DIAGNOSTIC PERFORMANCE AND THERAPEUTIC IMPACT OF LightCycler SeptiFast ASSAY IN PATIENTS WITH SUSPECTED SEPSIS

V. Herne*, A. Nelovkov, M. Kütt and M. Ivanova

Central Laboratory, East-Tallinn Central Hospital, Tallinn, Estonia

Received: December 10, 2012; Accepted: January 2, 2013

Rapid and reliable identification of pathogens is very important in the management of septic patients. We retrospectively evaluated the diagnostic accuracy and clinical utility of a multiplex real-time polymerase chain reaction (PCR) assay (SeptiFast (SF)) in patients with suspected sepsis in a tertiary care hospital in Tallinn, Estonia. A total of 160 blood samples from 144 patients were included in the study. SF results were compared with corresponding blood culture (BC) results. The concordance between SF and BC was 78.8%. The rate of positive results was significantly higher in SF than in BC (33.7% vs. 21.2%, respectively; $p < 0.001$). A total of 27 samples were found positive by both SF and BC, 27 by SF only, and seven by BC only. Of a total of 83 microorganisms detected SF identified 71, and BC 42 ($p < 0.001$). SF detected markedly more patients with candidemia: 11 patients were detected by SF compared to four patients by BC. Antimicrobial treatment was changed in 21 (38.9%) of 54 SF positive cases. In conclusion, our results demonstrated the high diagnostic accuracy of SF in detection of sepsis pathogens. In conjunction with its impact on therapeutic decisions, SF proved to be a useful adjunct to conventional blood culture in the diagnosis of sepsis etiology.

Keywords: bacteremia, bloodstream infection, fungemia, real-time PCR, sepsis, SeptiFast

Introduction

Bloodstream infection is a leading cause of morbidity and mortality in intensive care patients. Inappropriate antimicrobial therapy is one of the most important reasons of poor outcome of septic patients [1, 2]. A rapid and accurate etiological diagnosis is essential in the successful management of patients with bloodstream infections facilitating optimization of antimicrobial therapy [3]. The classical way to identify pathogens in bloodstream infections is conventional blood culture (BC). The disadvantage of BC is the long time to final results ranging in most cases from 2 to 5 days. The second limitation of BC is its low diagnostic sensitivity, especially in patients receiving antibiotic treatment [4].

Molecular diagnostic methods have a continuously growing importance in the detection of pathogens due to their rapidity and sensitivity. Different nucleic acid detection tests have been developed for detection of bacteremia and fungemia and have shown promising results in improving the diagnosis of bloodstream infections [5–11]. A multiplex real-time polymerase chain reaction (PCR) assay – SeptiFast (Roche Diagnostics) – is commercially

available for the detection of bacteria and fungi directly from blood [12]. The assay can be performed in less than 6 h and it identifies 25 most important pathogens causing bloodstream infections (Table 2). A number of studies have evaluated the SeptiFast assay in different patient populations [13–16]. However, few studies have addressed the clinical impact of the use of SeptiFast.

Therefore, in the present study, we aimed to evaluate retrospectively the accuracy of the SeptiFast assay (SF) for detection of bacteremia and fungemia compared to blood culture in patients with suspected bloodstream infection in routine clinical practice of our hospital. Then we evaluated the clinical relevance of discordant positive results by SF or BC and the impact of SF results on therapeutic decisions made by clinicians.

Materials and methods

This study was performed retrospectively using laboratory and clinical data of patients admitted to the East-Tallinn Central Hospital (tertiary care hospital with 492 acute care and 71 intensive care beds in Tallinn, Estonia) in period

*Corresponding author: Viive Herne, Central Laboratory, East-Tallinn Central Hospital, Ravi 18, 10138 Tallinn, Estonia;
Phone: +372 6 207 493; Fax: +372 6 207 210; E-mail: viive.herne@itk.ee

from March 2007 to July 2011. All patients providing blood samples for both BC and SF collected within up to 12 h apart from each other were included in the study. The patients with only for BC or SF collected samples were excluded. The decision of using SF was made either by the treating physicians or the infectious disease specialists. The main criteria for using SF were clinically suspected sepsis or septic shock and/or unavailability of other samples for microbiological investigation. The results of SF were used by clinicians along with other laboratory and clinical data for adjustment of antibacterial therapy.

The blood samples were collected by venipuncture according to standardized procedures. At least two sets of aerobic, anaerobic, and fungal blood cultures were taken (about 10 ml per bottle), each set, 0.5 to 1 h apart from each other during the acute febrile (elevation of body temperature $>38^{\circ}\text{C}$) or hypothermia episodes ($<36^{\circ}\text{C}$). Additionally, approximately 3 ml of blood was collected into K2EDTA tubes (Vacutainer[®], Becton Dickinson, UK) for the SF test.

