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Added value of multi-pathogen probe-based real-time PCR SeptiFast in the rapid diagnosis of bloodstream infections in patients with bacteraemia

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The commercial multi-pathogen probe-based real-time PCR SeptiFast (SF) was evaluated as a rapid and complementing tool for the microbiological diagnosis of bloodstream infections (BSIs) in a series of 138 matched blood samples from 65 patients with bacteraemia, hospitalized in an intensive care unit, when antibiotics had already been administered. SF was positive in 32.6 % of the samples, whereas blood culture (BC) was positive in 21.7 % ($P<0.05$). SF identified more pathogens (11 versus 5; specificity, 90.7 %) and reduced the time of aetiological diagnosis, with a mean of 16.3 versus 55.4 h needed for BC ($P<0.05$). SF enabled appropriate pathogen-oriented therapy in 72 % (36/50) of the BSI group of patients on the basis of epidemiological data. According to our data, the use of SF provided important added value to BC, in terms of earlier aetiological diagnosis of BSIs, enabling pathogen-oriented therapy in patients receiving empirical antibiotic treatment.

INTRODUCTION

Rapid and early detection of bacteria in blood is mandatory in the diagnosis and management of septic patients. An adequate treatment of aetiological micro-organisms within the first 6–12 h is critical for a favourable outcome in patients with bloodstream infection (BSI) (Dellinger *et al.*, 2008; Hall *et al.*, 2011). Delayed diagnosis and inappropriate antibiotic therapy are associated with increased morbidity and mortality in intensive care patients (Garnacho-Montero *et al.*, 2003; Zubert *et al.*, 2010). Blood culture (BC) represents the current gold standard for the aetiological diagnosis of bloodstream microbial pathogens (Kirn & Weinstein, 2013). However, BC is affected by low sensitivity, particularly when antibiotics have already been administered (Glerant *et al.*, 1999); moreover, prolonged incubation times for fungi and fastidious micro-organisms may have an impact on early management of patients (Bauer & Reinhart, 2010). BC results are reported to be negative in more than 50 % of true bacterial or fungal sepsis cases (Fenollar & Raoult, 2007).

Abbreviations: BC, blood culture; BSI, bloodstream infection; CoNS, coagulase-negative staphylococci; ICU, intensive care unit; SF, SeptiFast

Owing to their rapidity and sensitivity, molecular technologies are acquiring importance in the detection of DNA of bacterial and fungal pathogens (Dark *et al.*, 2009; Mancini *et al.*, 2010), thus allowing BSI diagnosis and appropriate management of septic patients (Livermore & Wain, 2013). The LightCycler SeptiFast (SF) test (Roche Diagnostics), approved for clinical use in Europe, is a multiplex real-time PCR designed to detect, from a single whole blood sample, in about 6 h, the most clinically important bacteria and fungi causing BSI (Lehmann *et al.*, 2008).

To date, the diagnostic performance of this test has been extensively evaluated in several clinical settings for the diagnosis of sepsis (Mancini *et al.*, 2008; Casalta *et al.*, 2009; Lehmann *et al.*, 2010; Pasqualini *et al.*, 2012; Herne *et al.*, 2013), and many of these papers have highlighted its great performance in shortening the time of diagnosis. On the contrary, data regarding the rapid microbiological documentation performed by SF in terms of appropriateness of the pathogen-oriented therapy are still limited (Lodes *et al.*, 2012).

The aim of this study was to assess the value of the SF test as an adjunct to BC for rapid microbiological diagnosis of BSIs in patients with bacteraemia in our hospital, focusing on timing in the adjustment of antibacterial therapy.

METHODS

Patient and samples. From March to December 2013, a total of 138 matched blood samples from 65 adult patients were collected at the Unit of Pathology of Cannizzaro tertiary care hospital, Catania, Italy.

All samples were from febrile patients hospitalized in the intensive care unit (ICU) suspected of having bacteraemia. For all patients, clinical and laboratory records were collected and formal consent was obtained. Patients were considered for inclusion if paired blood samples for SF and two sets of BC (two aerobic and two anaerobic bottles) were collected (within 1 h) at the same time for each febrile episode. Additional microbiological samples were also collected from other material at the suspected infectious site (urine, pus, bronchial lavage fluid, abdominal drainage, intraperitoneal fluid), when considered clinically relevant.

While SF and BC sampling was done prospectively, data analysis was conducted retrospectively.

BC. Blood samples were withdrawn from a single venipuncture at the onset of fever. For each BC set, a volume of 8–10 ml whole blood was inoculated into BacT/ALERT FAN aerobic and anaerobic bottles (bioMérieux), and then incubated in the BacT/Alert 3D automated system (bioMérieux) for up to 5 days for negative results. In the case of positive results, the bottles were removed from the instrument, and an aliquot was taken for Gram-staining and culture on solid media for subsequent analysis. Identification and sensitivity to antibiotics were determined with the VITEK 2 system (bioMérieux), and required at least 24–72 h, according to laboratory-defined standard procedures. A total of 552 bottles of BCs were evaluated.

