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Diagnostic Performance of a Multiple Real-Time PCR Assay in Patients with Suspected Sepsis Hospitalized in an Internal Medicine Ward

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Early identification of causative pathogen in sepsis patients is pivotal to improve clinical outcome. SeptiFast (SF), a commercially available system for molecular diagnosis of sepsis based on PCR, has been mostly used in patients hospitalized in hematology and intensive care units. We evaluated the diagnostic accuracy and clinical usefulness of SF, compared to blood culture (BC), in 391 patients with suspected sepsis, hospitalized in a department of internal medicine. A causative pathogen was identified in 85 patients (22%). Sixty pathogens were detected by SF and 57 by BC. No significant differences were found between the two methods in the rates of pathogen detection ($P = 0.74$), even after excluding 9 pathogens which were isolated by BC and were not included in the SF master list ($P = 0.096$). The combination of SF and BC significantly improved the diagnostic yield in comparison to BC alone ($P < 0.001$). Compared to BC, SF showed a significantly lower contamination rate (0 versus 19 cases; $P < 0.001$) with a higher specificity for pathogen identification (1.00, 95% confidence interval [CI] of 0.99 to 1.00, versus 0.94, 95% CI of 0.90 to 0.96; $P = 0.005$) and a higher positive predictive value (1.00, 95% CI of 1.00 to 0.92%, versus 0.75, 95% CI of 0.63 to 0.83; $P = 0.005$). In the subgroup of patients ($n = 191$) who had been receiving antibiotic treatment for ≥24 h, SF identified more pathogens (16 versus 6; $P = 0.049$) compared to BC. These results suggest that, in patients with suspected sepsis, hospitalized in an internal medicine ward, SF could be a highly valuable adjunct to conventional BC, particularly in patients under antibiotic treatment.

Sepsis is a common and potentially deadly condition in tertiary care hospital inpatients (3, 10), and early pathogen detection is crucial to decrease the related morbidity and mortality (1, 23). The diagnosis of sepsis relies heavily on blood culture (BC), which detects circulating living bacteria or fungi and tests susceptibility to antimicrobials. However, the sensitivity of BC is limited, particularly when antibiotics have already been administered (6, 17), and may not provide time-critical results that can impact on early management (2). Indeed, culturing, identification, and drug-susceptibility testing may require more than 48 h (7).

PCR technology can detect DNA of bacteria and fungi in blood rapidly (19), limiting the empirical use of broad-spectrum antibiotics and the development of drug-resistant organisms and superinfections. SeptiFast (SF; Roche Diagnostics GmbH, Mannheim, Germany), a commercially available PCR-based system, has been used in the molecular diagnosis of sepsis in patients from emergency room (14) and intensive care units (12, 15, 18), in neutropenic and immunocompromised febrile patients (9, 16, 20), as well as in patients with endocarditis (4). Data regarding the diagnostic usefulness of SF in the routine clinical management of patients with suspected sepsis hospitalized in the tertiary care units are still limited (5, 8, 21).

The aim of the present study was to prospectively evaluate this new PCR-based microbiological diagnostic tool in comparison with BC, in a group of consecutive adult patients with suspected sepsis hospitalized in an internal medicine ward.

MATERIALS AND METHODS

Patient selection. From March 2010 to August 2011 we enrolled 391 patients, hospitalized in the Department of Internal Medicine of the University of Perugia, Perugia, Italy, suspected of having systemic inflammatory response syndrome (SIRS) caused by bacterial or fungal infection and for whom BC was performed for causative pathogen identification.

SIRS was diagnosed when two or more of the following criteria were present: body temperature, >38°C or <36°C; heart rate, >90 beats per minute; respiratory rate, >20 breaths per minute; white blood cell count, >12,000 cells/μl or <4,000 cells/μl (3). Sepsis was defined as SIRS, defined as above, caused by bacterial or fungal infection (3).

