times necessary for the detection of pathogens with each technology. MALDI-TOF=matrix-assisted laser desorption ionisation-time of flight. POCT=point-of-care test.

	Number of blood cultures	Correct identification	Main findings
La Scola et al (2009)[128]	562	76%	Improved identification with formic acid extraction; poor performance for viridans streptococci (PPV=99·2%)
Prod'hom et al (2010)[129]	126	78·7%	99% of congruent identification at the species level
Stevenson et al (2010)[130]	212	80·2%	No identification in 19·8% of the isolates, probably due to a low bacterial load; misidentification of *Streptococcus mitis*
Christner et al (2010)[131]	304	87%	Mismatches for Gram-positive bacteria; turn-around time about 100 min
Ferroni et al (2010)[132]	388	90·8%	Profiles of *S mitis* and *Streptococcus pneumoniae* often indistinguishable
Ferreira et al (2011)[133]	330	31·8–96·9%	85% of correct identification at species level for Gram-negatives; unreliable detection of fungi
Lagacé-Wiens et al (2012)[134]	61	85·2%	No misidentification; mean turn-around time reduced by about 30 h
Clerc et al (2013)[135]	202	86·7%	Patients with Gram-negative bacteraemia; effect of MALDI-TOF on clinical management in 35% of patients
Clerc et al (2014)[136]	224	94·5%	Patients with *Staphylococcus aureus* bacteraemia; no false identification; MALDI-TOF combined with GeneXpert MRSA
Verroken et al (2014)[137]	925	81·1%	Use of a 5 h subculture protocol; correct identification of *Streptococcus* spp in only 65·4% of the cases

PPV=positive predictive value. MALDI-TOF=matrix-associated laser desorption ionisation-time of flight. MRSA=meticillin-resistant *Staphylococcus aureus*.

Table 1: **Summary of representative clinical studies using MALDI-TOF for the identification of microbial pathogens in positive blood cultures**

culture systems have substantially reduced the time needed to detect circulating bacteria. 90% of positive blood cultures are detected within 24 h of incubation, 95% after 48 h, and 99% after 72 h.[127] However, the sensitivity of blood culture continues to be suboptimum for fastidious pathogens and is substantially reduced in patients on antibiotic therapy at the time of blood draw.

In the past decade, several new technologies using either protein-based systems, such as matrix-associated laser desorption ionisation-time of flight (MALDI-TOF), or nucleic acid-based systems, such as PCR-based, transcription-mediated, loop-mediated isothermal or helicase-dependent amplifications of molecular targets, have emerged to speed up the identification of microbial pathogens. These new diagnostic platforms could be applied either on positive blood cultures or directly on whole blood (figure 3).

MALDI-TOF is deemed to be a major revolution in diagnostic microbiology, allowing rapid and accurate identification of bacteria and fungi on the basis of the generation of unique mass spectral protein fingerprint signatures. MALDI-TOF is typically used for routine identification of bacterial or fungal colonies on agar plates or broth media. Since 2009, several studies[128–137] have assessed the performance of MALDI-TOF for the identification of pathogens in positive blood cultures (table 1).

Overall, correct identifications of bloodstream pathogens are achieved in about 80–90% of the patients depending on the type of the isolate and the bacterial pellet extraction protocol that is used.[128–133] An observational study[138] suggests that routine use of MALDI-TOF helps reduce the time to appropriate antibiotic therapy, the length of ICU stay, and the mortality of patients with bacteraemia. Rapid detection of microbial resistance is crucial to reduce the risk of inappropriate therapy, which is known to increase morbidity and mortality or the unnecessary coverage of resistant pathogens.[135] MALDI-TOF coupled with a PCR-based rapid diagnosis of meticillin-resistant *Staphylococcus aureus* (GeneXpert MRSA) was shown to substantially reduce superfluous coverage of MRSA (29·2% in the control group and 17·1% in the intervention group).[136]

PCR-based technologies (such as singleplex or multiplex real-time, broad range, or digital PCR) coupled with endpoint melting curve analyses, sequencing, or mass spectrometry (ie, PCR and electrospray ionisation mass spectrometry), microarray hybridisation, and new generation sequencing platforms have been implemented to avoid time-consuming culture steps and increase sensitivity (reviewed in[139]). Several clinical studies[140–143] suggested that the use of the molecular tests (figure 3) on blood culture pellets reduced the turn-around time for the identification of the pathogen. However, except for drug resistance testing, hardly any value is added by implementation of nucleic acid tests on material derived from positive blood cultures because the tests are more expensive and time consuming than MALDI-TOF. To further reduce detection time, nucleic acid-based tests can be applied directly to blood samples (figure 3). However, the preanalytical processing of the specimen remains a big challenge in view of the low amount of pathogen present in blood. Volume restriction imposed by nucleic acid extraction protocols is one of the problems. But molecular methods have other downsides, including the risk of contamination at each step of the analytical procedure, especially when running open-system platforms, potential interference between microbial and human DNA, which is present in large

	Molecular assay	Number of patients or episodes	Type of patients	Main findings
Westh et al (2009)[144]	LightCycler SeptiFast Test (Roche)	359 patients, 558 episodes	Patients with SIRS with suspected infections, multicentre study	Higher rates of documented infections with molecular assay than with blood cultures (17% *vs* 26%); very large number of discordant results (n=148), representing 74% of the total number of isolates
Bloos et al (2010)[145]	LightCycler SeptiFast Test (Roche)	142 patients	Patients with severe sepsis, multicentre study	Higher proportion of documented infections with molecular assay than with blood cultures (34·7% *vs* 16·5%); patients testing positive with molecular tests had higher SOFA scores and a trend toward higher mortality than those who tested negative
Lamoth et al (2010)[146]	LightCycler SeptiFast Test (Roche)	86 patients, 141 febrile episodes	Patients with neutropenia	A third of false-negative results with molecular assay in patients with bacteraemia; large number of clinically questionable positive results for *Pseudomonas aeruginosa* and *Staphylococcus aureus*; identification of five *Candida* spp and one *Aspergillus* sp infections with molecular assay; improved documentation of infection in patients on antibiotics
Lehmann et al (2010)[147]	LightCycler SeptiFast Test (Roche)	436 patients, 467 episodes	Patients with sepsis, multicentre study	Discordant findings between molecular assay and conventional blood cultures in 26·9% of the septic episodes; the use of molecular assay substantially reduced the number of days on inappropriate antibiotic therapy
Yanagihara et al (2010)[148]	LightCycler SeptiFast Test (Roche)	212 patients	Patients with SIRS with suspected infections	Larger number of documented infections with molecular assay than with blood cultures (8·0% *vs* 1·3%); false-negative results with molecular assay, including five bacteraemia due to MRSA (n=2), *Enterococcus faecium*, *P aeruginosa*, and *Klebsiella* spp; improved documentation of infection in patients on antibiotics
Lucignano et al (2011)[149]	LightCycler SeptiFast Test (Roche)	803 patients	Neonates and children with suspected sepsis	Higher rate of positive results with molecular assay than with blood cultures (14·6% *vs* 10·3%) and in the subgroup of patients on antibiotics (14·1% *vs* 6·5%); more contaminants with blood cultures compared with molecular assay (5·8% *vs* 1·6%); compared with blood cultures, the molecular assay had a sensitivity of 85% and a specificity of 93·5%
Loonen et al (2014)[150]	Magicplex Sepsis Real-time Test (Seegene) and SepsiTest (Molzym)	125 patients	Patients with at least two SIRS criteria	Poor performance of both molecular tests with low sensitivities (MagicPlex: 11%, SepsiTest: 37%) and PPV (MagicPlex: 43%, SepsiTest: 30%), possibly related to the restricted volume (1 mL) of blood samples
Carrara et al (2013)[151]	Magicplex Sepsis Real-time Test (Seegene)	267 patients	Patients admitted in ICU, haematology, and emergency departments	Proportion of agreement between the molecular assay and blood cultures was 73% (Cohen's kappa test suggested fair to moderate agreement); similar sensitivities and specificities between the two tests (MagicPlex: 65% and 92%, blood cultures: 71% and 88%)
Jordana-Lluch et al (2013)[152]	PCR/EMI-MS	175 patients	Patients with suspected sepsis	94·2% and 77·1% overall agreements between molecular assay and conventional methods for blood samples or whole blood specimens, respectively; molecular assay sensitivities of 96·8% and 50% and specificities of 98·5% and 93·8% for blood samples or whole blood specimens, respectively
Laffler et al (2013)[153]	PCR/EMI-MS	906 patients of whom 464 were culture positive	Unspecified population of patients with suspected bloodstream infections	78·6% overall agreement between the molecular assay and blood cultures; 93·9% of 33 culture-negative, assay-positive specimens were deemed to be clinically relevant bloodstream infections; with assay, most of pathogens were detected within 8 h of blood sampling
Bacconi et al (2014)[154]	PCR/EMI-MS	331 patients	Patients with suspected bloodstream infections	Improved nucleic acid extraction system (5 mL of blood) and PCR conditions; twice as many positive specimens with assay compared with cultures (10·6% *vs* 5·4%); sensitivity and specificity of the assay: 83% (91%)* and 94% (99%)*

SIRS=systemic inflammatory response syndrome. SOFA=sepsis-related organ failure assessment. MRSA=meticillin-resistant *Staphylococcus aureus*. PPV=positive predictive value. ICU=intensive care unit. PCR/EMI-MS=PCR coupled with electrospray ionisation-mass spectrometry. *Value in ellipses obtained from replication testing.

Table 2: **Summary of representative clinical studies using molecular assays for the identification of microbial pathogens directly from blood**

excess, the absence of a gold standard, and the detection of non-viable microorganisms complicating the clinical interpretation of positive so-called DNAaemia signals.

Table 2 provides an overview of the characteristics and main findings of a selection of representative clinical studies that have used commercially available molecular assays for the identification of pathogens directly from blood. Most of the studies were done in a large number of patients with systemic inflammatory response syndrome, severe sepsis, or suspected bloodstream infections. Compared with conventional blood cultures, molecular assays produced shorter turn-around times and a higher number of positive results deemed to be clinically significant, especially in the subgroup of patients already on antibiotic therapy. Rates of agreement between conventional and molecular diagnostic methods were generally reasonable. Nevertheless, some studies[14,39,146,155–157] reported suboptimum sensitivities and specificities for molecular assays and a high proportion of discordant findings with some surprising false-negative results for non-problematic bacterial species, which could be related to insufficient blood volume sampling.

