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ARTICLE

Adjuvant role of Septi*Fast* to improve the diagnosis of sepsis in a large cohort of hematological patients

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Abstract

Febrile neutropenia and sepsis are common and life-threatening complications in hematological diseases. This study was performed retrospectively in 514 patients treated for febrile neutropenia at our institute, to investigate the clinical usefulness of a molecular tool, LightCycler® SeptiFast test (SF), to promptly recognize pathogens causing sepsis in hematological patients. We collected 1837 blood samples of 514 consecutive hematological patients. The time of processing is short. Overall, 757 microorganisms in 663 episodes were detected by molecular test and standard blood cultures (BC): 73.6% Gram-positive bacteria, 23.9% Gram-negative bacteria, and 2.5% fungal species. This large analysis demonstrated a significant episode-to episode agreement (71.9%) between the two methods, higher in negative samples (89.14%), and a specificity of 75.89%. Clinical variables that gave a statistically significant contribution to their concordance were absolute neutrophil count, ongoing antimicrobial therapy, timing of test execution, and organ localization of infection. The large analysis highlights the potential of molecular-based assays directly performed on blood samples, especially if implementing the detection of antibiotic resistance genes, which was lacking in the used study.

Introduction

Infections in patients with hematological malignancies are frequent and life-threatening complications [1]. Severe sepsis [2] and multiple organ failure are frequent cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT). Morbidity and mortality rates are even higher when Gram negative multidrug-resistant (GN-MDR) pathogens are involved. Recent improvement in supportive care have resulted in a considerable reduction of the treatment-related mortality [3], even though more effective prophylactic strategies and interventions are warranted [2, 4, 5]. Blood cultures (BC) are generally positive in only one third of cases; the culturing process and pathogen identification are lengthy, delaying the start of a pathogen targeted therapy. Thereby, a fast tool to rapidly detect pathogens causing sepsis could improve the diagnostic algorithm for sepsis [6, 7].

A preliminary experience with LightCycler® Septi*Fast* was previously conducted at our institution, demonstrating a good agreement with standard BC (83%) [8]. Others have

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Table 1 LightCycler® SeptiFast test (Roche molecular systems): microbial panel

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

[a]*S. epidermidis*; *S. haemolyticus*; *S. hominis*; *S. pasteuri*; *S. warneri*; *S. cohnii*; *S. lugdunensis*; *S. capitis*; *S. saprophyticus*; *S. saprophyticus*; *S. xylosus*

[b]*S. pyogenes*; *S. agalactiae*; *S. mitis*; *S. anginosus*; *S. bovis*; *S. constellatus*; S. cristatus; *S. gordonii*; *S. intermedius*; *S. milleri*; *S. mutans*; *S. oralis*; *S. parasanguinis*; *S. salivarius*; *S. sanguinis*; *S. thermophilus*; *S. vestibularis*

confirmed its capacity to quickly detect bloodstream infections in immunosuppressed patients [9–12] and its usefulness in the context of sepsis [7, 13] or febrile neutropenia [14].

We tested the ability of LightCycler® Septi*Fast* to hasten the diagnostic process of sepsis, first by comparing it with standard blood culture considered as gold standard, and then evaluating its potential as a more sensitive molecular method.

Materials and methods

This study was performed retrospectively by using laboratory and clinical data of hematological patients treated at the Hematology and BMT Unit of our institution, from 2009 to 2013. Antimicrobial prophylaxis and treatment of infectious complications were administered according to the institutional guidelines [15–17], following international recommendations upon written informed consent for all procedures.

Following the internal guidelines, samples were taken at fever onset and every 3 days at fever persistence. A new fever onset after a 72-h of apyrexia was considered as a new febrile episode. Clinical events, laboratory, and imaging results, were analysed retrospectively. At least two sets of aerobic and anaerobic bottles were taken during the acute febrile episode (elevation of body temperature >38 °C) from a central venous catheter (CVC) or a peripheral vein. Additionally, another sample of blood was concomitantly taken in a EDTA tube for the molecular test, performed in real time on fresh samples.

Cultural approach

Both aerobic and anaerobic samples were processed in a BacT/Alert 3D automated BC system. The monitoring of CO_2 production within each bottle was performed every 10 min, 24 h per day. All positive bottles were removed from the instrument, and an aliquot was used for Gram stain and culture on solid media for analysis. Antibiotic susceptibility was determined with the VITEK 2 system (bioMérieux), requiring not less than 24–36 h.

