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Routine use of a real-time polymerase chain reaction method for detection of bloodstream infections in neutropaenic patients☆,☆☆

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ABSTRACT

We examined the performance of a real-time polymerase chain reaction (PCR) test (Septi*Fast*) for early detection of bloodstream infection in febrile neutropaenic patients. Blood samples from 201 patients were screened for pathogens by blood culture and by PCR on the first day of fever. PCR results were available earlier (median 3 days for bacteria, 5 days fungal pathogens; $P \leq 0.01$). The sensitivity (0.74) and specificity (0.96) of the PCR test were acceptable for Gram negatives when culture was considered the gold standard, but sensitivity of the test was poorer for Gram-positive organisms (0.39). The PCR assay also led to 22.9% of invalid results. Septi*Fast* speeds the microbiological diagnosis of bloodstream infection in neutropaenic patients. However, the frequent failure of instrumental control procedures, the relatively poor sensitivity of the test, and the lack of phenotypic data on antimicrobial susceptibility associated with its high costs suggest that this assay cannot replace the blood cultures.

1. Introduction

Neutropaenia is a major risk factor for bloodstream infections (BSIs) in patients with haematological cancer. Fever develops in 65% of cancer patients receiving fluoroquinolone prophylaxis during the neutropaenic period, but a microbiologically documented diagnosis is made in only 22% of these cases (bacteraemia 18%) (Bucaneve et al., 2005).

Bloodstream infections are routinely diagnosed with blood cultures taken at the onset of fever (Hughes, 2005; Penack et al., 2006). However, one disadvantage of this method is the turn-around time of 2–6 days before the results are available (Bucaneve et al., 2005). Additionally, the sensitivity of blood culture is reduced in neutropaenic patients with haematologic malignancies because they often receive prophylactic antibiotics and are at risk for infections caused by cell-wall deficient bacteria and filamentous fungi, which are rarely detected by blood culture (Carrigan et al., 2004; Woo et al., 2001).

The detection of microbial DNA in blood by polymerase chain reaction (PCR) is a promising approach for diagnosing BSIs (Mancini et al., 2008). A common limitation in the assessment of novel molecular methods is the absence of a gold standard for detection of BSIs. In neutropaenic cancer patients in particular, the interpretation of PCR results is limited by negative blood culture results due to antibiotic treatment (Peters et al., 2004). Consequently, some authors have recommended that positive PCR results for blood culture–negative febrile episodes be interpreted based on corresponding clinical features of the infection rather than on purely microbiological results (Nakamura et al., 2010; Peters et al., 2004).

Previously, we assessed the clinical utility of a commercially available multiplex real-time PCR assay (LightCycler Septi*Fast* Test M^{GRADE}; Roche Diagnostics, Mannheim, Germany) for the microbiological diagnosis of BSIs in 100 severely immunocompromised patients (Varani et al., 2009). Since then, we have routinely used this procedure along with standard blood culture, evaluating in total 201 neutropaenic patients over the past 2 years. Based on this experience and on our analysis, we have identified advantages and limitations of this technology for diagnosis of infections in neutropaenic patients.

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2. Patients and methods

2.1. Study patients, settings, and definitions

In this prospective interventional study, we analysed 437 blood samples from 339 consecutive febrile episodes, obtained between June 1, 2008, and March 31, 2010. Blood samples were drawn from 201 severely neutropaenic (absolute neutrophil count <500/mm^3) patients (23 children and 178 adults) with haematological malignancies (105 acute myeloid leukaemia, 23 acute lymphoblastic leukaemia, 34 lymphoma, 15 multiple myeloma, and 8 chronic myeloproliferative disorders), severe aplastic anaemia (4 patients), solid tumours (9 patients), or other disorders (2 cases of autoimmune thrombocytopaenia, 1 case of haemophagocytic lymphohistiocytosis).

Patients were admitted to the Institute of Haematology and the Paediatric Oncology and Haematology Unit, St. Orsola-Malpighi University Hospital, Bologna, Italy. All patients enrolled were febrile (≥38.5 °C), neutropaenic, and received antibiotic and antifungal prophylaxis, as specified by in-house guidelines. Specifically, all adults received antibiotic prophylaxis with fluoroquinolones, and 91 patients who underwent allogeneic hematopoietic stem cell transplantation also received fluconazole as antifungal prophylaxis. All other patients received itraconazole or posaconazole. Paediatric patients received cotrimoxazole and, for those who were undergoing allogeneic haematopoietic stem cell transplantation ($n = 4$), prophylaxis with fluoroquinolones and fluconazole.

Clinical and microbiological data were used to judge the clinical relevance of a positive PCR or blood culture result by the attending physician caring for the patient. Specifically, coagulase-negative staphylococci (CoNS) or *Streptococcus* spp. identified by culture or PCR were not considered to be true pathogens in persistently neutropaenic patients if a) the neutropaenic patient defervesced in the absence of antibiotic therapy targeting the specific microorganism and/or b) only 1 set of blood cultures tested positive in concomitance of a PCR-negative finding.

