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Beyond Blood Culture and Gram Stain Analysis: A Review of Molecular Techniques for the Early Detection of Bacteremia in Surgical Patients

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Abstract

Background: Sepsis from bacteremia occurs in 250,000 cases annually in the United States, has a mortality rate as high as 60%, and is associated with a poorer prognosis than localized infection. Because of these high figures, empiric antibiotic administration for patients with systemic inflammatory response syndrome (SIRS) and suspected infection is the second most common indication for antibiotic administration in intensive care units (ICU)s. However, overuse of empiric antibiotics contributes to the development of opportunistic infections, antibiotic resistance, and the increase in multi-drug-resistant bacterial strains. The current method of diagnosing and ruling out bacteremia is via blood culture (BC) and Gram stain (GS) analysis.
Methods: Conventional and molecular methods for diagnosing bacteremia were reviewed and compared. The clinical implications, use, and current clinical trials of polymerase chain reaction (PCR)-based methods to detect bacterial pathogens in the blood stream were detailed.
Results: BC/GS has several disadvantages. These include: some bacteria do not grow in culture media; others do not GS appropriately; and cultures can require up to 5 d to guide or discontinue antibiotic treatment. PCR-based methods can be potentially applied to detect rapidly, accurately, and directly microbes in human blood samples.
Conclusions: Compared with the conventional BC/GS, particular advantages to molecular methods (specifically, PCR-based methods) include faster results, leading to possible improved antibiotic stewardship when bacteremia is not present.

Sepsis results from the severe dysregulation of the immune response triggered by infection [1] and is the leading cause of death in non-cardiac intensive care units (ICUs) [2–5]. In surgical ICUs, deaths from sepsis exceed deaths from pulmonary embolism and myocardial infarction [6]. The most common sources of infection in ICU patients arise from the lung, abdomen, urine, and blood; blood stream infections have the worst prognosis and an associated mortality rate as high as 60% [7–10]. Sepsis from all sources, however, is costly because of its high mortality rate and financial expense. The U.S. Centers for Disease Control and Prevention (CDC) reported a doubling of the number of hospitalizations for sepsis in the last decade, accounting for 1.1 million hospital admissions in 2008 [2], with an annual cost estimated at $24.3 billion [2,11]. Task forces focused on decreasing both the high mortality rate and cost of sepsis anticipate these goals can be achieved with the implementation of sepsis screening programs, treatment bundles, early resuscitation, prompt administration of empiric antimicrobial agents, and aggressive antibiotic stewardship [12,13]. Adherence to these recommendations has decreased mortality rates from sepsis [14,15]. Consequently, empiric antibiotic administration for patients with systemic inflammatory response syndrome (SIRS) and suspected infection is the second most common indication for antibiotic administration in ICUs [16]; however, it is not without risks. Of particular concern is the delay to receipt of diagnostic information and the opportunity for adverse side effects during that interval. Currently, blood culture followed by Gram stain analysis (BC/GS) is the most commonly used laboratory method and

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current gold standard for detecting bacteremia; however, it is suboptimal because of a number of unresolved issues. These include up to 5 d to confirm the presence or absence of bacterial pathogens, a high rate of contamination and low sensitivity because of slow-growing microbes, prior antibiotic exposure, and non-cultivatable pathogens [17,18]. Therefore, in cases in which no bacterial pathogens are present in the blood and no other source of infection suspected, antibiotic therapy is unnecessarily continued for at least 5 d. We postulate that earlier confirmation of the absence of bacterial pathogens is useful for improving antibiotic stewardship and decreasing the cost of sepsis.

This review contrasts the current BC/GS method used to detect the presence or absence of bacteremia to the potential opportunity of using polymerase chain reaction (PCR)-based methods for more rapid pathogen detection, particularly in cases in which no bacteria are present. The clinical criteria for patient selection for BC sample collection, sepsis screening techniques, and the technical details of PCR analysis are not included here as they are beyond the scope of this review [12,19–24].