LightCycler® SeptiFast test

The molecular assay required 1.5 ml K-EDTA-treated uncultured blood for each sample. Septi*Fast* (Roche molecular systems) is capable to detect 25 among the most common species isolated in BC (Table 1). The test makes use of dual fluorescent resonance energy transfer (FRET) probes directed against the species-specific internal transcribed spacer (ITS) regions, which represent non-coding sequences interspaced among highly conserved ribosomal RNA of bacteria and fungi. Results are obtained within two working days with a median time of 6 h. A more detailed description of this molecular test was reported in our previous experience [8].

Statistical analysis

Data are described as raw number (percentage) when reporting frequencies and mean ± standard deviation when reporting normally distributed continuous variables. We calculated the specificity, sensitivity, positive (PPV), and negative (NPV) predictive value of the test, referencing it against the gold standard (blood cultures) by using a 2×2 crosstab. If a polymicrobial finding was detected by one or both molecular and culture tests, the above mentioned analysis was calculated on the basis of the concordance of at least one of the detected pathogens [8]. We reviewed infections within 30 days of death to examine the proximate relationship of any infection to death (infection-related

Table 2 Patient baseline characteristics

Demographics	
Patients, No.	514
Episodes, No.	1837
Age, mean (SD), y	50 (15)
Males, No. (%)	312 (60.7)
Fever episodes characteristics	
Gender–Male, No. (%)	1165 (63.4)
Disease, No. (%)	
Acute leukaemias	1112 (60.5)
Others	725 (39.5)
Neutropenia grade, No. (%)	
IV	1106 (60.4)
Others	726 (39.6)
Last therapy, No. (%)	
CT	411 (22.4)
ASCT	196 (10.7)
SIB	133 (7.2)
MUD	209 (11.4)
MMRD	730 (39.7)
CB	34 (1.9)
Fever, No. (%)	1508 (82.1)
Test timing, No. (%)	
Onset	548 (29.8)
Ongoing	960 (52.3)
Known site of infection, No. (%)	999 (54.4)
Lung	646 (38.7)
Abdomen	115 (6.9)
Central nervous system	26 (1.6)
Central venous catheter	77 (4.6)
Soft tissues	135 (8.1)
Sepsis, No. (%)	1624 (88.4)
SIRS	1508 (82.1)
Severe sepsis	82 (4.5)
Septic shock	34 (1.8)
Ongoing therapy, No. (%)	1640 (89.3)
Prophylaxis	440 (24.0)
Therapy	1200 (63.5)
Invasive fungal infection, No. (%)	1675 (91.2)
No	1314 (71.5)
Possible	217 (11.8)
Probable	120 (6.5)
Proven	24 (1.3)

CT chemotherapy, *ASCT* autologous stem cell transplantation, *MUD* match unrelated donor, *SIB* sibling, *MMRD* mismatched related donor, *CB* cord blood, *SIRS* systemic inflammatory response syndrome

mortality, IRM) [18]. We analysed the possible influence on the concordance between tests of nine variables (underlying disease, neutropenia grade, ongoing antimicrobial therapy, test timing, and site of infection) using a logistic regression model. Results of the logistic regression are described as odds ratio (OR), *p* value, 95% confidence intervals (CI). We considered a significant *p*-value lower than 0.05. The statistical analyses of the study were conducted with IBM SPSS for Windows (IBM Corp. Version 20.0, Armonk, NY).

Results

A total of 1837 episodes from 514 patients, affected by febrile neutropenia, were collected between 2009 and 2013 (Table 2). The majority of patients had a severe neutropenia (60.4%) in the setting of an acute leukemia (60.5%).

Only 548 of the samples were collected at the onset of fever, while 960 were taken during an ongoing fever. The majority of tests (1200) were executed during the course of antimicrobial therapy. Only a small number of patients were tested in an afebrile state, mostly because of internal guidelines regarding CVC surveillance at hospital admission.

Together, Septi*Fast* and standard BC identified a total of 757 microorganisms in 663 out of the total 1837 episodes: 73.6% were Gram-positive bacterial species, 23.9% were Gram-negative bacterial species, and 2.5% were fungal species. Septi*Fast* gave positive results in 28.3% of cases, whereas 17.26% of total samples became culture positive (Fig. 1).

Out of the positive molecular samples, 464 were monomicrobial and 56 were polymicrobial. Among the culture-positive events, only 18 were polymicrobial. Overall, both methods detected a total of 580 and 333 microorganisms respectively (Fig. 2).

