Multiplex Blood PCR in Combination with Blood Cultures for Improvement of Microbiological Documentation of Infection in Febrile Neutropenia[▽]

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The frequent lack of microbiological documentation of infection by blood cultures (BC) has a major impact on clinical management of febrile neutropenic patients, especially in cases of unexplained persistent fever. We assessed the diagnostic utility of the LightCycler SeptiFast test (SF), a multiplex blood PCR, in febrile neutropenia. Blood for BC and SF was drawn at the onset of fever and every 3 days of persistent fever. SF results were compared with those of BC, clinical documentation of infection, and standard clinical, radiological, and microbiological criteria for invasive fungal infections (IFI). A total of 141 febrile neutropenic episodes in 86 hematological patients were studied: 44 (31%) microbiologically and 49 (35%) clinically documented infections and 48 (34%) unexplained fevers. At the onset of fever, BC detected 44 microorganisms in 35/141 (25%) episodes. Together, BC and SF identified 78 microorganisms in 61/141 (43%) episodes ($P = 0.002$ versus BC or SF alone): 12 were detected by BC and SF, 32 by BC only, and 34 by SF only. In 19/52 (37%) episodes of persistent fever, SF detected 28 new microorganisms (7 Gram-positive bacterial species, 15 Gram-negative bacterial species, and 6 fungal species [89% with a clinically documented site of infection]) whereas BC detected only 4 pathogens (8%) ($P = 0.001$). While BC did not detect fungi, SF identified 5 *Candida* spp. and 1 *Aspergillus* sp. in 5/7 probable or possible cases of IFI. Using SeptiFast PCR combined with blood cultures improves microbiological documentation in febrile neutropenia, especially when fever persists and invasive fungal infection is suspected. Technical adjustments may enhance the efficiency of this new molecular tool in this specific setting.

Febrile neutropenia is a frequent life-threatening complication in patients with hematological malignancies. Blood cultures (BC) identify a pathogen in only 20 to 30% of febrile episodes, and the rate of microbiological documentation drops to less than 10 to 15% for those receiving antibiotics at time of sampling (6, 39). The majority of episodes are thus uniquely managed based on the presence of fever and/or of a clinical site of infection. In addition, the low sensitivity of cultures for the detection of fungi is a major concern in these patients at high risk of invasive mycoses (13, 33). The diagnosis of invasive fungal infections (IFI) based on clinical, radiological, and microbiological criteria according to the European Organization for Research and Treatment of Cancer-Mycoses Study Group (EORTC-MSG) classification is often only presumptive and delayed (11). The persistence of fever despite broad-spectrum antibacterial therapy is observed in one-third of cases, and the lack of identification of the causal pathogen results in empirical modification of antibacterial therapy and adjunction of antifungal therapy (10, 18, 29). Additional tools are thus needed for the diagnosis of infection.

Molecular methods are able to rapidly detect microorganisms without incubation and despite ongoing antimicrobial

therapy (26, 28, 42). Homemade multiplex or broad-range PCR assays for the detection of bloodstream pathogens in cases of sepsis or febrile neutropenia have provided variable sensitivity and specificity compared with blood cultures (5, 8, 9, 28, 31, 42, 44). Their use is limited by the lack of standardized technical procedures and commercially available systems. The LightCycler SeptiFast test (SF) (Roche Diagnostics GmbH, Mannheim, Germany) is a real-time multiplex PCR amplification assay designed to detect a broad spectrum of bacteria and fungi in human blood from nonneutropenic patients with sepsis (21, 30). The internal transcript spacer (ITS) region of the bacterial and fungal genome is the target selected for identification of 25 bloodstream pathogens. The aim of this study was to assess the appropriateness and utility of SF for the microbiological diagnosis of infection in febrile neutropenic cancer patients.

MATERIALS AND METHODS

Patients. This prospective, observational study was conducted in the isolation ward of the Infectious Diseases Service at the University Hospital of Lausanne (Switzerland) between September 2006 and November 2007. Consecutive adult hematological patients undergoing induction or consolidation chemotherapy for acute leukemia or autologous hematopoietic stem cell transplantation were enrolled after written informed consent. The study protocol was approved by the Institutional Ethical Committee.

