Potential Clinical Usefulness of the Polymerase Chain Reaction Test to Detect Pathogens Causing Sepsis

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Abstract

Objective: A real-time polymerase chain reaction (PCR) test is expected for early and precise detection of pathogens in blood. In this study, we compared the ability of the PCR test and blood culture to detect pathogens in the blood of patients with sepsis.

Methods: Patients who were diagnosed as or suspected of having sepsis were included in this prospective observational study. A whole blood sample for PCR test was obtained serially simultaneously with the blood culture sample, and the results were compared.

Results: We obtained 93 samples from 26 patients; 69 samples were obtained during the septic condition, and 24 samples were from the non-septic condition. Origins of sepsis were pneumonia in 9 patients, necrotizing fasciitis in 5 patients, and other causes in 12 patients. In the septic condition, rates of positive results were 29.0% for the PCR test and 23.2% for blood culture. Sample contamination occurred in 1 PCR test sample and 5 blood culture samples.

Conclusion: In sepsis, the PCR test detected more bacteria than did blood culture even after administration of empirical antibiotics, which might contribute to precise diagnosis of the bacteremic cause of sepsis.

Keywords: Real-time polymerase chain reaction test; sepsis; blood culture

Introduction

Sepsis is a leading cause of death worldwide. Every year, more than 18 million people suffer from sepsis, and 1,400 people die of sepsis every day [1,2]. The Surviving Sepsis Campaign guidelines declared in 2004 pointed out the extremely high mortality rate of severe sepsis and septic shock and therefore emphasized the early recognition of sepsis and the beginning of broad-spectrum antibiotic treatment as early as possible to prevent deterioration of the patient’s condition [3]. This “early empirical therapy” is necessary until the origins of sepsis become clear; however, it would be better to know the precise target of therapy before administration of antibiotics so that the target microorganism is not missed and growth of antibiotic-resistant bacteria can be avoided.

To detect the origin of sepsis, Gram staining and culturing of samples from the infection site, as well as blood culture (BC), are the universal methods. Gram staining has been reevaluated recently as a quick, easy, and inexpensive way to detect the target microorganisms in infections [4,5]. However, because of the lower number of microorganisms in the blood in comparison with infection site, they are difficult to detect with Gram staining. In contrast, BC is the gold standard for detecting bacteremia and fungemia, but it takes at least several days to grow the microorganisms, and false negatives due to previous antibiotic use or the presence of fastidious intracellular pathogens are well known limitations of BC.

The polymerase chain reaction (PCR) test was developed as an attractive method to detect pathogens in blood within 5 hours, which is expected to aid in the early and precise diagnosis of the origins of sepsis. To date, significantly higher positive results from the PCR test than BC have been shown in multiple trials targeting sepsis [6,7,8]; however, antibiotic resistance determinants or interpretation of false-positive PCR tests are the major limitations preventing wide clinical use of this method. In their review in 2011, Pletz et al. [9] discussed the clinical advantages and cost effectiveness of PCR testing and stated that the PCR test 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* spp., *Aspergillus* spp., methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci, and extended-spectrum beta-lactamase and carbapenemase-positive Gram-negative microorganisms. However, in regard to the positive results of PCR tests, Bloos et al. [10] reported in 2010 that patients with positive PCR tests at enrollment had higher organ dysfunction scores and a trend toward higher mortality in comparison with those with negative PCR results. They concluded that PCR test results correlated with disease severity even if the BC remained negative and suggested that presence of microbial DNA in the bloodstream is a significant event.

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In the present study, we hypothesized that positive PCR test results would help to diagnose pathogens causing sepsis even if broad-spectrum antibiotics had already been administered before blood sampling. We therefore obtained serial blood samples from septic patients to compare the results of PCR testing and BC during use of broad-spectrum antibiotics.