In summary, molecular assays look very promising for the rapid detection of pathogens directly from the blood of patients with sepsis, especially in patients already on antimicrobial drugs. However, technological improvements are needed to improve DNA extraction procedures, to reduce contamination rates, and to reduce the detection threshold for difficult-to-grow, slow growing, or non-growing microorganisms. Other unmet medical needs include the ability to provide rapid microbial resistance profiles, the need for

***Panel 1*: The ideal profile of a sepsis biomarker**

- Fast kinetics
- High sensitivity and specificity
- Fully automated technology
- Short turn-around time
- Availability as point-of-care tests
- Low cost

	Type of study	Clinical application	Main findings
Jensen et al (2006) [161]	Prospective cohort study	Outcome prediction	Increasing concentrations of procalcitonin independently associated with 90 day mortality (hazard ratio 1·8) in critically ill patients
Bouadma et al (2010) [162]	Randomised open label trial	Reduction of antibiotic consumption and antibiotic stewardship	Procalcitonin-guided antibiotic therapy *vs* standard of care in critically ill patients; higher number of days without antibiotic therapy (14·3 *vs* 11·6) at day 28 in the procalcitonin group; non-significant increase in mortality at 2 months (30% *vs* 26%)
Jensen et al (2011) [163]	Randomised controlled trial	Guide for antibiotic therapy	Increased use of antimicrobial therapy and length of ventilation and ICU stay in the procalcitonin group; no increase in 28 day mortality (31·5% *vs* 32%)
Layios et al (2012) [164]	Randomised controlled trial	Reduction of antibiotic consumption	No signigicant reduction in antibiotic consumption (63% *vs* 58%)
Simon et al (2004) [158]	Meta-analysis	Diagnosis of bacterial infection	Procalcitonin had a sensitivity of 88% and a specificity of 81% for the diagnosis of bacterial infection
Tang et al (2007)[159]	Meta-analysis	Diagnosis of sepsis	Diagnostic odds ratio of procalcitonin for the diagnosis of sepsis in critically ill patients=7·8 (a value of less than 25 suggests poor accuracy); maximum joint sensitivity and specificity=73%, AUC 0·79
Wacker et al (2013)[160]	Meta-analysis	Diagnosis of sepsis	Pooled sensitivity (77%) and specificity (79%) of procalcitonin for the diagnosis of sepsis in critically ill patients (AUC=0·85)

ICU=intensive care unit. AUC=area under receiver operating characteristic curve.

***Table 3*: Summary of representative clinical studies and meta-analyses on procalcitonin as a sepsis biomarker**

multiplex diagnostic platforms (allowing identification of several pathogens on single specimens), the development of liquid-based and fully automated closed-system platforms (allowing point-of-care testing), and next generation sequencing technologies to provide fast genome analyses of clinically relevant microbial species. Finally, an unresolved issue is the clinical significance of DNAaemia, which might also show the presence of non-viable microorganisms.

Biomarkers

In the past two decades, biomarkers have been the subject of intense research with the goal to identify molecules that might be early indicators of sepsis or predictors of risk, severity, response to therapy, or outcome. A wide array of danger signals, blood cells, cell markers, acute phase reactants, soluble receptors, and countless mediators have been measured in the blood and in body fluids of patients with sepsis. Some of these biomarkers (ie, white blood cell counts, C-reactive protein, and procalcitonin) have even been incorporated into the definitions of systemic inflammatory response syndrome and sepsis.[14,39] Most biomarkers have been assessed in a small number of studies and few are in clinical use. New biomarkers will continue to emerge from studies on the pathogenesis of sepsis. Arguably, only a minority will ever match the ideal profile of a sepsis biomarker (panel 1).

Clinicians and sepsis investigators dream of molecules that might help them to discriminate infectious from inflammatory diseases. Infectious diseases specialists would also be keen on having specific markers for bacterial, fungal, or viral infections. How close are we to meeting these goals? A detailed review of such a vast topic is beyond the scope of this Commission, and we chose to focus on biomarkers that are in clinical practice and on some promising new ones.

C-reactive protein, a prototypic hepatic acute-phase protein, has been widely used to diagnose and monitor inflammatory and infectious diseases.[155] Although an association between serial C-reactive protein measurements and sepsis resolution or outcome was shown in some studies (reviewed by Simon and colleagues[158]), a slow kinetic with a peak reached 24–48 h after the initiating stimulus, a half-life of 19 h, and an absence of specificity are notable shortcomings of the use of C-reactive protein as a sepsis biomarker.

Procalcitonin, a precursor of calcitonin, has been studied extensively in sepsis for diagnosis,[158–160] outcome prediction,[161] and guidance of antibiotic therapy[162–164] (table 3).

Procalcitonin has interesting kinetics, not only sharply rising with an inducing stimulus and rapidly decreasing once the stimulus is cleared, but also rising in inflammatory states induced by surgery or cardiac arrest.[165,166] Meta-analyses[158–160] have shown that the diagnostic performance of procalcitonin was moderate in critically ill patients with sepsis (pooled sensitivity was 77%, specificity was 79%, and area under the receiver operating characteristic curve [AUC] was 0·85).[160] Unfortunately, the absence of a gold standard for the diagnosis of sepsis complicates the interpretation of many of the meta-analyses.

Several RCTs[167,168] have shown that procalcitonin-guided algorithms substantially reduced the use of antibiotics in patients with respiratory tract infections, without any associated safety concern. However, the proportion of patients on antibiotics was quite high in the control group and the duration of antibiotic therapy was longer than recommended. Clinical trials done in critically ill patients and those in the ICU with sepsis reported conflicting results on the effect of procalcitonin measurements on antibiotic usage. In a multicentre randomised trial done in eight ICUs in France, the duration of antibiotic treatment was reduced by 2·7 days in the procalcitonin intervention group.[162] However, the

trial algorithm was overruled in half of the patients and mortality was numerically higher, albeit not in a statistically significant manner, in procalcitonin-managed patients. Two recent studies[163,164] reported finding no clinical use for a procalcitonin-guided algorithm in the ICU. The procalcitonin-based strategy was even associated with a broad-spectrum of antibiotic use and prolonged mechanical ventilation and length of stay in the ICU. Pending further studies, the routine use of procalcitonin for the diagnosis and management of sepsis cannot be recommended.

Many study results[169] have shown associations between circulating concentrations of many cytokines and outcome of patients with sepsis, but the added value of cytokine measurement was often slight.[170,171] The study of cell surface markers and soluble receptors, such as the soluble triggering receptor expressed on myeloid cells (sTREM-1), has yielded interesting results. Monocyte expression of sTREM-1, a member of the immunoglobulin superfamily, is upregulated in patients with bacterial infections.[172] Although the diagnostic performance of measuring plasma concentrations of sTREM-1 for sepsis was quite high (AUC of 0·97) in the initial study by Gibot and colleagues,[173] it was substantially lower (AUC of 0·73) in a subsequent larger study done by the same investigators.[174] Type 1 immunoglobulin Fc receptor (CD64) is expressed by activated neutrophils and the AUC of CD64 expression on neutrophils was greater than 0·90 for the prediction of sepsis.[174,175] However, implementation of CD64 in clinical practice might be cumbersome because it is measured by flow cytometry. Blood concentration of pancreatic stone protein, a lectin-binding acute phase protein expressed by intestinal and pancreatic acinar cells, was associated with disease severity in patients with sepsis.[176–178] Pancreatic stone protein was better than procalcitonin and C-reactive protein for the prediction of in-hospital mortality.

In a review[171] published in 2010, Pierrakos and Vincent examined 3370 studies assessing 178 sepsis biomarkers. No single biomarker showed sensitivity and specificity greater than 90% for the diagnosis of sepsis or the prediction of outcome. Since then new sepsis biomarkers have been proposed, but none has yet reached the clinical arena. The guidelines of the Surviving Sepsis Campaign (SSC),[179] updated in 2012, included only one recommendation about biomarkers: to use procalcitonin to stop antibiotic therapy. However, the strength of the recommendation from the SSC scoring system (GRADE)[179] was weak (grade 2C) with a low quality of evidence for it.

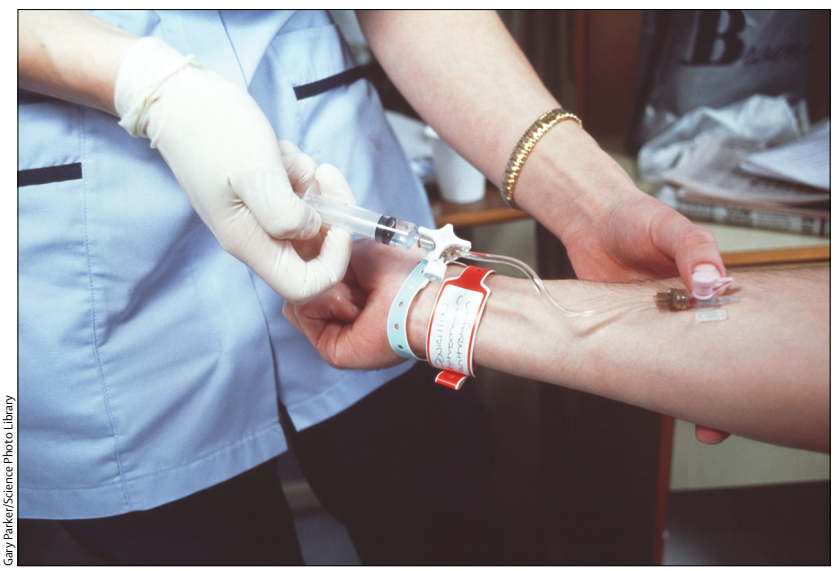
What kind of developments can be expected in the near future for biomarkers of sepsis? Omics technologies will probably deliver a vast array of new sepsis biomarkers. Although sepsis research should continue to be driven by a positive state of mind, in reality the search for a gold standard for sepsis diagnosis has been disappointing. In view of the complexity of the pathogenesis of sepsis, the diversity of clinical presentations, and the heterogeneity of patient populations, to believe that one biomarker will prove to be ideal is arguably unrealistic. At present, most biomarkers have high negative predictive values. However, to diagnose sepsis early in the clinical course, we need tests with high specificities and positive predictive values. This objective is more likely to be achieved by a combination of biomarkers in an Acute Physiology and Chronic Health Evaluation or SOFA score-like test.[174] Systems biology approaches might also help detect sepsis early as shown by a microarray study[180] based on the expression of 42 molecular markers or to predict death from sepsis on the basis of the analysis of plasma metabolome in combination with clinical variables.[181] As a final point, future biomarker studies will have to be multicentred, enrol large and homogenous patient populations with well defined infectious diseases entities, and be validated prospectively. For research to continue to rely heavily on the poorly defined sepsis phenotype might be an unwise approach.

