New Approaches in the Diagnosis of Sepsis

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Key words: sepsis, diagnosis, procalcitonin, polymerase chain reaction, hypophosphatemia

Abstract

In diagnosing sepsis the rapid identification of bacteremia at an early stage of the disease is critical for a favorable outcome. Furthermore, it is important that exact information on the stage of the disease be obtained rapidly in order to choose and initiate the appropriate therapy. In recent years many new techniques have been added to the diagnostic tools. In this review we will focus on three new methods for the early diagnosis of sepsis. These are: polymerase chain reaction, which offers the possibility to attain detailed information about the involved bacterial (or viral) species, and the laboratory markers procalcitonin and hypophosphatemia, which are indicators of the presence of infection with gram-negative bacteria. The approaches reviewed here were developed to expedite the diagnosis of especially early sepsis and might be a further step towards the improvement of therapy for sepsis.

IMAJ 2001;3:439–442

Sepsis is defined as the presence of bacteria or their toxic products in the circulation, together with clinical manifestations of infection, such as fever, leukocytosis and often hypotension. In severe cases, sepsis is even accompanied by septic shock. Critical for the initiation of an effective therapy for sepsis is the identification of bacteremia at an early stage of the disease, as well as identification of the bacterium. Also essential is information about the stage of the disease in these patients.

In the last few years many new techniques have entered into the early diagnostic process of sepsis. With the new tools the physician is able to rapidly acquire the prerequisite and detailed information about septic patients. In this review we discuss some of the new techniques and their role in daily clinical diagnostic procedures [Figure 1].

Identification of bacterial infection

The first and ultimate step in the diagnosis of sepsis is isolation of bacteria. This process usually requires 24 hours, but another 24 hours are needed to achieve more detailed information in order to identify the bacteria. Thus the identification of bacteria has to be accelerated, or other clinical and laboratory findings must be relied on to reach an initial diagnosis.

It is well known that a large proportion of patients (at least 15%) who appear to be clinically septic have negative blood cultures [1]. There are two major reasons to explain this phenomenon. One possibility might be that patients are indeed septic with bacteremia but the organisms in the sample did not grow under normal circumstances in the culture medium. The second possibility is that the apparently septic state may have resulted not from bacteremia but from pyrogenic cytokine activation – either by previous and transient bacteremia or derived from a non-bacterial origin. Because early and appropriate antibiotic treatment is a crucial factor for the patient's survival, the rapid and reliable detection of bacteria in the bloodstream has obvious clinical importance. Of the numerous techniques developed in recent years to detect bacteria in the bloodstream of patients, one of the most important is the polymerase chain reaction. Many studies have

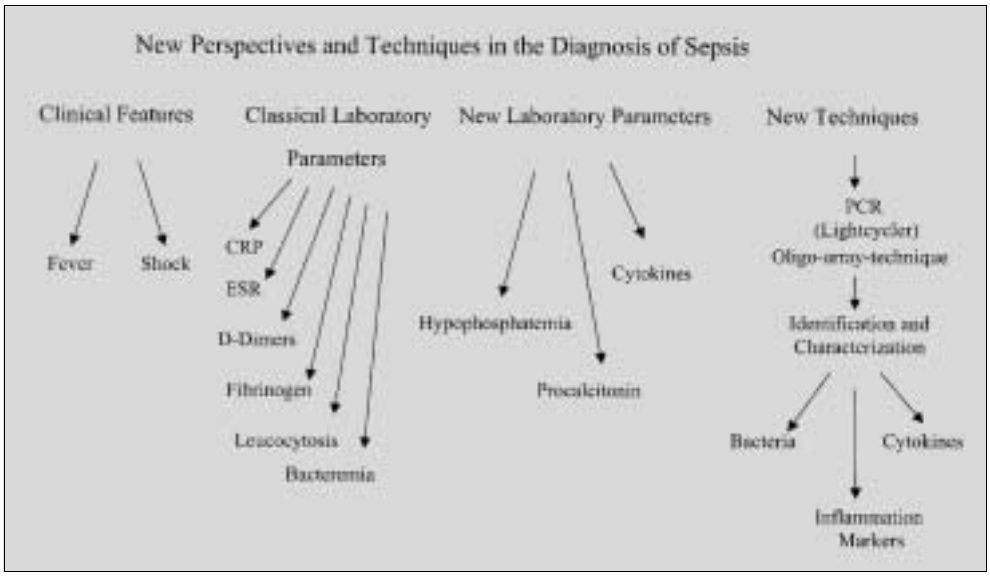

Figure 1. New perspectives and techniques in the diagnosis of sepsis.

shown the advantages of PCR compared to classical culture methods in identifying different types of bacteria. Since the number of these studies is too vast to relate to, in this review we will refer to only some of the recently published technical variations of PCR that might be an additional step towards the rapid detection of bacteria in sepsis.

