1
Unmet Medical Needs in Life-Threatening Infections – Caring for the Critically Ill

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1.1
Life Threatening Infections and Sepsis – Defining the Problem

The large number of infectious agents, complicated further by many varied pathogen- and host-specific characteristics, results in a broad spectrum of communicable diseases of which both prevention and control are challenging. While many infectious diseases are benign and are primarily treated in the community, severe infections may give rise to an urgent need to control the source of infection, to implement appropriate anti-infective therapy, and to provide supportive care to maintain homeostasis [1].

Under these conditions, the patient outcome from infection is determined not only by the invading pathogen which can be directly toxic and destructive to cells and tissues but also – or even primarily – by the host response. This host response may be inappropriately exaggerated, leading to severe tissue injury. Here, the effector molecules of immune cells, such as oxygen free-radicals and nitric oxide, cannot discriminate between microbial targets and host tissue [2]. Indeed, a novel concept has been proposed to describe the development of organ failure, that is, severe sepsis, as a disturbed "disease tolerance" where the eventual development of organ dysfunction is considered an inability to establish an appropriate equilibrium between direct pathogen damage and the ensuing host response (Figure 1.1) [3]. Patients with an uncontrolled focus of infection or an exuberant host response are particularly prone to develop organ dysfunction requiring care in a specialized "intensive care unit (ICU)." Such patients are referred to as *septic* (Figure 1.2).

Sepsis is defined and diagnosed by nonspecific alterations in temperature, heart and respiratory rate, and white cell count secondary to infection (Table 1.1) [4]. Unfortunately, in current clinical practice, neither the causative pathogen nor the specific cellular processes underlying deterioration of organ function that would be amenable to specific therapeutic intervention can be assessed in a way that would allow tailoring of anti-infective or immunomodulatory therapies to specific patient needs. This is particularly relevant given the pressing need to respond within the first few "golden" hours. These shortcomings regarding "point-of-care" diagnostics are in sharp contrast to the burgeoning development of sophisticated

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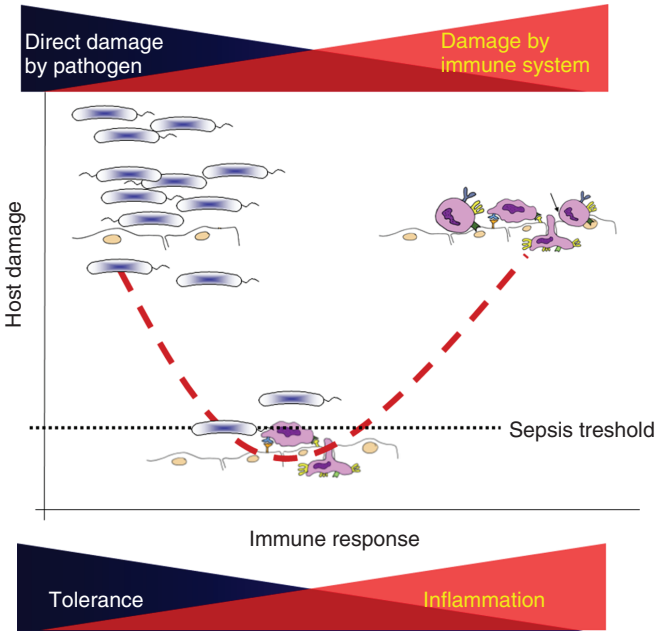

Figure 1.1 Evolving concepts of sepsis as a "host defense failure disease." The host response to invading pathogens requires a cytotoxic response that can result in a trade-off where tolerance of a pathogen may be associated with less organ injury.