BC was processed in microbiological laboratory using the BacT/ALERT[®] 3D (bioMerieux, Durham, NC) system for aerobes and anaerobes and BACTEC 9050 (Becton Dickinson, USA) system for fungi. Blood cultures for aerobes and anaerobes were incubated up to 7 days, those for fungi for up to 11 days. In case of positive blood cultures, microorganisms were identified according to standard laboratory procedures: (1) Gram staining, (2) subculture on non-selective and selective agar media according to the results of gram staining, (3) identification of pathogen by immunological, biochemical and enzymatic tests, and (4) susceptibility testing by disk diffusion test and/or gradient method for MIC detection accordingly to the identified pathogen and laboratory protocol. BC samples with growth of CoNS in only one bottle of a set were considered as contaminations and were included in the study as BC negative results.

The SF assay was performed in molecular laboratory every day according to manufacturer's instructions, as have been described previously [12]. The assay consists of specimen preparation by mechanical lysis and purification of DNA, real-time PCR amplification, and detection of bacterial and fungal DNA by hybridization probes, and automated identification of species and controls. Specimen preparation was performed in a laminar flow box; preparation of PCR reaction mix was performed in a UV box. All precautions to avoid DNA contamination were strictly followed. The volume of blood sample used for SF was 3 ml in the first year of study; 1.5 ml was used thereafter according to a change in manufacturer's instructions. Mechanical lysis of specimens was performed using the SeptiFast Lys Kit M^{GRADE} on the MagNA Lyser instrument. The negative control was used in each run, and the internal control was added to each sample before DNA purification. DNA amplification was performed on the LightCycler 2.0 instrument in three different PCR reactions: Gram-positive bacteria, Gram-negative bacteria, fungi using the internal transcribed spacer region (ITS) located between 16S and 23S ribosomal DNA sequences of bacteria, and 18S and

5.8S ribosomal DNA sequences of fungi as targets. The identification of species was performed by melting curve analysis using specifically designed SeptiFast Identification Software. SF results with negative internal control results were considered as indeterminate and were not included in the study.

The BC and SF results were compared separately by positivity of samples and by detected species of microorganisms/isolates. A retrospective analysis of clinical diagnoses and data of other laboratory assays was performed to confirm discordantly positive results. Additional laboratory data used in this retrospective analysis were the microbiological findings from other materials and from blood samples taken at a different time (± 3 days) within the same disease episode, as well as results of white blood cell counts (WBC), procalcitonin (PCT), and C-reactive protein (CRP), performed on the day of blood sampling. CRP was determined by CRP-particle enhanced turbidimetric assay (Cobas Integra, Roche Diagnostics), PCT was measured using either semiquantitative immuno-chromatographic assay (PCT-Q, BRAHMS Diagnostica) or electrochemiluminescence immunoassay (Cobas e411, Roche Diagnostics).

Additionally, the impact of SF results on the decisions of physicians concerning antimicrobial treatment was analyzed. The analysis was done retrospectively being guided by clinical records. The antibiotic therapy at the time of blood sampling and its' changes after receiving of SF results were recorded.

The McNemar's test was used to evaluate differences between SF and BC results. Inflammation marker levels according to SF and BC results were compared by the Wilcoxon rank sum test, for WBC and CRP, and by the Fisher's exact test, for PCT. A p value <0.05 was considered statistically significant.

Results

Patients

A total of 160 samples from 144 patients with severe infection were included in the study. A total of 109 (76%) patients were from intensive care department and 35 (24%) patients from other departments of hospital (abdominal surgery, orthopaedics, and cardiology). The patients had either clinically proven sepsis or septic shock or severe infection without known etiologic agent. Most frequent clinical diagnoses were acute pneumonia (43%), central venous catheter associated bloodstream infection (11%), acute peritonitis (12%), septic endocarditis (10%), acute pancreatitis (10%), and acute urinary tract infection (4%). A total of 108 (75%) patients had multifocal infection with concurrent diagnoses and polymicrobial etiology. The mean age of patients was 58 years (range 20 to 81 years). Eighty-three (57.6%) patients were men and 61 (42.4%) women. All but one patient received antimicrobial therapy during blood sampling.

Table 1. The comparison of SF and BC results by samples

	BC+	BC–	Total BC
SF+	27	27	54
SF–	7	99	106
Total SF	34	126	160

Comparison of SF and BC results

Of the 160 samples, 61 were positive by either SF and/or BC (Table 1) with a total 83 identified microorganisms/isolates (Table 2). The rate of positive results was significantly higher by SF than by BC: 54 samples were positive

by SF and 34 samples were positive by BC (detection rates 33.7% vs. 21.2%, respectively: $p < 0.001$). A total of 27 (16.9%) samples were positive, and 99 (61.9%) samples were negative both by SF and BC, resulting in a total concordance of 78.8% (27+99/160). Twenty-seven (16.9%) samples were SF positive but BC negative, and seven samples (4.4%) were BC positive but SF negative.

