Multiplex Polymerase chain reaction to diagnose bloodstream infections in patients after cardiothoracic surgery

Kevin Pilarczyk
imland Klinik Rendsburg managed by Sana GmbH Klinik für Intensivmedizin
Peter-Michael Rath
Universitatsklinikum Essen Institut für Medizinische Mikrobiologie
Jörg Steinmann
Universitätsklinik der Paracelsus Medizinischen Privatuniversität; Institut für Klinikhygiene Medizinische Mikrobiologie und Klinische Infektiologie
Matthias Thielmann
Universitätsklinikum Essen Klinik für Thorax- und Kardiovaskuläre Chirurgie
Maximillian Dürbeck
Universitatsklinikum Essen Klinik für Thorax- und Kardiovaskuläre Chirurgie
Stephan A. Padosch
Uniklinik Köln Klinik für Anästhesiologie und operative Intensivmedizin
Heinz G. Jakob
Universitätsklinikum Essen Klinik für Thorax- und Kardiovaskuläre Chirurgie
Fabian Dusse (✉ fabian.dusse@uk-koeln.de)
Uniklinik Koln https://orcid.org/0000-0001-7762-6966

Research Article

Keywords: Blood stream infection, blood culture, real time multiplex Polymerase Chain Reaction

Posted Date: December 21st, 2018

DOI: https://doi.org/10.21203/rs.2.137/v1

Version of Record: A version of this preprint was published on April 23rd, 2019. See the published version at https://doi.org/10.1186/s12871-019-0727-5.

Abstract

Background: Sepsis and other infectious complications are major causes of mortality and morbidity in patients after cardiac surgery. Whereas blood culture (BC) as the current diagnostic gold standard suffers from low sensitivity as well as a reporting delay of approximately 48–72 h, polymerase chain reaction (PCR) based technologies might offer a fast and reliable alternative for detection of bloodstream infections (BSI). The aim of this study was to compare the performance of real time multiplex-PCR "SeptiFast" (SF), a real-time multiplex PCR assay, with conventional BC testing in patients after cardiac surgery.

Methods: 279 blood samples from 168 individuals with suspected BSI were analyzed by SF and BC. Receiver operating characteristic (ROC) curves were generated to determine the accuracy of clinical and laboratory information for the prediction of positive SF results.

Results: Excluding results attributable to contaminants, 14.7% (n = 41) of blood samples were positive using SF and 17.2% (n=49) using conventional BC (p= n.s.). In six samples, SF detected more than one pathogen. Among the 47 microorganisms identified by SF, only 11 (23.4%) could be confirmed by BC. SF identified a significantly higher number of Gram-negative bacteria than BC (28 vs. 12, $\chi 2=7.97$, p=0.005). The combination of BC and SF significantly increased the number of detected microorganism, including fungi, when compared to BC alone (86 vs. 49, $\chi 2=13.51$, p<0.001). C-reactive protein (CRP) (21.7±11.41 vs. 16.0±16.9 mg/dl, p=0.009), procalcitonin (PCT) (28.7±70.9 vs. 11.5±30.4 ng/dl, p=0.015) as well as interleukin 6 (IL 6) (932.3±1306.7 vs. 313.3±686.6 pg/ml, p=0.010) was significantly higher in patients with a positive SF result. In addition, incidence of severe acute kidney injury (AKI) was higher in SF positive than in SF negative patients (31/42 [76%] vs. 125/237 [53%], p=0.01). Using ROC analysis, IL-6 (AUC 0.836) as well as CRP (AUC 0.804), but not PCT showed the best predictive values for positive SF results. Microbiological diagnostic information gained through SF led to 8 therapy adaptations.