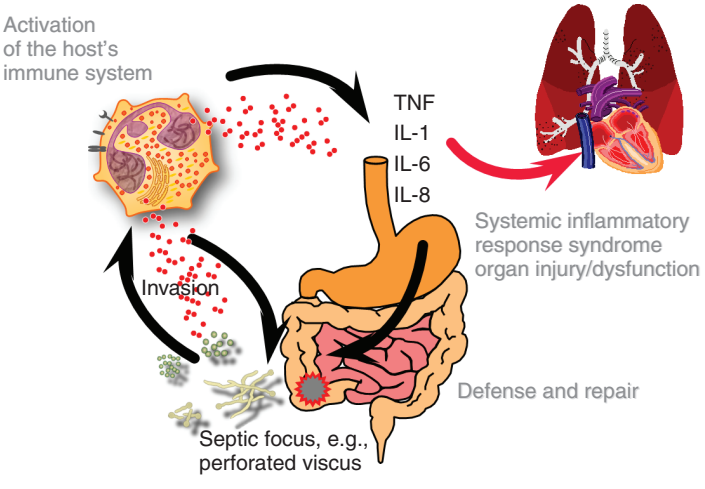

Figure 1.2 Activation of the innate immune system as a "double-edged sword." Activation of innate immunity reflects a prerequisite for defense and repair of a septic focus, such as a perforated viscus. However, this may lead to collateral damage if spillover of inflammatory mediators or release of activated cells into the systemic circulation occurs.

Table 1.1 Diagnostic criteria for the "systemic inflammatory response syndrome" (SIRS criteria).

- Temperature >38 or <36 °C
- Heart rate >90 beats/min
- Respiratory rate >20 beats/min or p_aCO_2 <32 Torr (4.3 kPa)
- White blood count >12 000 cells/mm^3 or <4000 cells/mm^3 or >10% immature (band) forms

molecular tools and the improved molecular and cellular understanding of the pathogen–host interaction via specific receptors and signaling cascades [5, 6]. As stated by Nathan [2]: "it makes no sense to use twenty-first century technology to develop drugs targeted at specific infections whose diagnosis is delayed by nineteenth-century methods." Thus, development of innovative diagnostic tests and strategies are needed to optimize treatment strategies, not only in selecting the correct anti-infective agent but also modulating inflammatory and other responses to fundamentally improve outcomes in a "personalized" manner.

The resulting diagnostic uncertainty regarding the causative pathogen reflects a central dilemma of intensive care physicians in treating life-threatening infections. On one hand, there is an important need to avoid delays in the initiation of appropriate antibiotics [7], yet this, in turn, triggers the overuse of "broad spectrum" antimicrobial agents creating a tremendous problem with multiresistant pathogens [8]. Likewise, many septic patients may already be in a state of overall immune suppression at the point of admission to intensive care, as anti-inflammatory systems are also activated in sepsis and these may outweigh the proinflammatory response. Introduction of an anti-inflammatory agent to such patients may arguably compromise the host even more.

1.2 Sepsis as a "Hidden Healthcare Disaster"

Sepsis arises from community-acquired infections but also, and more frequently, from healthcare-associated infections. It is a leading cause of morbidity and mortality worldwide. Its incidence is increasing, and the overall mortality is now in a similar range to that of myocardial infarction or stroke [9, 10]. This likely reflects changing demographics, with an aging population. In parallel, an ever-increasing number of invasive procedures, including those directly affecting the immune system, such as antineoplastic chemotherapy or organ transplantation, are performed in patients who would previously not have been considered for such procedures. As a consequence, the rate of hospitalization for sepsis in the United States increased from 221 per 100 000 population in 2001 to 377 per 100 000 in 2008 (Figure 1.3) [9]. A similar increase in the incidence of severe postoperative sepsis is also noted [11].

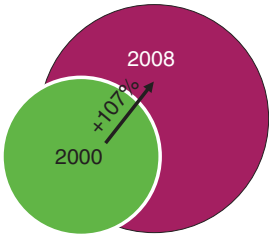

Figure 1.3 Sepsis – an underestimated and silently growing problem of modern healthcare: Because of multiple factors related to demographic changes, an increasing invasiveness of procedures in patients with inherent impaired immune function, and the advent of multidrug resistance in particular to Gram-negative pathogens, there is a silent but dramatic increase in the incidence of sepsis in healthcare systems across the world.

Sepsis has been called a "hidden public health disaster" [12]. Survivors carry an under-recognized risk of long-term cognitive and physical disability [13] and a more than twofold risk of dying over the next 5 years compared with appropriate controls [14]. The Center of Disease Control recently estimated that 15 billion dollars were spent on hospitalizations for sepsis alone in the United States and that inflation-adjusted aggregate costs for treating such patients increased annually by more than 10% [9].

