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EDITORIAL

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# The emerging power of molecular diagnostics: towards improved management of life-threatening infection

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Bacteraemia, characterised by the isolation of viable bacterial pathogens from the bloodstream, indicates an invasive infection that frequently leads to sepsis. The crude mortality of patients with bacteraemia ranges from 25% to 60%, with approximately one-third to half of fatal outcomes attributable to infection [1]. Several prospective studies have shown that early administration of appropriate antimicrobial therapy significantly improves the prognosis, with a twofold fatality rate in patients not receiving appropriate initial therapy [2]. Currently, initial treatment must be empirical, with selection of antimicrobial drugs based on the most likely pathogens and their local susceptibility profiles. Blood-culture specimens typically become positive within 8–36 h after sampling, and therapy can be adapted based on presumptive bacterial identification suggested by Gram-stain characteristics. By conventional methods, a complete microbial identification and susceptibility profile is not available until 24–48 h later. Some fastidious pathogens may be detected only later or not at all by blood culture, either because of slow growth with organisms such as yeasts or owing to inhibition by anticoagulants contained in culture medium, as seen with *Neisseria* spp. The increasing diversity of opportunistic pathogens causing bacteraemia in compromised hosts and the increase in acquired antimicrobial resistance make the empirical selection of appropriate antimicrobial therapy more complex. These difficulties drive the escalation in the use of broad-spectrum antibacterial agents, further fuelling the problem of resistance. Therefore, there is a need to improve the rapidity, sensitivity and accuracy of diagnosis of disseminated bacterial and fungal infections, both in terms of improving the prognosis of infected patients and of supporting more rational and parsimonious antibiotic prescribing.

Molecular diagnosis can contribute significantly to rapid detection and identification of pathogenic organisms in clinical samples [3]. PCR amplification of nucleic acid sequences characteristic of microbial pathogens using specific oligonucleotide primers has indeed proved useful for the early detection of slow-growing or fastidious pathogens when a specific diagnosis is clinically suspected, such as tuberculosis or bacterial meningitis. However, when a wide range of pathogens must be detected, as is the case for bacteraemia, the multiplex PCR approach that uses a combination of specific primers quickly reaches its technical limitations due to interference between multiple primer combinations and decreased sensitivity of the assay. This problem can be overcome by the broad-range PCR strategy. This technique relies on the use of a set of universal primers designed to amplify conserved segments of DNA from any bacteria present in the sample. Identification of the organism is performed by DNA sequence analysis [3, 4], hybridisation to species-specific oligonucleotide probes [5], restriction or conformation analysis [6]. The most commonly used target genes for broad-range PCR assays are the 16S or 23S rDNAs, which are increasingly used in bacterial taxonomy.

Sleigh and colleagues [7] further investigated the value of this rapid 16S rDNA universal PCR and sequence analysis method. They analyse blood samples taken in

parallel with blood cultures from critically ill patients presenting with sepsis. Based on multiple clinical and microbiological criteria to assess the significance of positive results, they have shown that this PCR-based method is about twice as sensitive (83%) as culture (45%) in the detection of disseminated bacterial infection. All true-positive specimens obtained by PCR which were concomitantly culture negative occurred in patients who were receiving antibiotics at the time of sampling. This finding, however, is difficult to interpret. Firstly, the authors unfortunately do not describe the type of blood culture media used with the Bactec system. This is important because resin-containing broth media proposed by this and other manufacturers have been shown to increase approximately twofold the yield of bacteria from bacteraemic patients under antibiotic therapy. Secondly, the clinical utility of this increased sensitivity of PCR in treated patients appeared limited in this series, considering that 57% of those patients had the same organism isolated from other blood culture specimens. The clinical utility of newer diagnostic approaches versus conventional methods is best evaluated by comparing the diagnostic yield by infectious episode and not only by specimen. In other words, how many aetiological diagnoses obtained by PCR would have been missed by the conventional culture method? In a large clinical evaluation of similar strategies by universal rDNA PCR/sequencing analysis of various body fluids and tissues, PCR was the only method to establish a diagnosis in 2.4% of infections, mostly those caused by fastidious pathogens or detected in patients already under antimicrobial therapy [4].

Moreover, Sleigh and colleagues [7] observed that a substantial proportion, i.e. 40%, of blood specimens with amplified bacterial DNA remained of indeterminate clinical significance after careful clinical evaluation. These unclear results were related either to DNA products of unsuitable quality for sequence analysis, or to bacterial DNA sequences inferred to represent sample contaminants or transient DNA from bacteria of questionable pathogenicity. Besides the technical limitations of this strategy, notably the difficulty in correctly identifying multiple sequences in mixed infection [6], these unexplained bacterial DNA signals found in the blood of septic patients with negative culture raise a number of intriguing questions that should be further examined in experimental and observational studies. What are the dynamics of bacterial DNA in the different body compartments during sepsis and multiple organ failure? Can bacterial DNA translocate through the damaged mucosa in the absence of multiplication in the bloodstream? What are the persistence and integrity of DNA released from non-viable or killed microorganisms in various tissues such as the reticulo-endothelial system and in body fluids such as blood? What is the clinical significance of these findings in terms of response to therapy and clearance of infection?

This report therefore provides encouraging results that confirm the sensitivity and potential clinical usefulness of broad-range PCR for the aetiological diagnosis of bloodstream bacterial infection. What should be done next? Firstly, we need to assess the turnaround time of test results and determine whether and how these results can be used in clinical decision-making algorithms. The rapid development of real-time PCR, high-throughput automated DNA sequencing systems and high-density probe micro-array technology should in the near future make these DNA sequence-based diagnostic approaches more suitable for continuous clinical monitoring. Outcome studies are the only way to establish the cost-effectiveness and cost-benefit of these diagnostic strategies. Important questions to be answered in this perspective include: can PCR-based assays significantly improve patient management, allowing shorter antibiotic courses in non-bacteraemic patients? Conversely, can these results lead to more frequent appropriate therapy and better cure rates in bacteraemic patients, with improved survival and shorter duration of intensive and inpatient care? These issues are complex and require close collaboration between clinicians, clinical microbiologists, molecular biologists and health economists. Priority should be given to studies in patient populations that are most likely to benefit from rapid diagnosis. Critically ill patients with life-threatening infection are obvious candidates for assessing whether these emerging molecular technologies will hold their promise of improved management.

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