SF real-time PCR. The LightCycler SeptiFast test (Roche Diagnostics) was used according to the manufacturer's instructions (Lehmann *et al.*, 2008). Briefly, DNA was extracted manually from 1.5 ml of EDTA-treated blood, using the SeptiFast Prep kit MGRADE (Roche Diagnostics). An internal control was added to each sample to check for false negatives. A negative control supplied by the manufacturer was included in each extraction series. DNA amplification was processed using three parallel multiplex primer mixes (Gram-positive, Gram-negative, fungi) of the SF kit MGRADE, and performed in a LightCycler 2.0 real-time PCR instrument (Roche Applied Sciences). Negative and positive controls were included in each PCR run. Melting temperature analysis of samples and controls in each channel, using specific hybridization probes, allowed species identification, and reports were generated using the SF identification software SIS (Roche Diagnostics).

The micro-organisms identified by SF have been reported in the SeptiFast Master List (Table 1). The analytical sensitivity of the assay, as determined by the manufacturer, is 30 c.f.u. ml^{-1} for most of the organisms detected by SF. Exceptions are coagulase-negative staphylococci (CoNS), *Streptococcus pyogenes*, *Streptococcus agalactiae* and *Candida glabrata*, for which the limit of detection is 100 c.f.u. ml^{-1} (Lehmann *et al.*, 2008).

Interpretation of results. A positive BC result was assumed to represent true bacteraemia according to previously published data (Weinstein, 2003; Pletz *et al.*, 2011). Whether a micro-organism identified with the SF test represented a true pathogen was evaluated by considering the identity of the micro-organism and the focus of infection as diagnosed by the clinician, and by comparing the BC results with other supplementary materials of the patient expected to be sterile under healthy conditions (i.e. bronchoalveolar lavage fluid, intraperitoneal fluid, pus, abdominal drainage, urine, etc.) and obtained ± 2 days from the onset of the bacteraemia.

The turnaround time of SF- and BC-positive samples was calculated as mean time (h) for the final microbial identification of pathogens. The results of SF were used by clinicians along with other laboratory and clinical data for adjustment of antibacterial therapy.

Data analysis. Statistical analyses were performed using GraphPad software. McNemar's test was used to assess differences between paired proportions. Statistical significance was assumed if a null hypothesis could be rejected at a P value of <0.05.

RESULTS

Patient population

In the study period, a total of 138 matched blood samples from 65 adult patients suspected of having BSIs were analysed with both conventional BC and SF. Clinical and laboratory records of the patients enrolled for the study are shown in Table 2. All patients were receiving empirical antibiotic treatment at the time of blood sampling.

Pathogens isolated

Of the 138 samples, 50 positive episodes of BSI (36.2%) were detected with the combination of SF and BC, with a total of 58 micro-organisms identified. Data regarding the micro-organisms detected by both methods are summarized in Table 3. The rate of polymicrobial infections was 16% (8/50). Among all pathogens, Gram-negative organisms were detected more frequently (74.1%, 43/58) than Gram-positive organisms and fungi (13.8%, 8/58, and 12.1%, 7/58) by both methods. *Klebsiella pneumoniae/oxytoca* was the most frequently detected pathogen (31%,

Table 1. SeptiFast Master List of pathogens detected

Gram-negative bacteria	Gram-positive bacteria	Fungi
Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
Klebsiella pneumoniae/oxytoca	CoNS	*Candida tropicalis*
Serratia marcescens	*Streptococcus pneumoniae*	*Candida parapsilosis*
Enterobacter cloacae/aerogenes	*Streptococcus* spp.	*Candida krusei*
Proteus mirabilis	*Enterococcus faecium*	*Candida glabrata*
Pseudomonas aeruginosa	*Enterococcus faecalis*	*Aspergillus fumigatus*
Acinetobacter baumannii		
Stenotrophomonas maltophilia		

Table 2. Clinical and laboratory records of the enrolled patients

Characteristic	Result
No. patients	65
Mean age (years)	54
Age range (years)	18–85
Men (*n*/%)	39/60
Women (*n*/%)	26/40
Mean WBC count (10^3 μl^{-1})	14.6±7.4
Mean C-reactive protei (mg dl^{-1})	12.5±9.5
Antibiotic therapy admission (*n*)	65
Infectious foci (*n*/%)	
Pulmonary	22/34.8
Abdominal	17/26
Urogenital	8/12.8
Skin	15/22.5
Systemic	3/4.6

18/58), followed by *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (19%, 11/58), and *C. parapsilosis* (6.9%, 4/58). A total of 20 micro-organisms (34.5%) were found in the SF group of patients. Based on post-hoc analysis, SF detected more pathogens (19%, 11/58) than BC (8.6%, 5/58), mainly owing to a higher rate of detection of fungal pathogens and of *Enterococcus faecium* and *Staphylococcus aureus*, while the number of CoNS was lower with SF. The remaining 9/58 pathogens were considered false positive. All differences were statistically significant ($P<0.05$).