Sample collection. Samples for BC and SF determination were collected at the same time point for each patient. Samples drawn only once from any given patient were considered for analysis. According to the clinical picture, different microbiological samples were also collected from suspected infectious sites (urine, sputum, bronchoalveolar lavage fluid, pus, and drainage fluids). Specific viral and fungal tests and detection of *Legionella pneumophila* and *Streptococcus pneumoniae* urinary antigens were performed when appropriate. Results from routine blood tests, imaging study findings, clinical events, and antimicrobial treatment were collected during the hospital stay.

Blood culture. For each sample, an aliquot of 5 to 10 ml whole blood was inoculated into BACTEC aerobic and anaerobic bottles (Becton Dickinson, Sparks, MD). BACTEC Plus bottles were used for patients under antibiotic therapy and standard bottles for untreated patients. Two sets

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from two different sites were collected at the same time. The bottles were then incubated in a 9240 automated blood culture system (Becton Dickinson). All bottles flagged positive were removed from the instrument, and an aliquot was taken for Gram stain and culture on solid media for subsequent analysis. Identification and determination of sensitivity to antibiotics were performed with conventional methods and with the Phoenix automatic system (Becton Dickinson).

SF real-time PCR. For real-time PCR, a 3-ml K-EDTA-blood sample was collected, and 1.5 ml was processed for the SF assay according to the manufacturer's instructions. To avoid contamination events, all molecular procedures were carried out according to the instructions included in the LightCycler SF package insert (Roche Diagnostics GmbH, Mannheim, Germany), including room separation and use of DNA decontamination with DNAZap (Applied Biosystems/Ambion, Austin, TX). Mechanical lysis using the SF Lys kit MGRADE and the MagNA Lyser was performed. Using the SF Prep kit MGRADE, DNA was extracted as described by the manufacturer. Hybridization probes were used. An internal extraction and amplification control, included in the kit, was introduced into each specimen soon after the mechanical lysis of the specimen, along with the lysis reagent, during the first steps of the extraction procedure. This control consists of a mixture of synthetic double-stranded DNA molecules with primer binding sites identical to those of the target sequences but differing in their probe binding sites, thus allowing differentiation of the amplified internal control and the target-specific amplicon. A negative control supplied by the manufacturer was included in each extraction series. Using the LightCycler SF kit MGRADE, real-time PCR was performed in a LightCycler 2.0 instrument (Roche Diagnostics). Three different primer mixes were used to amplify Gram-positive bacteria, Gram-negative bacteria, and fungi. The internal transcribed spacer region was the specific target for the detection of bacterial and fungal pathogens. Reagent controls provided in the kit were used as the positive control of the PCRs. The emitted fluorescence was measured in one of the four different detection channels (610, 640, 670, and 705 nm). An analysis of the melting curves was performed to further confirm the specificity of the products. Species identification (melting temperature analysis of specimens and controls in each channel) and report generation was obtained using the SF identification software SIS (Roche Diagnostics). The limits of detection were 100 cells per ml for coagulase-negative staphylococci, *Candida glabrata*, and *Streptococcus* spp. and 30 cells per ml for all other pathogens (LightCycler SF package insert; Roche Diagnostics). The microorganisms identified by SF have been listed elsewhere (11).

Definition of pathogen. Microorganisms detected by SF were considered to be pathogens if the results of the DNA kit coincided with the results of the BC analysis. If SF and BC were positive for different microorganisms, or if a microorganism was only detected by one of the two tests, culture results from other samples, taken from the suspected infection site, were evaluated. If these culture tests revealed the presence of the same organism, it was considered a pathogen. If a microorganism was detected in only one blood sample (SF or BC) without any other culture support, the clinical picture was considered: the microorganism was considered a pathogen if the patient suffered from sepsis, according to the ACCP/SCCM Consensus Conference Committee definition of infection (3) and if the species is generally accepted as a common etiologic agent of the patient's type of infection. Coagulase-negative staphylococci (CoNS) detected in only one bottle from two BC sets were not considered pathogens (13) in the absence of clinical and/or laboratory data suggesting their pathogenetic role.

