

Review

Bench-to-bedside review: The promise of rapid infection diagnosis during sepsis using polymerase chain reaction-based pathogen detection

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Abstract

Early infection diagnosis as the cause of a patient's systemic inflammatory syndrome is an important facet of sepsis care bundles aimed at saving lives. Microbiological culture provides the main route for infection diagnosis but by its nature cannot provide time-critical results that can impact on early management. Consequently, broad-spectrum and high-potency antibiotics are essential during the immediate management of suspected sepsis in critical care but are associated with the development of drug-resistant organisms and superinfections. Established molecular laboratory techniques based on polymerase chain reaction (PCR) technology can detect pathogen DNA rapidly and have been developed for translation into a clinical diagnostic setting. In the setting of sepsis in critical care, emerging commercial systems are now available for the analysis of whole blood within hours, with the presumed aim of adoption into the current care bundles. In this review, we consider the importance of early infection diagnosis in sepsis, how this is limited by culture approaches and how the emerging PCR methods are showing promise in early clinical observational studies. The strengths and weaknesses of culture and PCR pathogen detection in whole-blood samples will be highlighted and recommendations made for urgent appropriately powered diagnostic validation studies in advance of clinical effectiveness trials before these emerging PCR pathogen detection techniques can be considered for adoption in clinical practice.

Importance of rapid infection diagnosis in surviving sepsis

Sepsis is the clinical syndrome resulting from a host's systemic inflammatory response to infection and is a major international health care problem. The Surviving Sepsis Campaign promotes an important concept of early, goal-directed

management of sepsis as part of the evidence-based guidelines aimed at saving lives [1]. At the core of these guidelines are the early diagnosis of infection as a cause for the patient's systemic inflammatory response and the timely administration of appropriate antimicrobial chemotherapy.

The consensus definitions of infection in critical care require microbiological evidence of pathogens to make a probable diagnosis (for example, Gram stain) or culture to confirm the diagnosis [2]. The Surviving Sepsis Campaign guidelines advocate obtaining at least whole blood and, where possible, other supporting clinical samples for culture prior to the administration of antibiotics, all achieved within 1 hour in a patient with presumed severe sepsis [1]. Current opinion in critical care favours the early use of antibiotics, guided by local pathogen surveillance, usually of a broad spectrum and high potency, that is equally applicable to all clinical settings in patients with suspected severe sepsis [1]. There is evidence showing that the correct initial choice of antibiotic saves more lives than virtually any other intensive care unit intervention [1,3-5]. This may require broad-spectrum cover in the face of as-yet-unidentified infection because delaying antibiotic therapy in sepsis has been shown to increase mortality and morbidity [6,7]. Unfortunately, the widespread use of broad-spectrum antibiotics is implicated in the emergence of drug-resistant organisms and rising rates of infection with *Clostridium difficile* and fungi. Therefore, early de-escalation of antimicrobial agents is a key aim of Surviving Sepsis Campaign guidelines in order to reduce this problem [1].

CFU/mL = colony-forming units per millilitre; ELISA = enzyme-linked immunosorbent assay; MRSA = methicillin-resistant *Staphylococcus aureus*; PCR = polymerase chain reaction; SIRS = systemic inflammatory response syndrome.

Use and limitations of blood culture in sepsis diagnosis

Blood cultures have a central role in the detection of pathogenesis in patients with evidence of the systemic inflammatory response syndrome (SIRS), thus helping differentiate SIRS and sepsis [1]. Positive results enable antibiotic therapy to be rationalised once pathogen identification and antibiotic sensitivities are known. Blood cultures are considered to provide the clinical gold standard in the diagnosis of bloodstream infections [2], and an established evidence base for their appropriate use when assessing and treating suspected sepsis now exists [1]. In addition, blood cultures can have significant diagnostic value in settings for which the establishment of a microbiological diagnosis is otherwise difficult, particularly in deep-seated infections that would otherwise require invasive procedures for samples to be obtained for culture [2]. Surveillance data from blood cultures also constitute an important epidemiological tool on which to base empirical antimicrobial therapy [1].