Antibiotic therapy

Timing and choice of empirical therapy

Prompt initiation of appropriate antibiotics is crucial. The SSC guidelines[179] suggested that antibiotics should ideally be started within 1 h of the diagnosis of severe sepsis or septic shock (recommendations graded 1B and 1C). However, the strength of the inverse relation between the timing (ie, hour by hour) of antibiotic administration and mortality might not be as linear as suggested by some studies.[9,25,179,182,183] The empirical antibiotic regimen should be broad enough to cover all likely pathogens and be guided by local epidemiological data and the medical history of the patient, including previous infections, susceptibility profiles of colonising microorganisms, and recent exposure to antimicrobial drugs. Pharmacokinetic and pharmacodynamic considerations related to appropriate tissue penetration and the presence of hepatic or renal dysfunctions should also be taken into account. The empirical antibiotic regimen could rely on either one antibiotic (monotherapy) or on two or more antibiotics (combination therapy). Monotherapy consists typically of an extended-spectrum penicillin with or without a β-lactamase inhibitor, a third or fourth generation cephalosporin, or a carbapenem. Combination therapy is usually an association of a β-lactam with an aminoglycoside, a fluoroquinolone, an anti-Gram-positive drug, or an antibiotic active against multiresistant Gram-negative bacteria. Monotherapy and combination therapy were both given a grade 1B recommendation in the SSC guidelines (figure 4).

Whereas observational and retrospective studies[184,185] suggested a superiority of combination therapy to monotherapy in patients with Gram-negative sepsis, meta-analyses[186,187] suggested that monotherapy was as efficacious as and less toxic than combination therapy



Gary Parker/Science Photo Library

Figure 4: **Antibiotic treatment**

for the treatment of immunocompetent patients with Gram-negative bacteraemia or sepsis. In a Cochrane review[187] updated in 2014, all-cause mortality (risk ratio [RR] 0·97, 95% CI 0·73–1·30), clinical failure (RR 1·11, 0·71–1·01), and the development of bacterial resistance (RR 0·88, 0·54–1·45) were similar in patients with sepsis given β-lactam alone to those given β-lactam plus an aminoglycoside, whereas nephrotoxicity (RR 0·30, 0·23–0·39) was less frequent in patients with sepsis given only β-lactams. Two large multicentre studies[188,189] reported no superiority of a meropenem plus fluoroquinolone (ciprofloxacin or moxifloxacin) combination therapy to a meropenem monotherapy as empirical therapy for suspected ventilator-associated pneumonia or severe sepsis. Despite the intuitive appeal of combination therapy and the fact that it might increase the chance of appropriate therapy in the empirical phase of treatment, combination therapy is not better than monotherapy in terms of patient outcome and is associated with more adverse events.

Targeted therapy

Once the pathogen has been identified and antimicrobial susceptibility data are known, does evidence exist that favours the use of combination therapy for highly virulent or difficult-to-treat bacterial pathogens such as *Pseudomonas aeruginosa*? Several retrospective, small observational studies and subgroup analyses suggested some survival benefit by use of combinations of β-lactams and aminoglycosides in patients with *P aeruginosa* bacteraemia.[187] However, most of these studies had important methodological weaknesses, such as the use of different β-lactam antibiotics in experimental and control groups and insufficient power, casting doubts on the significance of these findings. In a post-hoc analysis[190] of 593 episodes of *P aeruginosa* bacteraemia published in 2013, 30 day mortality was similar in patients given one drug or with a combination of antibiotics. Similarly, in a meta-analysis[191] of 19 studies, no survival benefit was reported when patients were given a combination of a β-lactam plus an aminoglycoside or a fluoroquinolone versus being given β-lactam alone, either as empirical or as definitive therapy. The SSC guidelines[179] gave a weak grade 2B recommendation for the combination of an extended-spectrum β-lactam with an aminoglycoside or a fluoroquinolone in patients with *P aeruginosa* bacteraemia complicated by respiratory failure and septic shock. Adequately powered multicentre RCTs are obviously needed to resolve several long-standing controversies about the place of combination therapy in the management of patients with sepsis with microbiologically documented infections.

Intermittent versus continuous infusions

Optimisation of the use of antibiotics on the basis of their pharmacokinetic and pharmacodynamic properties could contribute to the improvement of the outcome of critically ill patients with sepsis. A large, prospective, multinational, pharmacokinetic point-prevalence study[192] published in 2014 on blood concentration of eight β-lactam antibiotics (ie, time-dependent antibiotics) suggested that 16% of the patients did not have free antibiotic concentrations higher than the minimum inhibitory concentration (MIC) during 50% of the dosing interval (50% *t*>MIC). In multivariate regression models, 50% and 100% *t* values greater than MIC were associated with positive outcome. Corroborating the previous work of several groups of investigators, these data suggested that personalised antibiotic therapy in critically ill patients might increase effectiveness. In a prospective, multicentre, double-blind RCT[193] in patients with severe sepsis given piperacillin-tazobactam, meropenem, or ticarcillin-clavulanate, plasma concentrations of antibiotic greater than the MIC (82% *vs* 29%; p=0·001) and clinical cure (70% *vs* 43%; p=0·037) were higher in the continuous infusion group than in the intermittent bolus group. Longer infusions were associated with lower mortality (RR 0·59, 95% CI 0·41–0·83) in a meta-analysis[194] of 14 studies (eight retrospective, three prospective, and three RCTs) comparing extended (equal to or greater than 3 h) or continuous (24 h) versus short-term (20 to 60 min) infusions of carbapenems and piperacillin-tazobactam in critically ill patients. However, two meta-analyses,[195,196] compiling 14 RCTs of β-lactam antibiotics in one and 29 RCTs of both time-dependent and concentration-dependent antibiotics in the other, did not report improved clinical results or outcome with continuous antibiotic infusions. The authors also pointed out major weaknesses and biases with regard to several important methodological elements (such as randomisation, concealment of allocation, blinding, doses of antibiotics, incomplete datasets, and selective reports of outcome variables). Additional information derived from

adequately powered, multicentre, randomised clinical studies are needed before recommendations can be made on the use of continuous administration of antibiotics.

De-escalation and duration of antibiotic therapy

Very few studies have assessed the clinical effect of de-escalation strategy in critically ill patients with sepsis. A 2012 Cochrane review[197] reported that insufficient evidence existed (no published RCT) to comment on the efficacy and safety of de-escalation therapy in patients with sepsis. A 2014 prospective observational study[198] of 712 ICU patients with sepsis showed that de-escalation was associated with a decreased 90 day mortality (odds ratio 0·55, 95% CI 0·34–0·87; p=0·011) both in the entire cohort of patients and in a subgroup with adequate empirical antibiotic therapy. Daily reassessment of antimicrobial therapy and de-escalation were given a grade 1B recommendation in the SSC guidelines.[179] In view of the vast heterogeneity of patients with sepsis, studies on the optimum duration of antibiotic therapy are unsurprisingly scarce, hence the very weak quality and strength (grade 2C) of recommendation for the 7–10 days treatment course proposed in the SSC guidelines.

Molecular targets and experimental therapies for sepsis

Conventional management principles with timely administration of intravenous fluids, oxygen, and antimicrobials combined with so-called source control, drainage of infectious foci, and advanced organ support in an intensive-care setting have reduced the overall mortality of severe sepsis to historically low rates (<20%).[2,7] Regrettably, long-term outcomes suggest that substantial residual morbidity and excess mortality risks persist for survivors after initial treatment for septic shock. The question remaining is whether therapeutic interventions that target specific molecular mechanisms implicated in the pathophysiological changes of sepsis might further improve this scenario, and potentially save additional lives.[36,199]

Principles of sepsis pathogenesis

Sepsis is a syndrome of organ failure that complicates the clinical course of patients with a primary, acute systemic infection or with a secondary infection after an initial sterile injury. In a small number of patients with sepsis, (eg, those with primary meningococcaemia, post-splenectomy sepsis), the acute release of TNF and other cytokines produces septic shock, a highly lethal syndrome. TNF-targeted treatment cannot be used to intervene in acute septic shock because TNF concentrations peak within 90 min of onset, a timeframe that is not amenable to targeted drugs.[200] However, most patients with sepsis do not develop acute septic shock. Rather, their clinical course is indolent and characterised by progressive and persistent organ failure that is refractory to treatment. Patients with sepsis that progress to persistent critical illness or multiple organ failure face short-term mortality of 20%, and 5 year mortality as high as 50%.[22] Unlike septic shock, the underlying pathogenesis of persistent critical illness has not been well studied in the laboratory or in clinical trials and its optimum treatment is ill-defined. Review of the experimental and clinical evidence suggests that neither inflammatory nor coagulation defects adequately explain the pathogenesis of organ dysfunction. Other explanations are needed to account for the progressive failure of organ function.[22,201]

To be useful, theories of sepsis pathogenesis will have to be corroborated by clinical evidence of cellular metabolic dysfunction, epithelial barrier failure, and endothelial capillary leakage syndrome, since these are ubiquitous in patients with sepsis with persistent critical illness. The entire septic process seems to be a manifestation of poor cellular and tissue barrier function, loss of specialised tissue actions, and a form of cellular hibernation. Tissues stop generating variability in their integrated circuitry and stop generating cycles of communication within and between tissues. Many investigators have noted the loss of specialised cell function and barrier function, suggesting that this constitutes a common host response to sepsis and other forms of critical illness.[22,201–204] A so-called magic bullet for sepsis is highly unlikely to be found, unless it is one that can correct these three principal defects: epithelial barrier dysfunction, endothelial leakage, and disrupted cellular metabolism. Lymphocyte apoptosis and compensatory anti-inflammatory responses have also been noted in patients with sepsis, but so far evidence that these processes are causal in the development of either organ dysfunction or mortality is insufficient.[22]

Recorded responses in the haemopoietic cell compartment during sepsis might represent immunometabolic responses to unknown signals that are also triggering parenchymal cell dysfunction.[205] By this reasoning, the immune cell responses that have been the target of exhaustive study might possibly, or even quite probably, be more equivalent to a biomarker than a causative mechanism. The host response in septic tissues at the cellular level is mainly dysfunction, not widespread necrosis, apoptosis, necroptosis, or other forms of cell death.[22] Individual cells' behaviour changes to a unicellular survival mode, restricting cell activity to minimise energy consumption, and becoming functionally incapable of doing their normally specialised, coordinated functions. The individual cells behave like de-differentiated, primitive, non-specialised, cells, resulting in organ dysfunction. Septic cardiac myocytes stop contracting normally—losing symmetry and synchrony with other cardiac cells. Kidney epithelial cells stop maintaining sodium–potassium gradients across tubular membranes. Endothelial cells retract and stop maintaining endothelial barrier function, leading to tissue hypoperfusion, tissue oedema, and so on. This general hyporeactive survival strategy during cellular

	Mechanism of action	Developmental status
Interleukin 22	Epithelial growth factor that promotes tight junctions and prevents epithelial permeability and apoptosis	Preclinical studies[214]
GM-CSF	Epithelial and myeloid cell growth factor, restricts colonic inflammation and helps injury repair	Preclinical and pilot studies in human beings[215]
Hepatocyte growth factor, interleukin 11	Epithelial cell growth factors that are cytoprotective for intestinal epithelial cells	Preclinical and pilot studies in human beings[213,216]
Drugs to regulate the microbiome	Tightens epithelial junctions, stimulates intestinal immunity, and limits growth of multidrug-resistant pathogens and *Clostridium difficile*	Preclinical studies[211,212,217–220]
Insulin-like growth factor-1	Reduces sepsis-induced excess gut permeability and bacterial product translocation and prevents apoptosis	Preclinical studies[217]
TNFAIP3	Prevents loss of tight junction occludin protein and membrane integrity during sepsis	Preclinical investigations[221]
Anti-HMGB1 monoclonal antibody	Blocks intestinal hyperpermeability and reduces cytokine response after ischaemia-reperfusion injury	Preclinical investigations[222]

GM-CSF=granulocyte-macrophage colony stimulating factor. TNFAIP3=tumour necrosis factor α-induced protein 3. HMGB1=high mobility group box 1.