Definition of Bacteremia

Bacteremia, which is defined as a blood stream infection (BSI), can be classified as primary or secondary. Primary BSIs lack an identifiable infection at another body site or the source of infection is because of an intra-vascular catheter. Secondary BSIs originate from another body system. This classification of primary and secondary BSI does not include transient or continuous low-grade bacteremia from procedures that release normal flora (e.g., toothbrushing or colonoscopy) or sites that contain an intra-vascular foci of bacteria (e.g., graft infection), respectively [25].

Empiric Antibiotic Therapy

Empiric antibiotic therapy initiated within the first hour of clinical suspicion of sepsis decreases mortality rates [14, 15,26], as shown primarily by retrospective or observational studies [27–30]. Conversely, empiric antibiotic administration can have multiple negative effects related to either adverse drug side effects or by altering the host environment, facilitating the development of opportunistic infections, or increasing selective pressure for antibiotic resistant and multi-drug-resistant (MDR) pathogens to arise [31,32]. It is clear that the problem of the increase in multi-drug-resistant (MDR) bacteria will not be addressed by the development of new antibiotics, as only three new antibiotics have been introduced for human use since 2000 [33]. The CDC Threat Report 2013 estimates that, in hindsight, up to 50% of antibiotics prescribed by physicians are unnecessary [32]. To our knowledge, no report has evaluated the impact of empiric antibiotic therapy on patients who have no identifiable source of infection. However, an observational cohort study performed by Hranjec et al. in surgical critically ill patients demonstrated lower mortality rates, more appropriate initial therapy, and a shorter duration of therapy in patients who had an objective diagnosis prior to administering antibiotics [34]. Therefore, although early administration of empiric antibiotic therapy has decreased sepsis-related mortality rates [14,15, 26], timely antibiotic stewardship of the currently available therapies is also important. This is specifically difficult when relying on BC/GS for guidance of therapy.

Blood Culture and Gram Stain Analysis Are Currently Used to Diagnose Bacteremia

The current, commonly used laboratory method for identification of bacteremia is growth in culture medium with subsequent GS analysis and sub-culturing of positive samples for determination of the organism(s) and antibiotic sensitivity [35]. Liquid culture medium inoculated with a 20 to 30 mL blood sample is placed into an automated device with a detector that indicates a positive sample by measuring CO_2 production using either a colorimetric or fluorescence sensor. This positivity is believed to be equivalent to 10^5 colony-forming units (CFU)/mL of bacteria; however, studies indicate that under standard conditions bacterial concentrations at the time of BC positivity are generally between 10^7 to 10^8 CFU/mL [36–39]. (One CFU/mL is equivalent to one viable bacterial cell in a milliliter of the sample.) Thus, the commonly held notion that the definition of clinically substantial bacteremia is the ability of the bacteria to grow to 10^5 CFU/mL is inaccurate.

The automated process generally requires from 1 d to 5 d for detection of a positive sample. As a result, samples are discarded if they do not achieve a positive level of growth within 5 d, although specific exceptions are made when particularly slow growing, fastidious, or intracellular pathogens are suspected [40,41]. The time to positivity is influenced by a number of factors including: the number of bacteria in the initial blood sample, the physiological state of the bacteria (i.e., antibiotic exposure may reduce or prohibit growth), and the identity of the bacteria as different bacteria have different growth rates [41,42] or may be unable to grow in the medium. Thus, it is impossible to predict the time to positivity based on the bacterial quantity at the start of a BC. However, it is likely that shorter incubation times are indicative of greater concentrations of bacteremia [43–45].

Only after a sample is deemed positive by the automated device is it then subjected to GS analysis. The GS analysis is relatively rapid (<15 min), inexpensive, and provides a determination of the presence or absence of bacteria and the classification of the bacterial pathogen as either gram positive, gram negative, gram variable, or gram indeterminate [46].