Conclusion: The real time PCR-based SF test might represent a valuable addition to the traditional BC method for rapid etiologic diagnosis of BSI in patients after cardiothoracic surgery. This powerful method furthermore applies in particular for individuals with fungal infections, Gram-negative bacteremia, AKI and/or elevated CRP and IL-6-concentration. However, due to the low performance in detecting Gram-positive pathogens and the inability to determine antibiotic susceptibility, it should always be used in combination with BC. [1]

Key words: Blood stream infection, blood culture, real time multiplex Polymerase Chain Reaction

Background

Nosocomial infections represent the main non-cardiac complication after cardiovascular surgery and are associated with substantial morbidity, increased mortality, prolonged hospitalization and, eventually, economic burden.[2, 3] Respiratory tract infections account for more than half of all nosocomial infections after open heart surgery followed by surgical site infections and bloodstream infections (BSI)

with a prevalence of approximately 20 %.[4] Patients with BSI have a 4.2-fold increased risk of death, compared with non-infected patients.[5] Current guidelines highlight the importance of rapid administration of the most appropriate antimicrobial treatment to improve the survival of patients with suspected BSI and sepsis.[6] The “gold standard” for the diagnosis of BSI is blood culture (BC) with pathogen identification and consecutive drug susceptibility testing. However, this process regularly requires at least 24 to 72 hours. Sensitivity of BC is low due to uncultivable or fastidious microorganisms, polymicrobial or invasive fungal infections, or administration of anti-infectives prior to blood sampling.[7] Lee *et al*. reported that 73% of pathogens were detected with the first blood cultures, 90% with two, 98% with three, and 99.8% with four different consecutive blood cultures.[8] In addition, discrimination between infection and potential contamination is sometimes difficult. Thus, there is an urgent need to establish a rapid, sensitive, and specific method for detection of bacterial and fungal pathogens to improve management of patients with suspected BSI. PCR-based technologies have emerged over the last two decades and could represent an appropriate diagnostic tool in terms of sensitivity and speed of pathogen detection, in particular in life-threatening infections.

LightCycler® SeptiFast (SF) is a multi-pathogen probe-based real-time PCR system targeting DNA sequences of 25 commonly observed bacteria and fungi present in blood samples within a few hours. However, data about the impact of PCR-based diagnostics on clinical decision-making process and modification of empirical antimicrobial therapy are very limited. A recently published prospective randomized trial demonstrated that in addition to a reduction in the time required for initial pathogen identification, the use of PCR was clearly able to reduce the time required for therapy modification from 38 to 19h, however without reaching statistical significance.[9] Currently, there are no data about the accuracy and the impact of PCR based detection of BSI in patients undergoing cardiac surgery. Therefore, the aim of our study was to compare the performance of SF with conventional BC system, to identify predictors for positivity of SF and analyze the impact of SF results on early adjustment of therapy in patients after cardiothoracic surgery.

Methods

Patients

Between January 2009 and February 2013, data were prospectively collected on all consecutive patients with SF at our Intensive Care Unit (ICU), Department of Thoracic and Cardiovascular Surgery, West German Heart Centre Essen, in our institutional database. The decision of using SF was made either by the treating physicians or the infectious disease specialists. A retrospective analysis was then performed. The study was approved by the Institutional Review Board according to the Declaration of Helsinki. All of the patients had previously granted permission for use of their medical records for research purposes. This written informed consent was obtained within the preoperative surgical written and verbal information conversation.

Patients were considered for inclusion in the study only if the met the following criteria:

- Suspected bacterial or fungal BSI
- Collection of paired blood samples for SF and at least 2 sets of BCs (2 aerobic and 2 anaerobic bottles) from a peripheral vein or a central venous line at the same time point (within 2 hours)

The BC and SF results were compared separately by positivity of samples and by detected species of microorganisms/isolates.

Blood cultures

Blood samples (at least 2 pairs of aerobic/anaerobic BC bottles, volume of 8–10 mL each) were collected by sterile venipuncture or from a central venous catheter CVC after disinfection of the connector and inserted into aerobic and anaerobic bottles, and were sent to the laboratory. Samples then were incubated into the Bactec 9240 Plus (Becton Dickinson, Heidelberg, Germany), an automated microbial detection platform based on the colorimetric detection of CO_2 produced by growing microorganisms, according to the Clinical and Laboratory Standards Institute Protocol. BC bottles were incubated up to 7 days.