Table 4: **New sepsis drugs targeting the epithelial barriers**

stress might preserve cellular energy status and maintain individual cell viability, but the loss of coordinated tissue function can be lethal to the host.[22]

Partly on the basis of these theories, new therapeutic drugs are being investigated in preclinical and clinical trials for patients with sepsis. These drugs are being designed to enhance cellular function or host defence against pathogenic invasion. New modalities to protect endothelium and epithelium, reverse immunometabolic dysfunction, and bolster host defences in patients with sepsis are now the target of novel sepsis drugs.

Novel therapeutics in development

Epithelial barrier functional impairment in sepsis as a therapeutic target

Gut epithelial dysfunction with loss of mucosal barrier function is a common concomitant event in septic shock and probably plays a central part in perpetuating systemic immune dysfunction in patients with sepsis.[206] Epithelial membranes throughout the body have an essential role in host survival. Epithelial cells segregate the host tissues from the external environment, are polarised with an apical surface and a basolateral surface, maintain a semipermeable membrane that regulates solute, water, and macromolecule flow, and interact with an endogenous microbiota now referred to as the resident microbiome.[207] The microbiome of the gut is the best known and studied but similar, although less complex, microbiomes are now evident in the respiratory tract, oropharynx, skin, and genitourinary tract.[208–210] The presence of the microbiome is important in normal health for epithelial structure and physiology. The microbiome of the gut alone contains 10^{14} cells: thousands of different species of bacteria, viruses, archaea, fungi, and protozoan parasites, and in its entirety, expresses 100 times more genes than the human host itself. Changes in the microbiome occur in critical illness and might change host responses and ultimate outcome. Attempts to regulate and re-establish a healthy microbiome are now underway as a novel treatment strategy in sepsis and persistent critical illness.[211,212]

Another therapeutic option is to repair damaged epithelial cells or reconfigure the structure and function of junctions between adjacent epithelial cells. In the alimentary tract epithelial cells are held together by intracellular peri-junctional actin networks, and highly regulated, dynamic, transmembrane structures, including occludin, ZO-1, and claudin.[206] Specific cytokines and growth factors can limit epithelial apoptosis, promote growth and repair, and strengthen epithelial tight junctions. Interleukin 11,[213] interleukin 22,[214] granulocyte-macrophage colony stimulating factor,[215] hepatocyte growth factor,[216] and regulators of TNF actions are potential targets for treatments under preclinical and clinical development. Insulin-like growth factor-1 is another promising treatment strategy to preserve epithelial barrier function in sepsis or septic shock.[217] A selected list of new epithelial barrier defensive strategies in the supportive care of the patient with sepsis is summarised in table 4.[213–222]

The vascular endothelium and haemostasis as a target for sepsis therapeutics

Endothelial membranes regulate inflammation and coagulation in the microcirculation in sepsis. Dysfunction of the endothelium disturbs the haemostatic balance in sepsis and contributes to the four major pathogenic pathways associated with coagulopathy in sepsis: tissue factor (TF)-mediated thrombin generation, dysfunctional anticoagulation, impaired fibrinolysis, and generalised platelet activation.[223] TF (also known as F3, thromoplastin, or coagulation factor III) is the main initiator of coagulation activation in sepsis. Activated monocytes and macrophages are major sources of TF in severe sepsis; however, endothelial cells also participate since activated endothelial cells exposed to TNF or interleukin-1β express TF at their cell surface. TF binds circulating factor VII and this complex activates factor X, stimulating generation of thrombin and fibrin.[224] TF can reside in microparticles shed from haemopoietic and endothelial cells. Microparticles have been implicated in activation of both coagulation and inflammation in sepsis.[225,226]

Coagulation is regulated by three main anticoagulant mechanisms: TF pathway inhibitor, APC, and antithrombin. In sepsis, endothelial cells can interfere with each pathway.[227] TF pathway inhibitor function is inhibited in sepsis by the reduced synthesis and degradation of glycosaminoglycans on the surface of activated endothelial cells. Likewise, antithrombin function is further compromised by the decreased production of glycosaminoglycans on the endothelial surface. Resting endothelial cells generate APC on the cell surface, produce tissue-type plasminogen activator to stimulate fibrinolysis,

and impede thrombin formation and platelet adhesion.[224] APC is formed from protein C when thrombin binds to the thrombomodulin receptor present on capillary endothelial cells and proceeds to proteolytically inactivate the acceleration factors of coagulation factors Va and VIIIa.[227] During sepsis, endothelial cell expression of thrombomodulin is impaired, restricting APC generation from circulating protein C. APC promotes fibrinolysis by inhibiting plasminogen activator inhibitor-1. Failure to generate APC on endothelial surfaces impairs fibrinolysis, leading to uncontrolled clotting and disseminated intravascular coagulation.

Platelet adhesion to activated endothelial cells is increased in sepsis by alterations in von Willebrand factor. Monomers of von Willebrand factor can be linked by disulphide bonds to form ultra-large multimers. Final assembly of von Willebrand factor multimers occurs on endothelial surfaces. In sepsis, large multimers of von Willebrand factor occur, which bind avidly to platelet glycoprotein Ibα under shear force stress.[224] These large von Willebrand factor multimers are normally cleaved by a protease termed a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13). Sepsis induces an acquired deficiency of ADAMTS13, resulting in ultra-large von Willebrand factor multimers and platelet adhesion to injured endothelium.[228] Expression of P-selectin on the platelet membrane mediates the adherence of platelets to leucocytes and endothelial cells, and further amplifies the expression of TF on monocytes.[229]

Thrombin and other circulating serine proteases can cleave a set of ubiquitously expressed, seven-trans-membrane receptors known as protease-activated receptors (PARs).[230] Four PARs are found in human beings and can either disrupt or protect endothelial barrier function, depending on which G-protein-linked intracellular signalling pathway is activated. Thrombin binds to PAR1, which is expressed on endothelial cells in the early phase of sepsis, contributes to endothelial dysfunction by Rho-dependent cytoskeletal derangements in endothelial cells, and induces endothelial cell contraction and rounding.[230,231] Endothelial cell contraction destabilises cell-to-cell contacts, causing an increase in vascular permeability, which facilitates the passage of large molecules (albumin and other plasma proteins) and leucocytes from the blood into the subendothelial compartment. Gaps in the endothelial barrier also expose the fluid compartment of blood to the basement membrane and vessel adventitia, which have abundant TF for clot initiation and collagen fibres for von Willebrand factor to polymerise and bind platelets, resulting in clotting.

After thrombin is linked to PAR1, thrombin-PAR1 complexes can transactivate PAR2 over time into a PAR1–PAR2 heterodimer, which has a protective role in endothelial barrier function and survival in sepsis models. This role reversal of PAR1 signalling from endothelial barrier disruption to barrier protection is mediated by PAR1 switching to PAR2, changing from the GTPase RhoA intracellular barrier disruptive pathway to the PAR2-Rac1-mediated intracellular signalling pathway.[232] Rac1 signalling results in actin polymerisation and improved barrier function.[233] This dual signalling system might be amenable to therapeutic intervention with drugs that target specific PARs. A study[234] published in 2011 reported that matrix metalloproteinase-1 (MMP1) cleaves PAR1 on endothelial cells thereby inducing endothelial barrier disruption. MMP1 activates PAR1 at a novel D_{39}–P_{40} site rather than the thrombin cleavage site at position R_{41}–S_{42}. MMP1 cleavage of PAR1 leads only to RhoA-mediated, endothelial barrier breakdown with no intracellular RAC1 signal switching.[235]

Endothelial barrier function prevents vascular leak, maintains an anticoagulant surface, and restricts movement of macromolecules (albumin, lipoproteins, immunoglobulins) out of the blood compartment. Cell–cell junctions include adherens junctions, mainly composed of vascular endothelial-cadherin, and tight junctions (the zona occludens), predominantly consisting of occludins and claudins.[233,236] The intercellular gaps are closed by intracellular actin filament networks and further stitched together by the transmembrane Robo4-slit proteins. Several therapeutic drugs that specifically target endothelial barrier dysfunction in sepsis are now in preclinical and clinical development (table 5).[35,237–246]

	Mechanism of action	Developmental status
C'5a inhibitors	Prevents excess endothelial permeability and apoptosis	Extensive preclinical and early clinical trials[237]
Anti-HMGB1 monoclonal antibodies	Prevents HMGB1-mediated loss of endothelial barrier function and restricts cytokine generation	Preclinical studies[238–240]
Fibrinopeptide $B\beta_{15-42}$	Fibrin split product that binds VE-cadherin and stabilises endothelial junctions	Phase 2 clinical trials[241,242]
Pepducins	Lipidated peptides activate PAR2-induced Rac-mediated barrier endothelial membrane stabilisation	Preclinical studies and early clinical studies[243,244]
VEGF receptor monoclonal antibodies	Prevents loss of VE-cadherin from endothelial junctions	Early clinical trials[245]
Recombinant human thrombomodulin	Prevents endothelial apoptosis; carboxypeptidase activity against C5a restricts neutrophil activation	Available in Japan, in phase 3 clinical trials[35]
Selepressin	Vasoconstrictor that binds to V1A receptors and restricts permeability	In phase 2 clinical trials of septic shock[246]

Rac is an intracellular signalling molecule from a subfamily of GTPases. C'=complement. HMGB1=high-mobility group box 1. VE=vascular endothelium. PAR2=protease-activated receptor 2. VEGF=vascular endothelial growth factor. V1A=vasopressin 1A receptor.