Shortcomings of BC/GS analysis

There are three major issues concerning the use of this culture-based approach to detect bacteremia. The first problem is the time to provide a result. Although some reports have indicated a time to positivity as rapid as 15 h [25], this does not include the time necessary to obtain the additional required information of species identification and antibiotic sensitivity, which generally requires another 24 to 48 h. Moreover, BC can take up to 5 d to provide a positive result, making it useless as an early guide to appropriate antimicrobial selection for a bacteremic patient. For patients with a SIRS response without bacteremia, the BC/GS analysis procedure requires at least 5 d to rule out the presence of bacteria in the sample. Therefore, the BC/GS analysis does not provide timely results for any patient and this is of particular concern for patients without bacteremia, who as a result are generally administered antibiotics unnecessarily for 5 d.

The second problem is the low sensitivity of BC/GS analysis. Blood culture/Gram stain analysis has an estimated overall positivity of only 30% to 60% despite application in the correct clinical context, standardized procedures, and optimal volume of blood collection [10,47–49]. These results suggest that 40% to 70% of the results are false-negatives. Two possible explanations for the false-negatives are the inability of many bacterial species to grow in standard laboratory culture media or concomitant/prior antibiotic administration [21,50].

The third problem is the opportunity for false-positives to be detected and lead to unnecessary antibiotic administration. False-positive results can be because of sample contamination from the skin flora of the patient or healthcare professional and is estimated to occur in 32% to 85% of clinical samples [51].

At present, the delayed time to result and low sensitivity and specificity with the BC/GS analysis demonstrate that this is suboptimal for routine clinical use and should cause clinicians to question the utility of this method for the detection of bacteremia and the fact that it is considered the gold standard.

Molecular Methods for Diagnosis of Bacteremia

The use of molecular methods for pathogen detection in bacteremia may have advantages in comparison to the traditional BC/GS analysis as some are more rapid, can identify and quantitate pathogens directly from clinical samples, and have reduced variability associated with organism-specific growth requirements. Molecular pathogen detection techniques have been developed that rely on mass spectroscopy, microscopy, or nucleic acid testing (NAT). Specific NAT techniques include traditional PCR and real-time PCR, which can be quantitative (qPCR), semi-quantitative, or qualitative.

Mass spectrometry

Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) is the process by which molecules are ionized and then detected after being separated by charge and mass [52]. MALDI-TOF MS is most commonly used for the identification and quantification for proteins and other biomolecules. It can be used clinically to identify bacterial pathogens based on the proteins present in cells either directly from positive BC or from bacterial colonies isolated from positive BC [53]. The time to detection is as short as 1–2 h; however, this cannot occur until the sample has been designated as positive (up to 5 d) and has colonized (up to 3 d). In addition, poor performance has been noted for the detection of Gram-positive organisms and polymicrobial samples. Other disadvantages include the high cost of the instrument, the requirement of a skilled technician [54,55], and its inability to reduce time for antibiotic sensitivity testing.

Electrospray ionization mass spectrometry (ESI-MS) is used as part of the analysis of PCR-generated DNA fragments (amplicons) in the Ibis Ples-ID® platform developed by Omnica Corporation (Irvine, CA), which is pending clinical trials. Specifically, it determines the molecular weight of amplicons from which a base composition is derived and compared with a large library of more than 750,000 entries for identification of bacteria, fungi, viruses, or parasites. Although this method is reliant on the quality of its database, it is rapid, can be used with polymicrobial samples from positive culture or whole blood, and can detect at least four antibiotic resistance genes. However, it appears that the high cost of the instrument and the requirement for a skilled technician will remain hurdles to clinical application and use.

Microscopy

The peptide nucleic acid fluorescent in situ hybridization (PNA-FISH) method developed by AdvanDx (Wobrun, MA) uses fluorescence microscopy to detect PNA probes that hybridize to organism-specific rRNA gene sequences. This method can reduce the time to result in positive BC; however, it is not useful in samples for which the type of bacteria present is unknown [56,57]. Furthermore, this method relies on a positive BC and cannot be performed directly on blood samples.