One approach to detect microbes is the use of conserved primers to amplify the 16S ribosomal DNA. This type of universal PCR is able to detect both gram-negative and gram-positive bacteria in one step. It effectively detected bacteria in the synovial fluid of patients with total knee arthroplasty [2], as well as in severely ill patients with clinical signs of sepsis [3]. In the latter study especially, the detection of gram-positive bacteria seems to be more sensitive with the PCR method than with classical culture methods. Although the cultures generally agreed with the DNA sequencing in determining the causative species, there was already a lack of specificity in identification of the exact type of species to which the bacteria belonged [3]. This inaccuracy was mentioned as a compromise between using the small highly conserved DNA sequences and using longer sequences that enable better organism identification but have less sensitivity. To overcome these problems a recently published study working with the same approach might be of interest [4].

In contrast to the 16S ribosomal region, which is highly conserved between different species, the 16S-23S ribosomal spacer region is highly variable within many species frequently containing tRNA genes. This great sequence variation has been used for typing of clinical isolates [5–7]. On this basis, the sequences of the 16S-23S ribosomal region can be used for analysis by hybridizing the labeled PCR products to an array of oligonucleotides immobilized on a solid support (membrane or glass slides). Thus, organisms of bacteremia could be identified directly from blood culture bottles by amplification of bacterial 23S ribosomal DNA, followed by reverse hybridization to an oligonucleotide array designed to differentiate the sequence variations of the species. The method is dependent on the successful amplification of bacterial DNA directly from the blood cultures and on the sensitivity and specificity of the oligonucleotide array. In a study conducted by Anthony et al. [4], the authors designed primers for the amplification of the 23S ribosomal DNA from a wide range of bacteria. Using published sequence data, they constructed an oligonucleotide array to interrogate PCR amplificons from a collection of blood culture isolates. The newly constructed array contains 30 different oligonucleotides of the 23S region of the respective bacterial strain. The primers for the amplification of the patients' samples were based on the conserved regions within the 23S region (region 6 and 10), as previously reported [8]. For detection of the PCR products the reverse primer was coupled to 5'digoxigenin. Hybridization of the samples on the oligonucleotide array was performed with the labeled PCR products and the colorimetric detection system determined the hybridization within 15 to 60 minutes. With 158 samples of blood culture broths included in their study, Anthony et al. [4] were able to achieve a positive predictive value of 100% for all organism groups except for streptococci (positive predictive value 50%) and *Corynebacterium* spp. (positive predictive value 96%). With this simple and rapid (4 hours) method, the availability of species identification from the majority of blood cultures might improve the patient's management and reduce the inappropriate use of antibiotics. Moreover, adding further oligonucleotides can easily expand the discriminatory power of this test method.

In the following section an additional variation of the use of PCR techniques is described for the diagnosis of viral infections, even in critically ill patients.

Light cycler techniques

Viral infections are one cause of severe infection in immunocompromised patients. Immunosuppressive-treated children with malignancies showed more severe viral infections than infections from bacteria [9,10], yet this might also result in severe illness and sepsis. One of the new techniques to diagnose viral infections early is the light cycler PCR. Herpes simplex virus is the virus most commonly detected in diagnostic laboratories [11]; however, since this virus replicates optimally in human diploid fibroblast in cell cultures, it is obvious that only samples with a high concentration of virus can be detected easily. Furthermore, other methods like enzyme-linked immunosorbent assay and latex agglutination assay as well as fluorescent antibody techniques fail when low titers are present in specimens that are inoculated in cell cultures [12–15]. Although the PCR has the capability to correctly and rapidly detect HSV, practical usage in the laboratory routine is difficult because of crossover contaminations between different probes from different patients.

These problems will be overcome with the light cycler technique in routine diagnostics. With this method, detection of amplified nucleoid acid products is accomplished in a closed system. Thus there is no opportunity for carry-over contaminations to occur. Furthermore, the amplification processes were measured in real time without transfer to some other detection system. In the detection of low titer HSV (and other viruses as well) this technique might be of advantage because the number of cycles needed to amplify the DNA can be controlled online and fitted for every sample. As recently shown [16], comparing classical methods of HSV detection with the routine light cycler method revealed a specificity of 100%, with the light cycler detecting even low titer specimens. This is especially important in the diagnosis of early sepsis.