While a number of clinical and technical factors may affect the isolation of the infecting organism [8], the volume of blood sampled is the most critical factor in the detection of bloodstream infection. The number of organisms present in adult bacteraemia is frequently low, often less than 10 colony-forming units per millilitre (CFU/mL) [9,10]. There is a direct relationship between blood volume and yield, with an approximately 3% increase in yield per millilitre of blood cultured [10]. National standard laboratories in Europe recommended that at least 20 to 30 mL of systemic blood be cultured from adults [11], which is reinforced by the Surviving Sepsis Campaign guidelines that promote at least two sets of cultures, which may also include additional blood sampling from established indwelling catheters to help delineate catheter-related infection [1].

Contamination of blood cultures giving a 'false-positive' result remains a significant problem that can limit diagnostic utility in the critically ill and is closely associated with poor patient-sampling techniques. Documented rates of contamination vary considerably between institutions, from 0.6% to over 6%, and the interpretation of these rates continues to be problematic [12]. Clearly, repeating blood culture sets increases sample volume and pathogen yield in the setting of bloodstream infection but is primarily recommended to assist in the recognition of contamination [12].

Despite laboratory techniques aimed at neutralising antimicrobial substances present in a blood sample, the sensitivity of blood cultures decreases greatly when taken after the initiation of antimicrobial therapy [13,14]. The use of prophylactic antibiotics and antifungal agents in immunocompromised neutropenic patients, who have a high risk of developing pathogenesis and who may subsequently show signs of SIRS, makes diagnosis challenging as blood cultures remain negative in many cases [15]. Such patients are also at

considerable risk of acquiring infection caused by slow-growing and fastidious organisms, including fungi, for which blood cultures are poorly sensitive.

The detection and identification of microorganisms based on traditional culture-based methods make time-critical decision-making rather difficult because of the significant time lags between patient sampling and results. This can take 2 to 3 days for bacteria and much longer for other fastidious organisms, and as a consequence, the time required to prove absence of infection by culture methods can exceed that of a treatment course of antibiotics [16]. Therefore, although blood cultures remain at the heart of the sepsis care guidelines, emerging alternative technologies aimed at complementing the deficiencies of culture, particularly related to improving time-critical diagnostics, are being investigated.

Polymerase chain reaction approaches to the diagnosis of pathogenesis

Molecular methods based on polymerase chain reaction (PCR) technology have been developed for infection diagnosis and pathogen identification. These methods offer a new approach based on detection and recognition of pathogen DNA in the blood, or indeed other clinical samples, with the potential to obtain results in a much shorter time frame (hours) than is possible with conventional culture. PCR-based pathogen detection depends on the ability of the reaction to selectively amplify specific regions of DNA, allowing even minute amounts of pathogen DNA in clinical samples to be detected and analysed. The DNA sequence that is amplified is determined by the design of oligonucleotide primers, short pieces of synthesised DNA that bind to either end of the sequence and form the starting point for DNA replication by DNA polymerase.

For bacterial pathogens, two basic approaches have been taken in assay design, using either specific primers that detect a particular organism or, more commonly, universal primers that bind to conserved sequences in bacterial but not human DNA. The latter approach has the potential to detect a large number of bacterial species in a given sample. Assays that are limited to the detection of a specific organism have often been developed to address a specific clinical need (for example, rapid confirmation of the presence of meningococci in patients with meningitis) [17]. In most cases, however, the detection of bloodstream infection requires assays capable of detecting a broad range of pathogens given that several microbial species may be involved, including infections with multiple organisms. To achieve this, the majority of studies have used primers targeting the 16S rRNA gene, with a smaller number focusing on the 23S rRNA gene, which also contains conserved sequences. Following PCR of these regions, the pathogen species present can be identified subsequently by sequencing of the amplified DNA followed by phylogenetic analysis using widely available nucleotide databases [18]. Since the 16S rRNA gene