Polymerase chain reaction-based methods for earlier detection of bacteremia

Over the last two decades many PCR-based assays for the detection or diagnosis of bacteremia have been developed in-house and by companies with marginal success [25]. Most of these assays isolate their DNA template from a positive blood culture or colony; however, recently a number of commercial methods have developed technologies to directly use any blood sample. All of these methods share four basic steps: lysis of the pathogen, extraction and purification of the DNA template, PCR amplification of the nucleic acid, and an identification method. The identification methods can include hybridization, DNA sequencing, or real-time detection with fluorescence.

Three general approaches have been developed based on the types of gene targets used and how they are employed: 1) pathogen-specific assays use species- or genus-specific targets, 2) multiplex assays allow for the parallel detection of different pathogens using species- or genus-specific targets, and 3) universal broad range assays use bacterial genome-specific targets (i.e., 16S ribosomal RNA [rRNA] gene) [25]. To date, commercial application all of these efforts have been restricted by assay-specific sensitivity, cost, increased labor requirements, inability to replace BC/GS analysis as a stand-alone diagnostic test, or limited clinical application.

Traditional PCR can evaluate if a patient is infected with a specific organism based on amplification with selected oligonucleotide primers of a single gene sequence [58]. Multiplex PCR uses a similar technology, but allows for multiple primer sets to be used within a single PCR reaction. Many methods can be used for the parallel differentiation of the different pathogens, including the production and separation of amplicons of varying sizes that are specific to different DNA sequences [22,35,59]. However, in all of these multiplex assays, a pre-determined set of bacterial species are queried, and the pathogen is only identified if it is within the specific set. Finally, qPCR couples the amplification process with detection of the original number of target DNA sequences in an unknown sample [60–62]. During the qPCR reaction, increasing fluorescent signals are detected as each newly synthesized strand interacts with a probe or dye. The starting concentration of the target sequence can be quantified based on a companion DNA standard [62]. An example

of a commercially available use of this technology is the SepsiTest™ (Molzym Corporation, Bremen, Germany).

Two commercial US-FDA approved applications of the multiplex approach, GeneXpert (Cepheid, Sunnyvale, CA), and StaphSR (BD MAX™ Geneohn, La Jolla, CA), use real-time PCR to identify and differentiate methicillin-resistant *Staphylococcus aureus* (MRSA) from methicillin-sensitive *Staphylococcus aureus* (MSSA) colonization in hospitalized patients. These methods can provide results within 3 h with a sensitivity and specificity ranging from 95.9%–98.3% and 85.3%–99.4%, respectively [25]. Similar methods to detect MRSA colonization have improved infection control [63]. In addition, this technology has been applied to the detection of specific bacterial species used in bioterrorism including *Bacillus anthracis*, *Francisella tularensis*, and *Yersina pestis* [58,64].

Alternatively, universal broad range assays that target bacterial genome-specific genes, such as the highly conserved 16S rRNA gene, can identify any previously isolated bacterium or its relative when the amplified DNA is then sequenced and compared with existing databases [65]. The 16S rRNA gene contains two types of regions; conserved regions are interspersed with nine unique variable regions. For this PCR reaction, primers are designed that anneal to any two different conserved regions that are separated by the variable regions. DNA sequence determination of the resulting amplicon confirms the sequence of the known conserved region and provides DNA sequence for the variable regions present in between, which can be compared with the extensive 16S rRNA gene databases [65]. The comparison to the databases may uncover the identity of the pathogen, define organisms to which the pathogen is related, or indicate a bacterium that was either previously unknown or has an unsequenced genome.

These PCR-based methods can be used to rapidly amplify specific bacterial genes to specifically or non-specifically detect the presence or absence of bacterial pathogens directly from clinical or environmental samples, in culture or after their isolation from culture [66,67]. Despite their successes, currently PCR-based approaches are only used as a supplement to, rather than a replacement for, the BC/GS analysis [68,69].