Table 5: **New treatment options to prevent or treat sepsis at the endothelial level**

Immune competence, immune suppression, and immune reconstitution as a target for sepsis therapeutics

Sepsis-induced immune dysfunction and evidence of immune depression in severe sepsis and persistent critical illness are increasingly being recognised in many studies and are now reaching the stage for formal clinical

	Mechanism of action	Developmental status
Interleukin 7	Promotes T-cell and B-cell differentiation and growth, inhibits apoptosis, and maintains TCR diversity	Preclinical investigations; early clinical trials in oncology[258]
Interleukin 15	Promotes T-cell growth and inhibits lymphocyte apoptosis	Preclinical investigations in sepsis; early clinical trials in oncology[259]
Monoclonal antibodies to PD1 or PD Ligand	Inhibits co-inhibitory signals at the T cell–APC level, maintains T-cell function, and is anti-apoptotic	Preclinical investigations in sepsis; early clinical trials in oncology[246]
Monoclonal antibodies to B-lymphocyte and T-lymphocyte attenuator	Inhibits co-inhibitory signals at the T cell–APC level, maintains T-cell and B-cell function, and is anti-apoptotic	Preclinical investigations in sepsis[260]
IMT 504 and other immunostimulatory oligodeoxynucleotides	Immunostimulatory nucleotides can activate T and B cells and promote mesenchymal stem-cell generation	Preclinical investigations; pilot studies in human beings[261]
Epigenetic regulators, RNAsi, and miRNA regulators	Alter transcription and translation of acute inflammatory cytokines and mediators	Preclinical studies in sepsis; clinical trials in oncology indication[262–264]
Interleukin-1 receptor antagonist	Blocks interleukin-1-driven macrophage activation syndrome (haemophagocytic syndromes)	In phase 2 clinical trials[254]
Vagal nerve stimulation and α 7 nicotinic agonists	Induces the cholinergic anti-inflammatory reflex, reduces cytokines and HMGB1 levels, and stabilises mitochondrial membranes	Preclinical studies[265,266]
AB103 peptide	Synthetic peptide that binds and inhibits CD28 interactions with APCs, limiting T-cell activation from bacterial superantigens	Preclinical and early phase 2 testing in necrotising soft tissue infections[256,257]

TCR=T-cell receptor. PD1=programmed cell death. APC=antigen-presenting cell. RNAsi=small inhibitory RNA. miRNA=micro RNA. HMGB1=high mobility group box 1.

Table 6: **Potential new treatment options to treat sepsis-induced immunosuppression or restrict selected immune activation states**

assessment as suitable targets for intervention.[247–249] Loss of adaptive immune function and downregulation of the host innate immune response have been well described and can cause adverse outcomes.[247] Expression of major histocompatibility complex class II antigens is substantially diminished in sepsis, as are the proliferative responses of lymphocytes, their functional capabilities, and their capacity to resist apoptosis.[248] Opportunistic viral infections are reactivated in most patients with severe sepsis,[250] in a way that is reminiscent of the occurrence of severe viral infections in organ transplant recipients.[251] These infections can cause clinically significant infections such as disseminated cytomegalovirus infection and herpes simplex infection. Opportunistic bacterial and fungal infections are substantially more frequent in patients with sepsis than in healthy individuals, with evidence of reactivation of several herpes viruses.[250] A crucial question is whether sepsis-induced immune suppression is a necessary compensatory defence mechanism to regulate the systemic septic host response, or a pathological state of immune suppression that increases the risk of opportunistic infections.[252] This question can only be answered by carefully controlled clinical trials in well characterised patient populations.[253]

In some clinical situations, targeted inhibitors of inflammatory cytokines might still be useful; one example is the role of interleukin 1 inhibitors in the macrophage activation syndrome (part of the continuum with the acquired haemophagocytic lymphohistiocytosis syndromes).[254,255] Additionally, in toxic shock syndromes and necrotising soft tissue infections, short peptide inhibitors of the CD28 homodimer interface between antigen-presenting cells and T lymphocytes look promising as drug targets and are in clinical trials.[256,257] A summary of novel immune modulation strategies to treat sepsis is found in table 6.[258–266]

The role of high-mobility group box 1 as a target for therapy in sepsis

High-mobility group box 1 (HMGB1) is a 30 kDa DNA-binding protein that is secreted from activated haemopoietic cells, and released from cells undergoing necrosis or necroptosis.[267,268] Three distinct isoforms exist that specifically interact and signal via distinct receptors: disulphide HMGB1, which binds and signals via myeloid differentiation 2 TLR4; thiol HMGB1, which binds and signals via the chemokine receptor CXCR4; and oxidised HMGB1, which is inert.[269] Analogous to Koch's postulates about the role of bacteria in disease, HMGB1 is a candidate therapeutic target because it is ubiquitously present in animals and human beings with sepsis, with increased blood concentrations reported to persist for weeks after disease clearance, administration or overexpression of HMGB1 in healthy animals produces multiple organ dysfunction that is similar to sepsis, and administration of HMGB1 monoclonal antibodies substantially improves organ function and prevents death in animal models of sepsis.[270] Engraftment of human haemopoietic stem cells in NOD/SCID/IL2Rγ$^{-/-}$ (non-obese diabetic, severe combined immunodeficiency, interleukin 2Rγ$^{-/-}$) mice without immune systems produces human lymphocyte apoptosis, cytokine storm, and death—which can be prevented by selectively blocking HMGB1.[271] This study[271] provides the only evidence directly suggesting that a human cytokine is necessary and sufficient to induce sepsis syndrome in mice.

HMGB1 is a key signalling molecule in human sepsis because it affects a myriad of host tissues for sustained periods during systemic inflammation. HMGB1 disrupts endothelial barriers, alters the actin filament cytoskeleton, impairs tight junctions, and promotes the release of large quantities of interleukin 1α and an array of other cytokines and chemokines. HMGB1 stimulates expression of cell surface adhesion components such as ICAM1 and VCAM1 on endothelial membranes.[238–240] HMGB1 induces major defects in gut epithelial barrier function and induces mucosal hyperpermeability in shock states.[222]

Anti-HMGB1 monoclonal antibodies are the leading experimental treatment in preclinical development. In experimental models, these antibodies have been used to prevent the onset of sepsis, reverse the organ damage during sustained or established sepsis, and, when given to survivors of sepsis, reverse cognitive impairments.[272] HMGB1 contains two DNA-binding domains, termed the A and B boxes. Truncated recombinant A box proteins are in development as therapeutic reagents. The anti-HMGB1 activity of A box was originally described in isolated macrophage systems in vitro, but in-vivo administration of recombinant A box substantially attenuates HMGB1 activity and prevents organ damage and lethality in sepsis.[273] The molecular mechanism of A box has been attributed to the protein competitively antagonising HMGB1 action on its cognate receptors. However, theoretically, A box might be interacting with as yet unidentified anti-inflammatory receptors to confer protection against HMGB1 in sepsis.

Two other anti-HMGB1-based experimental strategies that might be effective in sepsis include soluble receptor of advanced glycated end products (RAGE) and HMGB-binding, non-immunogenic oligodeoxynucleotides. RAGE has been implicated as an HMGB1-binding protein expressed on the surface of haemopoietic and other cells. Although the role of soluble RAGE in signal transduction is undefined, it is known to bind HMGB1 and sequester the DNA-binding protein from mediating signals via TLR4. Administration of soluble RAGE in animal models of sepsis can effectively prevent organ damage and lethality.[274] Oligodeoxynucleotides have been developed as HMGB1-binding molecules that substantially inhibit HMGB1-mediated activation, via intracellular TLR7 and TLR9, of innate immune responses. Administration of non-immunogenic oligo-deoxynucleotides to HMGB1 is protective in mouse models of sepsis and autoimmunity.[275]

Another strategy targeting HMGB1 is to suppress its release, and several experimental drugs are in study and development. The mechanisms of HMGB1 release require a two-step process because it is a nuclear protein devoid of a leader sequence to guide secretion. The first step begins with HMGB1 translocating from the nucleus and accumulating in the cytosol. This step is mediated by JAK-STAT-dependent signalling, which activates HMGB1 acetylation in lysine residues located in the nuclear localisation sequence.[276] The acetylated HMGB1 cannot re-enter the nucleus and therefore accumulates in the cytosol. Inhibition of JAK-STAT signalling prevents this step; researchers are investigating administration of JAK-STAT as a therapy for sepsis.[277] The second step of HMGB1 release from activated haemopoietic cells is extracellular release—a step that requires activation of double-stranded RNA-dependent protein kinase (PKR) and the inflammasome, which mediates secretion of leaderless cytokines, including interleukin 1, interleukin 18, and HMGB1. Inhibition of PKR prevents inflammasome activity, inhibits the release of HMGB1, and confers protection against sepsis in mouse models.[278]

Mitochondrial permeability is pathologically increased in cells isolated from animal models of sepsis, resulting in cytosolic accumulation of mitochondrial DNA.[279] This mitochondrial DNA binds to and activates the NLRP3 inflammasomes, mediating secretion of HMGB1. Early work had implicated signal transduction via α7 nicotinic acetylcholine receptors (α7 nAChR) in suppression of HMGB1 to effectively prevent organ damage and lethality in murine models of sepsis.[280] Selective α7 nAChR agonists are under study as therapeutic drugs for sepsis, an approach that has come under increased interest. The α7 nAChR is expressed on the mitochondrial membrane of haemopoietic cells. During inflammatory responses, the activated cells accumulate acetylcholine and other α7 nAChR agonists in their cytosol. These agonists interact with α7 nAChR expressed in mitochondria, stabilising the membrane permeability, decreasing cytosolic mtDNA, and inhibiting inflammasome activation.[280] These results are very interesting in the context of sepsis, because benefit might be derived from both the inhibition of HMGB1 release and the reversal of the defective mitochondrial function. Researchers are also developing purinergic receptor antagonists to inhibit receptor stimulation in response to extracellular ATP released by neutrophils or by necrotic and necroptotic cells.[281]

So-called bioelectronic medicine is a new specialty defined by the convergence of molecular medicine, neurophysiology, and biomedical engineering to develop devices to target inflammatory and metabolic diseases.[282–284] For example, recent clinical trials over the past 5 years using a vagus nerve stimulating device to activate the so-called inflammatory reflex successfully inhibited TNF and improved clinical outcomes in patients with rheumatoid arthritis. This success has launched additional efforts to develop bioelectronic medical therapy for other autoinflammatory diseases, including inflammatory bowel disease, irritable bowel syndrome, diabetes, and cancer. Preclinical studies of vagus nerve stimulation in murine sepsis models have been successful in the prevention of organ damage and lethality. The mechanism of action targets action potentials that travel via the vagus nerve to the coeliac ganglion, the origin of the splenic nerve. Activation of

