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REVIEW

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Will polymerase chain reaction (PCR)-based diagnostics improve outcome in septic patients? A clinical view

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Abstract Polymerase chain reaction (PCR)-based techniques allow more rapid and sensitive detection of pathogens compared with conventional blood culture. Nevertheless, the climate of opinion of relevant studies is that currently PCR can supplement but not replace blood culture. In numerous studies, combined detection rate of both methods was significantly higher compared with PCR or blood culture alone. Also, complete determination of antibiotic resistance can currently be performed only by blood culture. Further increase of the panel of multiplex PCR is complicated, because the vast majority of sepsis pathogens are already included, primer interactions leading to primer heteromers limit the amount of targets detectable within one PCR tube, and an array of too many individual PCR reactions for investigation of a single specimen leads to high cost and workload. Except for diagnostics of patients in whom unusual, not culturable, or fastidious pathogens are detected more often, such as immunosuppressed patients with suspected parasitic infection, etc., it might even not be necessary to further increase the spectrum of detectable species. If the primary aim of PCR diagnostics is to decrease inappropriate empirical treatment and improve patient outcome, detection should focus on those pathogens or resistance determinants that are not covered by guideline-recommended treatment regimens and that have been identified as the major cause of inappropriate treatment according to current studies. In our opinion, such a narrower assay is more cost effective, may achieve higher accuracy due to reduced intratest interference, and would better address current and emerging clinical needs.

Keywords Blood culture · *Candida* · Intensive care · *Enterococcus* · Antibiotics

The clinical problem

Despite technical advances in intensive care medicine, sepsis remains a disease with unacceptable high mortality. Sepsis is defined as an inflammatory host response (i.e., severe inflammatory syndrome) to infection. Sepsis can progress to severe sepsis and septic shock, which is associated with a drastic rise in mortality [1]. Thus, early and appropriate treatment of infection should interrupt this progression and improve patient outcome. Indeed, clinical studies have unequivocally shown that initial inappropriate treatment results in up to fivefold increased mortality, this increase being most dramatic in patients with septic shock [2]. Reasons for inappropriate treatment include (1) no coverage of the underlying pathogen, (2) antimicrobial resistance of the causative pathogen, which is mainly a problem in nosocomial infections and in infections by emerging multiresistant Gram-negative

bacteria, (3) choosing an anti-infective with poor penetration into the infectious focus, and (4) underdosing. Whereas all four reasons may be equally important, polymerase chain reaction (PCR)-based diagnostics can currently only assist with item 1.

Despite not being the focus of this review, items 3 and 4 will be briefly discussed here: The underlying focus in the majority of patients with severe sepsis and septic shock is pneumonia or an intra-abdominal infection [3]. To date, identifying the focus of sepsis is achieved by clinical expertise and imaging. An interesting development, which may assist in identifying the definite focus in near future, is the identification and evaluation of novel organ-specific biomarkers or biomarker profiles.

It is increasingly recognized that fixed doses of antibiotics are not helpful in intensive care unit (ICU) patients, because the individual clearance and volume of distribution of patients with severe sepsis and septic shock vary widely (e.g., due to volume substitution). Pharmacokinetic studies have demonstrated that fixed doses of antibiotics results in subtherapeutic antibiotic concentrations in many patients [4]. An Italian study recently demonstrated that antibiotic drug monitoring and individual dose adjustment of antibiotics to achieve predefined pharmacokinetic (PK)/pharmacodynamic (PD) parameters is clinically feasible and significantly reduces mortality [5].

Limitations of the current clinical approach

The current guideline-suggested strategy for empirical antimicrobial coverage of the underlying pathogen is the use of broad-spectrum antibiotics with antipseudomonal activity in patients with risk factors for *Pseudomonas* infection [6–9]. The disadvantage of this strategy is the increasing selection of antibiotic-resistant pathogens. In this context, ICUs have been identified in several studies as the main drivers of resistance development [10]. Early de-escalation after obtaining microbiological results is currently suggested as a solution to this dilemma. However, most clinicians are reluctant to change "the winning team" once the patient gets better [11].