Of 83 microorganisms detected, 30 (36.1%) were identified by both SF and BC, 41 (49.4%) were identified by SF only, and 12 (14.5%) by BC only. Significantly more microorganisms were identified by SF than by BC (71 versus 42, $p < 0.001$). In concordant positive cases, BC and SF identified the same microorganism in 26 cases. Of these, SF detected additional microorganism in six cases,

Table 2. The comparison of SF and BC by identified microorganisms according to SF detection list

Microorganism	Number of microorganisms detected			Total
	only by SF	only by BC	by SF and BC	
Included in SF detection list				
Gram-negative				
<i>Enterobacter cloacae/aerogenes</i>	3	2	2	7
<i>Escherichia coli</i>	3	1	2	6
<i>Klebsiella pneumoniae/oxytoca</i>	2		1	3
<i>Proteus mirabilis</i>				0
<i>Serratia marcescens</i>		1		1
<i>Acinetobacter baumannii</i>				0
<i>Pseudomonas aeruginosa</i>	5		3	8
<i>Stenotrophomonas maltophilia</i>	1	1		2
Gram-positive				
<i>Staphylococcus aureus</i>	5		9	14
Coagulase negative staphylococci*	3	1	2	6
<i>Streptococcus</i> spp.**	2	1		3
<i>Streptococcus pneumoniae</i>	3	2	1	6
<i>Enterococcus faecium</i>	2		3	5
<i>Enterococcus faecalis</i>	2		1	3
Fungi				
<i>Candida albicans</i>	7		4	11
<i>Candida tropicalis</i>	2	1	1	4
<i>Candida parapsilosis</i>	1			1
<i>Candida krusei</i>				0
<i>Candida glabrata</i>			1	1
<i>Aspergillus fumigatus</i>				0
Not included in SF detection list				
<i>Candida famata</i>		1		1
<i>Listeria</i> sp.		1		1
Total	41	12	30	83

*A group of staphylococci, including *S. epidermidis* and *S. haemolyticus*

**A group of streptococci, including *S. pyogenes*, *S. agalactiae*, and *S. mitis*

Table 3. Microbiological and clinical data and antimicrobial therapy in SF positive/BC negative cases

Patient no.	Pathogens detected by SF	Pathogens isolated by microbiological culture	Diagnosis	Adequacy of initial treatment	Change of anti-microbial therapy
1	<i>P. aeruginosa</i>		Meningitis acuta, ehrlichiosis	No	No
2	<i>P. aeruginosa</i>		Hepatic abscess, pneumonia	Yes	Yes
3	<i>E. faecalis</i>		Meningitis acuta, pneumonia, decubitus	No	No
4	<i>P. aeruginosa</i>	Pus: <i>P. aeruginosa</i> , <i>S. aureus</i> ; tracheal aspirate: <i>P. aeruginosa</i>	Pneumonia, sepsis	Yes	Yes
5	CoNS		Sepsis	Yes	No
6	<i>P. aeruginosa</i>		Sepsis	Yes	Yes
7	<i>S. pneumoniae</i>	Sputum: <i>S. pneumoniae</i>	Pneumonia, sepsis	Yes	Yes
8	<i>Streptococcus</i> spp.		Pneumonia, ileus, sepsis	Yes	No
9	<i>S. aureus</i>		Septic endocarditis	Yes	No
10	<i>E. faecalis</i>	Pus: <i>E. faecalis</i>	Flegmona of low extremity, sepsis	Yes	No
11	<i>S. aureus</i>	Tracheal aspirate: <i>S. aureus</i>	Pneumonia, sepsis	Yes	Yes
12	<i>E. coli</i>		Urinary tract infection, sepsis	Yes	Yes
13	<i>S. aureus</i> , <i>E. faecium</i>	Blood culture (day before): <i>S. aureus</i>	Cholecystitis, sepsis, myeloid leukemia	No	No
14	<i>C. albicans</i> , <i>C. parapsilosis</i>	Blood culture (3 days later): <i>C. albicans</i>	Sepsis	Yes	No
15	<i>S. pneumoniae</i>	Throat: <i>S. pneumoniae</i>	Pneumonia, sepsis	Yes	No
16	<i>P. aeruginosa</i> , <i>C. albicans</i>	Blood culture (day before): <i>C. albicans</i> ; tracheal aspirate: <i>P. aeruginosa</i>	Sepsis	Yes	No
17	<i>C. albicans</i>	Abdominal fluid, BAL, tracheal aspirate: <i>C. albicans</i>	Acute peritonitis, sepsis	Yes	No
18	<i>Streptococcus</i> sp.		Pneumonia, septic endocarditis	Yes	Yes
19	<i>E. cloacae</i> / <i>aerogenes</i>	Urine: <i>C. tropicalis</i> ; central venous catheter: <i>Morganella morganii</i>	Sepsis	Yes	No
20	<i>E. coli</i>	Eye fluid: <i>E. coli</i>	Septic endocarditis, spondylodiscitis, septic endophthalmitis	Yes	No
21	<i>K. pneumoniae</i> / <i>oxytoxa</i> , <i>E. cloacae</i> / <i>aerogenes</i>	Blood sample (2 days before): <i>C. glabrata</i> ; abdominal fluid: <i>Streptococcus</i> beta-hemolytic, <i>C. albicans</i> ; tracheal aspirate: <i>C. tropicalis</i> , <i>C. glabrata</i> ; wound secretion: <i>C. glabrata</i> , <i>C. albicans</i>	Perforation of duodenal ulcer, peritonitis, sepsis, gangrene of low extremity	Yes	No
22	<i>C. albicans</i> , <i>C. tropicalis</i>	Tracheal aspirate: <i>C. tropicalis</i> ; wound secretion: <i>C. albicans</i> , <i>C. tropicalis</i>	Perforation of duodenal ulcer, acute peritonitis, fungal sepsis	No	Yes
23	<i>S. pneumoniae</i>	Joint fluid: <i>S. pneumoniae</i>	Septic arthritis, septic meningitis, sepsis	Yes	No