In positive BCs, aliquots of blood were taken for Gram staining, plate culturing, and subsequent analysis. Identification and determination of antibiotic susceptibility were performed according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) standard with the VITEK MS, VITEK 2 (bioMerieux, Mercy l´Etoile, France), and WalkAway (Beckman Coulter, Krefeld, Germany).[10]

SeptiFast

The LightCycler® SeptiFast test M Grade (Roche Molecular Systems, Mannheim, Germany) is an *in vitro* nucleic acid amplification test for the detection of bacterial as well as fungal DNA in human blood. It allows the identification of 25 bacterial and fungal species (see table 1), being responsible for approximately 90% of all bloodstream infections. The analytical sensitivity of the assay, as indicated by the manufacturer, is between 3 and 100 colony forming units (CFU)/ml, depending on the microorganism. Following the manufacturer's instructions, DNA was extracted and was amplified by the LightCycler® in three individual reactions (Gram-positive bacteria, Gram-negative bacteria, and fungi) on the LightCycler® (version 2.0) instrument. To exclude false-negative results the test includes an internal control, provided by the SeptiFast kit. PCR products were simultaneously detected by fluorescence and melting temperature analysis, using specific hybridization probes and identification software.

Discrimination between infection and contamination in BC

Coagulase-negative staphylococci (CoNS), *Streptococcus* spp., *Corynebacterium* spp., or *Bacillus* spp. are frequent contaminants of BCs. To discriminate between true BSI and contamination, an algorithm based on a retrospective study with 654 non-neutropenic patients from a mixed ICU (surgical as well as medical patients) based on the presence of a CVC or other prosthetic material (heart valve, Left ventricular assist device (LVAD), pacemaker) and presence of systemic inflammatory response syndrome (SIRS) criteria was applied.[11] Positive findings for fungi were interpreted according to the European Organization for

Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group diagnostic classification of fungal infections.[12]

Discrimination between infection and contamination in SF

Isolates identified by PCR were considered to be pathogens or contaminants using a modified algorithm, combining microorganism pathogenicity, interpretation of blood culture results, and clinical, laboratory, and microbiological data.[13] The threshold of the SeptiFast software, based on the bacterial DNA amount, excluded CoNS and streptococci from the positive results and considered them contaminants. Fungal pathogens were categorized as described above.

Statistical analysis

Statistical analyses were performed with SPSS Statistics 19 (IBM, Chicago, IL). Continuous data were expressed as median ± 95% confidence interval (CI); categorical data were expressed as percentage. Comparisons between two groups were carried out using unpaired Student's t-test for normally or the Mann-Whitney Rank Sum Test for non-normally distributed data. Multiple groups were compared with ANOVA. Univariate analysis was performed on the quantitative variables using the Student t-test or Mann-Whitney test and on the qualitative variables using the Chi^2 test of Fisher's exact test.

To measure the sensitivity and specificity of laboratory and clinical data at different cut-off values, a conventional receiver operating characteristic (ROC) curve was generated. All variables showing a p-value of less than 0.1 between the two groups using Student t-test, Mann-Whitney test, Chi^2 test of Fisher's exact test were selected for ROC analyses. The optimal cut-off concentration was defined by the highest Jouden index (J = sensitivity + specificity – 1).Statistical significance was assumed for a p-value <0.05.

Results

During the study period, 279 matched blood samples from 169 patients suspected of having BSI were analyzed with conventional BC and SF. Out of these, 78% (132/169) were under antibiotic treatment at this time. Contaminants were significantly more frequent among blood cultures than SeptiFast (23 [8.2%] vs. 2 [0.71%], p<0.001).