Statistical analysis. SPSS statistical package, release 13.0 (SPSS Inc., Chicago, IL), was used for all statistical analyses. Values are expressed as means ± standard deviations (SD). Statistical significance was assumed if a null hypothesis could be rejected at a P value of <0.05. The McNemar test was used for testing the differences between paired proportions. Sensitivity is the number of positive samples correctly identified by SF or BC over the total number of samples positive for a pathogen. Specificity is the number of negative samples correctly identified by SF or BC over the total number of samples negative for a pathogen. Positive (or negative) predictive value is the number of positive (or negative) samples correctly identified by SF or BC over the total number of samples tested positive (or negative, respectively). Sensitivities, specificities, and predictive values were compared by means of the χ^2 test, using the 2-by-2 contingency table.

TABLE 1 Clinical and laboratory characteristics of the 391 enrolled patients

Patient characteristic	Value	No. of patients (%)
Median age, yr (IQR)[a]	73 (20–99)	
Males		215 (55%)
Body mass index (SD)	25 (±6)	
Concomitant disease		
History of CV[b] disease		88 (22%)
Malignancy		70 (18%)
Dementia		46 (12%)
Chronic lung disease		44 (11%)
Diabetes		41 (10%)
Chronic renal failure		28 (7%)
Immune deficiency		17 (4%)
Chronic liver disease		16 (5%)
Gangrene		3 (1%)
Patients fulfilling SIRS criteria		242 (62%)
Fever or hypothermia		287 (73%)
Leukocytosis ($>12.10^3/mm^3$)		155 (40%)
SBP[c] <120 mm Hg or 50 mm Hg decrease		101 (26%)
Respiratory rate >30/min		37 (9%)
Leucopenia ($<4 \times 10^3/mm^3$)		18 (5%)
Pulse rate >120/min		16 (4%)
Prior antimicrobial treatment >24 h		191 (49%)
Hospital death		48 (12%)

[a] IQR, interquartile range.
[b] CV, cardiovascular.
[c] SBP, systolic blood pressure.

RESULTS

Table 1 illustrates clinical and laboratory characteristics of the 391 enrolled patients. Two hundred forty-two patients (62%) fulfilled SIRS definition criteria (3). One hundred ninety-one patients (49%) were under antibiotic therapy for at least 24 h before sampling.

Eighty-five pathogens were identified in 391 samples. SF detected 60 pathogens and BC 57 pathogens. Concordant results were obtained in 82% of the cases: 289 samples were concordant negative and 32 samples concordant positive. Twenty-eight pathogens were detected only by SF (26 BC-negative and 2 BC-contaminated samples) and 25 only by BC (Table 2). No significant difference was found between the two methods in the rate of pathogen detection ($P = 0.74$), even after excluding the nine pathogens not included in the SF master list (11) ($P = 0.096$). No case of polymicrobial sepsis was observed. Table 3 summarizes the pathogens detected by SF and/or BC.

Sensitivity for pathogen identification was not significantly different between SF (0.71, 95% confidence interval [CI] of 0.60 to 0.79) and BC (0.67, 95% CI of 0.56 to 0.76; $P = 0.25$). SF had a significantly greater specificity than BC (1.00, 95% CI of 0.99 to 1.00, versus 0.94, 95% CI of 0.90 to 0.96; $P = 0.005$). The negative

TABLE 2 Comparison of SF and BC results in 391 samples

Result		No. of BC			
		Positive for a pathogen	Negative	Contaminated	Total
SF	Positive for a pathogen	32	26	2[a]	60
	Negative	25	289	17[b]	331
	Contaminated	0	0	0	0
	Total	57	315	19	391

[a] 1 BC contaminated by *Staphylococcus epidermidis* was positive for *Streptococcus* spp. with SF; 1 BC contaminated by *Propionibacterium acnes* was positive for *Enterobacter cloacae/Enterobacter aerogenes* with SF.
[b] 16 BCs contaminated by CoNS; 1 BC contaminated by *Corynebacterium amycolatum*.