Table 3. (cont.)

Patient no.	Pathogens detected by SF	Pathogens isolated by microbiological culture	Diagnosis	Adequacy of initial treatment	Change of anti-microbial therapy
24	<i>C. tropicalis</i>	Abdominal fluid: <i>C. tropicalis</i>	Perforation of sigmoidal diverticulum, peritonitis, sepsis	No	Yes
25	<i>E. coli</i>	Tracheal aspirate: <i>E. cloacae</i>	Hernia, pneumonia, sepsis	Yes	Yes
26	<i>S. aureus</i>	Wound secretion: <i>S. aureus</i>	Prosthetic joint infection, sepsis	Yes	No
27	<i>S. aureus</i> , <i>C. albicans</i>	Joint fluid: <i>S. aureus</i>	Spondylodiscitis, septic arthritis	Yes	No

whereas BC detected additional microorganism in 1 case. In two concordant positive cases BC and SF identified distinct microorganisms – SF detected CoNS and *Klebsiella pneumoniae/oxytoca* and BC detected *Streptococcus pneumoniae* and *Escherichia coli*, respectively.

SF identified 16 cases of polymicrobial infection: 15 cases with two microorganisms and one case with three microorganisms. BC identified eight cases with two microorganisms. The rate of polymicrobial infections was 29.6% for SF (16/54) and 23.5% for BC (8/34).

SF showed superior result in detection of many microorganisms, including *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and enterococci. Because of small number of results, these differences mostly did not reach statistical significance, except for *C. albicans*. *C. albicans* was detected by SF in 11 cases, compared with four cases by BC ($p < 0.05$).

The time from obtaining the sample to identification of microorganisms with SF ranged in our lab from 5 h to 22 h.

Table 4. Microbiological and clinical data and antimicrobial therapy in SF negative/BC positive cases

Patient no.	Pathogens isolated by BC	Diagnosis	Adequacy of initial treatment	Change of antimicrobial therapy
1	<i>C. famata</i>	Acute pancreatitis, abdominal abscess	Yes	No
2	<i>S. maltophilia</i>	Pneumonia, sepsis	No	No
3	<i>S. mitis</i>	Septic endocarditis	Yes	No
4	<i>S. marcescens</i> , <i>E. cloacae</i>	Pneumonia, sepsis	Yes	Yes
5	<i>Listeria</i> sp.	Listeriosis	No	Yes
6	<i>E. cloacae</i>	Acute pneumonia, sepsis, central venous catheter associated blood stream infection	No	Yes
7	<i>S. pneumoniae</i>	Pneumonia, acute pyelonephritis, sepsis	Yes	No

Table 5. WBC, CRP, and PCT values in SF and/or BC positive cases

	SF+/BC– (<i>n</i> = 27)	SF+/BC+ (<i>n</i> = 27)	SF–/BC+ (<i>n</i> = 7)
WBC (±SD) × 10 ⁹ /l	13.73 (±11.7)	11.63 (± 8.72)	6.37* (±4.4)
CRP (±SD) mg/l	197.7 (±138.9)	205.3 (± 99.5)	107.2** (±53) (<i>n</i> = 6)
PCT >2 ng/ml	89.5% (17/19)	73.9% (17/23)	16.7%*** (1/6)

WBC and CRP results are reported as means (±SD), PCT results as percentage of patients with PCT value over 2 ng/ml

