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Addressing unmet clinical needs in the early diagnosis of sepsis

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Summary

The incidence of sepsis and the number of sepsis-related deaths are increasing, making sepsis the leading cause of death in critically ill patients in Europe and the U.S.A. Delayed recognition of sepsis and inappropriate initial antibiotic therapy are associated with an increase in mortality and morbidity. Rapid and accurate identification of sepsis and its causative organisms are a prerequisite for successful therapy. The current gold standard for the diagnosis of sepsis is culture of blood and other body fluids or tissues. However, even in severe sepsis, blood cultures (BC) yield the causative microorganism in only 20–40% of patients. Moreover, at least 24 hours are needed to get preliminary information about the potential organism. Therefore, novel laboratory methods for the diagnosis of sepsis, such as multiplex real-time polymerase chain reaction (PCR), matrix-assisted laser desorption ionisation (MALDI) time-of-flight (TOF) mass spectrometry (MS) (MALDI-TOF MS) and calorimetry, have been developed and evaluated.

Key words: *diagnosis; sepsis; blood culture; multiplex real-time PCR; calorimetry*

Introduction

Sepsis is a life-threatening condition caused by the uncontrolled, systemic, inflammatory response to bacterial, viral or fungal infection [1–5]. Sepsis represents a substantial health burden. The incidence of sepsis and the number of sepsis-related deaths are increasing. The increase in sepsis is attributable to the aging of the population, the increasing longevity of patients with chronic diseases, and the relatively high frequency with which sepsis occurs in patients with AIDS [1–3]. The occurrence of sepsis in these patient groups may be especially harmful [4]. Increasingly aggressive cancer therapies and the increasing use of invasive devices, like cardiac pacemakers, valves and defibrillators, and procedures for a variety of medical conditions are likely to increase the number of sepsis cases over the next decade. In addition, the widespread use of broad-spectrum antibiotics has increased the rates of both antibiotic resistance and nosocomial fungal infections, which will have a direct impact on the incidence of sepsis. Sepsis is the leading cause of death in critically ill patients in Europe and the United States. In the US, severe sepsis (sepsis associated with acute organ dysfunction) and septic shock (sepsis with arterial hypotension despite adequate volume supplementation) develop in 750,000 people annually, and more than 210,000 of them die [1–5]. Moreover, sepsis is a costly disease. Sepsis costs as much as €50’000 per patient, resulting in an economic burden of nearly €17 billion annually in the US alone [1, 4–5].

Sepsis can be a response to any class of microorganism. Individual gram-negative or gram-positive bacteria account for 70% of these isolates, and the remainder are fungi or a mixture of micro-organisms. Microbial invasion of the bloodstream is not essential for the development of sepsis. In fact, blood cultures (BC) yield bacteria or fungi in only 20–40% of patients with severe sepsis [7–8]. In patients receiving prior antimicrobial therapy and in fastidious microorganisms, the sensitivity of BC is even lower. In patients whose blood cultures remain negative, the aetiologic agent has to be established by culture or microscopic examination of the infected material from a local site [6].

Diagnosis

Rapid identification of sepsis and its causative microorganisms is the basis for successful treatment. Unfortunately, both may be difficult with the current clinically available methods. Delayed recognition of sepsis and inappropriate initial antibiotic therapy are associated with an increase in mortality and morbidity [9–13]. The magnitude of the problem of inappropriate initial antibiotic therapy, even in experienced clinical centres, was recently highlighted in the Corticosteroid Therapy of Septic Shock (CORTICUS) study [14]. Of the 357 study patients with culture-positive sepsis, 86 (24%) were considered not to have received appropriate antimicrobial therapy by a clinical evaluation committee.

There is no specific diagnostic test for the septic response. Diagnostically important clinical findings in a patient with suspected or proven infection include fever or hypothermia, tachypnea, tachycardia, and leukocytosis or leu-

copenia. In addition, acutely altered mental status, thrombocytopenia, or hypotension also suggests the diagnosis. The septic response can be quite variable, however. In one study, 36% of patients had a normal temperature, 40% had a normal respiratory rate, and 33% had a normal white blood count [9]. Moreover, measurement of simple things such as ear temperature may be prone to error [15]. The systemic response of patients with other conditions than infection may be similar to that characteristic of sepsis. Non-infectious aetiologies of systemic inflammatory response syndrome (SIRS) include pancreatitis, burns, trauma, adrenal insufficiency, pulmonary embolism, myocardial infarction, dissecting aortic aneurysm, occult haemorrhage, anaphylaxis and drug overdose. Besides clinical findings, various laboratory markers, such as elevation of leukocytes, C-reactive protein, procalcitonin and copeptin, give useful diagnostic as well as prognostic information concerning sepsis [16, 17].

A definite aetiologic diagnosis of sepsis requires isolation of the microorganism from blood or a local site of infection. The current gold standard of bloodstream microbiological detection and identification is automatic, continuous monitoring of liquid culture, followed by Gram stain, subculturing and use of phenotypic methods to identify the organism and its susceptibilities. This process usually takes 1 to 5 days, which may result in substantial delays in the initiation of the appropriate treatment. Additional limitations of current culture methods include low sensitivity for fastidious organisms that are difficult to culture as well as uncertainty caused by antibiotics administered before the blood is sampled [6, 9].