Clinical management. Patients were hospitalized in positive-pressure high-efficiency particulate air-filtered isolation rooms. During the neutropenic period, no antibacterial prophylaxis was used and patients with mucositis and oral and/or gastro-intestinal tract *Candida* colonization received fluconazole prophylaxis. Diagnostic workup of fever and empirical antibacterial therapy were conducted according to guidelines of the Infectious Diseases Society of America (IDSA)

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TABLE 1. Analytical spectrum of the LightCycler SeptiFast test

Gram-positive bacterial species	Gram-negative bacterial species	Fungal species
<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
<i>Staphylococcus epidermidis</i> ^a	<i>Klebsiella pneumoniae</i>	<i>Candida tropicalis</i>
<i>Staphylococcus haemolyticus</i> ^a	<i>Klebsiella oxytoca</i>	<i>Candida parapsilosis</i>
<i>Streptococcus pneumoniae</i>	<i>Serratia marcescens</i>	<i>Candida krusei</i>
<i>Streptococcus pyogenes</i> ^a	<i>Enterobacter cloacae</i>	<i>Candida glabrata</i>
	<i>Enterobacter aerogenes</i>	
<i>Streptococcus agalactiae</i> ^a	<i>Proteus mirabilis</i>	<i>Aspergillus fumigatus</i>
<i>Streptococcus mitis</i> ^a	<i>Pseudomonas aeruginosa</i>	
<i>Enterococcus faecium</i>	<i>Acinetobacter baumannii</i>	
<i>Enterococcus faecalis</i>	<i>Stenotrophomonas maltophilia</i>	

^a For coagulase-negative staphylococci and streptococci, a semiquantitative analytical cutoff value has been set by the manufacturer for distinguishing between true pathogens and contaminants from the skin flora.

(18). Modification of antibacterial therapy and addition of antifungal therapy were based on the clinical course (persistent fever for >72 h, clinical deterioration, and/or a new or progressing focus of infection) and microbiological reassessment (18). Whereas clinical management was routinely based on BC results, SF results were not available in real time for therapeutic decisions.

Definitions. Standard definitions of neutropenia (neutrophil count < 500/mm³) and fever (measured once at $\geq 38.3^{\circ}\text{C}$ or twice at $\geq 38.0^{\circ}\text{C}$ for a 12-h period) were used (18). A new febrile episode was defined as a new onset of fever after a 72-h period of apyrexia. The etiology of febrile episodes was classified according to definitions of the International Immunocompromised Host Society (ICHS): microbiologically documented infections with bacteremia (MDI-B) or without bacteremia (MDI-NB), clinically documented infections (CDI), or fever of unexplained origin (FUO) (1). Standard definitions (CDC and WHO) were used for CDI classifications (15). Mucositis with a WHO score of >2 and diarrhea with a frequency of >8 episodes/day were considered to represent CDI (3). Invasive fungal infections (IFI) were classified according to the definitions of the EORTC-MSG (11).

Blood sampling. Sets of blood samples for cultures (BC) and for testing with the LightCycler SeptiFast test (Roche Diagnostics GmbH, Mannheim, Germany) (SF) were drawn at the onset of fever and every 3 days in cases of persistent fever. The same procedure was applied for each new febrile neutropenic episode. Blood (10 ml) was collected in each culture bottle (Bactec Plus aerobic/F and Lytic anaerobic/F; Becton Dickinson, Sparks, MD). Each set of samplings consisted of 4 pairs (i.e., 4 aerobic and 4 anaerobic bottles [80 ml]) of blood culture samples drawn simultaneously from the central venous catheter (2 pairs of samples consisting of 2 aerobic and 2 anaerobic bottles [40 ml]) and by peripheral venipuncture (2 pairs of samples consisting of 2 aerobic and 2 anaerobic bottles [40 ml]) according to the IDSA recommendations (18). For SF assays, 3-ml samples of blood were collected in EDTA tubes (Monovette K-EDTA; Sarstedt, Numbrecht, Germany). Each set of samplings consisted of 4 tubes (i.e., 12 ml) drawn immediately after blood cultures from the central venous catheter (2 tubes [6 ml]) and by peripheral venipuncture (2 tubes [6 ml]). SF samples were sent within 1 h to the laboratory and stored at 2 to 8°C.

Microbiological analyses. A Bactec 9240 automated blood culture system (Becton Dickinson, Sparks, MD) was used. The vials were incubated at 35°C for 5 days.