After excluding contaminants, SF identified 47, while BC identified 49 episodes of BSI (χ^2=0.05, P=0.822). As illustrated in figure 1, SF exclusively detected 36 pathogens that were missed by BC, whereas BC detected 39 pathogens in SF-negative individuals. Thus, 86 positive episodes of BSI were identified with the combination of both methods, being significantly higher than with SF or BC alone (χ^2=10.86, p<0.001 for SF and χ^2=12.35, p<0.001 for BC).

BC analyses resulted in 51% sensitivity, 83% specificity, 46.7% PPV, and 54.1% NPV whereas SF resulted in 51% sensitivity, 84% specificity, 46.4% PPV, and 54.4% NPV.

With BC, CoNS was the most frequently detected agent (13/49, 26.5%) followed by *E. faecium* (10/49, 20.4%) and *Candida* spp. (9/49, 18.4%). In contrast, the most frequently observed pathogen in SF was *Candida* spp. (9/47, 19.1%) followed by *Enterobacter* spp. (8/47) and *Klebsiella* spp. (7/49, 14.3%).

SF identified 7/33 (21%) Gram-positive bacteria, 28/35 (8%) Gram-negative, and 12/67 (67%) fungi, while BC identified 28/33 (85%), 12/35 (34%), and 9/18 (50%), respectively (Table 1).

Gram-negative detection rate was significantly higher with SF than with BC (χ^2=14.93; p<0.001), but for fungi, the difference to BC was not relevant (χ^2=1.03; p=0.3). In contrast, detection rate of Gram-positive bacteria was significantly higher with BC compared to SF (χ^2=25,09; p<0.001).

SF identified 36 pathogens that were not found in BC, while BC detected 39 pathogens in SF negative specimens. Microbial strains exclusively identified by SF were: *E. coli* (n=3), *Klebsiella* spp. (n=6), *E. faecium* (n=4), *Enterobacter* spp. (n=7), *E. faecalis* (n=1), *P. aeruginosa* (n=3), *A. fumigatus* (n=3), *C.* spp. (n=6), *S. marcescens* (n=3), *S. maltophilia* (n=1).

BC detected the following pathogens in SF negative samples: CoNS (n=13), *E. faecium* (n=10), *C. albicans* (n=6), *S. marcesens* (n=3), *Klebsiella* spp. (n = 2), *S. aureus* (n=1), *E. coli* (n=1), other *Streptococcus* spp. (n=1), *E. faecalis* (n=1), M. morganii (n=1).

Polymicrobial infections were observed in seven patients. Five episodes were detected by SF; while BC identified multiple agents in only four specimens.

Predictors for SF positivity

Several variables of the patients with and without pathogen identification in SF and BC were compared, respectively (table 2). Whereas baseline demographics, gender, BMI, EuroScore-2 and SAPS and TISS on the day of admission on ICU as well as type of surgery did not differ between the two groups, patients with positive PCR were significantly younger than patients with negative PCR (57 years [51.7-68.0] vs. 68.0 [64.3-70.0], p=0.01). In addition, prevalence of acute kidney Injury (AKI) with need for renal replacement therapy (RRT) was higher in SF positive patients (76% vs. 53%, p=0.01).

Laboratory markers of inflammation differed significantly between groups: C-reactive protein (CRP) (21.7 mg/dl ±11.41 vs. 16.0±16.9, p=0.009), procalcitonin (PCT) (6.6 ng/ml [2.7-16.4] vs. 3.1 [2.3-4.7], p=0.015) as well as interleukin 6 (IL-6) (235.0 pg/ml [83.5-1582.2] vs. 72.3 [46.5-104.7], p=0.010) were significantly higher in patients with positive SF result. In contrast, patients with negative PCR had a significantly higher WBC than patients with positive PCR (14.0 [13.0-15.0] vs. 12 [10.3-15.0], p=0.014).