Advantages of using PCR-based methods for bacterial detection directly from blood samples

There are a number of advantages to using PCR-based methods to detect bacteria directly from blood samples compared with BC/GS analysis, including smaller volume of collection (5 mL compared with 20–30 mL), the use of common anticoagulant (EDTA) tubes for specimen collection, increased sensitivity (fewer false negatives) and rapid result reporting. Real-time PCR has been proved a rapid and accurate tool for detecting specific bacteria [60,61]. If applied appropriately, PCR-based methods can verify the absence of bacteria, potentially allowing the early discontinuation of empiric antibiotics [21,47,48,69].

Challenges to using PCR-based methods for bacterial detection directly from blood samples

PCR is sensitive with a limit of detection that has been determined to be as low as one to 10 cells [70]. This ability to amplify fragments of DNA from a single bacterium may result in the false-positive amplification of the normal microbial flora from the host, contamination from the patient/health-care worker, or non-viable bacteria. Another challenge is choosing DNA target sequences for PCR analysis that are unique to the specific pathogen or region of the 16S rRNA gene [65]. Certainly, PCR analysis is especially useful for rapid identification of a single known bacterial pathogen (i.e., is there MRSA in this sample?). However, it becomes more challenging if a clinical sample is being tested for the presence of a bacterium among a list of pathogens (i.e., does this sample contain pathogens from this list of 25 specific pathogens?). The analysis is even more difficult with polymicrobial infections [68,69].

Polymerase chain reaction results must be interpreted with caution given that non-viable bacterial material may be detected in a patient who has already recovered from infection [24]. Animal experiments investigating the ability of PCR to amplify bacterial DNA from both viable and non-viable bacteria after extraction from host tissues indicate that the DNA from dead cells only persists for a few days in a live host [71]. Non-viable bacteria are not an issue in BC/GS analysis, as these cells obviously do not grow in culture media. Polymerase chain reaction identification of DNA from non-viable bacteria is one reason a sample may generate a positive PCR result and a negative BC result [72].

Interestingly, qPCR can provide an estimate of the number of bacteria per milliliter of the blood sample at the time of sample collection, based on the number of gene copies of the target DNA in the sample. This is particularly exciting as this information is impossible to determine from BC/GS analysis. As this will be new information, the threshold of clinical relevance is currently unknown and will need to be determined. Some studies suggest that lower bacterial counts may reflect an earlier phase of the infection and should not be disregarded [71,73]. Quantitative polymerase chain reaction may provide an opportunity to identify patients early in the course of infection and may be useful to follow the course of treatment to determine when antibiotics can be discontinued.

Clinical Studies of Pathogen Detection and Identification by Real-Time PCR Directly in Blood Samples

To date no commercially available multiplex real-time PCR system is FDA approved for clinical use in the United States. The most recent systematic review and meta-analysis of the performance of a multiplex real-time PCR assay (LightCycler® SeptiFast Test MGRADE, Roche, Germany) conducted by Chang et al. [69] included 34 studies and more than 6000 patients. The authors concluded that this assay was better at ruling-in than it was at ruling-out bacteremia, with a pooled sensitivity of 80% (95% CI 70%–88%) and specificity of 95% (95% CI 93%–97%). The associated positive likelihood ratio (LR) was 15.9, 95% CI 10.4–24.3, whereas the negative LR was 0.21 (95% CI 0.13–0.33). Included in this review was a large study by Lodes et al. [22], which evaluated this multiplex real-time PCR assay for the detection of bacteremia in ICU patients at risk for abdominal sepsis. In a cohort of 104 patients (148 blood samples), 77 microorganisms were identified by the LightCycler SeptiFast, whereas only 25 (32.5%) grew in culture [22]. Although the clinical relevance of the additional pathogens identified were not addressed, 16.9% of patients did

have their therapy adjusted based on the multiplex real-time PCR results [22]. Additionally included in this analysis was a study by Dierkes et al. [21], who reviewed the LightCycler SeptiFast performance in patients with presumed sepsis. Outcomes were measured by whether the initial treatment was maintained or adjusted. In the 101 blood samples (77 patients), 13% of pathogens were detected by the multiplex real-time PCR assay only and resulted in a change of antibiotic therapy in 8% of these patients [21]. The authors concluded that the addition of the multiplex real-time PCR to conventional BC/GS analysis has a beneficial clinical impact, as the molecular results were available an average of 21 h earlier than the corresponding BC/GS results [21].

