

Research on Diagnostic Value of Real-Time PCR in Comparison with Culture Method to Detect Agents for Sepsis

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Abstract

Introduction: Rapid detection of the pathogens and to start antibiotherapy accordingly affect the mortality rate in sepsis. The aim of the present study was to determine the effective microorganisms in blood samples of the patients who are admitted in intensive care units of our hospital and have suspicion for sepsis and to reveal the rates of sepsis.

Methodology: The present study was performed prospectively through the blood samples of the patients with preliminary diagnosis of sepsis sent to the microbiology laboratory. Sepsis panel was studied from the patients whom blood culture has been sent. Blood culture was taken into BacT-Alert (Biomérieux) full automated blood culture system. Vitek2 (biomérieux) full automated bacteria identification system was used to identify the bacteria. Presence of bacterial DNA molecularly was analyzed with multiplex real-time PCR (LightCycler SeptiFast, Roche Diagnostics).

Results: In our study, sensitivity of Real-time PCR method was detected as 50%, specificity was detected as 77%, positive predictive value (PPV) was detected as 53.1%, negative predictive value (NPV) was detected as 75% when compared with blood culture.

Conclusions: Although molecular tests are rapid and sensitive, they would not replace with conventional blood culture due to lack of antimicrobial resistance data, limitation of number of target pathogens and high costs. Improper antibiotic treatment, antibiotic resistance is caused to occur in the intensive care units (ICUs). Therefore, when combined with blood culture, molecular analysis will provide an urgent and appropriate antimicrobial treatment.

Keywords: Sepsis; Blood culture; Real-time PCR; Diagnosis.

Introduction

Sepsis is defined as a systemic inflammatory response against infection. Despite recent developments in medical technology and antimicrobial therapy, sepsis persists to remain a life threatening problem, especially in intensive care units (ICUs).

Spectrum of the microorganisms as a cause for sepsis is wide. *Staphylococcus aureus*, coagulase-negative staphylococci (CNS), *Escherichia coli* and other members of enterobacteriaceae family, *Pseudomonas aureginosa* and *Candida albicans* are most common causes for infection.

Primary infection locations for sepsis are usually the urinary system, genital system, respiratory system, skin and soft tissue, intraabdominal and intravascular catheters. The most common entry points for sepsis cases appeared out of the hospital are the respiratory system and urinary system whereas intravascular catheter and urinary catheter procedures are most common entry points for nosocomial sepsis cases. Nosocomial pneumonia stands as a primary infection location in intensive care units.

Therefore, early diagnosis and appropriate treatment of sepsis are clinically important. Blood cultures identify microbial etiology and plays a role for management of the treatment for the cases with suspicious infection [1 - 3]. Rapid detection of the pathogens and start an appropriate antibiotherapy affect the mortality rate in sepsis. Agents are identified by real-time Polymerase Chain Reaction (PCR) within 7 to 15 hours whereas bacterial culture results within 24 to 72 hours[4].

The aim of the present study was to determine the active microorganism in blood samples of the patients who were admitted to intensive care units of our hospital and suspicious for sepsis through culture method and Real-time PCR method and to reveal the rates for sepsis.

Methodology

The present study was approved by Ethical Board for Clinical researches of Medical Faculty with the resolution number of 2011/250. The project 121518004 was supported by Coordinatorship of Scientific Research projects of the university.

Blood culture samples of the patients who were admitted to intensive care units due to pre-diagnosis of sepsis referred to Central Microbiology Laboratory of Medical Faculty between November 2012 and March 2013 were enrolled into the study. Blood samples of the patients whose blood samples were referred to the laboratory for blood culture were transferred into EDTA tubes for simultaneous molecular tests. Demographic characteristics of the patients were obtained from automation management system of the hospital.

Blood culture samples were incubated by Bac T-Alert (Biomerieux) full-automated blood culture system. The sample was placed into the device and monitored inside the device periodically until positive or negative result was obtained. A voice alarm was provided by the device when reproduction was detected (a positive result). A negative result was reported in a sample when no microbial reproduction appeared within 5 days. The blood culture bottles with reproduction signal were removed from the device and approximately 1 ml blood sample was collected. The samples were planted into 5% sheep blood agar Biomerieux, Fransa), Eosin Metilen Blue (EMB) agar (Sigma) and Sabouraud dextrose agar(SDA). The plates were incubated in an incubator at 37°C for 24 to 48 hours. A negative result was reported in a sample when no microbial reproduction appeared within 5 days. Blood samples from blood culture bottles with a reproduction signal were planted into Sabouraud dextrose agar(SDA) which is a medium specific for yeast fungus at 37°C for 24 to 48 hours. The plates with reproduction in SDA medium were identified through API ID 32 C system.

DNA isolation was performed by MagNA Pure Compact Nucleic Acid Isolation Kit (Roche Diagnostic GmbH, Mannheim, Germany) and amplification was performed by Septi-Fast (Roche Diagnostic GmbH, Mannheim, Germany). Identification was carried out at 3 stages through LightCyler Septi-Fast Test;

- a) Homogenization (by MagNA Lyser System) and DNA isolation (MagNA Pure Compact System),
- b) Measurement of target DNA in 3 parallel reactions (Gram positive bacteria, Gram negative bacteria, fungus) via PCR amplification and specific hybridization probes,
- c) Automatic identification of the samples and controls.