Nevertheless, for the individual patient even this guideline-suggested empiric broad-spectrum approach might be insufficient in two scenarios:

1. Fungal infections: Empiric antimycotic coverage is recommended only in patients at risk (e.g., neutropenia, intra-abdominal infections) [8]. A recent analysis demonstrated that almost 30% of inappropriate empiric treatment was due to fungal infections [2]. It has—similar to the milestone study of Kumar et al. [12]—been shown, that delayed antimycotic treatment significantly increases mortality in patients with fungal infections [13–15]. In addition, the time to positivity for blood cultures in candidemia is usually much longer compared with bacteremia. In a recent study, median time to positivity was 32.1 h in one study, and this was associated with increased mortality due to a delay in treatment [16].
2. Infections by multi-resistant bacteria: With the exception of the American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) guideline for hospital-acquired pneumonia [9], most guidelines suggest empirical coverage of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecium* only in patients at risk [8, 17]. In addition, recommended empirical treatment does generally not cover extended spectrum beta-lactamase (ESBL) and carbapenemase-positive Gram-negatives, which are rapidly increasing in recent years [8, 17]. The aforementioned study found that a further 20% of inappropriate treatment was due to MRSA and 35% due to Gram-negative infections [2].

Currently licensed molecular methods and comparison with blood culture

Four different molecular diagnostic approaches for detection of bacterial and fungal DNA in whole blood samples are currently approved for clinical use by European regulatory authorities (i.e., CE certified):

1. A multiplex real-time PCR that simultaneously detects a predefined panel of the most important sepsis pathogens by species- or genus-specific fluorescent probes (SeptiFast®; Roche, Basel, Switzerland).
2. A eubacterial and panfungal real-time PCR that is able to detect nearly all known bacterial and fungal pathogens by a 16S and 18S ribosomal RNA (rRNA) gene-based universal PCR followed by sequencing of the amplification product for species identification (SepsiTest™; Molzym, Bremen, Germany).
3. A multiplex PCR that detects a predefined panel of the most important sepsis pathogens by electrophoretic separation of target-specific amplicons (VYOO®; SIRS Lab, Jena, Germany).
4. A eubacterial and panfungal PCR that is able to detect nearly all known bacterial and fungal pathogens by genome-specific targets followed by mass spectrometry for species identification (Plex-ID; Abbott, Wiesbaden, Germany).

To date, 19 studies investigating SeptiFast® and one study addressing the clinical utility of SepsiTest™ have been published. The clinical performance of YVOO® has been tested in ascitic fluid from patients with spontaneous bacterial peritonitis [18] and synovial fluid from patients with arthritis [19].

In most studies comparing PCR-based diagnostics with conventional blood culture, more pathogens were detected by PCR techniques. However, not all pathogens detected by blood culture were also found by PCR techniques despite these pathogens being included in the PCR panel [20–26]. In contrast to increasing amplification cycles, specific enrichment of prokaryotic DNA before multiplex PCR may be able to overcome these limitations of sensitivity without decreasing specificity. VYOO® uses a protein immobilized on a column (LOOXSTER® technology) that specifically binds to prokaryotic DNA for selective enrichment of bacterial DNA from mixed solutions with high amounts of human background (e.g., blood samples). A study on blood samples from septic patients showed that preanalytic DNA enrichment increased the ratio of bacterial DNA to human DNA from 26% to 74%, resulting in an at least 10-fold higher sensitivity [27]. In SepsiTest™, indirect enrichment of pathogen DNA is performed by application of a DNase that is specific for human DNA (Molysis® technology). By use of the Molysis® technology, sensitivity of bacterial DNA isolation from blood samples could be increased by nearly 100-fold to 50 cfu/ml of whole blood [28]. SeptiFast™ encounters the problem of the signal-to-noise ratio (i.e., increase in sensitivity decreases specificity) with lower detection limits for frequent contaminants such as coagulase-negative staphylococci (CNS), *Candida glabrata*, and *Streptococcus* ssp. (detection limit of 100 versus 30 cfu/ml in all other pathogens). Whereas the detection limit of 100 cfu/ml for CNS might help to decrease the likelihood of false-positive results (contamination), the limit of 30 cfu/ml is clearly inferior to the detection limit of blood culture (up to 1 cfu/ml).