	Mechanism of action	Developmental status
Specific bacterial vaccines	Promote antimicrobial clearance of common pathogens (*Meningococcal* spp, *Pneumococcal* spp, and *Haemophilus* spp)	In standard clinical practice to decrease bloodstream infection[286,287]
Vaccine against bacterial lipopolysaccharide	Prevents endotoxin-mediated activation of the host immune response	Preclinical investigations[288]
Vaccines against streptococcal or staphylococcal superantigens	Attenuates superantigen-induced activation of CD4+ T lymphocytes and antigen-presenting cells	Preclinical investigations[289]
Recombinant gelsolin	Protein that clears extracellular actin filaments, has immunomodulatory activity, and binds bacterial toxins	Early clinical trials in pneumonia and sepsis[290]
Polymyxin B perfusion columns, other blood purification strategies	Clears bacterial lipopolysaccharide, inflammatory cytokines, HMGB1, and other inflammatory mediators	In phase 3 clinical trials[291]
Immunonutrition strategies	Can induce inhibitory phenotype of macrophages and lymphocytes	Preclinical investigations[292]
Mitochondrial-sparing drugs	Improves cellular energetics and restricts apoptosis	Preclinical investigations[22,268]
Monoclonal antibodies to bacterial virulence factors and to common multidrug-resistant pathogens	Block bacterial virulence factors from invasive pathogens and promote immune clearance of pathogens	Early clinical trials[286,293]
Anti-HMGB1 monoclonal antibody	Prevents HMGB-1 mediated inflammatory effects and endothelial barrier breakdown	Preclinical investigations[268]
Soluble TREM-like transcript-1	Blocks TREM-1 signalling on innate immune cells, restricting leucocyte activation in sepsis	Preclinical trials[294]
Protease inhibitors; monoclonal antibody to PCSK9	Block proprotein convertases that activate endogenous proteases or impair lipopolysaccharide clearance by the LDL receptor	Preclinical studies and observational studies in human beings[295,296]
New formulations of intravenous immunoglobulins	IgM concentrates that have immune-modulatory effects, bacterial clearance, and C' clearance	Early clinical trials in pneumonia and sepsis[297]
Proresolving drugs	Lipooxygenase-derived lipidated mediators that promote resolution of inflammation, tissue repair, and clearance of damaged immune cells	Preclinical investigations[298]
Low-dose corticosteroids	Anti-inflammatory effects and reduce the synthesis of acute phase proteins	Large phase 3 trials[299]
β blockers for septic shock	Cardiac rate control during haemodynamic monitoring for myocardial protection	Phase 2 testing[300]
Orally administered protease inhibitors	Prevention of pancreatic enzyme-mediated gut luminal injury, resulting in increased intestinal permeability	In phase 2 trials[301]
Thymosin α 1	Short peptide that is a T-cell adjuvant and immunostimulant for sepsis-induced immunosuppression	Phase 2 clinical trials[302]
Mesenchymal stem cell therapy	Cells traffic to sites of injury and promote anti-inflammatory effects, and tissue repair by paracrine secretion of soluble factors	Phase 2 testing in other indications;[303] pilot studies in patients with acute respiratory distress syndrome and sepsis[304]

HMGB1=high mobility group box 1. TREM=triggering receptor expressed on myeloid cells. PCSK9=proprotein convertase subtilisin kexin type 9. IgM=immunoglobulin M. C'=complement.

***Table 7*: Other potential new treatment options to prevent or treat sepsis**

splenic neurons transmits signals to β2 adrenergic receptors expressed by a T-cell subset, which express choline acetyltransferase, the rate-limiting enzyme in acetylcholine biosynthesis.[285] Stimulated release of acetylcholine by these T cells culminates in signal transduction by interaction with α7 nAChR expressed in splenic macrophages. This transduction inhibits the release of mitochondrial DNA to attenuate inflammasome activation, and inhibits the nuclear translocation of NFκB. Preclinical studies investigating nerve stimulating devices to treat sepsis are in progress in several laboratories at present.

Other promising new therapeutic approaches to sepsis

In addition to HMGB1 inhibitors,[268] a substantial number of new strategies to treat sepsis are in various stages of preclinical and clinical development (table 7). These strategies include new vaccines[286,287] and monoclonal antibodies against common microbial pathogens,[287] microbial toxins,[288] virulence factors and superantigens,[289] gelsolin,[290] blood purification strategies,[291] mitochrondrial-sparing drugs,[268] immunometabolism approaches,[292] and other innovative strategies.[293,294] Specific protease inhibitors,[295,296] reformulated immunoglobulin preparations,[297] proresolving drugs,[298] and adjuvant therapies might also prove useful for the treatment of sepsis. Repurposed drugs with endothelial sparing and immunomodulation effects are in development for sepsis, including statins, angiotensin-converting enzyme inhibitors, angiotensin receptor-blocking drugs, old antibiotics, and APC derivatives.[201,223,224,305,306] The practical value of corticosteroids as a treatment for sepsis has been debated for decades and is now being put to the test in a large, phase 3, prospective trial[299] including nearly 4000 randomly assigned patients in Australia and New Zealand (the ADRENAL trial). β adrenergic blocking drugs might prove to be useful for heart-rate control and improved outcomes in a highly selected group of patients with septic shock. This innovative approach for haemodynamic support in septic shock is worthy of future investigation.[300] Oral administration of protease inhibitors such as tranexamic acid to protect the gut from protease-mediated excess permeability is now in clinical trials.[301] A short peptide thymic growth factor known as thymosin α1 showed promise for use in sepsis in a phase 2 trial in China published in 2013 and merits further clinical investigation.[302] The details and present status of a selection of other strategies for the treatment of sepsis that are in various stages of preclinical and clinical development are summarised in table 7.

Conclusions for new therapeutic interventions

Recovery and maintenance of epithelial and endothelial barriers are critical to survival in sepsis. Drugs are now in development that promote cell survival by targeting mitochondria and cellular energetics.[22,268,292] Novel interventions directed at re-establishing barrier function by the epithelium and endothelium are needed in sepsis research at this time when antibiotics are failing and recent clinical trials in sepsis have been disappointing.[22] We anticipate that intelligent use of these drugs with appropriate biomarker-driven trials using real-time

genomics and computational biology will succeed and bring a new generation of adjuvant therapies to benefit patients in septic shock.[36,304,307]

Breaking the mould

Failure of clinical sepsis trials

As we have noted earlier, although the outcome of patients with sepsis has improved substantially in recent years,[2,51] this improvement in outcome has not been accomplished by any of the many adjuvant therapies tested in more than 100 phase 2 and phase 3 clinical sepsis trials.[308,309] Rather, the improved sepsis prognosis is probably mainly caused by an increased recognition and faster intervention by health-care services for cases of sepsis than was previously achieved, possibly due to publication and adoption of the SSC guidelines[310,311] and improved care in the ICU in general.[2] Understandably, enthusiasm for the clinical development of innovative sepsis drugs seems to have diminished. This loss of enthusiasm poses a serious threat to further progress in the understanding of sepsis pathophysiology and eventually the care of patients with severe infections. Therefore, alternative approaches, encompassing both preclinical research and clinical trial design, are needed to move sepsis knowledge forward.

The reasons for the failure of so many clinical sepsis trials have been noted elsewhere in this Commission and discussed widely in the scientific literature.[36,308,309] Many drugs have tested very favourably in preclinical sepsis models, and sometimes even in early clinical studies. Several mutually non-exclusive explanations for the discrepant results obtained in these investigations and in phase 2 or 3 clinical sepsis trials have been suggested. Sepsis has long been thought to be the result of a hyperinflammatory reaction of the host to invading bacteria. This assumption was based on a large series of animal studies, in which either bacterial components, such as lipopolysaccharide, or viable bacteria were given intravenously at high doses, causing fulminant septic shock.[312] More than 100 different interventions have been shown to provide a survival benefit in animals exposed to lipopolysaccharide, but none of these have reached the status of registered sepsis drug.[309] Notably, such a hyperacute clinical presentation rarely occurs in patients with sepsis; more often, patients present with subacute disease associated with organ failure that develops more gradually over the course of days. Nonetheless, the reductionist model of acute inflammation induced by bolus injection of lipopolysaccharide, although not deemed a clinically relevant sepsis model, has been useful for dissection of mechanisms by which host mediator systems become activated. In healthy human beings, intravenous administration of low-dose lipopolysaccharide produced a global reprioritisation, affecting more than 80% of the cellular functions and pathways—as suggested by changes in whole genome transcriptional profiles in blood leucocytes[313,314]—and the response detected showed a resemblance to that reported in patients with sepsis or other types of acute injury, such as trauma.[21,314]

If we have learned one thing from the large body of preclinical research on the pathogenesis of sepsis and intervention studies in experimental models, it is that the host response to severe infection is very complex. In accordance, detailed observational studies in patients have revealed that the host response to sepsis entails concurrent proinflammatory and anti-inflammatory reactions, the extent and direction of which depend on the causative pathogen (load and virulence) and the host (genetic composition and comorbidity), with differential responses at local, regional, and systemic levels.[27] Preclinical research should adequately incorporate present knowledge of the pathogenesis of sepsis. The preclinical testing of experimental sepsis drugs should be expanded to investigate aged animals with comorbidity, thereby better resembling typical patients with sepsis. Additionally, while many different animal sepsis models have been used, including caecal ligation and puncture to induce faecal peritonitis, and instillation of live bacteria via the airways to induce pneumonia, careful comparison of responses across different infectious challenges is not generally done, and supportive care, including antibiotic therapy, is not usually included.[312] These omissions are not trivial, since specific host response pathways are essential for a protective innate immune response against invading pathogens, but in the context of overwhelming infection, such as that seen in patients admitted to the ICU (and thus given antibiotics and invasive supportive care), these host response pathways can contribute to organ damage.[315] A useful approach to sepsis drug research would be to monitor the effects and mechanism of action of new sepsis drugs in different animal models, ideally with biomarkers that could also be used in subsequent human studies. In view of the complexity of the host response to acute injury, an intervention targeting one host mediator or even one response pathway and providing benefit to a heterogeneous population of patients with severe sepsis is difficult to envision.

Sepsis research is troubled by the absence of accurate diagnostic methods that are able to stratify patients into biologically homogeneous subgroups. Sepsis trials almost invariably have used inclusion criteria based on the so-called Bone criteria[316] for sepsis—ie, the suspected presence of an infection together with at least two of four systemic inflammatory response syndrome criteria, including changes in body temperature, heart rate, respiration rate, and leucocyte counts. According to the Bone criteria,[316] severe sepsis is defined as sepsis complicated by acute organ dysfunction, and septic shock as sepsis complicated by hypotension refractory to fluid resuscitation. These diagnostic criteria are now widely deemed to have not been useful in the identification of individual patients or patient groups with sepsis that

might benefit from a specific intervention—ie, the Bone criteria of sepsis do not capture a biochemically or immunologically homogeneous population of patients—although the criteria are possibly helpful at the bedside for clinical decision making.[36,309] However, components of the host response to sepsis are not specific for infections; many host systems and pathways can be triggered not only by pathogens, but also by endogenous danger molecules named alarmins or damage-associated molecular patterns.[22,317] This non-pathogen-triggered response has led to the new idea that the derailed host response in sepsis is not fundamentally different from that seen in patients with non-infectious critical illness. These patients with non-infectious critical illness, who might benefit from specific immune modulatory therapies, have been systemically excluded from clinical sepsis trials because they are not infected.