As mentioned above, one major problem in the diagnosis of sepsis is differentiating between the bacterial species involved. This is essential before starting a suitable antibiotic treatment.

PCR = polymerase chain reaction

Procalcitonin

Recently, plasma procalcitonin levels were proposed as an indicator of the presence of infection. PCT might therefore be a laboratory marker that can compete with the standard parameters used in the diagnosis of sepsis, such as white blood cell count and C-reactive protein.

PCT is a 116 amino acid protein that is the precursor of calcitonin. PCT concentrations are very low in healthy individuals and were shown to markedly increase after endotoxin administration in healthy volunteers [17] and during severe systemic infection and septic shock [18]. Furthermore, PCT was shown to be elevated in bacterial, parasitic and fungal infections. Thus, PCT elevation may indicate bacterial infection before standard confirmation by positive bacterial culture is attained.

In patients with adult respiratory distress syndrome, those with an infectious cause for sepsis had a significantly higher serum level of PCT than patients with ARDS associated with a non-septic cause. The elevation of PCT remained detectable from an early stage of disease until 72 hours later, during which there was no overlap between septic and non-septic ARDS patients [19].

A recent observational study with 111 critically ill infectious patients demonstrated that the diagnostic combination of PCT and CRP increased the specificity for infection [20]. Similar results were obtained, even when compared to pro-inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6 [21]. Kuse et al. [22] showed that elevated PCT levels can help differentiate between acute rejection and infection with fever of unknown origin in patients after liver transplantation. Furthermore, PCT can serve as an indicator of severe sepsis and multiple organ dysfunction syndrome in patients with mechanical trauma, as shown in a retrospective study by Wanner et al. [23].

The actual role of PCT in sepsis is not known. In normal physiology, the only role established for PCT is as a precursor to calcitonin. Calcitonin is known to regulate bone and calcium metabolism and inhibit osteoclastic bone resorption. Regulation of calcitonin release is primarily influenced by plasma concentrations of ionized calcium. While this suggests a relationship to the hypocalcemic state in patients with sepsis, it has not been proved.

Also unknown is the site of PCT production, although some authors have proposed peripheral mononuclear cells as a possible site for PCT production during inflammation. PCT might be a helpful marker to differentiate between infectious and non-infectious causes of systemic inflammatory response syndrome.

Hypophosphatemia

Hypophosphatemia has long been reported to be associated with sepsis and gram-negative infections [24] and was proposed as a strong indicator of gram-negative bacteremia in sepsis [25]. Comparing serum phosphate levels from patients with blood culture-positive sepsis with the levels of blood culture-negative infected patients shows a significant difference, with hypophosphatemia as a strong indicator for infection. In addition to these findings it could be shown that about 80% of septic patients had hypophosphatemia associated with very high levels of TNFα, IL-6 and soluble IL-2 receptor [26]. It was shown that even in mice the injection of the respective interleukins (IL-6, IL-1β and TNFα) markedly decreased the phosphate serum levels [26], supporting the direct association between elevated cytokine levels and hypophosphatemia.

The results mentioned above were already confirmed in a study with patients from a surgical intensive care unit with a high incidence of hypophosphatemia (28.8%). In addition, survival of the patients with hypophosphatemia was significantly lower than in patients with normal blood phosphate concentration [27].

Taken together, both parameters – PCT and serum phosphate – might be additional helpful markers in the identification and characerization of the bacteria and the diagnosis of sepsis.

Conclusion

As reviewed here, the diagnostics of sepsis have become increasingly more sophisticated in the last few years. Especially regarding the identification of the involved bacterial species, the newly developed techniques like PCR and its variations can be very helpful. However, there are some limitations with regard to sensitivity and specificity, but it appears that in the not too distant future more rapid and very specific tools will be devised for the identification of the involved bacteria in infection and sepsis. Also, it has yet to be elucidated whether direct intervention, e.g., treatment of the hypophosphatemia, might favorably affect the outcome in these patients.

Finally, measuring laboratory parameters that determine the stage and severity of infectious diseases in septic patients will contribute to a better, faster and more specific treatment and will certainly enhance our understanding of the pathophysiological mechanisms involved in the pathogenesis of sepsis.

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PCT = procalcitonin
ARDS = adult respiratory distress syndrome
CRP = C-reactive protein

TNFα = tumor necrosis factor-alpha
IL = interleukin

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