1.3 Microorganisms and Types of Infection Triggering Sepsis

A recent global picture of infection and sepsis in ICUs worldwide is provided by the "Extended Prevalence of Infection in Intensive Care" (EPIC II) study. This reflects a 1-day, prospective, point prevalence study conducted on May 8, 2007, with subsequent follow-up [15]. Demographic, physiologic, bacteriologic, therapeutic, and outcome data were collected from approximately 14 000 patients in 1265 participating ICUs from 75 countries. These included 667 Western European ICUs, 210 Central and South American, 137 Asian, 97 Eastern European, 83 North American, 54 Oceanic, and 17 African. Sixty percent of participating ICUs were situated in university hospitals, 66% were mixed medical-surgical ICUs, and 94% had 24 h ICU physician coverage. On the study day, approximately half the patients were considered infected and 71% were receiving antibiotics. Infection was mostly of respiratory origin (66%), followed by abdomen (20%), bloodstream (15%), and renal tract/genitourinary system (14%). Microbiological cultures were positive in 70% of the patients with presumed infection, with 62% of positive isolates being Gram-negative organisms, 47% Gram-positive, and 19% fungi. The most common Gram-positive organism isolated was *Staphylococcus aureus* (20%), while the commonest Gram-negative organisms were *Pseudomonas* species (20%) and *Escherichia coli* (16%). Patients who had been in ICU for longer prior to the study day had higher rates of infection, especially with resistant and thus more difficult-to-treat pathogens, such as *Staphylococci*, *Acinetobacter*, *Pseudomonas*, and *Candida* species. Of note, ICU mortality (25% vs 11%) and hospital mortality (30% vs 15%) of infected patients was more than twice that of noninfected patients. Other, albeit smaller, surveys corroborate the EPIC II data and confirm the disease burden of infection in the critical care setting which increases with

the duration of stay as well as with shifting patterns of microorganisms [16]. Since 2007, a substantial increase in difficult-to-treat infections has been observed. This is primarily attributable to multidrug-resistant Gram-negative bacteria, a proportion of which are virtually untreatable, such as some carbapenemase-producing *Klebsiella* strains [17].

1.4 Emerging Problems Related to Resistance in Bacterial Infections

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains an important cause of healthcare-associated infections, and is endemic in most hospitals. Healthcare-associated MRSA infections are associated with increased morbidity and mortality compared to infections caused by methicillin-susceptible strains [18, 19]. MRSA is also an increasingly important cause of infection in the community setting. MRSA infections, both healthcare- and community-associated, are generally caused by a very limited number of (clonal) strains, suggesting that most cases result from direct or indirect person-to-person transmission of MRSA [20, 21]. The major reservoir for transmission is likely to be infected or colonized patients, with the vector being healthcare personnel or contaminated, shared equipment. With the introduction of a variety of bundled strategies including, but not restricted to, careful hand hygiene, there has been an associated reduction in the burden of MRSA infection in the healthcare setting. In 2005, there were an estimated 94 000 MRSA infections in the United States associated with nearly 18 000 deaths. Of these, 86% were associated with healthcare delivery, two-thirds of which had their onset outside the hospital setting [22].

Despite measures that have successfully prevented and controlled healthcare-associated MRSA, the number of Gram-negative bacterial infections continues to grow [23]. This is compounded by an increasing problem of antibiotic resistance of these pathogens which may result in higher mortality and morbidity [24].

There are thus conflicting recommendations. On one hand, there is advice to administer appropriate and early empirical antibiotic therapy, especially in patients with risk factors such as compromised immune function. In view of the growing resistance problem, multiple broad-spectrum antibiotics are often proposed. On the other hand, there are strong recommendations to limit antibiotic usage in general. This is a clear testimony for the need of diagnostic tests with fundamentally improved performance regarding sensitivity, specificity, and, most importantly, time-to-result.

1.5 The Role of Fungi and Viruses

Bacteria dominate as the type of pathogen responsible for most life-threatening infections in the "immune competent" host. However, immunosuppression

occurring in the later phases of sepsis [25], along with shifts in the host's commensal flora (primarily induced by antibiotics), contributes to overgrowth by fungi, most notably *Candida* species, in sterile body compartments. This can give rise to difficult-to-diagnose infections which may also contribute to the overall death toll. In a recent retrospective chart review study, we identified 999 patients with severe sepsis or septic shock from a total of 16 041 patients admitted to our 50-bed surgical ICU in a single center; hospital mortality was approximately 30% [16]. In total, data from 2117 blood cultures were available for analysis. Three phases could be described based on peaks in mortality. A third of all deaths occurred in the first 5 days following ICU admission. Of 882 blood cultures drawn within the first 5 days, only 15% were positive. Of note, 524 blood cultures were drawn in those patients staying >2 weeks and, while positive blood cultures were less frequently observed, the rate of opportunistic bacteria and *Candida* species doubled from 9% in the acute phase to 18% in this later phase.