Patients and Methods

This prospective observational study was performed from September 2007 to April 2008 in the medical and surgical intensive care unit in the emergency department of Osaka University Graduate School of Medicine, Osaka, Japan. Patients who were admitted to the intensive care unit and who were either diagnosed as having or were suspected of having sepsis were included. Patients who visited the emergency room only were excluded from this study. We diagnosed sepsis according to the definitions of the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) [11]. Samples of 1.5 ml of whole blood were collected from each patient for the PCR test together with a 10-ml whole blood sample for BC (5 ml for aerobic culture and 5 ml for anaerobic culture) via venipuncture, when BC was needed for diagnosis of sepsis or evaluation of antibiotic treatment. Blood culture was performed according to the standard technique in our clinical microbiology laboratory with the BacT/ALERT 3D system (BioMerieux; Hazelwood, MO, USA). Whole blood for PCR testing was collected into an ethylenediaminetetraacetic acid (EDTA) tube, and DNA extraction and amplification were performed separately to eliminate contamination. DNA extraction and amplification were performed according to the instructions of the SeptiFast test (Roche Diagnostics; Mannheim, Germany), and the details of the method are described elsewhere [12]. Briefly, microbe DNA from each patient’s sample was extracted using the SeptiFast Lys and Prep Kit M Grade (Roche Diagnostics). DNA amplification was performed on the Light-Cycler® 2.0 system (Roche Diagnostics) using a bacteria and fungi identification macro included in the Light-Cycler software V4.05. The SeptiFast mecA test to differentiate MRSA was performed after S. aureus was detected, except when coagulase-negative Staphylococcus (CNS) was found, because CNS also carries the mecA gene [13].

The medical or surgical staff treating the patients were blinded to the results of the PCR test. Tree medical staff members including an infection disease specialist reviewed the patient’s clinical records such as vital signs, laboratory results, antibiotics used, and the treatment course, and categorized each sampling point as occurring during the septic condition (sepsis) or during the non-septic condition (non-sepsis). Presence of CNS was regarded as contamination unless it was detected from 2 sets of BC bottles or detected from BC more than 2 times in a row or detected from other culture specimens obtained from aseptic sites (i.e., urine, catheter, wound, or body cavity). Infection was defined according to the International Sepsis Forum consensus [15]. The international definitions of the SCCM, ACCP, the European Society of Intensive Care Medicine (ESICM), the American Thoracic Society (ATS), and the Surgical Infection Society (SIS) were used as references to differentiate sepsis from non-sepsis for each sample [16].

This study was approved by the institutional review board of the Osaka University Graduate School of Medicine.

Results

Twenty-six patients were enrolled in this study. The patient population consisted of 16 men and 10 women, median age 66 (range 39-91) years. In 20 of the 26 patients, samples for both BC and PCR test were obtained serially during sepsis or to evaluate antibiotic effect, and then 93 samples from the 26 patients were collected (average 3.6 ± 2.9 samples for each patient). Sixty-nine samples were obtained during sepsis, and 24 samples were during non-sepsis. The origins of sepsis were pneumonia in 9 patients, necrotizing fasciitis in 5 patients, peritonitis in 3 patients, and other origins in 9 patients (Table 2).

The percentage of positive detections by PCR test and BC in samples obtained during sepsis and non-sepsis are shown in Figure 1. In sepsis, the PCR test detected pathogens in 20 of 69 (29.0%) samples, whereas BC detected them in 12 of 69 (17.4%) samples (8 samples were listed on the PCR test menu, and 4 samples were not listed on the PCR test menu). Neither PCR test nor BC detected pathogens in 24 non-sepsis samples. Contamination was determined in the BC in 4 samples from sepsis and 1 sample from non-sepsis, whereas no sample from sepsis and 1 sample from non-sepsis were determined to be contaminated in the PCR test. In the results from sepsis, 16 of 20 positive PCR samples, in which pathogens were all diagnosed as origins of sepsis, were obtained after the administration of broad-spectrum antibiotics. However, 7 positive BC samples were obtained before administration of antibiotics, and 5 samples, except for those determined to be contaminated, were obtained after the administration of effective antibiotics.

Comparison of PCR test and BC in cases with a positive PCR test and negative BC during antibiotics administration are shown in Figure 2. The details of each case are described in the figure caption. All blood samples in these cases were obtained during sepsis. In each case, the medical or surgical staff treating the patient were blinded to the results of the PCR test and BC. The PCR test detected pathogens in 11 of 16 (68.8%) cases, whereas BC detected them in 7 of 16 (43.8%) cases (8 samples were listed on the PCR test menu, and 4 samples were not listed on the PCR test menu). Neither PCR test nor BC detected pathogens in 5 cases. Conclusions were drawn from the comparison of PCR test and BC.

Table 1: Microorganisms Detected by the Polymerase Chain Reaction Test.

| Gram positive | Gram negative | Fungi          |
|---------------|---------------|----------------|
| Staphylococcus aureus | Escherichia coli | Candida albicans |
| CNS | Klebsiella (pneumoniae/ oxyt.) | Candida tropicalis |
| Streptococcus pneumoniae | Serratia marcescens | Candida parapsilosis |
| Streptococcus spp. | Enterobacter (cloacae/ aerog.) | Candida kruusei |
| Enterococcus faecium | Proteus mirabilis | Candida glabrata |
| Enterococcus faecalis | Pseudomonas aeruginosa | Aspergillus (fumigatus) |
| MRSA (mecA gene) | Acinetobacter baumannii | Stenotrophomonas maltophilia |

CNS, coagulase-negative Staphylococcus aureus; MRSA, methicillin-resistant Staphylococcus aureus.