Patients with proven BSI in SF suffered from a more complicated postoperative course with prolonged ICU stay compared to SF-negative patients (ICU stay [days]: 26.1±16.2 vs. 19.4±12.8, p=0.019). Comparing patients with positive and negative BC, demographics, inflammatory markers and organ function did not differ whereas ICU-stay was longer in individuals with positive blood culture (16 days [15-19] vs. 18.5 [14.0-26.2], p=0.044).

Using ROC analysis, IL-6 (AUC 0.836, sensitivity 78.6%, specificity 75.9% for a cut-off 184 pg/ml) as well as CRP (AUC 0.804, sensitivity 71.4%, specificity 75.9% for a cut-off 15.25 mg/dl) showed the best predictive values for positive SF results. In contrast, PCT and leukocytes were associated with poor predictive capacity.

Impact of SF on antimicrobial therapy

In eight out of 37 cases with pathogens solitarily identified by SF (21.6%) microbiological diagnostic information led to therapy adaptations (table 3). Only one of these pathogens was detected by blood culture whereas the other seven remained undetected with conventional diagnostics. In three patients, detection of *A. fumigatus* in SF led to the addition of antifungal therapy with voriconazole, in another three patients therapy was escalated with fluconazole and caspofungin, respectively. In one patient, vancomycin was added due to *E. faecium* identification in SF.

50% of patients could be discharged home whereas four patients died during the further hospital course.

Discussion

Our data demonstrate that the PCR-based SF test might represent a rational adjunct tool to the traditional BC method for rapid etiologic diagnosis of BSI in patients after cardiothoracic surgery. SF detects significantly more Gram-negative microorganisms than BC whereas BC was superior regarding Gram-positive pathogens

Early and reliable diagnosis of BSI and identification of bacteria and fungi is essential to initiate appropriate therapy in septic patients within one hour after sepsis as recommended by current guidelines.[6] For decades, detection of pathogen microorganisms in patients with suspected BSI was mainly based on BC. However, the classical BC procedure per se has two intrinsic limitations: Firstly, this method is limited by the delay of identification of the pathogen and the antimicrobial susceptibility profile of 24 to 72 hours in most of the cases. Although blood cultures incubated in modern instrumented systems typically signal positive in a median time of 12–36 h. In addition, approximately 30% of pathogens remain undetected by BC and the time to positivity from collection to detection is longer for some fastidious bacteria, anaerobes, and fungi or under antimicrobial therapy.[14] Thus, there is an urgent need to improve the diagnostic tools for an improved management of patients with BSI or sepsis. Molecular methods offer distinct advantages over blood cultures, including increased sensitivity and rapid diagnosis. However, diagnostic accuracy and cost–effectiveness should be established before implementation in clinical practice. SF runs on the LightCycler® instrument, the first real-time PCR-based system to be awarded a Conformité Européenne (CE) mark for pathogen detection and identification in suspected bloodstream infection and is, to date, most intensively investigated in clinical studies.[7, 15]

A meta-analysis including a total of 34 studies enrolling 6012 patients with suspected sepsis reported a high specificity with a modest and highly variable sensitivity.[16] Recently published studies revealed a low sensitivity of the PCR method accompanied with a limited utility for the diagnosis of healthcare-

associated BSI in critical care patients.[17] In contrast, another study including 104 critically ill patients suffering from SIRS showed that in 25 cases (16.9%, n=148) rapid identification of involved pathogens by multiplex-PCR led to adjustment of therapy.[18] A recently published randomized controlled trial enrolling 78 adults with suspected pulmonary or abdominal source of a systemic infection demonstrated a significant reduction in the time required for initial pathogen identification with SF compared with standard BC.[8] Taken together, the results of studies available so far about the usefulness of the SF for rapid detection of BSI in critical ill patients are divergent. According to the review of Dark, the performance of SF in different patient cohorts and clinical settings may vary significantly requiring careful and specific evaluation of the use of SF in respect of the clinical situation.[19] Patients after cardiothoracic surgery significantly differ from other cohorts: The use of cardiopulmonary bypass leads to a damage of the gastrointestinal mucosa, subsequent increased permeability, possible bacteremia, and the activation of a self-limited inflammatory response. The incidence of fungal infections especially in transplant recipients is, due to immunosuppression, higher than in the general ICU population. Commonly used biomarkers for bacterial infection, e.g. procalcitonin, might not work properly in the cardiothoracic population.[20]