Novel laboratory methods

Novel laboratory methods have been developed and evaluated in clinical pilot studies that may, to some extent, address the unmet need to shorten and improve current laboratory procedures for the detection of micro-organisms responsible for blood stream infections [19–31]. These methods extract, purify and then amplify nucleic acids that appear in blood following bacterial and/or fungal lysis. Ultimately, these methods may be helpful in the early diagnosis and prognosis of patients with suspected sepsis.

Septi*Fast*

Septi*Fast* is an innovative, real-time, multiplex, polymerase chain reaction (PCR) test (Roche Diagnostics, Rotkreuz, Switzerland) designed to detect and identify the most important bacteria (19) and fungi (6, Candida species and Aspergillus fumigatus) causing bloodstream infections from whole blood within hours. This assay reportedly identifies the 25 organisms that account for more than 90% of the culturable pathogens associated with sepsis [19–28]. The SeptiFast procedure involves extraction of nucleic acid from 1.5 ml of whole blood using mechanical lysis with ceramic beads, and manual spin column-based nucleic acid purification under a contamination-controlled workflow [18, 27]. After extraction of microbial nucleic acid from blood, three PCR amplification runs have to be performed on the Roche LightCycler instrument in parallel: one for gram-positive bacteria, one for gram-negative bacteria, and one for fungi (yeasts and molds). If methicillin-resistant staphylococci and/or vancomycin-resistant enterococci are under consideration and are suspected, additional LightCycler tests can be run for the detection of the resistance genes in question. The time required to conduct the SeptiFast analysis is less than 6 hours [18]. However, the time until the final result can be communicated to the treating physician in clinical routine may be significantly longer and will depend largely on logistic details and on how the SeptiFast method can be incorporated into the routine workflow of the laboratory [24–26]. Preliminary clinical results have been reported by different groups, including the current authors, regarding sensitivity and detection time [20–26]. The findings showed that SeptiFast gave a positive result slightly more often compared to blood culture. Overall, the available evidence suggests that blood culture and SeptiFast should be considered complementary methods. While both methods agreed and detected the same pathogen in the majority of positive cases, both methods missed cases that were detected with the other method and deemed clinical relevant. In one analysis based on 212 patients presenting with suspected sepsis, SeptiFast seemed to be particularly beneficial among patients pretreated with antibiotics, in whom SeptiFast had a significantly higher detection rate compared to blood culture [21]. Several disadvantages of SeptiFast have to be taken in account. The amplification-based assays may potentially lead to detection of transient bacteraemia and fungemia due to translocation from naturally colonised surfaces and even non-replicating bacteria. Such results are medically irrelevant but may be misleading and difficult to judge in clinical settings. The reported detection limit of SeptiFast is 30–100 CFU/ml (detection limit of blood cultures: 1 CFU/ml), which is above the usual bacterial burden in sepsis of <10 CFU/ml [27]. Therefore, SeptiFast may be not sensitive enough for detecting bacteraemia. In addition, SeptiFast does not offer broad susceptibility testing.

An important confounding variable for the detection of pathogen DNA is the presence of human DNA in circulating white blood cells. When whole blood is treated to extract and purify pathogen DNA, human DNA is co-isolated in great excess relative to pathogen DNA. The burden of human DNA is reduced by DNase treatment in the SeptiTest (Molzym, Bremen, Germany) method. Ongoing clinical studies will define the clinical benefit of this approach.

MALDI-TOF MS

Matrix-assisted laser desorption ionisation (MALDI) time-of-flight (TOF) mass spectrometry (MS) (MALDI-TOF MS) is an even more sophisticated method which couples broad-range PCR amplification to electrospray ionisation/mass spectrometry [19, 29]. This technique uses primers designed to genomic regions highly conserved across the bacterial and fungal domains of life. Preliminary results suggest that MALDI-TOF may rapidly (within 1 hour of detection of positive blood cultures) and accurately identify bacteria [19, 28–30].

Calorimetry

Calorimetry is a non-specific technique for the direct measurement of complex biological processes in the cell, resulting in thermal changes over time (i.e. heat flow-time curve). All living organisms produce heat as a result of metabolism. Compared to normal human cells (or the degradation process of inorganic substances), rapidly dividing cells, such as bacteria, fungi or protozoa, produce a significantly larger amount of heat (≈1–40 picowatts per cell). Medically important bacteria replicate with a doubling time of 20–30 minutes, making the detection of microbial heat flow an attractive diagnostic approach in medical microbiology. The clinical use of calorimetry was previously hindered by insufficient sensitive instrumentation and was lacking software. During the last years, such instruments have become available. Preliminary results obtained at the University Hospital Basel using blood, platelet concentrates, ascites and cerebrospinal fluid were promising in some settings, but disappointing for use in patients presenting with suspected sepsis to the emergency department [31–34].

Conclusion

Several long-term benefits can be anticipated from improvements in the diagnosis of sepsis. Rapid detection and identification of organisms in blood and other primarily sterile body fluids is one of the most important tasks of the clinical microbiology laboratory in order to initiate an appropriate antimicrobial treatment. Currently, the standard methods for the diagnosis of infection involve liquid (e.g., blood culture bottle) and solid growth media such as agar plates. Typically, the average time to detect a positive culture ranges from 1–5 days. Early detection of infection and the causative microorganism by real-time multiplex PCR, MALDI TOF MS or calorimetry would offer unique opportunities to improve patient outcomes. In addition, excluding an infection would prevent the overuse of antibiotics, save costs and prevent development of antibiotic resistance, which is an increasingly important epidemiological problem in hospitals and the community. Blood products, donor tissues and organs, medical devices and special food may be tested with the new techniques for the presence of microorganisms before infusion or transplantation.

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