When compared to blood cultures, we found Septi*Fast* to have a sensitivity of 50.69%, a specificity of 75.89%, a PPV of 28.27%, and a NPV of 89.14%.

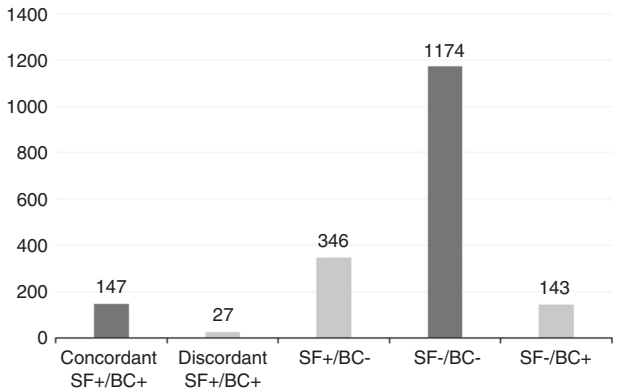

Fig. 1 Concordance between Septi*Fast* and blood cultures. Discordant positivity to Septi*Fast*/blood culture includes cases of isolation of different germs by the two methods. Concordance rate in microbial identification: 71.9% (discordant positivity not included). SF Septi*Fast*, BC blood culture

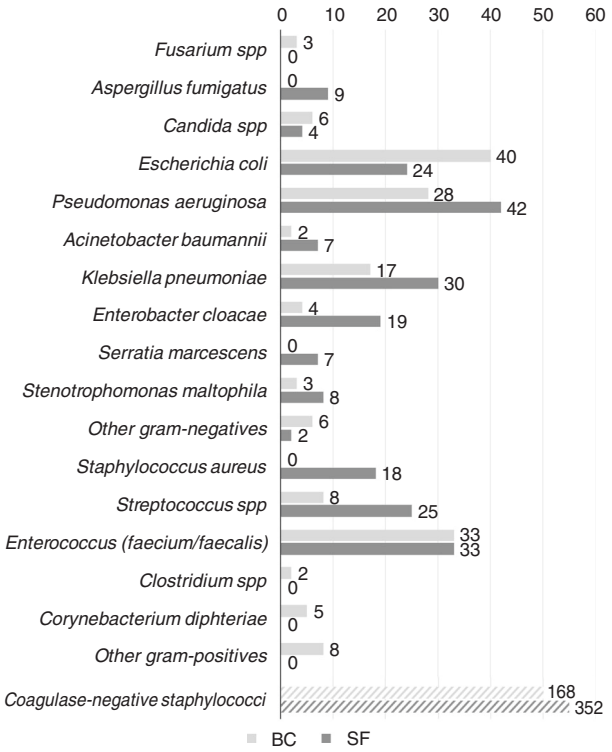

Fig. 2 Microorganism identification. The figure shows the absolute number of fungi, Gram-negative bacteria, and Gram-positive bacteria isolated by blood culture and Septi*Fast*. The striped columns does not respect the axis label. SF Septi*Fast*, BC blood culture

Table 3 Comparison between Septi*Fast* and blood culture results

Septi*Fast*	Blood cultures		Total
	Positive	Negative	
Positive	147	373	520
% within Septi*Fast*	28.3%	71.7%	100.0%
% within blood cultures	50.7%	24.1%	28.3%
Negative	143	1174	1317
% within Septi*Fast*	10.9%	89.1%	100.0%
% within blood cultures	49.3%	75.9%	71.7%
Total	290	1547	1837
% within Septi*Fast*	15.8%	84.2%	100.0%
% within blood cultures	100.0%	100.0%	100.0%

Discordant positivity to Septi*Fast*/blood culture was considered as false positive since blood cultures remain the gold standard so far

Sensitivity: 50.7%, Specificity: 75.9%, Positive predictive value: 28.3%, Negative predictive value of 89.1%