Concordance of SF and BC

Twenty-five of the 138 samples (18.1%) were concordant positive in the SF and BC systems. Eighty-eight (63.7%) were negative by both methods. Thus, an overall agreement between methods was obtained in 81.9% of all samples. Forty-five of the 138 samples (32.6%) were positive by SF and 30/138 (21.7%) were positive by BC ($P<0.05$). Data regarding the samples detected by both methods are summarized in Table 4. Twenty-five discordant cases were found. In 20 samples with a negative BC, pathogens were identified only by SF. In additional microbiological samples from the same patient, the identical pathogen was isolated in 11 cases (three bronchial lavage, one wound swab, two intraperitoneal fluid, one abdominal drainage, one pus and three urine). In five samples with a negative SF, pathogens were identified only by BC. On the basis of a post-hoc analysis, SF showed a higher positive result than BC: 36/138 (26.1%) were positive by SF and 30/138 (21.7%) were positive by BC. Because of the small number of results, these differences did not reach statistical significance.

Time to microbiological diagnosis and treatment adjustment

With regard to the timing of the aetiological diagnosis of BSI, we estimated the turnaround time, from the arrival of the sample, for BC compared with that for SF simultaneously. In our hospital, the SF analytical session is performed once daily, 6 days a week. The mean time for an SF result was 16.3 h [confidence interval (CI) 95%; range 14.4–18.2], while a mean of 55.4 h (CI 95%; range 47.2–72.8) was needed for BC.

Early detection by SF and epidemiological data allowed our clinicians to adjust the empirical antimicrobial treatment in 60% of patients. In the group of patients with BSI, the percentage of pathogen-oriented therapy was up to 72% (36/50), calculated only on the SF true-positive results. In the 11 SF true-positive/BC-negative cases, representing the added value of the molecular approach, therapeutic

Table 3. Pathogens detected by SF and by BC analyses

Pathogens	*n*	Strains detected		
		Only by SF	Only by BC	Both methods
Gram-negative bacteria	43	12	3	28
K. pneumoniae/oxytoca	18	3	0	15
Acinetobacter baumannii	11	2	2	7
Pseudomonas aeruginosa	11	6	1	4
Stenotrophomonas maltophilia	2	1	0	1
Escherichia coli	1	0	0	1
Gram-positive bacteria	8	4	2	2
Staphylococcus aureus	3	2	0	1
Enterococcus faecium	2	2	0	0
CoNS	3	0	2	1
Fungi	7	4	0	3
C. albicans	3	1	0	2
C. parapsilosis	4	3	0	1
Total	58	20	5	33

Table 4. Comparison of SF and BC results

Test	BC positive	BC negative	Total SF
SF positive	25	20	45
SF negative	5	88	93
Total BC	30	108	138

changes of antimicrobial treatment were further analysed. These data are summarized in Table 5.

DISCUSSION

Healthcare systems spend considerable resources collecting and processing BCs for the detection of bloodstream pathogens. Missed opportunities to document the true cause of BSIs can adversely affect patient outcome, leading to the unnecessary administration of antimicrobial agents, often broad-spectrum, with increased cost, toxicity and risk of inducing resistance or selecting multidrug-resistant clones (Sharma & Kumar, 2008). Despite BC remaining crucial in BSI diagnosis, several limitations negatively affect its clinical utility (i.e. time lag and low sensitivity) (Glerant *et al.*, 1999; Peters *et al.*, 2004).

Here, we examined the utility of the molecular test SF for the early identification of BSI-causative organisms compared with BC in 65 febrile adult patients hospitalized in an ICU suspected of having bacteraemia, focusing on timing in the adjustment of antibacterial therapy.

Concordance for positive and negative results between BC and SF (81.9%) was comparable with other studies performed in ICUs (Lehmann *et al.*, 2010; Dark *et al.*, 2011). Our data showed that the combination of both tests yielded a microbiological identification of BSI in a significantly higher proportion (36.2%) of samples compared with results obtained with BC or SF alone, in keeping with other recent studies (Lucignano *et al.*, 2011; Pasqualini *et al.*, 2012; Paolucci *et al.*, 2013).

Specifically, in our series, SF showed better detection power for *Enterococcus faecium*, *Staphylococcus aureus* and *C. parapsilosis*. This finding is supported by a study by Fernández-Cruz *et al.* (2013) demonstrating an eightfold higher risk of developing BSI complication due to a longer persistence of Gram-positive cocci in patients with discordant SF and BC results. On the contrary, fewer CoNS specimens were identified by SF than by conventional BC.