Since the 2013 publication by Chang et al., Talfeski et al. evaluated the impact the LightCycler SeptiFast on infection management in 78 ICU patients with either suspected pneumonia or abdominal sepsis. Each group had blood drawn for both traditional BC/GS and for processing by the LightCycler SeptiFast; however, in the intervention arm ($n=41$), the samples were processed immediately, whereas the control samples ($n=37$) were stored for later evaluation of the LightCycler SeptiFast performance. The addition of this multiplex real-time PCR technology shortened the duration from biological sampling and availability of microbiological results by telephone to the ICU physicians (15.9 ± 5.9 h compared with 38.1 ± 11.6 h, $P < 0.001$). Additionally, the availability of this microbiological diagnostic information led to changes in therapy in four (9.8%) of the intervention group and five (13.5%) in the control group. Although the study was underpowered to detect a decreased duration to change antimicrobial therapy in the intervention group, the mean of the intervention group was 18.8 ± 5.6 h compared with 38.3 ± 14.5 h in the control group (non-substantial findings).

These findings suggest that the addition of this multiplex real-time PCR technology may shorten the time to appropriate antimicrobial therapy. Finally, the sensitivity for a positive LightCycler SeptiFast test result was 58.3% (seven of 12 samples) with a corresponding specificity of 74.2% (49 of 66 samples) [74]. Schreiber et al. [75] compared LightCycler SeptiFast to two other PCR-based methods, SepsiTest and VYOO® (SIRS-Lab, Jena, Germany), and BC/GS analysis in a prospective, observational study consisting of 50 patients with clinical signs and symptoms consistent with sepsis. SepsiTest is a qPCR technology that uses broad-range primers to identify bacterial and fungal pathogens by amplification of their 16S rRNA or 18S rRNA genes, respectively, followed by DNA sequence analysis [76]. VYOO is a multi-step multiplex-PCR assay, which can potentially identify 34 bacteria, seven fungi, and five resistance genes. Many patients (72%) in this cohort were treated with antibiotics prior to enrollment; a 26% positive BC rate was observed. Only 25% of the clinically relevant pathogens identified by BC/GS analysis could also be identified by all three PCR assays, with 64% showing concordant negative results. At present, there is insufficient evidence to support the use SepsiTest or VYOO in clinical applications.

These studies all demonstrate the superiority of the LightCycler SeptiFast in comparison with other PCR-based technologies for direct bacterial pathogen detection. Unfortunately, the inability of the LightCycler SeptiFast to rule out bacteremia limit its clinical usefulness, particularly in patients that receive empiric antibiotics while waiting 5 d for a negative BC/GS result to discontinue therapy.

Current clinical trials

Current clinical trials involving PCR-based technology for detection of specific pathogens include studies quantifying bacterial load in vancomycin-resistant Enterococcal (VRE) infections [77], detection of MRSA in blood cultures and wound swabs [78], and identifying patients colonized with MRSA, VRE, and extended spectrum beta-lactams (ESBL) producing organisms upon ICU admission (MOSAR-ICU) [79].