Overall, the concordance rate between the two methods was 71.9% (Table 3). The majority of concordant cases were double negative, showing high agreement (89.14%) in negative samples, whereas 8% of the events were concordantly double positive (Fig. 1). Among the 373 molecular-positive and culture-negative events, 203 tests were performed during an ongoing fever. Analyzing these false-positive episodes in a multivariate analysis, no variables were predictors. Among the 346 molecular positive/blood culture negative episodes, the mainly represented bacteria were coagulase-negative *Staphylococci* (70.52%), *Streptococcus spp* (6.36%), *P. aeruginosa* (5.78%), and *E. faecium/faecalis* (5.49%). Importantly, nine samples resulted positive for *Aspergillus fumigatus*, generally hardly detected by the cultural assays, in patients with a possible invasive fungal disease (host factor and clinical features detected by CT scan; galactomannan negative) as defined by EORTC/MSG Consensus Group [19]. The false negative (molecular negative/blood culture positive) events were mostly represented by *coagulase-negative Staphylococci* (60.14%) detected in a single blood culture bottle, and therefore of limited clinical relevance. Other potential contaminants not directly included in the SF panel were *Micrococcus luteus* and *Bacillus massiliensis*. However, a substantial number of discordances was observed also for clinically more relevant pathogens, including *E. coli* in 11.89%, and by *E. faecium/faecalis* in 8.39% of the episodes.

We performed direct logistic regression to determine the influence of different factors on the possibility to have concordant results between the two tests. We separately analyzed nine independent variables (underlying disease, neutropenia grade, ongoing antimicrobial therapy, test timing, and different sites of infection). The strongest predictors of concordance between the two methods were the abdomen involvement (OR 2.99, CI 1.56–5,72, $p = 0.001$), and the presence of grade IV neutropenia, with an OR of 1.59 (CI 1.26–2.03, $p < 0.001$). We also documented a significant association between concordance and test execution at fever onset ($p = 0.041$), though the effect is modest (OR 1.27, CI 1.01–1.60). Similarly, a slight increase in the likelihood of having concordant results can be seen in case of ongoing prophylaxis during the tests execution (OR 1.37, CI 1.07–1.77, $p = 0.014$). After analyzing the factors influencing the overall concordance between tests, we sought to analyze the settings in which the molecular result could be regarded as reliable. Accordingly, we separately analyzed the double positive and double negative tests. We considered the ability of the previously identified factors to influence the concordance with a double positive test result. In order to do this, we excluded from the analysis the double negative tests. We found that both testing during an ongoing fever and while receiving therapy increased the chances of the tests being concordant (OR 2.1 and 2.05, CI 1.39–3.16 and 1.38–3.06, respectively, $p < 0.001$ for both). We then considered the possible influence on the concordance with a double negative test result. Thus, we excluded from the analysis the double positive tests. We found that testing a patient in antimicrobial prophylaxis (OR 1.44, CI 1.13–1.84, $p = 0.003$), testing at the onset (OR

1.31, CI 1.04–1.66, $p = 0.22$), abdominal involvement (OR 1.69, CI 1.14–2.52, $p = 0.01$), and grade IV neutropenia (OR 1.87, CI 1.50–2.33, $p < 0.001$) all increased the chances of concordance.

In 26 out of the 514 consecutive patients (5%), infections represent a primary cause of death (IRM). Infection-related deaths included 13 cases of pneumonia, 2 viral and 2 fungal infections. Septic shock was documented in 6/26 cases; Septi*Fast* resulted positive in all samples (2 *Pseudomonas aeruginosa*, 2 *Klebsiella pneumoniae*, 1 *Escherichia coli*, 1 *Stenotrophomonas maltophilia*), while concomitant BC in only three of them.

Discussion

Prompt diagnosis is fundamental to prevent infections. Recently, a growing incidence of Gram-negative rods was reported in most cancer centres [20–24]. The potential adjuvant role of molecular assays in the diagnostic algorithm of infection is a matter of intense debate and of great interest to the clinical community [6, 9].

We analysed the clinical value of Septi*Fast* in a large cohort of patients, evaluating the settings in which its performance is more reliable.

This analysis confirmed the capacity of a molecular test to detect pathogens in sepsis, since Septi*Fast* gave positive results in 28.3% of cases, whereas only 17.4% of total samples were culture positive. We confirmed also a high episode-to episode agreement (71.9%) between Septi*Fast* and blood culture with a higher specificity (75.89%) than sensitivity (50.69%). Other groups have reported similar results, as expected from a molecular test [25].