While the role of invasive fungal infection is increasingly acknowledged, the contribution of viral infections to initiate or maintain a systemic inflammatory response syndrome SIRS is poorly defined. The 2009 influenza A (H1N1) pandemic did not significantly affect ICU occupancy rates and, compared with community-acquired pneumonia of other origins, H1N1 pneumonia was associated with the same risk of death when potential confounders were taken into consideration [26]. However, this pandemic more commonly affected young people, many of whom developed severe respiratory failure requiring extracorporeal lung assist support. As the pathogen underlying ICU admission for community-acquired pneumonia is rarely identified with conventional diagnostics, viral infections are probably underdiagnosed. Viruses may play an important role in complicating the course of defined ICU patient populations, such as cytomegalovirus (CMV) in immunocompromised patients. While reactivation of dormant virus within the critically ill host likely occurs, it remains unclear to what extent they cause secondary infections. Antiviral treatment may improve outcome [27], but they do carry their own toxicity. Multicenter trials that address this problem are ongoing. Ganciclovir is frequently used as both first-line prophylaxis and systemic disease therapy against CMV, but resistance is increasingly occurring and this is associated with worse outcomes. Thus, implementation of rapid and sensitive techniques for the early detection and monitoring of CMV and ganciclovir resistance is clearly desirable to support patient management [28]. Furthermore, strategies to individualize the therapy of life-threatening infection by viruses such as CMV and Epstein–Barr virus may include, in addition to antiviral agents, either a reduction in immunosuppressant therapy (as these infections occur frequently in post-transplant patients [29]), or even immunoactivating agents such as Granulocyte macrophage colony-stimulating factor GM-CSF and interferon-gamma. Here, a reliable point-of-care monitoring of the host's immune system would potentially allow a correct selection of agents to improve clearance of infection while at the same time reducing the risk of, for example, graft failure in transplant patients [30].

1.6 The Need for New Approaches in Diagnostics of Life-Threatening Infection and Sepsis

The presence of an infectious focus is currently identified and confirmed by a combination of clinical examination and imaging techniques. Whenever possible, specimens are obtained from the site of infection in addition to blood cultures for conventional microbiology. An example is taking specimens during lung washings during bronchoscopy for suspected lower respiratory tract infection. At present, these techniques, though time consuming, allow a better determination of the infecting pathogen and antibiotic resistance patterns [31].

The polymerase-chain reaction (PCR) technique can directly amplify pathogen DNA from a suspected focus [32, 33] or a blood sample. This carries the potential to increase the sensitivity of pathogen detection and to decrease the result turnaround time in routine clinical practice [34]. At present, PCR testing is generally considered as supplementary to culture-based techniques, particularly for fastidious, resistant, and difficult-to-culture pathogens. Some experts suggest that the focus for such tests should be on detection of pathogens or resistance factors that fall outside guideline-recommended antibiotic coverage, as well as for specific at-risk populations, for example, transplant recipients [35]. However, with improvements in technology, point-of-care PCR testing of blood and other body samples may not only shorten the time to diagnosis but also reduce the number of patients receiving inappropriate empirical antibiotics.

Although inappropriate anti-infective therapy is seemingly associated with excess mortality [36, 37], a liberal or "aggressive" strategy with early initiation of (combined) antibiotics to cover a very broad spectrum of pathogens may also be associated with similar increases in mortality [38]. Although not fully understood, there is increasing support for the concept that concomitant release of host intracellular "danger-associated molecular patterns (DAMPs)" or "alarmins" [39] can signal, via the Toll-like and other receptor systems, a similar pathophysiological cascade culminating in multiple organ failure as that induced by "pathogen-associated molecular patterns (PAMPs)" released from bacteria and other pathogens [40, 41]. Indeed, DAMPs may be released even in the absence of pathogens or their PAMPs. The use of anti-infective agents themselves carries multiple adverse effects (Table 1.2); this is further compounded by altered handling of these drugs by the dysfunctional liver and/or kidney, which not only

Table 1.2 Problems associated with antibiotic use in individual patients.