Test samples were collected from the patients at the onset of the febrile episode and before empirical antibiotic therapy was administered. Blood was drawn from peripheral veins in adults. In paediatric patients, blood was drawn from central venous catheters (CVC), as venous access is often difficult in this population (Hall and Lyman, 2006).

2.2. Microbiological methods

All blood samples were processed at the hospital microbiology unit. For culture, blood was collected twice at the onset of fever within a 30-min interval, while 3 samples were taken if CVC was present. Five to 10 mL of blood was put into each aerobic and anaerobic culture bottle (BacT/Alert 3D system, BioMerieux Italia, Florence, Italy), according to the Clinical and Laboratory Standards Institute protocol (Wilson et al., 2007). The cultures were incubated up to 132 h before assessing a negative result. Positive blood cultures were smeared and Gram stained. Subcultures were simultaneously started by seeding on both nutritive and selective agar medium to obtain isolated colonies. The isolated bacteria were biochemically identified (Vitek2 instruments and panel, BioMerieux), and antimicrobial susceptibility testing (AST) was performed by automated instruments, as follows: Gram-positive bacteria were tested using the Sensititre Aris system (Trek, Cleveland, OH, USA), and Gram-negative bacteria were tested with the Vitek2 instrument (BioMerieux).

For PCR testing, 3 mL of blood was sampled and processed for patients who weighed ≥45 kg and 1.5 mL for those who were <45 kg. Specimens were collected once at the onset of the febrile episode and then processed by LightCycler Septi*Fast* Test M^{GRADE} as described (Varani et al., 2009). Samples for blood culture and PCR testing were collected through a single venipuncture.

The PCR assay is based on 3 principal steps: 1) specimen preparation by mechanical lysis and purification of DNA from whole blood; 2) real-time PCR amplification of target DNA in 3 parallel reactions (Gram-positive bacteria, Gram-negative bacteria, fungi); and 3) detection by specific hybridization probes and automated identification of species and controls, as described (Lehmann et al., 2008). A defined volume of internal control was introduced into each specimen to verify the amplification reaction. Target bacterial (Gram-positive and Gram-negative) and fungal DNA were simultaneously amplified as reagent controls. When amplification of internal control or reagent control failed, the results of PCR were considered invalid.

Blood cultures were accepted for automated incubation from 8:00 a.m. to 7:00 p.m. (Monday to Saturday). PCR was performed once per day from Monday to Friday (samples were received by 12:00 p.m.). Blood samples intended for PCR analyses arriving to the laboratory after 12:00 p.m. were stored at 4 °C until the next PCR session was programmed (time limit for storage was 72 h, as suggested by the manufacturer).

This study was conducted according to the regulations of the St. Orsola-Malpighi University Hospital Ethical Committee.

2.3. Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) plus 95% confidence intervals (CI) were calculated for the PCR assay using blood culture results as a "gold standard" reference excluding nonevaluable PCR test results. The percentage of positive tests was compared in 2-by-2 contingency tables using the chi-quare of Fisher's test. Time to positivity for PCR and culture results was defined as the time interval from blood collection to the complete identification of germs, including AST data. The distribution of time to positivity for PCR versus cultures results was compared using *t* test or Mann–Whitney test, when appropriate. A 2-sided P value of < 0.05 was considered statistically significant. Analysis was performed using the SPSS 20 Statistical package (IBM, Armonk, NY, USA).

3. Results

Of the 437 samples evaluated by blood culture and PCR, 100 (22.9%, corresponding to 75 febrile episodes) yielded a technically invalid result by PCR because of the failure of the internal control or reagent control to amplify and were excluded from further analysis. The remaining 264 febrile episodes (corresponding to 337 samples) were studied. The microorganisms identified are summarized in Table 1.

In the 49 febrile episodes that were positive by both blood culture and PCR, organisms were detected on average 2.5 days earlier by PCR versus blood culture ($P < 0.01$); in 159 febrile episodes, PCR yielded a negative result 5 days before blood culture ($P < 0.01$) (Fig. 1). The overall concordance between the 2 tests was 79% (Table 2).

With blood cultures as the gold standard, the PPV of PCR assay ranged from 0.39 (95% CI 0.0.25–0.53) for Gram positive, 0.74 (95% CI 0.53–0.88) for Gram negatives to 50% (95% CI 0.03–0.97) for fungi, and 67% (0.13–0.98) for mixed infections (Table 2). The relatively poor sensitivity of the test for Gram-positive pathogens (0.39, 95% CI 0.25–0.53) reflected the occasional failure of the PCR test to detect CoNS growing in culture. However, CoNS may reflect contamination during venipuncture rather than true infection, especially in cases where neutropaenic patients defervesced without antimicrobial therapy active against CoNS. The PCR test appeared to be more useful for detecting the presence of Gram-negative pathogens, with a more acceptable sensitivity of 74%. Estimates of the sensitivity, specificity, PPV, and NPV were less precise for fungi and mixed infections, given the small number of cases (Table 2).