Diagnosis of infective endocarditis: comparison of the LightCycler SeptiFast real-time PCR with blood culture

Infective endocarditis (IE) is a life-threatening condition, in which identification of the causative pathogen is pivotal for subsequent effective therapy. Blood culture (BC) is considered the gold standard for microbiological diagnosis of IE (Watkin et al., 2003) but in up to one third of patients with suspected IE, BC can be negative due to a number of possible factors, such as prior antimicrobial therapy, the presence of fastidious microorganisms, or a low bacterial load (Lamas & Eykyn, 2003; Naber & Erbel, 2007). Identification of pathogens from heart valve tissue by real-time PCR has been shown to be a reliable technique (Lang et al., 2004; Mencacci et al., 2011). Several studies have demonstrated the diagnostic value of PCR in IE patients (Kühn et al., 2011; Zaloudiková et al., 2011), but few studies (Casalta et al., 2009) have evaluated the diagnostic utility of the commercially available PCR-based system SeptiFast (SF, Roche Diagnostics) for testing blood specimens. The SF system is able to detect and differentiate between a wide range of Gram-negative and Gram-positive bacteria, as well as some fungi, commonly involved in systemic infections (Lehmann et al., 2008). The aim of this study was to assess the diagnostic performance of the SF system, compared with BC, using blood specimens from patients with IE.

Seventy-nine patients admitted to the Infectious Diseases ward of the University of Perugia with suspected IE between June 2008 and January 2011 were evaluated. Of these patients, 23 were diagnosed as having definite IE according to the modified Duke’s criteria (Durack et al., 1994). For each IE patient, two sets of blood samples were obtained from different veins on admission together with two 3 ml K-EDTA-blood specimens for use in the SF system. Of the 23 patients, 15 had two major Duke’s criteria (endocardial involvement and positive blood cultures). The remaining eight patients tested negative by BC, but tested positive by echocardiogram for infective endocarditis and had at least three minor Duke’s criteria (glomerulonephritis, Osler’s nodes, rheumatoid factor, conjunctival haemorrhages).

For BC, a mean of 5–10 ml blood was inoculated into BACTEC aerobic and anaerobic bottles (Becton Dickinson). Plus and Standard BACTEC bottles were used for patients undergoing or not undergoing antibiotic therapy, respectively. The bottles were then incubated in a BACTEC 9240 Blood Culture System (Becton Dickinson). All bottles that were flagged as positive were removed from the instrument and Gram staining, culture on solid media and identification of isolates were performed using conventional methods and with the Phoenix Automated Microbiology System (Becton Dickinson). An evaluation of the SF real-time PCR test and details of the SF Master Panel List have been described elsewhere (Casalta et al., 2009). SF-PCR was performed within 8 h of sampling, in accordance with the manufacturer’s instructions, and species identification and report generation were performed using SF identification software (SIS, Roche Diagnostics). The study was performed according to good clinical practice and following the Declaration of Helsinki principles, and consent was obtained from each patient. For statistical analysis, patient age was compared between different groups using the Mann–Whitney U-test for independent samples (Siegel & Castellan, 1998) and categorical variables were analysed by the McNemar test (McNemar, 1947). The significance level was 0.05.

The study population was composed of 23 patients, including 13 males and 10 females. Mean age was 65.5 ± 13.1 years (interquartile range 38–87). No significant difference of age was found between males and females (P=0.828). The BC technique detected 15 pathogens (five Staphylococcus aureus, two Streptococcus gordonii, two Streptococcus bovis, one Streptococcus anginosus, one Staphylococcus hominis, one Staphylococcus epidermidis, one Enterococcus faecalis, one Kyotococcus sedentarius and one Fusobacterium isolate) with a sensitivity of 65 %. The SF system also detected 15 pathogens [seven Staph. aureus, five Streptococcus spp. from the SF Master List, two coagulase-negative staphylococci (CoNS) from the SF Master List and one E. faecalis isolate] with a sensitivity of 65 %. For each patient, identical results were obtained with the SF system in each of the two specimens collected. Concordant results between the BC method and the SF system were obtained from 11/23 specimens (nine positive for the same organism and two negative samples), while 6/23 were BC-negative/SF-positive and 6/23 were BC-positive/SF-negative. In particular, two Staph. aureus, two CoNS, and two Streptococcus spp. were detected by SF only, while one isolate each of K. sedentarius, Fusobacterium sp., Strep. gordonii, Strep. bovis, Staph. hominis and Staph. epidermidis were detected by BC only. Comparison of the two methods showed that there was no statistically significant agreement between these results [Cohen’s Kappa −0.150; 95 % confidence interval (CI) −0.238–0.538; P=0.472]. Conversely, the combination of BC and SF detected a higher number of pathogens in comparison to each method alone (21 vs 15; P=0.031), with a sensitivity of 91 %.