- Overgrowth of (multi)-drug resistant bacteria and fungi
- Jarisch–Herxheimer reaction: release of bacterial products, such as endotoxin potentially triggering a vigorous host response with associated side effects on organ function
- Effects on critical cellular effector functions: immunomodulatory effects, impairment of mitochondrial function
- Typical drug-related side effects: for example, rashes, liver, and renal dysfunction

makes pharmacokinetics and dosing unpredictable but also increases the risk of direct drug-induced toxicity [42].

1.7 Rapid and Sensitive Culture-Independent Strategies to Identify Blood Stream Infection

The basic principles regarding the diagnostics of infection hold particularly true for blood cultures, which is the current gold standard for identifying primary or secondary bloodstream infection. However, this is far from being an ideal gold standard, as a positive result is obtained in only a subset of severely septic patients and results are frequently obtained too late to influence clinical decision making [43, 44].

Molecular approaches to improve conventional culture-based identification may range from strategies to shorten the time from positivity of the blood culture to identification of the pathogen to complete culture-independent, direct microbial nucleic acid amplification techniques.

Important developments to improve the performance of the blood culture approach include fully automated instruments for handling and culture and the use of matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry, which can decrease the time-to-result to approximately 1 h after a positive culture is recognized [44].

An alternative strategy, that is, extraction and amplification of microbial nucleic acids from a positive blood culture and subsequent hybridization on a microarray platform to detect the pathogen and certain resistance genes (gyrB, parE, and mecA) among 50 bacterial species (Prove-it Sepsis, Mobidiag, Helsinki, Finland), was recently evaluated [45]. A total of 2107 positive blood culture samples taken from 3318 blood samples from patients with suspected sepsis were analyzed; 86% of positive blood culture samples included a pathogen covered by the molecular assay which had an overall 94.7% sensitivity and 98.8%, specificity; both increased to 100% for identifying MRSA bacteremia. On average, the assay was 18 h faster than conventional blood cultures, providing proof of the concept that molecular assays can shorten the time-to-result. Shortcomings included an incomplete coverage of pathogens, an inability of the test to be applied directly to a biological sample, and restricted information regarding antimicrobial susceptibility (primarily regarding multiresistant Gram-negative bacteria) despite an excellent performance for detecting MRSA. While PCR-based detection of MRSA (and also Vancomycin-resistant Enterococci VRE) is feasible because of a limited number of resistance genes, the need to identify the large and continuously evolving set of genotypes encoding extended-spectrum β-lactamases renders a molecular approach difficult and a conventional PCR-based approach unreliable.

These shortcomings, obviously with the exception of the need for prior culture, also hold true for PCR-based approaches to directly amplify microbial nucleic acids from the bloodstream. This led to the view discussed earlier that PCR tests should only supplement but not replace blood culture (BC). However, such a

combined diagnostic approach has been documented in various clinical studies to yield higher detection rates of an alleged pathogen compared to each method alone and to decrease the time-to-result [34]. This appears to particularly hold true for well-defined at-risk populations and for fungal infection [46, 47].

PCR improves the identification of bloodstream infection because of typically easy-to-culture bacteria such as *Streptococci* and *Staphylococci*, but also in polymicrobial infection including difficult-to-culture species such as fungi [48–53]. As a result, and despite its inherent limitations, PCR may offer a promising approach to decrease the time-to-result, which may favorably influence antibiotic decision making in the critically ill septic host. Data from patients with sepsis, endocarditis, or bloodstream infection indicated an up to 2.5-fold increase in positive test results compared to blood culture. Concordance was moderate to good with respect to recovery of blood culture positive cases by PCR in most but not all studies applying the best validated technique, the LightCycler/Septifast® [1, 33]. In specific conditions, for example, infective endocarditis, concordance of results may be higher as the focus is within the bloodstream. It is noteworthy that a significant proportion (approximately 20–30%) of blood culture findings was not reproduced by PCR [54]. In addition to the limited use of PCR to comprehensively assess resistance patterns and the inherent high costs of broad, multiplexed PCR tests, these findings prompted the recommendation that PCR be viewed at present as a supplementary test rather than as a replacement for conventional blood culture testing [35].