For SF assays, DNA was extracted from the EDTA whole-blood tubes within 48 to 72 h after sampling. Specimen preparation and DNA amplification and detection were performed in a dedicated laboratory according to the manufacturer's recommendations (21, 30). Each specimen included an inhibition control, and each run included positive, negative, and extraction controls. The SF analytical spectrum includes the most common Gram-positive and Gram-negative bacteria, as well as pathogenic fungi (Table 1). For coagulase-negative staphylococci and streptococci, a semiquantitative analytical cutoff value has been set by the manufacturer in order to avoid false-positive results due to contamination by colonizing skin flora (21, 30).

Data and statistical analyses. Positive results for SF and BC were compared for neutropenic patients at the onset of fever (day 0) and during persistent fever lasting for 3 days or more (days 3, 6, 9, etc.). Discordant results were interpreted according to clinical assessment and radiological plus microbiological findings. Microorganisms recovered by BC (including coagulase-negative staphylococci) in at least one of the four pairs (at least one of the four aerobic and four anaerobic bottles) of the sets of samples drawn at the same time were classified

as true pathogens according to the recommendations for management of febrile neutropenia (2, 18, 34).

In BC-negative episodes, microorganisms identified by SF were interpreted as true pathogens in the presence of at least one of the following criteria: (i) the same microorganism was recovered at the same time by cultures from a clinically relevant site other than blood (e.g., central venous catheter, urine, sputum, or bronchoalveolar lavage [BAL] fluid or from another normally sterile site) or (ii) a site of infection was documented clinically and/or radiologically in concomitance with the positive SF finding and the microorganism identified by SF was consistent with a potential pathogen according to the site of infection (28). In the absence of the criteria listed above, the significance of a positive SF result remained indeterminate. Positive SF findings for fungi were interpreted according to the EORTC-MSG diagnostic classification of fungal infections (11).

Fisher's exact test and the nonparametric Mann-Whitney rank-sum test were used for the analysis of proportions and continuous variables, respectively. A two-sided *P* value < 0.05 was considered statistically significant.

RESULTS

141 febrile neutropenic episodes occurred in 86 consecutive patients, with a median of 1 per patient (range, 1 to 6). A total of 237 sets of blood samples for cultures (BC) and SeptiFast PCR (SF) were obtained, with a median of 2 sets per patient (range, 1 to 8); of those sets of samples, 144 (61%) were drawn while broad-spectrum antibiotic therapy was ongoing. The characteristics of patients and febrile episodes are shown in Table 2.

Febrile episodes were classified according to the ICHS definitions as follows: 35 (25%) were MDI-B, 9 (6%) were MDI-NB, 49 (35%) were CDI, and 48 (34%) were FUO (Table 2).

Diagnostic performance of blood cultures and SeptiFast blood PCR at the onset of fever (day 0). At the onset of fever, 44 microorganisms (21 Gram-negative and 23 Gram-positive bacteria; no fungi) were detected by BC in 35/141 (25%) febrile episodes. SF identified 46 species of microorganisms (29 Gram-negative and 13 Gram-positive bacteria and 4 fungi) in 35/141 (25%) episodes. Together, BC and SF allowed the identification of 78 species of microorganisms in 61/141 (43%) episodes (*P* = 0.002 compared with BC or SF alone): 12 were detected by both BC and SF, 32 by BC only, and 34 by SF only (Table 3).

Among the 32 species of microorganisms detected by BC only, 13 (41% [10 Gram-positive and 3 Gram-negative bacteria]) were not included in the SF analytical spectrum. Among the remaining 19 isolates, 7 were *Streptococcus* spp., 3 were coagulase-negative staphylococci (2 of them were recovered in only 1 of 4 pairs of bottles), and 6 were *Escherichia coli*. A clinically or radiologically documented focus of infection was present in 18 (56%) cases (13 cases of gastrointestinal mucositis, 2 catheter-related infections, 2 pneumonias, 1 urinary tract infection), while the 14 (44%) remaining pathogens were associated with a primary bacteremia without a documented source. These 32 species of microorganisms were all considered true pathogens according to the definitions described in Materials and Methods.