sequence is ubiquitous in bacteria, this approach has the potential to detect virtually any bacterial pathogen, although some shortcomings have been identified [19]. As early as 1999, Cursons and colleagues [20] showed that PCR directed against the 16S ribosomal region was effective in detecting pathogenesis in a cohort of critically ill patients, although careful optimisation of pathogen DNA extraction was necessary, particularly for the detection of Gram-positive organisms. It was concluded that PCR was more sensitive than conventional blood culture, with a significant proportion of patients being PCR-positive and blood culture-negative. Jordan and colleagues [21] used a similar approach to detect sepsis in neonates but with the addition of pyrosequencing, a technique that enables a more rapid identification of the bacterial species by sequencing short fragments (approximately 30 bases) of the 16S rRNA.

The 23S rDNA region shows more sequence variation between bacterial species than 16S, potentially making the former more suitable for discriminating the different blood-borne pathogens encountered in critical care. The few studies that have targeted the 23S region show that it is effective in detecting a range of bloodstream infections [22,23]. More recently, the gene sequence between the 16S and 23S regions, the so-called internal transcribed region, has been targeted because it contains more variable regions than either 16S or 23S, allowing even better discrimination of bacterial species [24,25].

Perhaps the single most important advance in molecular diagnosis of infection has been the application of real-time PCR. In this technique, the products formed during PCR are monitored continuously as the reaction progresses, using either fluorescent dyes that bind nonspecifically to double-stranded DNA or fluorescently labeled probes that bind to specific sequences. The whole process of PCR amplification, product detection and analysis is achieved in a single reaction vessel. Furthermore, several sequence-specific probes with different fluorescent reporters can be added to the reaction, allowing simultaneous determination of multiple products. This process is therefore ideally suited to infection diagnosis in which a variety of pathogen species could be involved. Another advantage of real-time PCR is that, unlike conventional PCR, it offers the potential to quantify the amount of pathogen DNA present in a clinical sample. Real-time PCR has now been applied in a number of clinical pilot studies. Jordan and Durso [26] recently described a real-time assay based on 16S rDNA which was capable of detecting a range of common pathogens encountered in neonatal sepsis, and when the assay was applied to 85 blood samples from culture-proven sepsis, they reported a 94% agreement. Subsequently, a number of small studies have indicated the analytical effectiveness of using real-time PCR to detect infection in an adult intensive care setting [25,27-29], although the clinical utility of such measurements requires further evaluation.

The ability to rapidly detect the presence of antibiotic-resistant organisms is an increasingly important consideration in intensive care. Conventional antimicrobial susceptibility tests require culture of the organism from the clinical sample with a further delay of at least 24 to 48 hours before results are available. Once the genetic differences that underlie drug resistance in a particular species are known, it is feasible to develop PCR assays able to rapidly identify resistant organisms directly in clinical samples [30]. This approach has been successfully applied to the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in various clinical samples with assays aimed at the *mecA* and *femA* genetic elements that cause resistance. A study of tracheal aspirates from mechanically ventilated patients showed concordance between PCR and conventional culture in 57 out of 60 MRSA-positive samples [31]. Furthermore, of the three discordant results (PCR-positive, conventional-negative), all three were later shown to be MRSA-positive through complementary bacteriological testing. Similarly, Louie and colleagues [32] showed diagnostic sensitivity and specificity of 99% and 100%, respectively, for PCR in a study of 306 patients with MRSA bacteraemia with PCR data available within 3 hours. More importantly, it has now been shown that the introduction of PCR screening for MRSA in critical care has a significant impact on transmission rates, with an associated relative risk reduction of 0.65 and a 95% confidence interval of 0.28 to 1.07, in a cohort of 1,305 critically ill patients [33]. While PCR may be appropriate in identifying resistance in specific organisms, it is unlikely in general to be the most effective way of screening for drug resistance given the hundreds of known resistance genes. The use of other genetic techniques (such as DNA microarray) which allow simultaneous analysis of a large number of drug-resistance genes in diverse bacteria is under investigation [34].