On evaluating the six BC-positive/SF-negative specimens, we found that in two cases (K. sedentarius and Fusobacterium sp.) the pathogens were not included in the SF Master List, in two cases (Strep. gordonii and Strep. bovis) the SF system gave a result of Streptococcus spp. DNA traces [610 nm channel, Gram-positive, melting temperature (Tm) 50.54 and 50.75 °C, respectively], and in another two cases (Staph. hominis and Staph. epidermidis), the SF system gave a result of CoNS DNA traces (640 nm channel, Gram-positive,
In 15 patients affected by definite IE, the level of sensitivity for the SF system was similar to that of BC for streptococci, enterococci and \textit{Staph. aureus} (Fernández et al., 2010), whereas Casalta et al. (2009) found that in a group of 19 definite IE patients positive for BC on admission, SF showed a sensitivity as low as 42.1 %. Conversely, in a patient who died due to septic shock from endocarditis, with branched fungal structures on autopsy, the SF system detected the presence of \textit{Aspergillus fumigatus} DNA in blood specimens, in spite of several negative BCs (Palomares et al., 2011).

In this study, 12 out of the 23 patients were already under antibiotic therapy before sampling, but the comparison of SF and BC results in pre-treated or untreated patients did not show a statistically significant difference (data not shown). However, it is noteworthy that 4/6 pathogens detected by SF only (one \textit{Staph. aureus}, one CoNS, two \textit{Streptococcus} spp.) were from antibiotic pre-treated patients.

To the best of our knowledge, very few studies have compared the SF system to BC as a diagnostic tool for the diagnosis of IE. In 15 patients affected by definite IE, the level of sensitivity for the SF system was similar to that of BC for streptococci, enterococci and \textit{Staph. aureus} (Fernández et al., 2010), whereas Casalta et al. (2009) found that in a group of 19 definite IE patients positive for BC on admission, SF showed a sensitivity as low as 42.1 %. Conversely, in a patient who died due to septic shock from endocarditis, with branched fungal structures on autopsy, the SF system detected the presence of \textit{Aspergillus fumigatus} DNA in blood specimens, in spite of several negative BCs (Palomares et al., 2011).

The data presented here demonstrate that the SF system shows a good diagnostic performance, overlapping BC in terms of sensitivity, and, when used in combination with BC, significantly improves sensitivity by 26 %. Our results show that the SF system can be a valuable method for the detection of pathogens, particularly Gram-positive bacteria, in culture-negative IE patients. Indeed, staphylococci and streptococci account for more than 80 % of all cases of endocarditis and, among them, \textit{Staph. aureus} is the leading cause of endocarditis worldwide (Murdoch et al., 2009), while CoNS are the third leading cause of IE after streptococci, mostly in native valves (Chu et al., 2008).

Interestingly, in four BC-positive/SF-negative specimens, the molecular method detected DNA traces of \textit{Streptococcus} spp. and CoNS, which were excluded as not significant by the SIS. This may be because, when SIS is set with a minimum sensitivity of 100 c.f.u ml$^{-1}$ to avoid false-positives due to skin contamination by streptococci or CoNS (LightCycler SF package insert; Roche Diagnostics), pathogen detection may be hindered. These results suggest that, in cases of IE, the SIS cut-off for diagnosis of sepsis from streptococci or CoNS should be modified, reducing the detection limit to a lower threshold so that DNA traces are read as clinically relevant. To rule out contamination in cases where DNA traces are detected, results should be confirmed in at least two separate specimens, as is the case with BC methods.

**Fig. 1.** Melting curves referring to \textit{Strep. gordonii} (a), \textit{Strep. bovis} (b), \textit{Staph. hominis} (c) and \textit{Staph. epidermidis} (d) isolates from patients with infective endocarditis. Arrows indicate specimen spikes, excluded by the SeptiFast identification software as not significant, or spikes of corresponding positive controls.
for the microbial diagnosis of IE (Durack et al., 1994).

One important limitation of the SF system is the absence of endocarditis-causing fastidious, unusual, and HACEK group (Haemophilus, Actinobacillus actionymetemcomitans, Capnocytophaga, Cardiobacterium hominis, Eikenella corrodens, Kingella kingae) bacteria in the SF Master List.

The SF system has been shown to be superior to BC in antimicrobial pre-treated patients with sepsis (Vince et al., 2008). In IE patients, Casalta et al. (2009) found that the SF system detected an aetiological agent in three out of 20 patients with negative BC due to antibiotic pre-treatment. Similarly, we found that four out of six SF-positive/BC-negative specimens were from antibiotic pre-treated patients, but the difference between the two methods in treated or untreated subjects was not statistically significant, possibly due to the low number of patients included in the study. Although further studies are needed to address this issue definitively, these data strongly suggest a role for the SF system in determining the aetiology of IE in culture-negative patients that have been pre-treated with antibiotics.

In conclusion, the SF system, together with BC, could represent a valuable tool for diagnosis of IE from clinical blood specimens. Reduction of the detection limits set for CoNS and streptococci could improve the sensitivity of the SF test.

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