* $p = 0.023$ (compared to SF+/BC–)

** $p = 0.024$ (compared to SF+/BC+)

*** $p < 0.05$ (compared to other groups)

Diagnostic accuracy of SF

Clinical findings and laboratory data (clinical diagnosis, inflammation markers, and microbiological data from other materials or additional blood samples) supported the etiology of sepsis in 26 out of 27 patient cases positive by SF only (Table 3). The levels of WBC, CRP, and PCT were significantly elevated in the group with SF positive but BC negative cases, with mean values statistically not different from those in the group with both method positive (Table 5). In one case positive by SF only (Table 3: patient 1), the SF result – *P. aeruginosa* – did not correlate with laboratory and clinical data and was considered false positive. Culture results from other materials confirmed the SF results in 15 (58%) of 26 cases. In 12 of these cases, the same microorganism was isolated from other material (tracheal aspirate, throat swab, pus, sputum, abdominal fluid, eye fluid, joint fluid, and wound secretion), and in three cases, confirmation came from a BC but taken at different time points.

In concordantly positive cases with additional microorganisms detected by SF only, confirmation was obtained in two cases with *C. albicans* isolated from tracheal aspirate and from catheter.

In all seven patient cases positive by BC only, the blood stream infection was supported by laboratory results and clinical data (Table 4).

In concordantly positive cases with additional microorganisms isolated by BC only, the culture results from other patient samples supported these findings in two cases.

Finally, we defined all cases in which both method gave positive results as well as all cases in which a positive result in either SF or BC was considered clinically relevant as true positive results; in addition, all other cases were defined as true negatives. Using this definition, the sensitivity and specificity of the SF assay were 88.3% and 99%, respectively.

Therapeutic changes

Therapeutic changes of antimicrobial treatment were analyzed in 54 SF positive cases. In 33 (61.1%) of these, the antimicrobial treatment remained unchanged (Table 6).

Antibiotic treatment was changed in 21 cases (38.9%). In 14 cases (25.9%), antimicrobial treatment was escalated by changing the antibiotic to one with wider antibacterial spectrum or by addition of another antibiotic. In only four cases (7.4%) was the antimicrobial treatment de-escalated: in three cases by reducing the antibacterial spectrum of the antibiotics used, and in one case by reducing the number of antimicrobial drugs.

Discussion

A few years ago, a commercial broad-range real-time PCR assay – LightCycler SeptiFast – has become available. It is a relatively rapid molecular method allowing detection of sepsis pathogens in approximately 6 h. There are several studies to date about diagnostic feasibility and potential clinical utility of SF [10–24]. Fewer studies have evaluated this assay in routine clinical practice regarding impact on therapy [25, 26].

By implementation of SF, we accelerated the diagnostics of sepsis, minimizing the time of pathogens detection to 5 h, while the blood culture method takes at least 36 h to identify pathogens. In some cases, time to SF result has been about 20 h because we perform SF from 8 am to 4 pm only (7 days of the week). In comparison, BC takes up to 11 days to give a definite negative result.

Our study showed good concordance rate between SF and BC results, which is comparable with other studies [10, 14, 17, 18]. The majority of non-concordant results consisted of SF positive but BC negative results. Compared to a definition of true positive cases, the sensitivity of SF was 88.3%, while the sensitivity of blood culture was only 56.7%. Our findings correlate with those from other studies [10, 11, 13, 16, 21, 22, 26]. A likely reason for the higher sensitivity of SF is antibiotic treatment – in most cases in our study, blood was sampled from patients on broad spectrum antibacterial treatment, which is well known to affect the sensitivity of blood culture but not that of SF [11, 16, 22].

The molecular methods are especially useful in detection of slow growing species such as fungi [27]. Our study also indicates the high potential of SF in the diagnosis of candidemia – we observed a significantly higher detection rate of SF for *C. albicans*.

Table 6. Impact of SeptiFast on antimicrobial therapy

Number of antimicrobials	Spectrum of antimicrobial activity			
	Minimized*	Changed**	Maximized***	Not changed
Increased	0	0	2	0
Not changed	3	2	5	33
Decreased	1	0	7	1

*Treatment de-escalated to the antibacterial(s) with narrow spectrum of activity

**Treatment changed to the antibacterial(s) with another antibacterial spectrum

***Treatment escalated to the antibacterial(s) with broad spectrum of activity

SF detected noteworthy more positive cases with *P. aeruginosa*, *S. aureus*, and *Enterococcus* spp., although statistically not significantly. The higher detection rate of these microorganisms by SF was documented previously [16, 17, 20, 28, 29]. A possible explanation for higher detection rate of *P. aeruginosa* is that in cases of bacteraemia, it is almost always isolated from the aerobic blood culture bottles only, thereby significantly decreasing the likelihood of detection [30]. For enterococci, rapid disappearance (sterilization) from blood after initiation of antibacterial therapy may be a cause [19].