However, regarding the utility of SF for the detection of BSI in patients after cardiothoracic surgery there is a paucity of data: Using SF for analyzing heart valve tissue of patients with active infectious endocarditis, the sensitivity of the SF was 100% and SF could identify pathogens in cases where BC tested negative.[21] In a recently published observational study analyzing 130 blood samples from 30 thoracic allograft recipients (23 heart and 7 lung transplants) showed a superiority of PCR compared to BC with a significantly higher number of positive samples in SF.[22]

In accordance with previous studies, the results of the present study demonstrate that SF, compared to BC, provided a better management of contaminants and a lower contamination rate. .[23] In respect of CoNS interpretation and discrimination in BC clinical judgment must be used due to a lack of objective criteria. In contrast, in SF an automated software is used to identify contaminants, which explains the lower rate of contaminants.

In accordance with recently published data, we observed a clear superiority of SF in detecting Gram-negative organisms compared to conventional BC. [24]

The reason for the discrepancy between the detection rate of Gram-negative and Gram-positive pathogens is unclear. Recently published studies could demonstrate that the superiority of SF over BC is particularly observed in very ill patients with severe sepsis.[25] In our cohort, patients with Gram negative BSI had higher concentration of CRP, IL-6 and PCT as well as a higher incidence of AKI with need for RRT compared to those with Gram-positive pathogens. Therefore, it might be hypothesized that SF is superior in detecting Gram-negative pathogens not in general but in critically ill patients with severe infections.

BSI caused by Gram-negative bacteria is associated with a 7-fold increased risk of early mortality after cardiac surgery after adjusting for other covariates, compared with no BSI.[5] In contrast, BSI caused by Gram-positive bacteria other than *S. aureus* was only associated with a 2.2-fold increased risk of

mortality. These data are consistent with other studies reporting an increased mortality associated with BSI due to Gram-negative bacteria and lower attributable mortality associated with BSI due to Gram-positive ones.[26] Against this background, the early detection of Gram-negative bacteria in SF is of tremendous clinical relevance and the identification of additional pathogens with SF might help to improve survival of patients with Gram-negative bacteremia.

Since invasive fungal infections with Aspergillus are frequently associated with high morbidity and mortality, in particular immunocompromised patients benefit from prompt initiation of anti-fungal therapy.[27] However, the Surviving Sepsis Campaign does not recommend the routine use of empirical antifungals, based on the relatively low frequency of fungal causation of sepsis (~5% of cases), although this is likely to rise. In our cohort of patients, a notably but not significant higher number of *Aspergillus* amplicons were detected by PCR as compared with blood culture.

Due to the long incubation times required and the lack of sensitivity early diagnosis of fungal infections by BC is often difficult and may delay therapy. Therefore, using SF could improve patient outcome as a result of rapid and accurate fungi detection and the consecutive timely initiation of appropriate therapy.[28] Hence, one important clinical impact of SF seems to be the identification of otherwise undetected fungal BSI.

However, SF was inferior to BC in detecting Gram-positive bacteria including *S. aureus* representing an important pathogen associated with high mortality.

Coagulase-negative staphylococci (CoNS) are a major constituent of human skin commensal flora, which were once considered relatively apathogen and a likely contaminant. But in patients with prosthetic valves, pacemakers, defibrillators, ventricular assist devices, intravascular catheters, or other foreign bodies these organisms have increasingly been recognized as a cause of clinically significant infections. Due to the propensity of these organisms to colonize foreign material to form a biofilm and to display resistance to multiple antibiotics, infections with CoNS are difficult to treat. Thus, due to the significant number of infections that would be missed, it does not appear SF could replace blood culture for the identification of bloodstream infections, especially in patients after cardiac surgery.