Several avenues have been pursued to address the opposite problem, that is, a PCR positive but blood culture negative result. One study analyzing microbiological specimens other than blood confirmed that PCR performed on DNA prepared from whole blood did increase the number of presumably true-positive results in septic patients [55]. However, a proportion of positive PCR results still remained unconfirmed by other tests. In this study, 200 adults with presumed infection presenting to an emergency room had 45 positive PCR tests compared to 37 positive blood cultures. Sixty-eight percent of the positive PCR results were confirmed by blood, urine, or catheter cultures. Consistent with earlier discussed results, all MRSA bacteremia episodes were detected.

Assessing the association of PCR status with measures of morbidity and mortality reflects a complementary strategy to validate the significance of an amplicon in the absence of a concomitant blood culture result. We studied cases with severe sepsis, and found 34.7% of PCRs tests were positive compared to 16.5% of blood cultures [54]. Seventy-eight percent of positive cultures had a corresponding PCR result, while only 23% of PCR results were confirmed by blood culture. Compared to patients with a negative PCR, those testing positive had higher organ dysfunction scores and a trend toward higher mortality (39.1% vs 25.3%; $p = 0.115$). This held true particularly for predefined cohorts of patients with a fungal amplicon. These were observed twice as frequently as cultures that were positive for fungi; both results were associated with an extremely high mortality (90%). These data lend support to the concept that the presence of a pathogen-associated DNA amplicon reflects a meaningful event in severe sepsis. As a consequence, PCR

warrants further investigation as to its suitability to guide anti-infective therapy in the light of the conflicting findings of initiation of early "aggressive" antibiotic therapy [37, 38]. Such studies are currently under way. Thus, PCR may serve to close the gap regarding those infections not covered by guideline-recommended initial treatment [35], but most likely will not allow a fundamental improvement in diagnostics such that antibiotic therapy can be safely narrowed in terms of spectrum of activity or even withheld.

Although recovery rates of PCR are generally higher than those obtained from conventional culture, in most cases a causative microorganism cannot be identified, even in the most severely sick septic patients. This has prompted efforts to improve pre-analytical handling of blood samples to increase sensitivity. Whole blood as a template for PCR faces limitations because of its very high human DNA background. Increasing the ratio of pathogen to host DNA may improve the diagnostic performance by amplifying pathogen DNA against the eukaryotic background. Discrimination of "self" as opposed to bacterial DNA is achieved by immunocompetent cells via species-specific cytidylate-phosphate-deoxyguanylate (CpG) motif recognition via TLR9 (toll-like receptor). This process is not restricted to TLR9 but is also observed, for example, for a human cytidylate-phosphate-deoxyguanylate-binding protein (CXXC Finger Protein 1 or hCGBP) [56, 57]. This mammalian transcriptional activator avidly binds unmethylated CpG dinucleotid motifs by recognition of the sequence [A/C]CG[A/C] [57] with an even higher number of potential binding sites compared to TLR9 [58, 59]. A cloned, truncated protein derived from CXXC Finger Protein 1 can be used for affinity chromatography to decrease the human DNA background in blood samples taken from septic patients. When comparing these results to blood culture, approximately a threefold increase in sensitivity was achieved in a limited cohort of patients [60]. Time-to-result for episodes of bacteremia or "DNAemia" was also reduced, as in other PCR-based approaches, to <8 h despite the additional preanalytic step. However, the full potential of this test, which requires substantial "hands-on-time," can only be exploited if staffing of the laboratory allows unscheduled access, or automated systems become available [34].

1.8 Beyond Infection – Profiling the Immune Response of the Septic Host

In addition to identifying the alleged pathogen, biomarkers reflecting the response of the innate immune system are required to consolidate a diagnosis of "sepsis." These biomarkers may range from individual biomolecules, for example, proteins such as procalcitonin (PCT), to multiplexed signatures of biomolecules including transcriptomic, proteomic, or metabolomic profiles [61]. Profiling of the host response can provide valuable information regarding the pathogen, prognosis, and potential response to treatment [62]. PCT is the best validated single protein marker to date; while this marker can be used to identify a population at risk and

to guide anti-infective therapy [63], it is far from perfect, so there is clear space for superior technologies.