Ample evidence exists to suggest that the so-called one size fits all approach in sepsis trials—ie, one intervention tested in a very heterogeneous group of patients with sepsis defined by clinical criteria—is part of the reason why so many trials have not shown clinical benefit. The absence of unequivocal criteria to enrol patients based on specific biochemical or immunological features restricts generalisability across trials and the ability to identify patients most likely to benefit from a specific therapy. For example, early trials examining the effect of lipopolysaccharide neutralisation reported increased mortality in patients with Gram-positive infections.[318] Additionally, although none of the 16 different trials investigating the efficacy of anti-TNF showed a survival benefit in the overall population of patients with sepsis,[319,320] evidence suggests that specific subpopulations might be harmed by TNF inhibition,[320,321] thereby diluting a potential advantage in other patients. This interpretation is supported by results obtained in animal models of sepsis, wherein anti-TNF drugs improved outcome in some infections, but was associated with increased lethality in others.[322]

Some sepsis drugs might have failed in clinical trials because they were not (fully) active or because of inadequate dosing, or both. For example, a monoclonal antibody directed against lipopolysaccharide that had been tested in clinical trials proved not able to neutralise lipopolysaccharide,[323] and a monoclonal antibody directed against TNF did not seem to be capable of neutralising TNF activity in patients with sepsis.[324] Additionally, researchers have debated about whether or not the very positive results of recombinant human interleukin-1 receptor antagonist in a phase 2 sepsis trial[325] could not be reproduced in phase 3 studies because of changes in the biological activity of the drug.[326,327] Dosing of novel sepsis drugs is another area of concern, since it is based mainly on animal data and restricted pharmacokinetic studies in patients. These pharmacokinetic studies mainly use traditional biochemical metrics, whereas a more detailed understanding of the immunomodulating effects of the drug along its presumed mechanism of action, preferentially in different body compartments, would provide much more meaningful information. In this respect, an important realisation is that clear and easy to measure read-outs for biological activity of experimental sepsis drugs are scarcely available, and reports of analyses of host response pathways targeted by these compounds in patients are insufficient. Generally speaking, biomarkers that can assist in the monitoring of a drug's effect on the purported target pathway do not exist. As such, many of the sepsis treatments assessed in clinical trials are given with restricted knowledge of whether the intervention affected the host systems it intended to target.

Alternative trial design

Patient heterogeneity is a hallmark feature of populations enrolled in sepsis clinical trials. Several strategies have been proposed to overcome this important drawback, such as the design of very large trials in tens of thousands of patients, the performance of small trials in patients with organ-specific or pathogen-specific infections, and parallel trials with identical study protocols in different regions of the world.[36] At present, these alternative strategies are difficult to implement for several reasons, including insufficient funding and an absence of sensitive and rapid tests to detect causative pathogens. Adaptive trial design has been suggested as an alternative approach for clinical sepsis trials.[328] With this design, several treatment groups can be run simultaneously and the effect of a new drug is assessed early in the trial. The initial results obtained can be used to modify the trial in accordance with those findings. Modifications could include the type and dose of the drug, the sample size, and patient selection criteria. The aim of an adaptive trial design is to find more quickly than conventional trial designs interventions with therapeutic efficacy and to focus on patient groups for which the drug is effective. Thus, ideally adaptive trial design can lead to early cessation of non-effective drugs, while different dosing regimens for potentially effective drugs can be assessed concurrently. The first computer simulation of a phase 2 sepsis trial using an adaptive trial design was published in 2013.[329] Future sepsis trials could test adaptive trial designs to establish whether these trials advance therapeutic sepsis research. However, the heterogeneity in patients with sepsis remains a concern even in these alternative trial designs.

Most phase 3 sepsis trials used data from small phase 2 trials for definition of inclusion criteria and drug dosing. Usually driven by a non-significant trend towards improved survival in a phase 2 study, sponsors and investigators have been reluctant to change the phase 3 trial design. In view of the many negative phase 3 studies, alternative strategies could be implemented. For example, a study could have several early trials with different designs to guide the optimum design for a large phase 3

trial. These early trials could also be used to assess potential biomarkers that provide insight into the biological activity of the drug under investigation. Generally, larger phase 2 studies will probably result in a lower risk of studying inactive treatments in phase 3 studies than smaller phase 2 studies might.

Most published sepsis trials used 28 day mortality as a primary endpoint. However, this arbitrarily set time period might not be the best measure of success, since many patients are still in the hospital at day 28 and important outcome measures could be missed. For example, tight glucose control in critically ill patients did not affect 28 day mortality but was associated with increased lethality at 90 days,[10] whereas the harmful effects of fluid resuscitation by a low-molecular-weight hydroxyethyl starch in patients with sepsis only became apparent beyond 28 days.[330] With the diminishing case-fatality rate of sepsis,[2,51] larger studies than those done so far are needed to show statistically significant improvements in short-term (up to 90 days) survival. The need to enrol additional patients will lead to the participation of additional clinical sites, which is expected to increase heterogeneity in care. Although this increased heterogeneity could improve the generalisability of trial results, it could also jeopardise the goal to reproduce phase 2 results. More importantly, short-term mortality might not be the optimum endpoint for sepsis trials. Survivors of sepsis have a substantially reduced quality of life and long-term sequelae, including worsening of chronic comorbidities, and cognitive and physical impairments.[331] Additionally, survivors of sepsis are at risk of early death after hospital discharge, with 5 year mortality as high as 75%.[121] Therefore, consideration of late endpoints that include physical and cognitive disabilities might be more patient-centred than endpoints measured earlier in a trial's progress. Since modern intensive care management has reduced early sepsis mortality quite successfully, now might be the time to at least partly change the focus for drug development in sepsis to interventions that seek to affect the late, rather than the early, sequelae of sepsis.

The role of regulatory agencies

So far, regulatory agencies such as the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) have taken a conventional approach to the implementation of clinical trials in severe sepsis or septic shock and have favoured a frequentist-probability-based investigational model. For registration purposes and after exploratory phase 2 dose-finding studies, two adequately designed, well controlled, confirmative (phase 3) trials would generally be needed to establish the efficacy of a novel therapeutic drug in the treatment of severe sepsis or septic shock (one controlled study with statistically compelling and clinically relevant results would constitute the minimum requirement). The absence of any licensed active comparator necessitates that the trials be done with a superiority testing design, comparing the investigational product to placebo when added to best standard of clinical care.

In the investigation of patients with acute, life-threatening illnesses such as severe sepsis or septic shock, the standard primary endpoint of these trials is usually defined as the 28 day all-cause mortality.[332] However, in view of the earlier cited limitations in the implementation of such studies,[36,308,309]—ie, uncertainties surrounding the host response phase of the enrolled individuals, insufficient precision in sepsis definitions, absence of predictive biomarkers, and the fact that multicentre trials now need to include a large number of patients to allow sufficient statistical power to detect a significant difference in outcome between treatment groups—regulators appreciate the need to instead consider alternative investigational models that are expected to pose fewer burdens to clinical development, albeit without lowering scientific and regulatory standards, could be worthwhile. For example, compared with a conventional study design, adaptive design models—if carefully planned—are generally accepted to have the potential to offer study information in a more efficient way, sparing useful resources; increase the chance of success of the study objective; and be more informative about the treatment effects, which in turn could lead to more efficient designs for subsequent studies.[333]

One of the regulators' concerns in the use of alternative investigational methods, such as the Bayesian model, is related to the fact that the integrity of the data could be affected by the introduction of potential bias. With the implementation of the adaptive design method, interim analysis of efficacy endpoints, which could result in early termination of the study because of insufficient efficacy or a change in design for the remainder of the study, needs careful prespecification, with appropriate methods to control the overall study-wide type I error, thus minimising the probability of incorrect rejection of the true null hypothesis. To reduce potential bias, and in accordance with the International Conference on Harmonisation guidelines,[334] establishment of an independent data monitoring committee and an independent statistical data analysis centre are important prerequisites. Additionally, to reduce potential bias, any approach to adaptive design should be consistent with guidance issued by both FDA and EMA, providing details of the proposed study design, decision criteria, and sample size considerations.[333,335]

Some of these aspects of trial modification remain controversial because they increase the probability of changes to the trial's integrity (eg, modification of a primary endpoint), but other aspects such as blinded sample-size re-estimation and starting, stopping, and continuing rule adaptations are more readily acceptable from the regulatory viewpoint. Additionally, trial adaptations are less of a concern during the early phases

(phase 1 and 2 trials) of the clinical development than phase 3 trials, since early trials are deemed exploratory. However, for phase 3 trials, which supposedly confirm earlier hypotheses, regulators prefer that changes are preplanned well in advance with clear justification and the number of design modifications restricted. Additionally, seamless transition from a phase 2 to a phase 3 clinical trial has been proposed as a further option,[36] although this approach has been criticised because of the lost opportunity to pause and gather useful insight after analysis of phase 2 trial results.[333,336]

In view of the various challenges implicated in the clinical investigation of new therapies in severe sepsis or septic shock, academia and trial sponsors are, as a rule, advised to take advantage of available development support offered by the main regulatory agencies. This advice seems even more important if innovative methods or technologies are to be explored. In this respect, notably, in a changing and challenging drug discovery environment, drug regulators have increasingly recognised their dual role, not only as gatekeepers of public health—by ensuring patients' access to safe and effective drugs—but also as enablers of pharmaceutical innovation. As such, drug regulators have become part of a multi-stakeholder effort, impartially contributing to innovative drug development.[337] This approach (eg, under the remit of the EMA Innovation Task Force[338]) allows participation of relevant experts at early stages—eg, involving the Biostatistics Working Party in issues regarding novel study designs and analyses methods. These early exchanges could be particularly helpful for developers, informing and preparing them for subsequent scientific advice to be obtained from the regulatory authority. Such advice serves as the main platform for assistance on scientific topics during drug development and has proven to predict increased probability of regulatory success.[339] By obtaining scientific advice offered by the main regulatory agencies in a formal procedural context, study designers can address important questions that could cover a wide range of preclinical and clinical aspects of the development programme—eg, choice of optimum animal models, qualification and use of biomarkers, possible co-enrolment of special populations in the primary trial, selection of an optimum primary endpoint in relation to the phase 3 trial, and inferences to be made from subgroup analyses.[340] Additionally, in view of increasingly complex regulations for drug trial design, regulatory authorities have forged closer international collaborations with each other than they have previously, thus increasingly aligning their requirements for drug approval. As such, the further possibility to obtain so-called parallel scientific advice from the FDA and EMA could allow more streamlined regulatory expectations that can be achieved faster than would be possible without collaboration between these authorities. Early input from health technology assessment bodies could increase the probability that approved drugs get reimbursed and thus ultimately reach patients.