Cases with positive result only by SF assay should always be interpreted with caution since we cannot rule out the possibility that circulating DNA is not reflecting infection. Our retrospective analysis of laboratory data showed that the levels of inflammation markers were significantly elevated in the group with only by SF positive results, being similar to those in the group with SF and BC results both positive (Table 5). According to this, and considering patients' clinical findings, we concluded that all but one of the SF positive but BC negative results were clinically relevant. It is interesting to note that patients with only BC positive results had significantly lower levels of markers of inflammation (Table 5).

Microbial cultures from other patient samples confirmed the SF results in 57.7% (15/26) of cases, which is consistent with findings in other studies [10, 13]. In this regard, Tsalik et al. recently determined in a cohort of patients presenting in emergency rooms that the presence of DNA in blood as detected by SF is indeed an indicator of infection [14].

In one sample, SF detected *P. aeruginosa* which was not supported by BC or other results. When reviewing the amplification pattern, we noticed abnormal melting curves and therefore suspect this to be an artefact in the PCR reaction rather than contamination from other sources. SIS interpreted it as positive because the criteria for positive result have been met.

Thus, after resolving the discordant cases, our results show that the SF assay increased the rate of detection of clinically relevant pathogens by 16.2% (26/160).

Cases where different methods identified different pathogens were very interesting. In one case, BC identified *S. pneumoniae* whereas SF detected CoNS. In this case, the analysis of melting curve data of SF revealed a peak in the curve for *S. pneumoniae* but the interpretation of the reaction yielded a negative result because of the cut-off for streptococci imposed by the assay-specific software (used to rule out contamination). BC but not SF yielded CoNS in one set of blood samples, and this result was classified as a probable contamination. The cause of detection of discordant microorganisms in a second case, SF detected *K. pneumoniae/oxytoca* and BC detected *E. coli*, remained unknown; of interest, there is evidence of misidentification of strains within the enterobacteriaceae [12, 31].

The most intriguing cases were those with BC positive but SF negative results. In two cases, *Candida famata* and *Listeria* sp., these microorganisms are not included in the

SF panel. In one additional case (*Streptococcus mitis*), the LC software revealed a peak, but the crossing point was higher than that of the "cut-off" thus ruling the sample negative. In four cases, the reason of negative SF result remained unknown. In these cases, we have to consider the greater volume of sample used for BC in comparison with SF.

In contrary to other studies which found considerably higher rates of polymicrobial infections by SF compared with BC [10, 14, 26], we only noticed a moderate difference in this respect.

According to our analysis, the impact of SF on treatment decisions is relatively low. In 21 (38.9%) of 54 SF positive cases, the antimicrobial treatment was changed to some extent, and in 33 cases, it remained the same. These findings correlate with results of other studies which show even lower impact of SF on antimicrobial treatment [23, 25, 26]. One possible reason for this could be the empiric antibiotic therapy with broad spectrum antibiotics (antipseudomonal penicillins or carbapenems). In this regard, in only four cases was the antimicrobial treatment de-escalated to antimicrobial drugs with a narrower spectrum of action. These results are not a surprise since de-escalation of broad spectrum antimicrobial treatment in severely ill patients is rarely based on results of an individual test. Furthermore, SF also has the disadvantage of only allowing the detection of resistance against MRSA but not against other pathogens as does BC. An intriguing finding is that in five cases the initial antibacterial treatment was evaluated as inadequate (according to the isolated pathogen and the spectrum of antibacterial coverage of prescribed antibiotic), but the treatment was not changed after receiving SF result. Obviously, detected by SF pathogens were suspected by clinicians to be contaminants at the moment of receiving SF results. One of these pathogens was *P. aeruginosa*, detected only by SF, which we considered to be false positive (as was mentioned above). In one case, SF detected *Enterococcus faecium* as additional pathogen to concordantly positive *S. aureus*, but this finding did not lead to change in antibiotic therapy – in this case *E. faecium* might be real contaminant. In the case with *E. faecalis* detected only by SF, the antimicrobial treatment did not cover this pathogen, although the markers of inflammation were elevated – the reason of it remains unclear. In two cases, with *P. aeruginosa* and *C. glabrata* detected, the preliminary clinical diagnosis did not support findings of SF, and the antibiotic therapy was changed only after receiving confirmation by BC.

In the end, based on all our findings we conclude that SF assay is a useful additional rapid diagnostic tool in detection of sepsis pathogens. However, further studies are needed determining the benefit of PCR assay in outcome of septic patients.

Acknowledgements

The authors thank Oliver Liesenfeld for his help in improving the article. The authors thank also Marika Tam-

maru for her help in carrying out the statistical analysis, Katrin Lang for useful remarks on the text, and all colleagues who contributed to this study.