Statistical analysis was performed by SPSS 15.0 (SPSS Inc, ABD, IL) Version and *ap value* < 0.05 was accepted significant. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated through McNemar test.

Results

Blood culture samples of 100 patients whose blood samples were referred to Central Microbiology Laboratory of Medical Faculty by pre-diagnosis of sepsis between November 2012 and March 2013 were enrolled into the study. The patients included 47 (47%) females and 53(53%) males. The patients were between 11 and 93 years old (age average 59.84 years; SD=21.2). Distribution of the samples included into the present study according to the clinics was shown in the table (Table I).

Majority of the samples (40%) was observed to be referred from emergency intensive care unit. No reproduction was detected in 63 blood samples for culture included into the study whereas a microorganism was detected in 37 (37%) samples. Distribution of the microorganisms detected in the blood culture was shown in the table (Table II).

A bacteria was isolated in 37 (37%) of the patients whose blood culture were analyzed. The most common agent identified was CNS (in 11 samples). However, 6 of 11 cases mentioned above were evaluated as contamination because of skin contamination appeared during blood collection.

Similarly, agents causing sepsis were analyzed through molecular method. No agent was detected in 64 (64%) of 100 patients and one and/or two agents were detected in 36 (36%) samples (two agents were detected in four samples). The *Aspergillus fumigatus* detected in two cases was accepted as contamination caused by the environmental distribution of bacteremia agents detected through molecular method was shown in the table (Table III).

The most common microorganism detected through molecular method was found *K.pneumoniae* (18.4%). Similar results were obtained in 68 of 100 samples through blood culture and molecular method and the p value was found 0.860 when results of both methods were compared statistically (McNemar test) indicating no difference. Distribution of the microorganisms in percentage detected by blood culture and PCR was shown in the graphic (Figure 1).

No agent was detected in 51 (51%) of 100 samples by both tests. Same agent was detected by PCR in 17 (53%) of 32 samples which were accepted as pathogen in the blood culture. Negative detection ratio of both tests was found highly consistent when compared with positive detection ratio by McNemar analysis.

In 17 samples without any pathogen detected in the culture, only one and/or two agents were detected through molecular method; however, one agent was detected in 15 samples where no pathogen could be detected by molecular method.

Twenty-one microorganisms were detected molecularly in other 17 samples without any reproduction in the blood culture. A secondary microorganism was detected in 5 samples by PCR method in addition to the agent detected in the culture (Table IV).

The agents detected by both methods were accepted as pathogens and evaluated as a single microorganisms [5].

Discussion

Sepsis is defined as a systemic inflammatory response against infection. The presentation of sepsis appeared after 48 to 72 hours following hospitalization is defined as nosocomial sepsis. Despite recent developments in medical technology and antimicrobial therapy, sepsis persists to remain a life threatening problem, especially in intensive care units (ICUs). Sepsis is 13th cause of death in United States and at second rank in ICU. Sepsis appears to be an important infection problem for hospitalized patients, especially in ICU and a significant portion of the patients dies because of sepsis [6]. Bacteraemia corresponds to 30% to 40% of all cases with septic shock and severe sepsis and is an important cause for morbidity and mortality. The infections in the blood should be diagnosed rapidly to start an efficient antimicrobial therapy and to make the patient's prognosis better [7]. Mortality due to bacteraemia is reported between 20% to 50%. Mortality is lower in community-acquired bacteraemia whereas higher in nosocomial acquired bacteraemia. Furthermore, existence of the agents with different resistance profiles in different hospitals affects the prognosis[8, 9].

It is reported that the delay to start treatment significantly increases the mortality rate. For instance, a study reported that a delay in treatment of candidemia longer than 12 hours significantly increased the mortality [10]. Due to the noticeable correlation between the diagnosis and the treatment, different diagnostic methods have been used to provide results rapidly. The blood culture methods where the agent could be detected directly and agent-oriented in vitro sensitivity tests could be run is still accepted as a gold standard for diagnosis of blood circulation infections [11]. However, longer incubation period causes time loss. LC-SF (LightCycler SeptiFast) test is the first DNA-based test used to determine the microorganisms in the blood directly without need for a preliminary incubation.

In the present study, blood samples were obtained from 100 patients for both blood culture and LC-SF test simultaneously. When distribution of the blood culture samples according to the clinics referred was evaluated, most of the samples were referred from emergency medicine intensive care unit (n: 40, 40% and reanimation unit (n: 26, 26%). Such clinics are the services where critical patients admitted for a long period requiring intensive care. These are the patient group that nosocomial infections are detected most. In the present study, all of the patients were selected from hospitalized patients; however, source of bacteraemia-fungemia was not searched whether nosocomial.