Personalised medicine

The strongly expanding knowledge of the host response to sepsis, and the failure of so many clinical sepsis trials with similar inclusion criteria and design, should prompt investigators to reconsider the (clinical) concept of sepsis and its therapeutic approach. The assumption that the host response in severe sepsis is extreme and should be suppressed is unlikely to be true for many patients; patients with severe sepsis presenting with similar signs and symptoms might express very different disturbances in homoeostasis, varying from inflammation-driven organ injury to profound immune suppression, and mixtures thereof.[27,247] The heterogeneity of sepsis populations is a major factor in the absence of advancement in the establishment of new sepsis therapies. The specialty of oncology has made tremendous progress with the development of well-defined stratification systems that assist in prognostication and identification of subgroups of patients that are likely to benefit from a specific therapy. Medical oncologists make use of specific biomarkers that aid them to choose and monitor targeted therapies. The sepsis specialty is devoid of clinically useful stratification systems, and is in need of biomarkers that show the mechanism of action of a drug and thus can be used for inclusion and monitoring.

Although many different biomarkers have been tested in patients with sepsis, so far none has been proven useful in clinical practice and decision making.[171] Systems biology could provide means to identify sets of biomarkers that can stratify patients with sepsis into subgroups that are more likely to benefit from an intervention.[341] Ideally, such analyses, which could include both RNA molecules and proteins, would not only provide information about the specific host systems or pathways that are disturbed in an individual patient, but also offer the opportunity to monitor the effect of targeted therapies.[342] Computational methods for modelling inflammatory responses have become increasingly advanced and are ready to be used in the clinical arena.[343] Several companies are investing in manufacturing bedside tests for host responses that can deliver results within hours without hands-on involvement of specialised laboratory personnel. The near future will tell whether this approach will advance interventional sepsis research.

Conclusion

Now is the time for innovation in sepsis research and clinical trial design. The many failures of sepsis clinical trials assessing therapies directed at modifying the host response have rightly prompted sepsis researchers to reconsider the relevance of animal models of sepsis for the pathogenesis of this syndrome and for drug development. Alternative inclusion criteria, including those that would capture patients with more homogeneous host response features, and alternative trial designs, including those that would allow more rapid discrimination between effective and non-effective

therapies than the present criteria would greatly enhance the sepsis specialty. In this respect, biomarkers that can assist in the stratification of patients with sepsis into subpopulations that might benefit from a specific intervention and that can help to monitor treatment responses are urgently needed. Sepsis researchers face great challenges in the years ahead to accomplish these new goals.

Sepsis: a call to action

In conceiving and writing this Commission, our intent has been two fold. The first was to describe the present status of what is one of those most challenging medical disorders in routine clinical practice, and the second, to identify those areas in which we think research is most crucial in driving forward transformational change. This second intent, what we have called the roadmap for the future, has informed this call to action (panel 2). We acknowledge that we have intentionally left untouched some aspects of the subject, notably aspects of critical care support and neonatal sepsis. However, we believe that many of the themes we have identified will apply to these areas as well.

***Panel 2*: Sepsis—a call to action**

We call for everyone associated with sepsis care and research—patients, clinicians, academics, public health agencies, industry, and regulators—to

- Agree on a working case definition of sepsis, which should be used in all future clinical trials
- Persuade WHO and its partners to include sepsis in its Global Burden of Disease report, and encourage the establishment of national registries to find out the population-based incidence of the disorder
- Improve training of front-line clinical staff, and develop public awareness campaigns
- Improve access to timely, aggressive, and high-quality supportive care interventions, adapted where needed to low-resource settings
- Increase investment in both basic and translational clinical science, with two specific goals: to identify new treatment targets and strategies, focusing on those that can help patients the world over, including patients with sepsis who live in low-income and middle-income countries; and to develop the application of the principles of personalised medicine to the treatment of sepsis
- Encourage pharmaceutical companies and regulatory agencies to work with the academic community to take a fresh look at clinical trial methods and endpoint requirements to ease exploratory studies of new drugs for sepsis
- Support patient groups and encourage academics to improve their understanding of the physical and psychological effects of sepsis, and to develop new methods of prevention and management of these problems

Defining sepsis and estimating its global burden

Establishment of a robust case definition is the fundamental bedrock of all epidemiological studies, yet the academic sepsis community continues to struggle with this basic prerequisite.[1,26] Issues as apparently simple as whether sepsis necessarily implies the presence of infection have caused debate and disagreement, and more importantly, difficulty in the ability to make accurate comparisons between clinical studies. We believe that the sepsis community needs to make a concerted effort to agree a working case definition for sepsis and (crucially) to then use that definition as the basis for all future clinical trials.

In support of this approach, we make three observations. First, this working case definition of sepsis must not (and need not) in any way impede fundamental research into the pathogenesis of the disorder and should not constrain innovative approaches to thinking about the causes, and ultimately the treatment of the disorder. Second, a rigorous case definition used for epidemiological reasons could be different from a more pragmatic description for routine clinical practice. Researchers broadly agree that early recognition of sepsis is a key to improved outcomes.[3,4,4] Development of a simple bedside algorithm to help the early clinical recognition of the disorder will improve survival; meanwhile, a robust and more complex case definition used in clinical trials will form the basis of new approaches to management that can ultimately be rolled out into clinical practice. The third consideration is the urgent need to obtain a better estimate of the global burden of disease than exists at present. Remarkably little is known about sepsis outside the context of clinical practice in major critical care centres in developed countries. As the Global Sepsis Alliance has pointed out the absence of sepsis (other than neonatal sepsis) from the Global Burden of Disease report is a major impediment to the encouragement of reporting nations to focus on the disease and to develop new strategies to identify and treat it. Agreement of a case definition of sepsis would be a fundamental first step in persuading WHO and the global health community of the importance of this issue.

For more on the **Global Sepsis Alliance** see www.globalsepsisalliance.com

Sepsis diagnosis and therapy: a role for personalised medicine

We welcome the huge advances that have been made in the microbiological diagnosis of sepsis, notably in non-culture-based diagnostics such as MALDI-TOF or nucleic acid-based systems such as PCR-based, transcription-mediated, loop-mediated isothermal, or helicase-dependent amplifications of molecular targets. Within just the past few years these advances in microbiological diagnosis of sepsis have genuinely brought about a profound change in the speed with which bacteria can be identified and their antimicrobial susceptibility pattern known. However, the fact remains that unless the clinician suspects sepsis early on in the disease course, the microbiological samples will not be obtained and the diagnosis will be delayed. We believe that a lack of awareness of sepsis, both in clinicians[3,4,4] and the general public, contributes to the fact that sepsis is often recognised when its clinical course is already established and as such, the effectiveness of treatment is diminished. In the case of meningococcal meningitis,

For the **Meningitis Research Foundation** see http://www.meningitis.org/symptoms

for example, campaigns by organisations such as the Meningitis Research Foundation to emphasise the clinical importance of the non-blanching spot have been highly effective at increasing public awareness. We believe that improved training of front-line clinical staff would make a substantial contribution to the improvement of the outcome of sepsis.

Sepsis is a complex disorder that can occur in many clinical contexts, be caused by a wide range of different microorganisms, and is almost certainly driven by the interplay of a diverse range of pathological processes. Each of these processes probably operates in different ways, at differing rates, and to differing extents, depending on the patient, and is driven—at least partly—by varying genetic susceptibility. This complexity is fertile ground for the development and assessment of personalised medicine, an approach that has so far had little or no effect on the usual clinical approach to patients with sepsis. Some have argued[26] that the wish to make the entry criteria for sepsis trials as broad-based as possible (often inevitably driven by commercial considerations), has been one of the main hindrances to the development of new drugs for sepsis.

Development of new treatments for sepsis

A seeming paradox that is frequently noted is the fact that advances in the knowledge of the basic science of sepsis have not been matched by the introduction of new drugs for sepsis. A more accurate assessment of the situation would be that many new drugs have been developed, but none has shown a reproducible benefit to patients in clinical trials. Frustrating as this conclusion is, we are clear that it should in no way dampen the enthusiasm for increased investment in fundamental studies of the biology of the disease with the goal of identifying new drug targets. Of equal priority is to use this knowledge to improve our ability to target new therapies with increased precision at the patient population most likely to benefit—the principle of personalised medicine. One of the challenges of this approach will probably be the need to move away from the so-called all-comers clinical trial towards a more nuanced approach to patient selection, something that will need the support and encouragement of both industry and regulators.

At the same time, we need to acknowledge that antibiotics will remain a cornerstone of the treatment of sepsis for the foreseeable future. As has been amply discussed elsewhere,[345] the challenge of antibiotic resistance will be as keenly felt among those treating sepsis as in many other areas of medicine, and we strongly endorse the recommendations of others that tackling this issue, both in terms of the development of new drugs and the improved use of existing drugs, is a very high priority.

Dealing with the long-term complications

Patients with sepsis are frequently cared for in ICUs, either because sepsis itself led to their admission or because sepsis developed as a complication of their admission for other reasons. However, clinicians have only in recent years become fully aware of the long-term morbidity associated with admission to ICUs, sometimes referred to as post-ICU syndrome.[346,347] Sometimes the issues are obvious, such as the tragic cases of children who need amputations as a complication of meningococcal sepsis. But less obvious are the physical malaise, muscle wasting, and nutritional problems that have been described, and the psychological morbidity associated with the sometimes alienating environment of critical-care units or the life-threatening disease that brought them there.[348] Efforts to study this issue systematically and importantly, to develop therapeutic interventions are only just beginning;[349] we endorse these attempts.

Contributors

Each group of authors takes responsibility for the text and views expressed in their individual parts. JC and J-LV wrote the introduction; NKJA, FRM, and DCA wrote the section on epidemiology; TC, KJ, SG, and JD wrote the section on diagnosis and therapy; SO and KT wrote the section on targets and experimental therapies; TvdP and EP wrote the breaking the mould section; and JC wrote the call to action section. JC conceived the Commission. All authors approved the final manuscript.

Declaration of interests

The views expressed in this Commission are the personal views of the authors and must not be understood or quoted as being made on behalf of or representing the position of the European Medicines Agency or one of its committees or working parties. We declare no competing interests.

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