Conversely, SF detected 34 species of microorganisms (11 Gram-positive and 19 Gram-negative bacteria and 4 fungi) which were not recovered by BC in 4/35 (11%) cases of MDI-B, 2/9 (22%) cases of MDI-NB, 12/49 (24%) cases of CDI, and 8/48 (17%) cases of FUO. Of the 34 species of microorganisms detected, 22 (65%) were interpreted as true pathogens, i.e., the presence of the same microorganism recovered by culture at a site other than blood (*n* = 1 [urine]) or

TABLE 2. Characteristics of patients and febrile episodes

Febrile neutropenic patient characteristic (<i>n</i> = 86)	Value ^a
Male/female.....	53 (62)/33 (38)
Median age in yr (range)	54 (17–71)
Hematological malignancies	
Acute myeloid leukemia/acute lymphoblastic leukemia.....	37 (43)/9 (10)
Lymphoma.....	12 (14)
Multiple myeloma.....	22 (26)
Other.....	6 (7)
Chemotherapy	
Induction/consolidation for acute leukemia.....	45 (52)
Autologous HSCT ^b	37 (43)
Other.....	4 (5)
Median no. of days of neutropenia (range).....	11 (1–140)
Febrile episodes (<i>n</i> = 141)	
First febrile episodes.....	85 (60)
Recurrent febrile episodes	56 (40)
Ongoing antimicrobial therapy at onset of fever	51 (36)
Microbiologically documented infections	
(MDI).....	44 (31)
Bacteremic.....	35 (25)
Single Gram-positive bacterial species	13 (37)
Single Gram-negative bacterial species	15 (43)
Polymicrobial.....	7 (20)
Nonbacteremic ^c	9 (6)
Clinically documented infection (CDI)	
Upper/lower gastrointestinal tract.....	36 (73)
Upper/lower airways	6 (12)
Catheter/skin/soft tissues	7 (14)
Fever of unexplained origin (FUO).....	48 (34)
Episodes with persistent fever for ≥3 days	52 (36)
Invasive fungal infections (EORTC-MSG criteria)	
Probable.....	7 (5)
Possible	2
	5

^a Values represent number (percent) except where otherwise indicated.

^b HSCT, hematopoietic stem cell transplantation.

^c Four cases of enterocolitis (*Clostridium difficile* infection), one of pneumonia (*Pneumocystis jirovecii*), and four of urinary tract infections (two *Escherichia coli*, one *Enterobacter cloacae*, and one *Klebsiella pneumoniae*). *Clostridium difficile* and *Pneumocystis jirovecii* are not in the SF analytical spectrum. In one of the four urinary tract infections, SF detected the same microorganism (*K. pneumoniae*/K. *oxytoca*) in blood.

of microorganisms consistent with a clinically or radiologically documented site of infection (*n* = 22 [2 cases of oral and 10 of gastrointestinal mucositis and 3 cases of respiratory tract, 2 of urinary tract, and 5 of catheter-related or skin or soft tissue infections]). The pathogenic role of the 12/34 (35%) remaining species of microorganisms was undetermined in the absence of a consistent site of infection. *Pseudomonas aeruginosa* and *Staphylococcus aureus* accounted for 4/12 and 3/12 of those remaining microorganisms.

The number of positive SF tubes per sampling (i.e., 4 tubes) was significantly higher when the same microorganism was also identified by BC (median, 3 [range, 1 to 4]) compared with BC-negative episodes (median, 1 [range, 1 to 4; *P* < 0.0001])

TABLE 3. Microorganisms detected at the onset of fever (day 0) by blood culture and/or the LightCycler SeptiFast test (SF)^a

Species	No. of species detected by:		
	Blood culture only	Blood culture and SF	SF only
Gram-negative bacteria			
<i>Escherichia coli</i>	11	10	19
<i>Klebsiella pneumoniae</i> /K. <i>oxytoca</i>	6	5	2
<i>Enterobacter cloacae</i> /E. <i>aerogenes</i>	1	2	4
<i>Pseudomonas aeruginosa</i>			1
<i>Acinetobacter baumannii</i>		1	11
<i>Stenotrophomonas maltophilia</i>	1	2	1
<i>Capnocytophaga gingivalis</i> ^b	1		
<i>Sphingomonas paucimobilis</i> ^b	1		
<i>Leptotrichia</i> spp. ^b	1		
Gram-positive bacteria			
<i>Staphylococcus aureus</i>	21	2	11
<i>Staphylococcus epidermidis</i> /S. <i>haemolyticus</i>	3	1	7
<i>Streptococcus mitis</i> /S. <i>agalactiae</i>	7		3
<i>Streptococcus salivarius</i> ^b	1		
<i>Enterococcus faecium</i>	1	1	
<i>Enterococcus faecalis</i>			1
<i>Enterococcus</i> spp. (other) ^{b,c}	3		
<i>Gemella</i> spp. ^b	2		
<i>Bacillus cereus</i> ^b	2		
<i>Bacillus</i> spp. (other) ^b	1		
<i>Corynebacterium</i> spp. ^b	1		
Fungi			
<i>Aspergillus fumigatus</i>	0	0	4
<i>Candida albicans</i>			1
<i>Candida tropicalis</i>			2
Total	32	12	34