Table 2: Number of Patients According to the Origin of Sepsis.

| Infection | Number of patients |
|-----------|--------------------|
| Pneumonia | 9                  |
| Necrotizing fasciitis | 5                |
| Peritonitis | 3                 |
| Retroperitonitis | 2                |
| Meningitis | 2                  |
| Phlebitis | 2                  |
| Cholangitis | 1                 |
| Mediastinitis | 1                |
| Sinusitis | 1                  |
| Total     | 26                 |
especially when BC results were negative [17]. In their multicenter trial the interpretation of positive PCR test results was not consistent, compared with BC is reported in many recent studies [6,7,8]; however, could be obtained from BC. The higher sensitivity of this method nucleic acids within 5 hours, which would make it possible to obtain detect bacteria and fungi in whole blood by the amplification of their time PCR to diagnose the cause of sepsis. This method is designed to antibiotic therapy are still challenges to be overcome. In the present of the target microorganism and early determination of definitive diagnosis of sepsis by other samples obtained from the site of infection. Because rowther et al. [19] reported the usefulness of PCR testing combined treatment. To distinguish true bacteremia from contamination, Rowther et al. [19] reported the usefulness of PCR testing combined with serum procalcitonin. They reported that positive PCR results with elevated procalcitonin levels suggested true sepsis, and negative PCR results with reduced procalcitonin levels suggested non-sepsis. Additional examination and careful evaluation is needed to decide between contamination and bacteremia in these septic patients.

Another concern regarding positive PCR results is contamination. In this study, only one sample was determined to be contaminated by S. aureus in PCR testing, whereas 5 samples were contaminated by CNS according to the BC results. One reason for lower CNS detection is the higher threshold of analytical sensitivity of CNS than for other pathogenic microorganisms in the PCR test. CNS is usually considered as contamination, but Casalta et al. [18] pointed out that this higher threshold of the PCR test for CNS risks low-grade bloodstream infections, such as endocarditis, being overlooked during antibiotic treatment. To distinguish true bacteremia from contamination, Rowther et al. [19] reported the usefulness of PCR testing combined with serum procalcitonin. They reported that positive PCR results with elevated procalcitonin levels suggested true sepsis, and negative PCR results with reduced procalcitonin levels suggested non-sepsis. Additional examination and careful evaluation is needed to decide between contamination and bacteremia in these septic patients.

One limitation of the present PCR test was its inability to detect microorganisms that were not listed on the test menu. There were 4 samples in which bacteria detected by BC were not on the PCR test menu but were the cause of sepsis. Citrobacter, which is now increasingly a cause of sepsis in compromised hosts [20,21], is one of them. The other 3 samples included anaerobic bacteria, Bacteroides species, and Fusobacterium species, which have been highlighted recently as causes of severe sepsis [22]. In the case of anaerobes in particular, they take more time to grow in BC due to their specialized culture requirements, and it is therefore desirable to include these bacteria in the PCR test menu in the future. The second limitation of the PCR test was the lower sensitivity of the mecA gene to distinguish MRSA from methicillin-sensitive S. aureus. Although we reexamined these samples, the mecA gene was detected in only 1 sample. Information about multidrug