In addition, in SF pathogen identification is restricted to the 25 tested microorganisms and, moreover, susceptibility testing is not possible of and. Therefore, SF cannot replace BC but represents an adjunct tool in combination with BC. Even though in our study antimicrobial therapy was escalated due to the results of SF in eight patients, no de-escalation was done. As most of our patients were already on broad spectrum antibiotics and several blood cultures were drawn before choosing SF as diagnostic tool, empirical antibiotic therapy was considered to be adequate for most of the pathogens detected in SF. It might be hypothesized that the impact on therapy may be even more pronounced if SF would have been used earlier in the treatment course. De-escalation of therapy based on PCR might be difficult in general due to the inability of susceptibility testing.

Recently published studies could demonstrate that use of new PCR based technologies in the management of septic patients lead to a significant reduction in treatment costs with a an average net saving of 9970 € per patient.[29] This economic benefit is mainly based on shortening of intensive care unit stay and the use of fewer antibiotics. However, the costs of SeptiFast (approximately 200-300 USD) are high compared to Blood Culture (approximately 30 USD). Therefore, we assessed the predictive value of clinical and laboratory data to restrict the SF assay to clinical cases with a high probability of positive SF results. There is only limited data available in the literature about predictors for SF positivity:

Mencacci investigated the predictive role of procalcitonin in patients with suspected sepsis for positive test results in BC and PCR and revealed an area under the curve of 0.927 for SF positivity.[30] When applying a cut-off value of 0.37 ng/ml, the number of SF assays could be reduced by 53.9% with identifying 96.4% of pathogens. Leli et al. identified increased procalcitonin or white blood cells, fever >38°C, and low serum albumin as independent predictors of positive SF results in blood samples taken within 12h after the onset of fever in 285 patients.[31] A prospective observational cohort study including neurosurgical patients with external ventricular drainage (EVD) identified the intrathecal concentrations of IL-6 to be a reliable predictor of pathogen identification in SF. [32]

In our cohort, IL-6 as well as CRP was good predictors for SF positivity. Although measurement of PCT concentration are considered to be the gold standard of systemic inflammatory markers for diagnosis as well as for evaluation of the treatment effectiveness, PCT only showed moderate predictive capabilities. The discrepancy could be due to the following: It is well established that aortic cross clamp and cardiopulmonary bypass related perioperative stress is associated with elevated PCT after cardiac surgery.[33] Against this background, several studies showed a poor correlation between elevated PCT concentration and bacterial infections or sepsis after major cardiac surgery.[34] In addition, renal function is a major determinant of procalcitonin concentration and the value of PCT might be limited in patients with renal impairment or RRT.[35] Another aspect is that in our study 12 out of 47 positive SF results identified fungal pathogens. Thus, PCT as marker of bacterial infections is, anyway, not suitable for prediction of SF positivity in our cohort even more. Although the correlation of biomarkers and SF results are not very strong, in respect of the high costs it might be helpful in the decision to perform SF or not.

Limitations

There are several potential limitations to this study. First, our study suffers from the general limitation of a single-center, retrospective investigation: patients were recruited from one cardiothoracic ICU of a university hospital, and consequently, the results may not be applicable to other clinical settings with different patient characteristics, resources, and laboratory procedures. In addition, due to the small number of specific pathogens, the power to detect a difference between the groups is limited.