TABLE 3 Pathogens detected by SF and/or BC in 391 samples

Pathogen	No. of pathogens detected		
	Only by SF	Only by BC	Both methods
Staphylococcus aureus	3	3	8
Streptococcus pneumoniae	3	0	0
Streptococcus spp.	2	1[a]	2
Enterococcus faecalis	1	2	1
Enterococcus faecium	2	1	0
Enterobacter cloacae/Enterobacter aerogenes	2	0	1
Escherichia coli	6	7	12
Klebsiella pneumoniae/Klebsiella oxytoca	4	1	3
Pseudomonas aeruginosa	3	0	1
Coagulase-negative staphylococci	1	1[b]	4
Candida albicans	1	0	0
Subtotal	28	16	32
Clostridium perfringens	ND[c]	1	0
Hafnia alvei	ND	1	0
Peptostreptococcus spp.	ND	1	0
Listeria spp.	ND	1	0
Leuconostoc spp.	ND	1	0
Leifsonia aquatica	ND	1	0
Aeromonas sobria	ND	1	0
Salmonella group D	ND	1	0
Streptococcus dysgalactiae	ND	1	0
Total	28	25	32

[a] *Streptococcus bovis*.
[b] *Staphylococcus haemolyticus*.
[c] ND, not included in the SF master list (11).

predictive value did not differ between the two methods (SF, 0.92, 95% CI of 0.88 to 0.94; BC, 0.91, 95% CI of 0.87 to 0.93; $P = 0.25$), while positive predictive value was significantly higher for SF (1.00, 95% CI of 1.00 to 0.92) than it was for BC (0.75, 95% CI of 0.63 to 0.83; $P = 0.005$).

The combination of the two methods resulted in a significantly increased rate of pathogen detection compared with either method alone (both methods, 85 pathogens, versus SF alone, 60, or BC alone, 57; $P < 0.001$).

Additional sensitivity analyses were performed in prespecified subgroups in order to compare the diagnostic yield of the two tests in different clinical conditions. Among the 242 patients with confirmed SIRS, no significant difference were found between SF and BC (24 versus 15 pathogens; $P = 0.2$). Among the 191 patients in which blood samples were drawn at least 24 h after starting antibiotic therapy, SF detected a greater number of pathogens compared to BC (16 versus 6 pathogens; $P = 0.049$). In this group of patients, compared with the whole group, SF had also a significantly greater sensitivity (0.81, 95% CI of 0.62 to 0.92, versus 0.50, 95% CI of 0.32 to 0.67; $P < 0.01$) and negative predictive value than BC (0.96, 95% CI of 0.91 to 0.98, versus 0.90, 95% CI of 0.84 to 0.94; $P = 0.05$).

DISCUSSION

We studied an unselected group of adult patients hospitalized in an internal medicine ward for suspected sepsis and for whom a BC was ordered. Our study shows that SF in addition to BC leads to the identification of more pathogens and could be a highly valuable tool in the diagnosis of sepsis. SF also exhibited a significantly higher positive predictive value than BC because of the absence of contaminations. While no significant differences in the two methods were found in the overall population, SF showed a higher sensitivity than BC in the subgroup of patients under antibiotic therapy for more than 24 h.

In agreement with previous studies performed in intensive care units (12, 22), in emergency rooms (14), and in neutropenic patients (9) the combined use of SF and BC significantly improved the detection of bloodstream pathogens also in an unselected population admitted for sepsis to an internal medicine ward.

Our results seem to coincide with a previous multicenter trial, performed in tertiary care units. In 359 patients clinically suspected for bacterial or fungal sepsis, Westh et al. found that SF showed a higher positivity rate and a lower contamination rate than BC (21). A large study at an infectious diseases department showed an SF specificity of more than 97% with an suboptimal sensitivity and positive predictive value, especially for *S. pneumoniae* and other *Streptococcus* species (8). In a small study, Dierkes et al. did not find a significantly higher sensitivity of SF, although SF allowed an adequate antibiotic therapy in an additional 8% of patients (5).