Otherwise, a considerable proportion of blood cultures are obtained from pretreated patients or patients developing sepsis despite antibiotic prophylaxis (e.g., patients after solid organ, stem cell or bone marrow transplantation). In these patients, PCR-based methods should still be able to detect DNA from nonviable pathogens that cannot be detected by culture. Clinical studies provide inconclusive data in this regard: in a Danish study, 52% of all causative pathogens were detected by SeptiFast® despite effective antimicrobial treatment compared with a 37% detection rate by blood culture [29]. In addition, several smaller studies revealed results in favor of SeptiFast® compared with blood culture when samples were obtained during antimicrobial exposure [23, 24, 30, 31]. Only in one study, by Tsalik et al. [32], did blood culture identify more cases of septicemia than PCR among patients with an identified infectious etiology (66 and 46, respectively; $P = 0.0004$). The authors did not provide an explanation for these unexpected results.

The SepsiTest™ approach has been evaluated for blood samples to date only in one clinical study in 187 patients with systemic inflammatory response syndrome, sepsis, or neutropenic fever [33]. Compared with blood culture, the diagnostic sensitivity and specificity of the SepsiTest™ were 87.0% and 85.8%, respectively. The concordance of PCR and blood culture for both positive and negative samples was 86.0%. In this study, results of other microbiological samples apart from blood cultures as well as clinical data of the patients were considered, and in 25/31 PCR/sequencing-positive but blood-culture-negative patients the PCR/sequencing result was judged as possible, probable or true bacteremia [33]. The high concordance between blood culture and PCR may be explained by the unlimited spectrum of pathogens that can be identified by the sequencing approach compared with the defined panel of multiplex PCR. However, a study directly comparing multiplex PCR and post-PCR sequencing has not yet been reported. The first clinical experience with VYOO® in sepsis on the ICU showed that VYOO® was able to detect sepsis in 36% of suspected sepsis cases, compared with 12% for blood culture; in addition, causative fungal pathogens were detected by VYOO® in 11% of patients, compared with 0% for blood culture [38]. Currently, a large multicenter clinical trial is ongoing to evaluate the utility of VYOO® in blood from septic patients.

Published studies investigating Plex-ID® are not available yet.

PCR-guided treatment—what is the evidence?

Numerous studies have been published that compare diagnostic performance of PCR-based diagnostics with conventional blood culture. In contrast, studies addressing the potential therapeutic impact or even reporting on clinical outcomes after adjustment according to PCR results are rare [21, 23]. These studies are, however, urgently needed, since comparison studies between PCR- and blood-culture-based diagnostics have important limitations: although blood culture is considered the gold standard of microbiological diagnosis of sepsis, it has an apparently low sensitivity, it only detects viable pathogens, and the medium is not optimized for culture of fungi and fastidious bacteria. Thus, a positive PCR result that is not confirmed by blood culture may reflect a real bacterial or fungal infection, although contamination of the investigated sample by commensal flora can never be fully excluded.

There is one randomized trial in 309 patients after allogenic stem cell transplantation: Hebart et al. [34] compared empirical and/or (noncommercial) PCR-based amphotericin B treatment versus empirical liposomal amphotericin B treatment. Amphotericin B was empirically started after 120 h of febrile neutropenia refractory to broad-spectrum antibacterial therapy. In addition, in the intervention group, therapy was started after one positive PCR result. Survival curves showed better

survival until day 30 when close PCR monitoring was performed (mortality 1.5% versus 6.3%; $P = 0.015$), but there was no difference at day 100.

There are no randomized interventional trials investigating the clinical impact of SeptiFast®, SepsiTest™, YVOO® or Plex-ID. In an observational study by Dierkes et al., altogether in 8 of 101 samples (8%) from 77 patients an adjustment of therapy would have been triggered by the SeptiFast® results. Three of those adjustments would have been made earlier (before availability of blood culture results), while five would have been missed by conventional blood culture. In six patients an antifungal drug was added to the initial regimen, and in three other patients vancomycin was added for coverage of *Enterococcus faecium* (in one patient both modifications were made) [21].

In a study by Wallet et al., in only 8/72 ICU patients organisms were detected by SeptiFast™ (negative blood culture) and in 4 of these patients antibiotic treatment was changed [25]. Outcome of patients after treatment adjustment was not reported in these studies.