Competing interests

The authors declare that they have no competing interests.

References

- Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D, Kumar A, Cheang M: Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 34(6), 1589–1596 (2006)
- Garnacho-Montero J, Garcia-Garmendia JL, Barrero-Almodovar A, Jimenez-Jimenez FJ, Perez-Paredes C, Ortiz-Leyba C: Impact of adequate empirical antibiotic therapy on the outcome of patients admitted to the intensive care unit with sepsis. *Crit Care Med* 31(12), 2742–2751 (2003)
- Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, Reinhart K, Angus DC, Brun-Buisson C, Beale R, Calandra T, Dhainaut JF, Gerlach H, Harvey M, Marini JJ, Marshall J, Ranieri M, Ramsay G, Sevransky J, Thompson BT, Townsend S, Vender JS, Zimmerman JL, Vincent JL: Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock. *Crit Care Med* 36, 296–327 (2008)
- Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vanderbroucke-Grauls CM: New developments in the diagnosis of bloodstream infections. *Lancet Infect Dis* 4, 751–760 (2004)
- Peters RPH, Agtmael MA, Simoons-Smit AM, Danner SA, Vandenbroucke-Grauls CM, Savelkoul PHM et al.: Rapid identification of pathogens in blood cultures with a modified fluorescence in situ hybridization assay. *J Clin Microbiol* 44(11), 4186–4188 (2004)
- Jordan JA, Durso MB: Real-time polymerase chain reaction for detecting bacterial DNA directly from blood of neonates being evaluated for sepsis. *J Mol Diagn* 7, 575–581 (2005)
- Wellingtonhausen N, Kochem AJ, Disque C, Mühl H, Gebert S, Winter J, Matten J, Sakka SG: Diagnosis of bacteremia in whole-blood samples by use of a commercial universal 16S rRNA gene-based PCR and sequence analysis. *J Clin Microbiol* 47(9), 2759–2765 (2009)
- Tissari P, Zumla A, Tarkka E, Mero S, Savolainen L, Vaara M, Aittakorpi A, Laakso S, Lindfors M, Piiparinen H, Mäki M, Carder C, Huggett J, Gant V: Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: an observational study. *Lancet* 375, 224–230 (2010)
- Mancini N, Carletti S, Ghidoli N, Cichero P, Burioni R, Clementi M: The era of molecular and other non-culture-based methods in diagnosis of sepsis. *Clin Microbiol Rev* 23(1), 235–251 (2010)
- Westh H, Lisby G, Breusse F, Bøddinghaus B, Chomarat M, Gant V, Goglio A, Raglio A, Schuster H, Stuber F, Wissing H, Hoeft A: Multiplex real-time PCR and blood culture for identification of bloodstream pathogens in patients with suspected sepsis. *Clin Microbiol Infect* 15, 544–551 (2009)
- Bloos F, Hinder F, Becker K, Sachse S, Dessap AM, Straube E, Cattoir V, Brun-Buisson C, Reinhart K, Peters G, Bauer M: A multicenter trial to compare blood culture with polymerase chain reaction in severe human sepsis. *Intensive Care Med* 36, 241–247 (2010)
- Lehmann LE, Hunfeld KP, Emrich T, Haberhausen G, Wissing H, Hoeft A, Stüber F: A multiplex real-time PCR assay for rapid detection and differentiation of 25 bacterial and fungal pathogens from whole blood samples. *Med Microbiol Immunol* 197(3), 313–324 (2008)
- Louie RF, Tang Z, Albertson TE, Cohen S, Tran NM, Kost GJ: Multiplex polymerase chain reaction detection enhancement of bacteremia and fungemia. *Crit Care Med* 36(5), 1487–1492 (2008)
- Tsalik EL, Jones D, Nicholson B, Waring L, Liesenfeld O, Park LP, Glickman SW, Caram LB, Langley RJ, Velkinburgh JC, Cairns CB, Rivers EP, Otero RM, Kingsmore SF, Lalani T, Fowler VG, Woods CW: Multiplex PCR to diagnose blood stream infections in patients admitted from the emergency department with sepsis. *J Clin Microbiol* 48(1), 26–33 (2010)
- Lamoth F, Jaton K, Prod'homme G, Senn L, Bille J, Calandra T, Marchetti O: Multiplex blood PCR in combination with blood cultures for improvement of microbiological documentation of infection in febrile neutropenia. *J Clin Microbiol* 48(10), 3510–3516 (2010)
- Lucignano B, Ranno S, Liesenfeld O, Pizzorno B, Putignani L, Bernaschi P, Menichella D: Multiplex PCR allows rapid and accurate diagnosis of bloodstream infections in newborns and children with suspected sepsis. *J Clin Microbiol* 49(6), 2252–2258 (2011)
- Dierkes C, Ehrenstein B, Siebig S, Linde HJ, Reischl U, Salzberger B: Clinical impact of a commercially available multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis. *BMC Infectious Diseases* 9, 126 (2009)
- Mancini N, Clerici D, Diotti R, Perotti M, Ghidoli N, Marco DD, Pizzorno B, Emrich T, Burioni R, Cicci F, Clementi M: Molecular diagnosis of sepsis in neutropenic patients with haematological malignancies. *J Med Microbiol* 57, 601–604 (2008)
- Casalta JP, Gouriet F, Roux V, Thuny F, Habib G, Raoult D: Evaluation of the LightCycler® SeptiFast test in the rapid etiologic diagnosis of infectious endocarditis. *Eur J Clin Microbiol Infect Dis* 28, 569–573 (2009)
- Lehmann LE, Alvarez J, Hunfeld KP, Goglio A, Kost GJ, Louie RF, Raglio A, Reguero BJ, Wissing H, Stüber F: Potential clinical utility of polymerase chain reaction in microbiological testing for sepsis. *Crit Care Med* 37(12), 1–6 (2009)
- Lehmann LE, Hunfeld K-P, Steinbrucker M, Brade V, Book M, Seifert H, Bibgold T, Hoeft A, Wissing H, Stüber F: Improved detection of blood stream pathogens by real-time PCR in severe sepsis. *Intensive Care Med* 36, 49–56 (2010)
- Yanagihara K, Kitagawa Y, Tomonaga M, Tsukasaki K, Kohno S, Seki M, Sugimoto H, Shimazu T, Tasaki O, Matsushima A, Ikeda Y, Okamoto S, Aikawa N, Hori S, Obara H, Ishizaka A, Hasegawa N, Takeda J, Kamiyama S, Sugahara K, Asari S, Murata M, Kobayashi Y, Ginba H, Sumiyama Y, Kitajima M: Evaluation of pathogen detection from clinical samples by real-time polymerase chain reaction using a sepsis pathogen DNA detection kit. *Critical Care* 14, R159 (2010)

