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Diagnostic utility of LightCycler SeptiFast and procalcitonin assays in the diagnosis of bloodstream infection in immunocompromised patients

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ABSTRACT

Sepsis is an increasingly prevalent cause of death, and management in the early stage is a critical issue. However, microbiological findings are generally obtained late during the course of the disease. In this study, we evaluated the clinical utility of procalcitonin (PCT) in improving the diagnosis of bloodstream infections and the potential utility of the SeptiFast (SF) test, a multiplex pathogen detection system, in the etiological diagnosis of immunocompromised patients. Seventy-nine hospitalized immunocompromised patients were included in this study. Our results demonstrate that while the PCT value correlates highly with sepsis, the results do not discriminate adequately enough to justify its independent use as a diagnostic tool. The SF test, combined with blood cultures, improves microbiological data in immunocompromised patients, especially in cases of previous antibiotic therapy and invasive fungal infection.

1. Introduction

Sepsis is a serious medical condition caused by microbial pathogens in the blood and is associated with high rates of morbidity and mortality (20–70%) (Barnato et al., 2008; Dombrovskiy et al., 2007). The identification of microorganisms in the blood is crucial for early and appropriate antimicrobial therapy (Leibovici et al., 1998). Blood cultures (BC) are considered the "gold standard" for the identification of bacterial and fungal bloodstream infections, although this method is limited by high-volume requirements to maximize its sensitivity and often long incubation times (at least 24 to 72 h for positive results and 5 days for a negative results). Furthermore, several factors, such as empirical therapy, initiated before blood sampling, or the presence of fastidious pathogens, may have a negative impact on the diagnostic yield of BC even when a bloodstream infection is strongly suspected. Thus a large proportion of patients (at least 15%) who appear clinically septic produce negative BC results (Bone, 1992; Hugonnet et al. 2004; Lamas and Eykyn, 2003). In an effort to address some of these limitations to improve the sensitivity and decrease the time to pathogen's identification, new markers for differentiating between the presence and absence of infection, such as procalcitonin (PCT), and new molecular techniques for etiological diagnosis, such as the LightCycler SeptiFast (SF) test, have been developed. PCT, a peptide produced ubiquitously in response to endotoxins or mediators released in response to bacterial infections, has been suggested to have superior diagnostic utility compared to other biomarkers for suspected sepsis (Assicot et al. 1993; Müller et al., 2000). The SF test is capable of detecting genetic material belonging to several bacterial and fungal pathogens, representing approximately 90% of the species, and has been recently used for the molecular diagnosis of sepsis in hospitalized patients with suspected bloodstream infections (Avolio et al., 2010; Luoie et al., 2008; Mancini et al., 2009; Varani et al., 2009). In this study, we aimed to compare SF and PCT results with BC results in immunocompromised patients and to assess the clinical validity of both methods in predicting septicemia.

2. Materials and methods

2.1. Patients inclusion criteria

Over 8 months, 79 hospitalized patients were included in the study: 41 male and 38 female, between the ages of 5 and 68 years. We included all immunocompromised patients, which was defined as patients with any of the following: neutropenia (neutrophil count $<1 \times 10^3/\mu L$), exposure to immunosuppressive agents, hematological malignancy, or solid tumor. Blood samples were obtained from individual patients, with all, but 4, having BC drawn before starting antimicrobial therapy. The underlying clinical conditions were as follows: 21 acute lymphoblastic leukemia, 2 Wilms' tumor, 3 hepatocellular carcinoma, 2 allogenic stem cell transplantations, 18 non-Hodgkin's lymphoma, 4 colon cancer under chemotherapy, 2

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ovarian cancer, 1 Leishmania visceral, and 32 exposure to glucocorticoids for autoimmune disease (rheumatoid arthritis, ulcerative colitis, and autoimmune thyroid). The average white blood cell count was 6.8 ± 11.41 × 10^3/μL, and neutrophil count was 3.19 ± 3.99 × 10^3/μL. The patients were in an immunocompromised state and hospitalized in the Department of Pediatric Oncology and Department of Internal Medicine, with suspected bloodstream infections and at least 2 criteria for systemic inflammatory response syndrome (SIRS): temperature >38 °C or <36 °C, heart rate 90 beats/minute, $PaCO_2$ <32 mmHg, or white blood cell count >12,000 or <4000 cells/μL.

2.2. Blood culture

At the onset of fever, 1 set of blood cultures (aerobic/anaerobic and fungal) was taken by sterile venipuncture and processed using Bactec 9240 (Becton Dickinson, Heidelberg, Germany) and followed up after 30 min with a second set of blood cultures. From 8 am to 6 pm, when the BC gave a positive signal, Gram staining was carried out. An aliquot of positive BC was plated onto solid media and incubated for 24/48 h, and identification was carried out with a Vitek 2 system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's protocol.