An agent may not be detected in the blood cultures of all septic cases. An agent may not be detected due to some conditions such as empirical antibiotherapy, bacteriae requiring special conditions for reproduction, false negative results in the blood culture (this condition is associated with less sample quantity, keeping the sample at room temperature longer before placing into the incubator and failures of collection technique) or due to blood culture samples collected empirically without any finding of bacteraemia-fungemia. Mehli et al.[12] detected reproduction in 26.26% of the samples in their study. Willke et al.[13] reported reproduction in 12% of their samples. Such ratio was found 32% in the present study.

Possibility of exogenous contamination was reduced significantly by technical developments like PCR. Although this molecular method provides results faster than the blood culture, current version of this method requires an effort of a technician in a laboratory environment, except the PCR stage. Consequently, to perform the analyses in groups is recommended. A particular care should be taken to prevent contamination for determination of *Aspergillus fumigatus* in the blood in laboratory environment [4]. Only Mancini et al.[14] detected *A. fumigatus* in one patient by LC-SF test. Exogenous contamination was detected in 2 cases during the present study. No exogenous contaminant was detected in the study conducted by Wallet et al. [4]. The researchers linked this with a great attention during preparation stage, changing the clothes during work and decontamination of the devices with laboratory surfaces.

When results obtained with blood culture are compared with those detected by molecular method, similar results were obtained in 68 of 100 patients and the compliance was found 68%. The compliance detected was observed higher (51%) in negative samples and lower (17%) in positive samples. West et al. [15] identified 50 samples detected in the culture as equivalent species with those detected by PCR. Totally 558 sample pairs obtained from 359 patients were analyzed and no agent was detected in 382 samples by both methods. The compliance between both methods was calculated as 71%. Grif et al.[16] found 51

(71.8%) samples negative by both analyses. Positive results were obtained in 5 samples by both analyses and the compliance rate between blood culture method and PCR analysis were detected as 78.9%. Dierkes et al.[17] detected positivity in 14 (13%) samples by blood culture and PCR together and negativity in 69 (65%) samples.

Since DNA may be detected without requirement of any alive bacteria by molecular methods, antibiotherapy does not affect the sensitivity of molecular methods. Therefore, more microorganisms were detected by molecular method in many studies when compared with the blood culture. An agent was detected in 32 (32%) of 100 samples by blood culture whereas the agent was detected in 38 (38%) samples by PCR in the present study. Lehmann et al.[11] detected a microorganism in 58 (12.8%) samples by culture and in 114 (25.2%) molecularly. Dierkes et al.[17] detected an agent in 23 (22%) and 28(26%) samples by blood culture and PCR, respectively in a similar study. In a study conducted by Mancini et al.[14], 21 (20.4%) and 34 (33%) of 103 samples were detected positive by blood culture and PCR respectively.

In the present study, an agent was found in 15 samples which did not present any agent molecularly by blood culture. Although molecular tests provide rapid and sensitive results, the factors that affect the test negatively include different inhibitors preventing amplification of the DNA which is tried to be detected. These inhibitors include anticoagulants such as heparin, hem molecule within hemoglobin and wide range of different DNA in the sample. Furthermore, among the microorganisms detected by blood culture, those which are not included in the PCR panel cannot be detected by PCR [18]. Since the agents detected by the blood culture in the present study were within PCR test panel, the cause was considered as inhibition of PCR. It was concluded that inhibition would be associated with other factors, because the tubes include EDTA.

Lehmann et al.[11] detected an agent in 18 samples by blood culture method where no agent was detected by PCR. In a similar study carried out by Dierkes et al.[17], an agent was detected in 9 cases with blood culture only. In line with the present study, Matsushima et al.[19] detected a microorganism in 12 samples which could be detected by PCR.

Wallet et al.[4] detected 11 agents in their study by PCR whereas 74 microorganisms which could not be detected by blood culture were detected in the study of Lehmann et al.[11]. The cause for such excessive count was that Lehmann et al. analyzed 453 samples. Dierkes et al.[17] detected an agent in 14 of 106 samples by PCR only. In line with the present study, Matsushima et al.[19] detected an agent in 16 samples by molecular method only.

Consequently, sensitivity, specificity, positive predictive value, negative predictive value of Real-time PCR method were detected 50%, 77%, 53.1%, 75% according to the blood culture. Wallet et al.[4] found the following analytic parameters of the molecular method; sensitivity, specificity, PPV, NPV as 40%, 88%, 27% and 93%, respectively. Lehmann et al.[20] detected the analytic values of the test as 39%, 81%, 35% and 95%, respectively. Grif et al.[16] found negative predictive value as 94%, sensitivity as 63% and specificity as 81% for PCR analysis in a similar study. Specificity of the molecular method has similar values in the aforesaid studies. Although Real-time PCR method cannot

detect all pathogens of sepsis, it has detected very important pathogens which cannot be detected by blood culture analysis. However, use of such method is limited because no antibiotic sensitivity test could be run and certain microorganisms were not included into the panel. However, combination of molecular and blood culture analyzes would significantly increase the rate for pathogen detection. This molecular method which detects pathogens rapidly complements conventional blood culture analysis. Timely detection of the active pathogens is especially required before antibiotherapy.

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