In the present study, 28 pathogens were detected only by SF and the clinical relevance of these organisms was subsequently confirmed through an appropriate diagnostic workup which included microbiological, clinical, and imaging findings. Among SF-positive/BC-negative samples, 3 samples were positive for *Staphylococcus aureus*, concordant with the diagnosis of endocarditis, and 3 samples revealed *S. pneumoniae*, concordant with clinical picture, urine antigen test, and radiological findings of pneumonia. Two samples positive for *Streptococcus* spp., 1 sample positive for *Enterococcus faecalis*, and 1 sample positive for CoNS were from patients with endocarditis. Two *Enterococcus faecium* strains were detected in samples from patients with intestinal occlusion. Two samples positive for *Enterobacter cloacae/Enterobacter aerogenes*, 5 samples positive for *Escherichia coli*, 4 samples positive for *Klebsiella pneumoniae/Klebsiella oxytoca*, and 3 samples positive for *Pseudomonas aeruginosa* were associated with concordant findings in urine culture. One sample positive for *E. coli* was consistent with a diagnosis of acute cholecystitis. Finally, 1 sample positive for *Candida albicans* was concordant with culture from a patient's bedsore infection.

On the other hand, SF failed to detect 25 pathogens identified only by BC. Among them, 9 were not detectable because they are

not included in the SF master list (11), but 16 have to be considered SF false-negative results. Among the 16 BC positive/SF negative samples, 2 *S. aureus* samples were from patients with septic shock and 1 sample was from a patient with pacemaker pocket infection; 1 *S. bovis* sample and 1 *E. faecalis* sample were from patients with endocarditis. One *E. faecalis* sample, 1 *E. faecium* sample, 4 *E. coli* samples, and 1 *K. pneumoniae* sample were concordant with urinary infection; 2 *E. coli* samples were from patients with aspiration pneumonia and 1 sample was from a patient with cholangitis; 1 *S. haemolyticus* sample was from a patient with endocarditis. The SF false-negative samples may be attributable to a number of factors, including microbial load below the detection threshold of the test, low sample volume, inappropriate sample preparation, or genetic variability/mutation of the target site. Seventeen out of the 19 contaminated BCs were due to CoNS, which were not detected by SF. Indeed, the presence of low concentrations of CoNS and *Streptococcus* spp. DNA may reflect contamination of workflow at different stages and, therefore, it is excluded as not a significant result by the SF SIS software. Overall, in our study, SF had a greater specificity and a higher positive predictive value than BC, while maintaining a similar sensitivity and negative predictive value.

Another finding of the present paper deserves to be commented. The diagnostic performance of SF was significantly superior to BC in patients under antibiotic treatment. As expected (6, 17), the results of BC were affected by the antibiotic pretreatment, a very common situation in febrile patients admitted to an internal medicine ward. Indeed, in our population, 49% of the patients had been receiving antibiotics for $\geq$24 h before sampling. In this subgroup of patients, SF identified a significantly larger number of pathogens than BC.

It is likely that rapid detection of pathogens, also in noncritically ill patients, such as those hospitalized in an internal medicine ward, may have a favorable impact on the clinical outcome of patients with suspected sepsis. The present study was not designed to address the above hypothesis, which deserves to be tested in further prospective studies.

Blood culture remains a mainstay for the identification of microorganisms in bloodstream infections in patients with suspected sepsis and has the unique ability to identify drug susceptibility. Although suboptimal sensitivity, cost issues, limited availability, and the need for trained laboratory personnel limit the routine use of SF in unselected patients with suspected sepsis, the test could be considered in selected patients as an additional tool aimed at improving diagnostic accuracy whenever a quick etiological diagnosis is required for appropriate and timely clinical management. Our data support the utility of adding SF to the diagnostic workup of patients admitted to an internal medicine ward for suspected sepsis, especially if pretreated with antibiotics.

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