Fungal infections are also targets for the development of molecular diagnostic techniques. Infections with *Aspergillus* and *Candida* species are increasingly important in intensive care and are associated with high morbidity and mortality. However, current diagnostic procedures like mycological culture or microscopy require either long growth periods or suffer from poor sensitivity. Several PCR assays aimed at identifying specific fungal species have been developed, although this approach has limitations given that in a proportion of cases infections may be polymicrobial. A recent review showed that, in the case of *Aspergillus*, most PCR assays were targeting the two most prevalent types, *Aspergillus fumigatus* and *A. flavus*, which represent only 2 out of the 20 or so *Aspergillus* species that have been shown to cause opportunistic infections in humans [35]. Studies comparing PCR methods with the enzyme-linked immunosorbent assay (ELISA) test for *Aspergillus* antigen and other markers used in the diagnosis of invasive pulmonary aspergillosis have reported diagnostic sensitivity of 79% for PCR compared with 58% and 67% for ELISA and other

markers, respectively; diagnostic specificity was 92% for PCR compared with 97% for ELISA and 84% for the other diagnostic tests, respectively. Importantly, results of PCR were available more quickly than those of the other diagnostic tests [35]. More recently, broad-range real-time PCR assays for fungal species have been developed [36,37]. Schabereiter-Gurtner and colleagues [37] used a combination of universal primers and group-specific probes to identify 11 clinically relevant *Aspergillus* and *Candida* species in a range of clinical samples, including blood, tracheal aspirates and cerebrospinal fluid. The assay was able to detect fungal infections with high analytical sensitivity (for example, 5 to 10 CFU/mL blood).

In terms of clinical utility in sepsis, the most effective PCR approach would be one in which both bacterial and fungal species could be detected and identified in a single assay. Roche Diagnostics (Basel, Switzerland) has introduced the SeptiFast™ platform following recent European regulatory approval. SeptiFast™ is a commercial real-time PCR diagnostic kit that is designed to detect and identify 25 bacterial and fungal species that make up greater than 90% of the pathogens causing bloodstream infections in critical care, with a sensitivity of between 3 and 30 CFU/mL [25]. To date, there has been only limited published evidence of the clinical utility of SeptiFast™. A recent study by Louie and colleagues [38] in 200 patients with clinical suspicion of sepsis recruited from a mixture of intensive care and general medicine acute wards showed that SeptiFast™ detected more instances of pathogenaemia than did blood culture, with results available potentially within 6 hours. However, that study also reported several false-negatives in which culture detected an organism that was not present on the SeptiFast™ PCR panel, a limitation of the current platform. Broadly similar findings with SeptiFast™ have been reported in a group of haematology patients with suspected sepsis [39]. A second commercial system for diagnosis of bloodstream infection, SepsiTTest™ (Molzym GmbH & Co. KG, Bremen, Germany), recently gained European regulatory approval. This test takes a different approach, using universal primers to report the presence of a bacterial or fungal DNA in blood with species identification relying on post-test sequencing of the products. To date, there have been no published studies evaluating the use of SepsiTTest™ in a clinical setting.

Key issues in the application of polymerase chain reaction to the diagnosis and management of sepsis

The application of PCR techniques to detect and identify pathogens has the potential to revolutionise the diagnosis and management of sepsis. Unlike microbiological culture, a PCR diagnosis confirming the absence or presence of a pathogen, along with species identification, could be available to the clinician in a few hours. However, there remain a number of unresolved questions about the interpretation of PCR clinical data and the significance of circulating pathogen