The choice to compare Septi*Fast* to BC, still accepted as the standard for the detection of bloodstream infections, could be a limitation, particularly in the setting of ongoing antimicrobial therapy. Thus, the estimated overall specificity of Septi*Fast* is high but still limited by the comparison with the low accuracy of blood culture in detecting such events. In fact, reflecting on the hypothetically greater diagnostic power of Septi*Fast*, the above-called false positive could also represent a true microbial identification not elicited by the blood culture. However, in the absence of a molecular-to-molecular test comparison, the presence of false positive molecular tests may result in inappropriate modification to the initial empirical regimen and overtreatment in some episodes. The molecular approach could be particularly helpful for the diagnosis of polymicrobial infections or in case of persistent fever, when the patient has already received antibiotics without any microbial detection. A comparison with another PCR-based technique could be more reliable because of its major sensitivity, the presumed characteristic of molecular approaches.

The presence of false-negative PCR results (in presence of positive BC) could be explained in two ways. Firstly, the bacteria detected by the blood cultures could be contaminant agents that were not present in the Septi*Fast* sample. In fact, unfortunately because of the retrospective nature of the study, we were unable to ascertain where the Septi*Fast* sample was taken, and in particular whether it originated from the same site of collection of positive cultures. Therefore, the possibility of a contamination of the blood culture sample by opportunistic microorganisms in some cases still cannot be excluded.

A negative Septi*Fast* could result in the under-treatment of some cases, determining discontinuance of antibacterial therapy in the setting of febrile neutropenia, underlying the need to associate standard blood cultures to the molecular assay. The incorporation of a wider panel of pathogens within a molecular test may be expected to reach greater diagnostic sensitivity [26] (fewer false negative results), providing a greater confidence in case of a negative test result. Moreover, a majority of Gram-positive bacteria (*coagulase-negative staphylococci*) were detected in the cases positive only by BC, helping clinicians to differentiate a sample contamination from the isolation of real pathogens.

In our analysis, we found Septi*Fast* to be more concordant with blood culture in case of absolute neutropenia, during prophylaxis, at fever onset and if abdomen was involved as site of infection. Examining more in depth our data, we concluded that, in the setting just described, only negative Septi*Fast* resulted to be more concordant with blood culture. In contrast, concordant positive results were more likely to be related to the ongoing fever and antimicrobial therapy. These results were strongly influenced by the double negative cases (1174); double positive ones were only 147.

Interestingly, the clinical setting associated to either double positive or negative results can give suggestions on whether Septi*Fast* is worthy to be executed in a particular patient on the basis of its reliability. Moreover, even the outpatient management can be influenced by the execution of this test. The time advantage was extremely important for negative samples, as in the case of a patient presenting at the onset of febrile neutropenia during prophylaxis, when Septi*Fast* negativity could allow doctors to be sure at about 70% not to admit the patient because of the expected concordant negativity in blood culture.

The value of this molecular approach is the rapidity of processing (6 h from the beginning of the extraction phase [8]), remarkable even if compared to the new fast blood cultures, which take at least 48–72 h. This data in the setting already described can compensate for the higher costs [27, 28]. In fact, for example, the expense can be balanced by a hospital admission sparing, as previously described. In

addition, in critical patients, in whom a prompt specific therapy is fundamental (particularly during an ongoing fever and prolonged antimicrobial therapy scenario, in which a positive concordance between the two methods is significant), Septi*Fast* could help in early sparing useless and/or potentially more toxic antibiotics. Recently, Mancini et al. conducted an observational propensity score-matched study to evaluate the costs and clinical outcomes of episodes of suspected sepsis in haematological patients, comparing a retrospective cohort managed with standard assays and a prospective cohort managed with the addition of a molecular assay. Interesting findings were observed in the prospective group, such as a reduction of the costs for antifungal therapy, favouring a more rational use of economic resources [19]. Anyway, the clinical context may impact the ultimate management plan more than a laboratory test, requiring a prospective trial to better address this issue. The retrospective nature of this study prevent us from drawing a conclusion on the value of adding this molecular approach in patient management.

Another aspect that is worth underlining is the Septi*Fast* ability in detection of fungi (2.5% in our study). In fact, some fungal species (e.g., *Aspergillus fumigatus*) are not recognised by standard blood culture, and can be consequently difficult to detect. Importantly Seti*Fast* was able to detect *Aspergillus spp* even in case of serial negative galactomannan antigen tests, thus allowing an early diagnosis, promptly established targeted therapy and severe fungal infection control [29].

The study underlines the potential of molecular-based assays directly performed on blood samples, especially if implementing the detection of antibiotic resistance genes, which is lacking in the used study.

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Author contributions R.G, M.C.B, N.M, M.C, and F.C. conceived the study, analyzed data and wrote the manuscript. All authors carried out the collection of patient information and provided clinical care. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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