As mentioned above, microorganisms bear well-conserved structures known as PAMPs. These bind to the pattern recognition receptors (PRRs) located either on the cell membrane or in the cytosol of host cells to initiate the host response. Well-recognized PAMPs include lipopolysaccharides (LPSs) found in the outer membrane of Gram-negative bacteria, peptidoglycan, and muramyl dipeptide (MDP), which are cell wall constituents of Gram-positive bacteria, as well as bacterial flagellin and nonmethylated CpG DNA motifs. The various families of PRRs currently comprise TLRs, NOD-like (nucleotide-binding oligomerization domain-like) receptors (NLRs), and RIG-like (retinoic acid-inducible gene-like) receptors (RLRs). While not totally specific, TLR2 recognizes peptidoglycan monomers, TLR4 recognizes bacterial LPS, TLR5 recognizes bacterial flagellin, TLR9 recognizes CpG DNA motifs, and NLRs recognize MDP [41]. The current pathogenetic view of sepsis is based mainly on the modulation of proinflammatory and anti-inflammatory cytokine production by the interaction of PAMPs with these PRRs, which, in turn, is modulated by the host genomic background.

Emerging data lend support to the notion that compound markers utilizing parallel "omics" approaches may describe the host response in a more comprehensive way to individualize treatment [64–66]. Recent data from transcriptomic, proteomic, and metabolomic profiling indicate that marker panels are superior to single genes or markers, and can differentiate noninfectious from sepsis-associated systemic inflammation [67, 68]. This is achieved by reflect signaling via the various PRRs that are characterized by shared or "common host response" patterns as well as by specific responses to the pathogen. Thus novel and robust "biomarkers" are feasible and are urgently needed to identify patterns associated with infection as the underlying cause of a systemic host response in a correct and timely manner. Proof-of-concept studies show that algorithms can be developed to describe gene-expression signals, for example, as continuous, nondimensional scores, or as trajectories to assess infectious or noninfectious causes for organ dysfunction.

1.9 Host Factors Contributing to Pathogenesis of Sepsis

The prognosis of the patient depends on both the virulence of the pathogen and the individual host's response to infection that is directed to kill the invading pathogens, but is also affected by host (genetic) factors [69]. None of the numerous interventions developed to modulate the host response, such as cytokine-neutralizing therapies, that were documented to improve survival in preclinical models of sepsis have proved successful in subsequent clinical studies [50]. Although inclusion of patients into these trials has not been stratified according to biomarkers, *post hoc* analysis suggests that a signal indicating a beneficial effect of neutralizing mediators of inflammation may be associated with markers of activation of the immune system, such as IL-6. Concepts referred

to as *theragnostic strategies*, using a biomarker (set) to guide therapeutic decision making, are promising but in their infancy. Currently discussed or available interventions include a broad range of drugs that can interfere with innate or adaptive immunity to either inhibit an overwhelming inflammatory response or to enhance this vital defense mechanism. A current paradigm holds that a (late) phase of immune paralysis follows an initial "cytokine storm" [70, 71]. Acknowledging that reasons for failure are likely to be multifaceted, the inability to individually stratify patients by their host response is most probably a key factor. Indeed, the same nonspecific SIRS criteria, which essentially rely on a clinical physiological phenotype, have been used to include septic patients into trials of immunomodulatory strategies. This approach is increasingly viewed as fundamentally flawed. Novel and robust "biomarkers," most likely panels of biomolecules, are urgently needed to correctly and timely describe the nature of the host response and the immune status of an individual patient. Specifically, there is an imperative to monitor multiple biomarkers related to pro- as well as anti-inflammatory gene products. This is supported by the ever-increasing evidence base from transcriptomic studies showing that events are not tightly associated with "early" and "late" phases of the sepsis disease continuum. Activation of inflammatory innate immunity occurs alongside impaired adaptive immune responses [72].

A phenotypic characterization to enable individualization of therapy may also require a description of organ function beyond current strategies that simply measure markers of injury, such as enzymes released upon cellular demise, or metabolites, such as creatinine or bilirubin, that monitor organ dysfunction as a hallmark of severe sepsis. These indicators of injury and function are rather