^a Microorganisms included in the SF analytical spectrum that cannot be distinguished at the species level have been coupled (e.g., *K. pneumoniae*/K. *oxytoca*, *E. cloacae*/E. *aerogenes*, *S. epidermidis*/S. *haemolyticus*, *S. mitis*/S. *agalactiae*).

^b Pathogens not included in the SF analytical spectrum.

^c *Enterococcus gallinarum*, *Enterococcus durans*, and other *Enterococcus* spp.

and in cases of CDI compared with FUO (a median of 1 and a range of 1 to 4 versus a median of 1 and a range of 1 to 2; *P* = 0.03).

Episodes with persistent fever for ≥3 days. Persistent fever for ≥3 days despite ongoing antimicrobial therapy was observed in 52/146 (36%) febrile episodes (19 MDI, 23 CDI, and 10 FUO). The median duration of fever in these episodes was 7 days (range, 3 to 25).

Additional species of microorganisms were detected by BC on or beyond day 3 of persistent fever in 4 (8%) episodes. In contrast, the yield of SF was significantly higher, with 28 new bacterial or fungal species identified in 19 (37%) episodes of persistent febrile neutropenia (*P* = 0.001). The four species detected by BC (one *Stenotrophomonas maltophilia*, two *S. epidermidis*, and one *S. haemolyticus*), two of which were also detected by SF, were resistant to ongoing antibacterial therapy. The 28 additional species of microorganisms detected by SF and not by BC were 15 Gram-negative bacteria, 7 Gram-positive bacteria, and 6 fungi. Twenty-five (89%) of them were identified in the presence of a consistent clinical site of infection: 2 oral and 19 gastrointestinal cases of mucositis, 3 respi-

TABLE 4. Microorganisms identified by the LightCycler SeptiFast test (SF) only in samples from patients with blood culture-negative neutropenic episodes and with persistent fever on day 3 or beyond 3 days after onset of fever according to the clinically and/or radiologically documented site of infection

Site of infection	Species identified on indicated day of fever (no. of episodes)	
	3	> 3
Upper gastrointestinal tract	<i>P. aeruginosa</i> , <i>S. aureus</i>	
Lower gastrointestinal tract	<i>P. aeruginosa</i> (4), <i>S. aureus</i> , <i>E. faecium</i> , <i>A. fumigatus</i> , <i>C. parapsilosis</i>	<i>E. coli</i> (3), <i>Klebsiella</i> spp., ^a <i>Enterobacter</i> spp., ^a <i>P. aeruginosa</i> , <i>E. faecium</i> (2), <i>C. albicans</i> (2), <i>C. tropicalis</i>
Lung	<i>P. aeruginosa</i> , <i>S. pneumoniae</i>	<i>C. albicans</i>
Catheter/skin	<i>S. aureus</i>	
No site	<i>P. aeruginosa</i> , <i>A. baumannii</i>	<i>P. aeruginosa</i>

^a *Klebsiella* spp. and *Enterobacter* spp. cannot be distinguished at the species level by SF.

ratory tract infections, and 1 catheter-related or soft tissue infection (Table 4).

Invasive fungal infections (IFI). IFI was diagnosed according to the EORTC-MSG criteria (11) in 7 (5%) febrile episodes: 2 probable and 1 possible cases of aspergillosis, 3 possible cases of candidiasis (2 of which were associated with seroconversion of antimannan antibodies), and 1 possible IFI. While no fungemia was detected by BC, SF detected fungi in 5/7 IFI cases (4 candidiasis and 1 aspergillosis) (Table 5). Fungi were detected by SF at a median of 9 days (range, 6 to 15) after the onset of fever, while IFI was diagnosed by EORTC-MSG criteria after a median of 23 days (range, 8 to 106) and empirical antifungal therapy had been started after a median of 7 days (range, 6 to 8). SF diagnosis preceded EORTC-MSG diagnosis in 3 cases. SF was negative in two cases of probable pulmonary aspergillosis, with positive bronchoalveolar lavage (BAL) galactomannan and negative blood galactomannan test results. In addition, SF was positive for fungi in 4 febrile episodes that were not classified as IFI according to EORTC-MSG criteria (Table 5). Enterocolitis (CDI) and gastrointestinal tract colonization with *Candida* spp. were documented in 2 cases (positive SF results for *C. albicans* and *C. tropicalis*). In the remaining 2 cases, no clinical symptoms or signs of infection were found.