In the patient series of Bloos and Kortgen, 40% of positive VYOO® results led to a change in anti-infective therapy [35]. Of six patients with fungemia who did not have antifungal therapy started after a positive VYOO® result, four died, whereas only one of four who had antifungals started based on the VYOO® result died [36].

On the other hand, it has to be considered that false-positive PCR results may potentially increase unnecessary utilization of antibiotics. Therefore, a PCR result has to be critically judged in the clinical and microbiological context of the patient, and the identified species of the pathogen has to be taken into account; for instance, single detection of DNA of typical contaminant should not immediately prompt antibiotic therapy.

PCR false positives—an unresolvable question?

A major problem in studies comparing PCR-based diagnostics with blood culture is interpretation of pathogens detected by PCR that are not found in corresponding blood cultures. To distinguish between false- and true-positive results, most investigators analyzed additional information available on these patients such as additional microbiological tests and levels of inflammatory parameters: The majority of pathogens detected by PCR but not by blood culture could be identified as true positive because they were found by culture of specimen obtained from the infectious focus [e.g., bronchoalveolar lavage fluid (BALF), wound swabs]. For comparison of a novel diagnostic method with a poor gold standard (i.e., blood culture), there is even a statistical model called latent class analysis, which has unfortunately not been applied by the aforementioned studies [37].

A recent prospective controlled study in three surgical intensive care units enrolling 142 patients with severe sepsis and 63 surgical controls found that, in severe sepsis, 34.7% of samples analyzed by SeptiFast® were positive compared with 16.5% of blood cultures ($P < 0.001$) [26]. Compared with patients with negative PCR at enrollment, those tested positive had higher organ dysfunction scores [SOFA, median (25–75th percentile) 12 (7–15) versus 9 (7–11); $P = 0.023$] and a trend toward higher mortality (PCR negative 25.3%, PCR positive 39.1%; $P = 0.115$).

In 353 patients with community-acquired pneumonia, Rello et al. [38] detected pneumococcal DNAemia by real-time PCR in 58 of 353 patients. Only in 29/58 patients did blood cultures reveal bacteremia. However, they were able to demonstrate an almost linear relation between an increase in log numbers of pneumococcal copies and the risk for septic shock and/or the need for mechanical ventilation.

These results suggest that presence of microbial DNA in the bloodstream is a significant and prognostic event even if the accompanying blood culture remains negative. Further studies are, however, necessary to confirm these observations.

Clinical needs not addressed to date

Despite promising results with current PCR-based diagnostic tests, full determination of antibiotic resistance can currently only be performed by blood culture. This becomes increasingly important in the context of rising broad-spectrum resistance in Gram-negative rods, particularly broad-spectrum beta-lactamase- and carbapenemase-positive Enterobacteriaceae and multidrug-resistant *Acinetobacter*. These pathogens are resistant to almost all antibiotics except colistin and to a lesser degree tigecycline [39, 40], which are not contained in the recommendations of current major guidelines for empiric treatment. A recent study from Greece, the most important source of VIM-1 metallo-beta-lactamase-expressing Enterobacteriaceae, clearly showed that initial inappropriate treatment of these pathogens results in a twofold increase in mortality [41]. Because carbapenemases, e.g., NDM-1, encoding genes are located on plasmids and can therefore spread over species barriers, utility of PCR diagnostics based on species identification is limited [42].

Whereas fast identification of methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant enterococci (VRE) may be feasible by molecular methods since methicillin resistance in MRSA or vancomycin resistance in VRE are encoded by a limited number of genotypes, extended-spectrum beta-lactamases are encoded by a large and still evolving variety of genotypes,

Table 1 Limitations and advantages of sepsis PCR assays

Limitations
High assay cost (mostly >100 € per sample)
Special laboratory equipment necessary for some systems and highly trained personnel required
Real time to result often >24 h despite analytical time to result of <8 h
Sensitivity for detection of intracellular pathogens not defined
Determination of antimicrobial susceptibility not possible for the majority of pathogens
Different spectrum of detectable sepsis pathogens compared with blood culture
Only detection of the most prevalent sepsis pathogens with some systems
Benefit for patients in terms of mortality (except for *Aspergillus* PCR [34]) not yet determined
Advantages of PCR assays over blood culture diagnostics
Detection of nonviable pathogens
Detection of fastidious and nonculturable pathogens
Faster detection of slow-growing pathogens such as fungi

making prediction of resistance by molecular methods unreliable.