There are currently five clinical trials evaluating the clinical use of this PCR-based technology for the broad detection of bacterial pathogens. The first, ''A New Method for Detection of Bacteria in the Bloodstream,'' will evaluate the efficacy of qPCR compared with BC/GS for detection of bacteremia in burn ICU patients [80]. The remaining four will specifically evaluate the clinical performance of the LightCycler SeptiFast in special patient populations. The ''Optimal Antibiotic Treatment of Moderate to Severe Bacterial Infections'' trial will evaluate the impact on 30-d survival, clinical stability, and appropriate antibiotic use in hospitalized patients with bacterial infections when the shortened duration to pathogen identification via the LightCycler SeptiFast is combined with a computerized decision support system (TREAT) for antibiotic selection; this study is not yet recruiting [81]. The ''Value of the LightCycler® SeptiFast Test MGRADE for the Pathogen Detection in Neutropenic Hematological Patients'' is a Phase 4, randomized open label trial to assess the clinical value of the LightCycler SeptiFast as an adjunct to traditional microbiological assays for the early detection and identification of a potential pathogen, specifically in patients with neutropenia [82]. The ''Benefit of SeptiFast Multiplex PCR in the Etiologic Diagnosis and Therapeutic Approach for Onco-hematology Patients Presenting Sepsis'' (SEPTIFAST) trial is a prospective, observational cohort study in patients with hematologic diseases to evaluate the ability of LightCycler SeptiFast to identify pathogens not identified with BC/GS and whether or not it changes the therapeutic plan [83]. The ''Diagnosis of Septicaemia by Detection of Microbial DNA in Blood in Severe Infections'' (EVAMICA) trial will evaluate the ability of LightCycler SeptiFast compared with BC/GS to identify the presence of bacteremia and fungemia in patients with febrile neutropenia and endocarditis or severe sepsis [84]. In addition, the trial will compare the time to positivity with the LightCycler SeptiFast and BC/GS in these neutropenic patients. At the time of publication of this text, the trial in neutropenic hematological patients, SEPTIFAST and EVAMICA trials had been completed but results had not yet been published. There are no current ongoing clinical trials for SepsiTest or VYOO.

Application of qPCR to Discontinue Empiric Antibiotics

In patients with a SIRS response, qPCR can be a valuable adjunct to BC/GS analysis especially in patients on empiric antibiotic therapy [18,21,85]. However, the impact of qPCR methods on therapeutic decisions and outcome has only been studied in observational settings. The common theme of all previous clinical qPCR technologies has been to identify the presence of specific bacterial or fungal organisms in blood,

and this has been met with limited success [69]. A more simple application of qPCR is via universal broad range assays, utilizing the bacterial-specific 16S rRNA targets to detect the presence or absence of bacterial pathogens. In the case that no bacteria are present and no other site of infection is suspected, empiric antibiotics could be discontinued much sooner than the current BC/GS analysis would allow because of the lengthy 5 d necessary to confirm a negative result.

Conclusion

Sepsis remains a serious medical problem with a high mortality rate [2,3]. Successful patient outcomes are heavily reliant on the appropriate and timely initial antibiotic administration, [47,48] which has resulted in empiric antibiotic administration for patients with SIRS and suspected infection as the second most common indication for antibiotic administration in ICUs [16]. Empiric antibiotic administration is not without harm, and appropriate antibiotic stewardship should be included in the efforts to reduce the cost and mortality rate from sepsis. The traditional BC/GS analysis method requires up to 5 d before a negative result is confirmed, and during this time patients are potentially administered antibiotics unnecessarily, contributing to MDR and secondary infections. As a result, the continued use of the historical BC/GS analysis technology for the identification of bacteremia in patients with suspected sepsis is suboptimal. The current application of multiplex real-time PCR has been met with limitations because of assay-specific decreased sensitivity, particularly in polymicrobial samples, unknown clinical relevance of the detection of DNA from non-viable pathogens and inability to replace BC/GS as a stand-alone test. Currently there are no FDA-approved multiplex real-time PCR systems available for clinical use for the detection of bacterial pathogens in the United States. The proper application of PCR-based technology could be used to rule out the presence of bacteremia soon after the administration of antibiotics, improving appropriate treatment decisions, including antibiotic stewardship.

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