DISCUSSION

The performance of the LightCycler SeptiFast test (SF) in combination with blood culture (BC) was assessed with a large sample of febrile neutropenic patients. When used together, SF and BC significantly increased the rate of microbiological documentation at the onset of fever compared with the use of BC or SF alone. While the yields of BC alone and SF alone were similar at the initial presentation of fever, SF provided a high number of positive results in cases of BC-negative persistent neutropenic fever by identifying new bacterial and fungal microorganisms consistent with a clinically documented site of infection.

Studies performed with nonneutropenic patients with sepsis showed that SF may be used in addition to BC to improve the timing and performance of the etiological documentation of infection (4, 7, 12, 14, 21–23, 27, 35, 36, 38, 41, 43). Few data are available on the utility of SF in febrile neutropenia, although recent studies have suggested that it may be a helpful supplementary tool for the detection of bacteria or fungi (24, 37, 40). BC typically fail to detect pathogens when antimicrobial therapy is ongoing. Our study identified BC-negative episodes—in particular, those occurring in patients with persistent neutropenic fever—as the clinical subset in which this

TABLE 5. Characteristics of febrile episodes with invasive fungal infections (IFI) and/or positive LightCycler SeptiFast test (SF) results for fungi^a

IFI (EORTC-MSG criteria)	SF result	Persistent fever (>3 days)	Gastrointestinal tract mucositis and <i>Candida</i> colonization	Serological marker (GM, Mn, anti-Mn) ^b	CT finding consistent with IFI	Ongoing antifungal therapy ^c
Probable aspergillosis	—	+	—	Pos GMn in BAL ^d	+	+
Probable aspergillosis	—	+	—	Pos GMn in BAL ^d	+	+
Possible aspergillosis	<i>A. fumigatus</i>	—	—	Neg	+	+
Possible candidiasis	<i>C. albicans</i> / <i>C. parapsilosis</i>	+	+	Pos anti-Mn ^d	+	—
Possible candidiasis	<i>C. albicans</i>	+	+	Pos anti-Mn ^d	+	+
Possible candidiasis	<i>C. tropicalis</i>	+	+	Neg	+	+
Possible IFI	<i>C. albicans</i>	+	—	Neg	+	+
None	<i>A. fumigatus</i>	+	—	Neg	— ^e	—
None	<i>C. albicans</i>	—	+	Neg	—	—
None	<i>C. tropicalis</i>	+	+	Neg	—	+
None	<i>C. tropicalis</i>	—	—	Neg	— ^e	—

^a +, condition present; —, condition absent.

^b GMn, galactomannan; Mn, mannan; anti-Mn, anti-mannan antibodies; BAL, bronchoalveolar lavage fluid; Pos, positive; Neg, negative.

^c Values represent those seen at the time of a positive SF result or of an IFI diagnosis (EORTC-MSG criteria) when the SF result was negative.

^d Positive galactomannan, index ≥ 0.5 ; positive anti-mannan, ≥ 10 arbitrary units/ml.

^e Computed tomography (CT) not done.

molecular diagnostic tool may best complement cultures for the etiological diagnosis of bacterial and fungal infections. These data suggest that SF is an efficient tool for the early diagnosis of IFI when combined with clinical, microbiological, and radiological criteria. As the time window of SF positivity overlapped with the start of empirical antifungal therapy, SF may have an impact on therapeutic decisions. Moreover, the detection of circulating microbial DNA after prolonged appropriate anti-infective therapy in a patient with persistent signs of infection suggests a sustained release from the infectious focus. This finding and those reported from recent studies on molecular diagnosis performed in critically ill patients may provide a new insight into the natural history of infection in these high-risk settings (8, 22, 42, 43). Although SF does not provide information on the susceptibility of microorganisms, the species identification may be useful for the reassessment of the appropriateness of ongoing antimicrobial therapy when infection is not responding to treatment.