DNA as a marker of infection. There are several possible reasons for frequently reported so-called 'false' positives in which PCR shows evidence of pathogen DNA in the absence of culturable organisms. Given the sensitivity of the PCR technique, it is important to rule out the possibility that a 'false' positive occurs as a result of environmental contamination, although these events can be minimised by adoption of strict procedures for sample collection and processing [25]. PCR may also give a positive result in the absence of intact pathogens since it does not distinguish between DNA associated with viable bacteria and DNA originally from intact bacteria in the circulation which have been destroyed as a result of host immune responses and/or recent antibiotic administration. Further studies are needed to monitor the kinetics of bacteria DNA appearance and clearance from the blood during infection and antibiotic treatment. It is important to consider that 'false' positives may have biological significance and provide information of diagnostic value. For example, freely circulating pathogen DNA may be a biomarker of infection at extracirculatory sites due to shedding of pathogen DNA into the circulation. With this in mind, the adoption of techniques that separate intact organisms in blood, or other fluids, prior to DNA extraction [40] would allow discrimination between the presence of DNA associated with intact pathogens and free pathogen DNA. Such DNA separation may help investigators to understand the high rates of 'false-positive' PCR results in blood from patients with suspected sepsis and potentially provide valuable information on the broader infection phenotype in an individual. In recognising the potential limitations of PCR for detecting infection, it is also important to balance these against uncertainties in the current 'gold standard' of microbiological culture.

Despite the significant potential benefits in reducing unnecessary antibiotic use in critical care, there has been little discussion so far regarding the value of a negative PCR result from blood ruling out sepsis in patients who are subsequently shown on culture to have no evidence of infection. However, here again, careful validation of pathogen PCR is required since instances of negative PCR results associated with positive blood culture are reported, although these are relatively infrequent and, in many cases, appear to be the result of the organism's not being included in the particular PCR panel [41]. Similar considerations apply to the application of PCR to other clinical samples such as sputum for the diagnosis of nosocomial respiratory infections such as ventilator-associated pneumonia. However, additional sample-specific issues may arise such as the high background levels of commensal organisms likely to be present in the respiratory tract which may make clinical interpretation of PCR data more difficult.

Conclusions

There is real promise of rapid infection diagnosis in suspected sepsis using PCR pathogen detection as evidenced

by its inclusion as an emerging technology in recent Surviving Sepsis Campaign publications and by the early commercialisation of these molecular laboratory techniques for application in critical care. While culture techniques offer the best practice today, limitations, particularly in terms of their time constraints and their sensitivities in patients already exposed to antibiotics, are significant. However, the promise of rapid PCR-driven single-blood-sample diagnostics in this arena has yet to be realised, and only a few peer-reviewed observational studies using the emerging commercially available systems have been published. Therefore, we have the opportunity now to drive a thorough health technology assessment process of the available PCR systems from laboratory diagnostic validation through to appropriately powered, well-designed clinical validation and effectiveness studies in populations of critically ill patients who would most likely benefit. We anticipate that, if PCR technologies prove to have acceptable clinical diagnostic validity, subsequent clinical effectiveness studies are most likely to prove cost-efficient when comparing standard culture with PCR in delivering early antibiotic de-escalation. Until such processes have been completed and reported, we cannot recommend technology adoption. We also believe that a systematic approach to the investigation of pathogen DNAemia during critical illness will help delineate patient phenotypes in the SIRS/sepsis spectrum and provide a firmer understanding of the biology of pathogen DNA that underpins rapid PCR-based diagnostics. Furthermore, the feasibility of determining antibiotic sensitivities at a gene level for a range of pathogens associated with sepsis remains to be investigated but should not delay the assessment of the currently available commercial platforms aimed at pathogen detection and identification.

Competing interests

PMD has received rail travel costs from Roche Diagnostics to allow the presentation of PCR-based pilot data of infection diagnosis at a UK national education meeting. The research laboratories of GW and PMD have performed service evaluations of SeptiFast™, SepsiTst™ and noncommercial multiplex PCR platforms; SeptiFast™ was provided free of charge for this purpose by Roche Diagnostics UK. We have not received any funding or sponsorship for this article. This article represents our views, based on our experiences within the National Health Service, of this and other PCR-based assays and not necessarily the views of Roche Diagnostics.

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