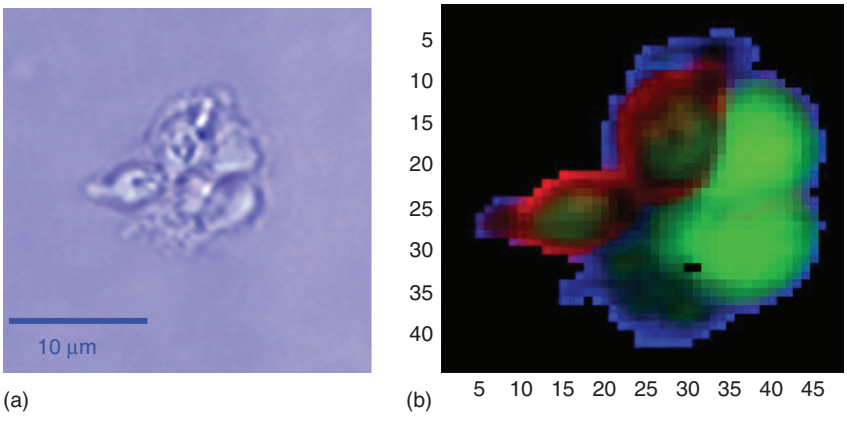

Figure 1.4 Biophotonic strategies to study infection and ensuing host response. Micro-Raman spectroscopic image of *Candida albicans* phagocytosed by a neutrophil. This allows the identification of the pathogen and its dimorphic behavior (hyphal as opposed to yeast phenotype) that impacts on its pathogenicity. Such information can be retrieved label-free on a single-cell level without the need for culture. (a) White-light image and (b) false-color Raman image constructed by end-member analysis using the N-finder algorithm (blue: neutrophil, red: phagocytosed Candida in hyphal form, and green: phagocytosed candida in yeast form).

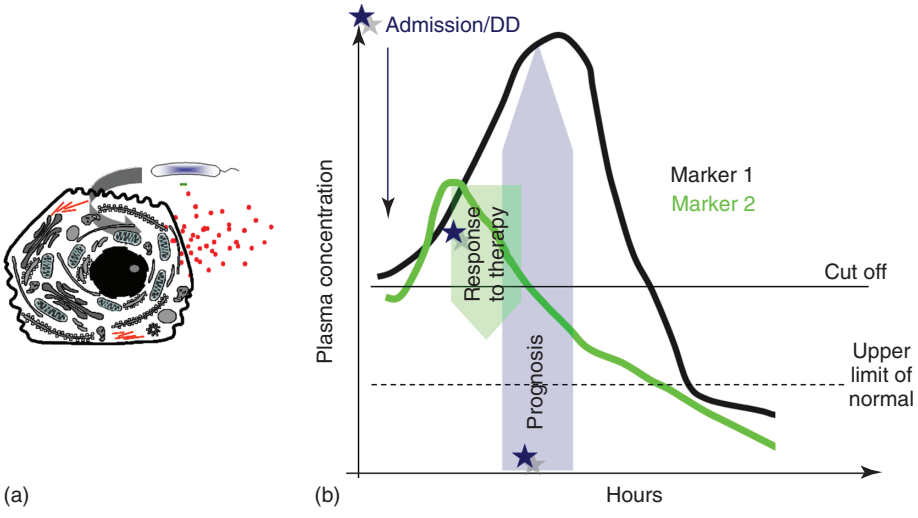

Figure 1.5 The use of "biomarkers" released upon contact of host cells with pathogens to diagnose sepsis and guide therapeutic interventions. (a) Biomarkers are released in response and, thus, indicate contact of pathogen(s) or pathogen-related compounds with host cells. (b) Kinetics of release of two virtual biomarkers (marker 1 and 2). Because of their differing release kinetics, these markers could be used to derive information regarding the nature of disease, prognosis, and response to treatment. These data could then be used to guide therapeutic interventions, such as initiation or discontinuation of antibiotics in case of infection.

insensitive compared to metabolomic parameters that are increasingly available with the advent of mass spectrometry in the routine laboratory.

Taken together, despite the moderate progress achieved with quality management programs such as the "Surviving Sepsis Campaign" [31, 73], sepsis remains an underappreciated killer. As current improvement strategies are directed, in the light of diagnostic uncertainty, at early administration of broad-spectrum antibiotics, development of (multi)-drug antimicrobial resistance has become a major concern. Moreover, the host response is subject to significant variability, with a vulnerable trade-off between disease tolerance and pathogen elimination. Unmet medical needs and strategies to improve patient care will most likely be achieved via improved diagnostics to identify the pathogen and to describe the immune status early and in a way that tailored use of available anti-infective and immunomodulatory therapeutics is made possible (Figures 1.4 and 1.5).

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