The occurrence of about one-third false-negative SF results has been reported in studies of nonneutropenic patients (22, 43). While SF failed to detect frequent bloodstream pathogens such as *E. coli*, coagulase-negative staphylococci, and streptococci, about 40% of the false-negative SF results in our analysis occurred with microorganisms not included in the SF analytical spectrum. The smaller blood volumes used for SF compared with BC may have contributed to the failure to detect some pathogens. The low sensitivity of SF for coagulase-negative staphylococci and streptococci is possibly associated with the semiquantitative analytical cutoff value specifically set in order to distinguish contamination from infection in nonneutropenic patients with sepsis (21). Finally, the presence of high bacterial loads might have resulted in a paradoxical inhibition of the molecular detection.

The absence of a reliable diagnostic gold standard is a common limitation for the assessment of new molecular techniques: in particular, negative BC results due to ongoing antimicrobial therapy or small circulating microbial loads and positive BC results due to contaminations represent a major limitation for the interpretation of positive or negative SF results (28). In this context, the calculation of sensitivity, specificity, positive and negative predictive values, and likelihood ratios as well as of the efficiency of the SF may be inappropriate. Experts have recommended the interpretation of positive PCR results for BC-negative febrile episodes according to the clinical context (26, 28). In the present analysis, the majority of the microorganisms detected by SF could be considered to represent the etiological cause in the presence of a clinically or radiologically documented site of infection. These results may be particularly significant for patients with persistent or progressing signs of infection, who may benefit from a modification of empirical antimicrobial therapy (4, 26, 35). Our data also suggest that the number of positive SF tubes may contribute in discriminating true-positive from false-positive results. Although the detection of *P. aeruginosa* and *S. aureus* in blood cultures usually reflects true infection, the unusually large proportion of these bacteria in a single SF tube in the absence of a documented focus may suggest a potential contamination. Despite the use of MagNA Pure water, contamination of PCR assays by waterborne bacteria such as *Pseudomonas* spp. is frequently reported (16, 19, 20). Although all the assay-specific

internal control results were negative in our cases, low-level DNA contamination cannot be ruled out. Blood sampling from indwelling vascular catheters or by venipuncture may represent another source of contamination by cutaneous flora. On the other hand, the transient presence of viable bacteria or degraded bacterial DNA may be detected in bloodstream samples due to the breakdown of the protective barriers of skin and gastrointestinal tract mucosal membranes and/or due to the effect of ongoing efficacious antimicrobial therapy.

Although SF looks promising for the early diagnosis of IFI, the small number of probable or possible IFI and the absence of microbiologically or histopathologically proven cases are limitations of this data set that do not allow the estimation of the test's diagnostic performance. The failure of SF to detect two cases of probable pulmonary aspergillosis with negative galactomannan antigenemia might suggest that its utility for the diagnosis of localized infections is limited. These results are consistent with the limited sensitivity and specificity of blood PCR methods for the diagnosis of aspergillosis reported in previous studies (25). SF detected *Candida* DNA in four cases of probable or possible invasive candidiasis, while BC results remained negative. Minor differences among BC systems with respect to detection of *Candida* spp. do not explain the discrepancy discussed above, which is consistent with the identification by PCR of the circulation of unviable fungal components or of very low viable fungal loads (17, 32). However, not having used BC bottles containing a specific fungal medium may have potentially limited the detection of yeasts by cultures.

The SF has been designed for the detection of pathogens in septic nonneutropenic patients, and adjustments of the microbial spectrum and the analytical technique may improve its efficiency in febrile neutropenic patients (21). In particular, reassessment of the semiquantitative analytical cutoff threshold for coagulase-negative staphylococci set by the manufacturer in order to distinguish true pathogens from contaminants for intensive care unit (ICU) or emergency department patients might be appropriate for febrile neutropenic cancer patients, for whom a central line is very frequently used; the interpretations and implications for management of the recovery of coagulase-negative staphylococci from blood may substantially differ from those in other clinical settings, and the absence of neutrophils in the analyzed blood may influence the detection of microorganisms. In addition, the technique in its current version is work-intensive: automating the test protocols would facilitate its cost-effective use on a routine basis.

In conclusion, the LightCycler SeptiFast test gives new insights into the natural history of infection during neutropenia. These data suggest that this multiplex PCR technique, when combined with BC, provides clinically relevant information for the diagnosis of infection in cases of BC-negative febrile neutropenia, particularly in persistent fever despite antibacterial therapy, when a nonresponding bacterial infection or an invasive fungal infection is suspected.

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