23. Maubon D, Hamidfar-Roy R, Courby S, Vesin A, Maurin M, Pavese P, Ravel N, Bulabois CE, Brion JP, Pelloux H, Timsit JF: Therapeutic impact and diagnostic performance of multiplex PCR in patients with malignancies and suspected sepsis. *J Infect* 61, 335–342 (2010)
24. Regueiro BJ, Varela-Ledo E, Martinez-Lamas L, Rodriguez-Calvino J, Aguilera A, Santos A, Gomez-Tato A, Alvarez-Escudero J: Automated extraction improves multiplex molecular detection of infection in septic patients. *PLoS One* 5(10), e13387 (2010)
25. Wallet F, Nseir S, Baumann L, Herwegh S, Sendid B, Boulo M, Roussel-Delvallez M, Durocher AV, Courcol RJ: Preliminary clinical study using a multiplex real-time PCR test for the detection of bacterial and fungal DNA directly in blood. *Clin Microbiol Infect* 16(6), 774–779 (2010)
26. Lodes U, Bohmeier B, Lippert H, König B, Meyer F: PCR-based rapid sepsis diagnosis effectively guides clinical treatment in patients with new onset of SIRS. *Langenbecks Arch Surg* 397(3), 447–455 (2012)
27. Pemán J, Zaragoza R: Current diagnostic approaches to invasive candidiasis in critical care settings. *Mycoses* 53(5), 424–433 (2010)
28. Dubská L, Vyskočilová M, Minaříková D, Jelínek P, Tejkalová R, Valík D: LightCycler SeptiFast technology in patients with solid malignancies: clinical utility for rapid etiologic diagnosis of sepsis. *Crit Care* 16(1), 404 (2012)
29. Deschaght P, Schelstraete P, dos Santos Santiago GL, Van Simaey L, Haerynck F, Van Daele S, Wachter E, Malfroot A, Lebecque P, Knoop C, Casimir G, Boboli H, Pierart F, Desager K, Vaneechoutte M, De Baets F: Comparison of culture and qPCR for the detection of *Pseudomonas aeruginosa* in not chronically infected cystic fibrosis patients. *BMC Microbiology* 10, 245 (2010)
30. Enoch DA, Simpson AJH, Kibbler CC: Predictive value of isolating *Pseudomonas aeruginosa* from aerobic and anaerobic blood culture bottles. *J Med Microbiol* 53 (11), 1151–1154 (2004)
31. Claeys G, De Baere T, Wauters G, Vandecandelaere P, Verschraegen G, Muylaert A, Vaneechoutte M: Extended-spectrum beta-lactamase (ESBL) producing *Enterobacter aerogenes* phenotypically misidentified as *Klebsiella pneumoniae* or *K. terrigena*. *BMC Microbiol* 4, 49 (2004)