The main advantage of PCR over blood culture is the faster availability of results, 6–8 h versus 2–4 days, thus allowing focused treatment earlier. However, this technical advantage can only be fully exploited when immediate analysis by a 24 h service is possible, which is not available in most institutions. Another drawback of molecular diagnostics in comparison with conventional blood culture is the higher cost, especially if individual samples have to be analyzed on a 24 h service basis, and the need of specifically trained personnel for test performance but also interpretation of results. Therefore, for most microbiology laboratories, testing of individual samples is not feasible.

To overcome these aforementioned shortcomings, a completely automated test for individual sample analysis may substantially improve clinical utility. In a recent study, an automated extraction system was used instead of the labor-intensive and time-consuming manual DNA extraction of SeptiFast®. The results showed that SeptiFast® with the alternative automatized MagNA Pure compact extraction not only shortened the complete workflow to 3.6 h, but also increased sensitivity of the molecular assay for detecting infection as defined by positive blood culture confirmation [43]. Completely automated systems from extraction to read out (point-of-care tests) are currently also under development but have not yet been evaluated in clinical studies.

Table 2 Studies on PCR-guided therapy

Study and assay	Study population	Design and study protocol	Outcome measures	Results
Hebart [34] (*Aspergillus* PCR)	Postallogenic stem cell transplantation ($n = 309$)	Start of amphotericin B treatment in dependence on positive fungi PCR or standard protocol	Mortality at day 30 and 100	Mortality at day 30: 1.5% (PCR based) versus 6.3% (control); $p < 0.015$ mortality at day 100: no difference
Dierkes [21] (SeptiFast®)	Clinical suspicion of sepsis ($n = 77$)	Retrospective analysis of impact of PCR result on clinical management	Change to adequate antimicrobial therapy based on PCR result	PCR positive but blood culture negative in 13 patients, adaption of therapy recommended and done in 5/13 patients, treatment adjustment possible earlier in 3/14 blood-culture-positive patients
Wallet [25] (SeptiFast®)	ICU patients ($n = 72$)	Prospective observational study	Change to adequate antimicrobial therapy based on PCR result	PCR positive but blood culture negative in 8 patients, adaption of therapy recommended and done in 4/8 patients
Kortgen [36] (VYOO®)	ICU patients ($n = 98$)	Retrospective analysis of PCR results with respect to detection of fungal DNAemia	Mortality of patients with fungal DNAemia	Detection of fungal DNAemia in 10 patients by PCR (all corresponding blood cultures negative), 4 patients treated with fluconazol, 1 died, 6 patients not treated with antimycotics, 4 died

Conclusions

PCR-based techniques allow more rapid and sensitive detection of pathogens compared with conventional blood culture. Nevertheless, the climate of opinion of the above-mentioned studies is that currently PCR can supplement but not replace blood culture. Combined detection rate of both methods was significantly higher compared with PCR or blood culture alone [22, 23, 26, 31]. Also, complete determination of antibiotic resistance can currently be performed only by blood culture. Therefore, de-escalation strategies can currently not be based on PCR results.

Further increase of the panel of multiplex PCR is complicated because the vast majority of sepsis pathogens are already included, primer interactions leading to primer heteromers limit the amount of targets detectable within one PCR tube, and an array of too many individual PCR reactions for investigation of a single specimen leads to high cost and workload. Except for diagnostics in immunosuppressed patients, who are usually infected by a very wide range of pathogens, it might even not be necessary to further increase the spectrum of detectable species: if the primary aim of PCR diagnostics is to decrease inappropriate empirical treatment and improve patient outcome, detection should focus on those pathogens or resistance determinants that are not covered by guideline-recommended treatment regimens and that have been identified as the major cause of inappropriate treatment according to current studies: *Candida* ssp., *Aspergillus* spp., MRSA, VRE, and extended-spectrum beta-lactamase and carbapenemase-positive Gram-negatives [2]. In our opinion, such a narrower assay would be more cost effective, may achieve higher accuracy due to reduced intratest interference, and would better address current and emerging clinical needs (Tables 1, 2).

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