Discussion

Early diagnosis and proper use of antibiotics is essential to treat sepsis. Empirical broad-spectrum antibiotics were recommended by the Surviving Sepsis Campaign guidelines; however, precise diagnosis of the target microorganism and early determination of definitive antibiotic therapy are still challenges to be overcome. In the present study, we have shown some value in the new method using real-time PCR to diagnose the cause of sepsis. This method is designed to detect bacteria and fungi in whole blood by the amplification of their nucleic acids within 5 hours, which would make it possible to obtain information on bacteremia and fungemia quite earlier than that which could be obtained from BC. The higher sensitivity of this method compared with BC is reported in many recent studies [6,7,8]; however, the interpretation of positive PCR test results was not consistent, especially when BC results were negative [17]. In their multicenter trial to compare PCR test and BC, Bloo et al. [10] reported 34.7% positive results with the PCR test in comparison with 16.5% positive results for BC in severe sepsis. Their study showed higher organ dysfunction scores and a trend toward higher mortality in patients with positive PCR results, and they concluded that positive PCR results (presence of microbial DNA in the bloodstream) were a significant event even if the BC remained negative. We also showed a higher rate of positive results for the PCR test versus BC in sepsis (20/69 [29.0%] vs. 12/69 [17.4%], excluding contamination), and all positive PCR samples except 1 with contamination were confirmed as detecting the bacteria causing the sepsis. Of the 20 positive PCR samples in sepsis, 16 were collected after broad-spectrum antibiotic administration had started. These included cases of meningitis, cholangitis, mediastinitis, and retroperitoneitis, in which it is sometimes difficult to obtain samples from the site of infection. In such cases, broad-spectrum antibiotics are administered immediately after diagnosis on the basis of clinical findings and/or radiographic images along with sampling for BC. The surgical approaches of resection or drainage would be undertaken, but the original microorganism that caused the sepsis is not always detected in the presence of broad-spectrum antibiotics. As shown in Figure 2, the PCR test was positive in sepsis in the presence of broad-spectrum antibiotics even if the BC was negative. The microorganisms identified in the positive PCR results were confirmed as the origins of sepsis later from samples obtained at the site of infection. Although we did not inform the medical or surgical staff of these results because of the requirements of the study protocol, it is possible that if the PCR test results can be obtained within 5 hours, the strategy to treat sepsis or antibiotics used could be changed earlier in the treatment course. Although the sample size in the present study is small, we believe our results showed some benefit of the PCR test in clinical use, especially when broad-spectrum antibiotics are being used.
Figure 2: Comparison of polymerase chain reaction (PCR) test and blood culture (BC) in cases with positive PCR test in which antibiotics were administered. Case 1: A 45-year-old man with retroperitonitis due to pancreatitis. *Enterobacter* spp. and *Klebsiella* spp. were detected in samples obtained by surgical procedure. Case 2: A 63-year-old woman with cholecystitis. *Escherichia coli* was detected in purulent bile. Enteritis due to MRSA became a complication after day 7. Case 3: A 63-year-old man with mediastinitis due to pharyngitis. *Streptococcus* spp. was detected in samples obtained by surgical procedure. Case 4: A 67-year-old woman with meningitis. *Streptococcus pneumoniae* was detected in cerebrospinal fluid. Case 5: A 69-year-old man with retroperitonitis due to pancreatitis. *Pseudomonas aeruginosa* was detected in samples obtained by surgical procedure. Case 6: An 89-year-old woman with urinary tract infection complicated by pneumonia. *E. coli* was detected in a urinary sample. Case 7: A 61-year-old woman with necrotizing fasciitis due to pharyngitis. *Streptococcus pneumoniae* was detected in cerebrospinal fluid. Case 8: A 58-year-old man with abdominal abscess due to traumatic perforitis. *S. aureus* was detected in samples obtained by surgical procedure. Case 9: A 67-year-old man with pneumonia. *P. aeruginosa* was detected in samples of tracheal aspirate.
In conclusion, the PCR test detected more bacteria during sepsis, even after administration of empirical antibiotics, than did BC. The PCR test might contribute to precise diagnosis of bacteremic cause of sepsis.

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Table 3: Summary of PCR Test and BC Results According to Pathogen.

| Pathogens on the PCR test menu | PCR(+) BC(+) (n) | PCR(+) BC(-) (n) | BC(+) PCR(-) (n) |
|------------------------------|----------------|----------------|----------------|
| Coagulase-negative Staphylococcus | 0 | 0 | 5 |
| Staphylococcus aureus | 2 | 5 | 2 |
| Streptococcus species | 1 | 2 | 0 |
| Streptococcus pneumoniae | 1 | 0 | 1 |
| Escherichia coli | 1 | 2 | 0 |
| Enterobacter aerogenes/cloacae | 0 | 2 | 0 |
| Klebsiella pneumoniae/oxytoca | 0 | 2 | 0 |
| Pseudomonas aeruginosa | 1 | 2 | 1 |

Pathogens not on the PCR test menu

| Pathogens not on the PCR test menu | PCR(+) BC(+) (n) | PCR(+) BC(-) (n) | BC(+) PCR(-) (n) |
|-----------------------------------|----------------|----------------|----------------|
| Citrobacter species | 0 | 0 | 1 |
| Bacteroides species | 0 | 0 | 2 |
| Fusobacterium species | 0 | 0 | 1 |

PCR, polymerase chain reaction; BC, blood culture.