2.3. LightCycler SeptiFast polymerase chain reaction

Immediately after the blood was drawn for the BC, a 1.5-mL EDTA blood sample was collected for the molecular method. The LightCycler SeptiFast test M Grade (Roche Molecular Systems, Mannheim, Germany) is an in vitro nucleic acid amplification test for the detection of bacterial and fungal DNA (16S–23S and 18S–5.8S internal transcribed space regions of rRNA genes, respectively) in human blood. DNA was extracted from the 1.5 mL of blood collected in EDTA that was lysed according to the manufacturer's instructions, in a dedicated pre–polymerase chain reaction (PCR) room to prevent contamination. After purification, the DNA was processed in 3 parallel multiplex real-time PCR reactions: Gram-positive bacteria, Gram-negative bacteria, and fungi. The melting profile of amplified products was calculated by a specific dedicated software, thus allowing the identification of the pathogens at the genus or species level. The detection limit ranged from 3 to 100 CFU/mL (Lehmann et al., 2008), whereas the turnaround time was approximately 6 h.

2.4. Procalcitonin

The plasmatic level of PCT was measured with an enzyme-linked fluorescence assay (VIDAS© BRAHMS PCT assay; bioMérieux, Lyon, France), according to the manufacturer's instructions. The upper limit of the reference interval used in this study was 2 ng/mL, as suggested by the manufacturer.

2.5. Interpretation of the data

Significant bacteremia or fungemia was defined by microbial growth in 1 or more BC sets. The importance of the isolation of a potentially contaminating microorganism in a single set of BC or the detection of CoNS DNA in blood by the SF assay was evaluated according to clinical suspicions. Discordant results between the assays were resolved on the basis of isolation of the same microorganisms from other sites of the same patient, clinical conditions, or on the basis of the other assay (i.e., serum galactomannan assay).

2.6. Statistical analysis

Plasmatic PCT mean levels (±SD) were calculated for subjects with bacterial infection and in all other patients. The significance of the differences between the means for the 2 groups was assessed using Student's *t* test.

3. Results

3.1. Performance and clinical value of the PCT assay

The final diagnosis was microbiologically documented (BC positive) as bacterial sepsis in 30 of the 79 patients (37.9%); 2 patients had fungal sepsis and 47 had no bloodstream infections. Plasmatic PCT concentrations were significantly higher in patients with bacterial infection (21.36 ± 22.7) compared to all other patients (5.27 ± 10.63) ($P < 0.01$; Fig. 1). A cut-off value of 2 ng/mL was associated with 75% specificity and 90% sensitivity. Among all patients with documented sepsis, 2 had PCT value of <2 ng/mL. Of the 10 patients with nonbacterial sepsis and a PCT value above the cut-off, 1 patient had visceral leishmaniasis (PCT = 44.53 ng/mL), 2 patients had fungal infections (*Candida albicans*, PCT = 4.63 ng/mL; *Candida parapsilosis*, PCT = 9.96 ng/mL). No microorganisms were isolated in the remaining patients.

3.2. Performance and clinical value of LightCycler SeptiFast assay

Blood samples from 79 cases were examined. Thirty-two samples were associated with a positive BC, with 3 of these (9.4%) yielding 2 or more isolates. Of the 79 sample examined, 32 samples had positive results with SF test, which was concordant with BC results in 21 cases (65.6%) (Table 1).

Negative results in both tests were obtained in 42 cases, an agreement of 81%. The remaining 15 cases were discordant. Discordant results between the assays were resolved on the basis of another assay (i.e., serum galactomannan assay) and available clinical and microbiological data (i.e., response to therapy, culture from the other sites). Of the 15 discordant cases, 3 episodes concern microorganisms not detectable by the SF test (*Listeria monocytogenes, Morganella morganii, Providencia stuartii*; Table 1). Table 2 shows the discordant results in samples with a single microorganism. In 2 cases, *Escherichia coli* was missed by the SF test. In 1 case, the presence of *Candida krusei* DNA in the SF test was deemed clinically meaningless; the same microorganism was not identified from the other culture. In 2 cases, *Klebsiella pneumoniae* was detected by the SF test, whereas the BCs were negative: the blood samples were drawn after starting antimicrobial therapy for suspected urosepsis. In 1 case, the SF results were determined to be true on the basis of isolation of the same microorganisms from another site in the same patient: *C. parapsilosis* was detected by the SF test and in the catheter. Furthermore, *C. parapsilosis* was recovered from a second set of BC drawn 24 h later.

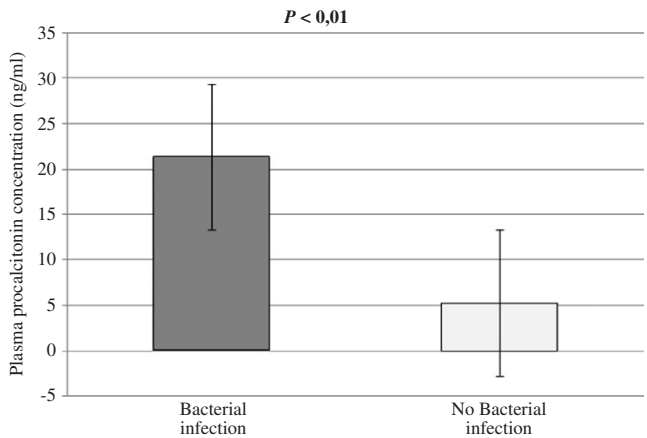

Fig. 1. Procalcitonin levels in patients with bacterial bloodstream infections and in patients with no bloodstream infections.