With regard to SF-positive, BC-negative samples found in our patient cohort, we want to point out that most pathogens detected only by SF were also isolated in other microbiological specimens from the same patient, suggesting a clinical relevance in line with expert recommendations (Pletz *et al.*, 2011).

In particular, we considered one *Stenotrophomonas maltophilia*, one *Candida albicans* and two *Acinetobacter baumannii* samples true-positive on the basis of subsequent isolation from additional microbiological samples (three urine and one wound swab); these cases demonstrate the ability of SF to anticipate BC results even when the bacterial load is very low. In nine other cases, we judged six *Pseudomonas aeruginosa* and three *K. pneumoniae/oxytoca* to be false positives due to transient bacteraemia, explained by the possible translocation of these micro-organisms from naturally colonized surfaces; no pathogens were isolated from any of the other patient samples. Finally, the identification of two *Enterococcus faecium*, two *Staphylococcus aureus* and three *C. parapsilosis* was confirmed by the isolation of the same micro-organisms in two intraperitoneal fluid, one abdominal drainage, one pus and three bronchial lavage samples.

Table 5. Therapeutic changes of antimicrobial treatment in the 11 SF true-positive/BC-negative cases

Patient no.	Diagnosis	Empirical treatment	Pathogens detected only by SF	Pathogen-oriented therapy	Outcome of therapy
1	Polytrauma	β-Lactam + aminoglycoside	*Acinetobacter baumannii*	Polymyxin	Responsive
2			*C. parapsilosis* and *Enterococcus faecium*	Echinocandin + glycopeptide	Responsive
3			*Staphylococcus aureus*	Glycopeptide	Responsive
4			*C. parapsilosis*	Echinocandin	Responsive
5			*Acinetobacter baumannii*	Polymyxin	Responsive
6			*C. parapsilosis*	Echinocandin	Responsive
7			*Staphylococcus aureus*	Glycopeptide	Responsive
8			*C. albicans*	Azole	Responsive
9			*Stenotrophomonas maltophilia*	Co-trimoxazole	Responsive
10			*Enterococcus faecium*	Glycopeptide	Responsive

In five cases with positive BC, the SF test gave negative results. These discrepancies were obtained for two *Acinetobacter baumannii*, one *Pseudomonas aeruginosa* and two CoNS. In the first three cases, the reason for the negative SF result remained unknown, owing to negative results from all other cultures. One possible explanation is that in these samples a lower volume of blood was used. For the CoNS it is reasonable to consider contamination, according to laboratory-defined procedures. The LC (LightCycler) software revealed a peak, but the crossing point was higher than that of the cut-off ($C_p > 20$ cycles), thus ruling the sample negative.

Whether the lower detection rate of BC was influenced by concomitant antibiotic therapy could not be addressed in our study. However, several studies have reported this issue, particularly when antibiotics have already been administered (Glerant *et al.*, 1999; Peters *et al.*, 2004).

Nevertheless, the adoption of strict procedures for sample collection and processing minimizes the possibility that a false positive occurs as a result of environmental contamination (Dark *et al.*, 2009).

In the present study we highlighted once again a significant reduction in the time taken for microbiological diagnosis using SF, in keeping with two recent studies (Chang *et al.*, 2013; Schaub *et al.*, 2014). Specifically, the SF turnaround time advantage is more important for negative samples since a 5 day turnaround is needed for BC results (Bouza *et al.*, 2004; Baron *et al.*, 2005).

Finally, since SF results are given on a daily basis, the early detection allows a specific pathogen-oriented antibiotic treatment to be rapidly delivered. According to our analysis, the use of SF, in combination with additional microbiological information related to the clinical context and epidemiological data, allows clinicians to confidently begin appropriate antimicrobial therapy. Lodes *et al.* (2012) advocated the high value of SF for the rapid detection of pathogens in suspected sepsis, thus supporting our findings.

However some shortcomings negatively affect the implementation of this molecular technique for routine laboratory diagnostics, i.e. cost, the need for special equipment and the lack of antibiotic susceptibility testing; moderate overtreatment due to some false-positive results cannot be excluded.

Even if SF improves the detection of BSI agents, BC cannot be replaced by molecular testing (Mancini *et al.*, 2008; Lucignano *et al.*, 2011; Paolucci *et al.*, 2013). Upcoming technological improvements, including resistance detection, are mandatory in order to gain full agreement to molecular detection of pathogens.

In conclusion, even with the limitation of the low number of samples, the present study, confirming the great potential of a molecular approach for diagnosis of BSI in combination with traditional BC, showed its added value in improving the microbiological documentation for pathogen-oriented therapy, especially in patients receiving empirical antibiotic treatment.

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