In interpreting the results of this study heterogeneity in the methods of drawing blood samples for BC must be considered as a limitation. It could not be ensured that all collected samples complied with the guidelines for drawing blood samples for BC, what can affect both for sensitivity and specificity.[36]

A major limitation is the fact that there were no predefined criteria for performing PCR e.g. presence of more than two SIRS criteria. The decision to perform SF was made by the consultant in charge of the ICU at the time or the infectiologist. The algorithms used in this study to differentiate between contamination and infection of BC and SF were not evaluated in the cardiothoracic population. Therefore, the reliability of this algorithm in this setting is uncertain. However, as there is no published algorithm for cardiothoracic patients we modified the originally published algorithm to incorporate specific characteristics of our patient's cohort e.g. the presence of prosthetic heart valves or other extracorporeal devices.

Due to the retrospective nature of our study we could not ensure that the same blood sample was used for SF and BC.

It has to be mentioned that the SF test is not available in the United States yet.

Conclusion

The PCR-based SF test might represent a valuable addition to the traditional BC method for rapid etiologic diagnosis of bloodstream infections in patients after cardiothoracic surgery. This applies in particular for individuals with Gram-negative bacteremia, AKI and/or elevated CRP and IL-6 - concentration. The most important clinical impact appears to be the identification of fungal BSI, especially Aspergillus that often was not detected by BC. However, because SF missed a certain number of Gram-positive pathogens, can only detect 25 pathogens and is unable to determine antibiotic susceptibility, it should always be used in conjunction with traditional blood culture methods.

Abbreviations

ANOVA: analysis of variance; AKI: acute kidney injury; AUC: Area under the Curve; BC: blood culture; BSI: bloodstream infections; CE: Conformité Européenne; CFU: colony form-ing units; CI: confidence interval: CoNS: Coagulase-negative staphylococci; CRP: C-reactive protein; CVC: central venous catheter; DNA: deoxyribonucleic acid; ICU: Intensive Care unit; IL-6: interleukin 6; LVAD: Left ventricular assist device; NI: Nosocomial infections; NPV: negative predictive value; PCR: polymerase chain reaction; PPV: positive predictive value; RRT: renal replacement therapy; ROC: receiver operating characteristic; SF: SeptiFast; SIRS: systemic inflammatory response syndrome

Declarations

Authors' contributions: KP, PMR, FD initiated the study. KP, PMR, JS, MT, HJ, FD contributed to the study design. KP, MD, FD acquired the data. KP, PMR, JS, FD analyzed and interpreted the data. KP, MD, SAP, FD drafted and revised the manuscript. MT, SAP, HJ critically revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate: The study was approved by the Institutional Review Board of the University Hospital Essen according to the Declaration of Helsinki. All of the patients had previously granted permission for use of their medical records for research purposes. Written informed consent was obtained within the pre-operative surgical written and verbal information conversation.

Consent to publish: Not applicable in that the manuscript does not contain data from any individual person.

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Availability of data and materials: The data of the current study are available from the corresponding author on reasonable request

Competing interests: The authors declare that they have no competing interests.

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not for-profit sectors.

Acknowledgements: None.

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Tables

Table 1: Analytical spectrum of the LightCycler® SeptiFast test

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

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

Table 2: Detected microorganisms after exclusion of contaminations

		Number of isolates				
Pathogens	Total	Detected by PCR	Detected by BC	PCR pos / BC pos	PCR pos / BC neg	PCR neg / BC pos
Gram-positive bacteria	**33**	**7 (21%)**	**28 (85%)**	**2 (6%)**	**5 (15%)**	**26 (79%)**
S. aureus	2	1 (50%)	2 (100%)	1 (50%)	0 (0%)	1 (50%)
CoNS	13	0 (0%)	13 (100%)	0 (0%)	0 (0%)	13 (100%)
S. pneumoniae	0	0	0	0	0	0
other Streptococcus spp.	1	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)
E. faecalis	3	2 (67%)	2 (67%)	1 (33%)	1 (33%)	1 (33%)
E. faecium	14	4 (29%)	10 (71%)	0 (0%)	4 (29%)	10 (71%)
Gram-negative bacteria	**35**	**28 (80%)**	**12 (34%)**	**6 (17%)**	**22 (63%)**	**7 (20%)**
E. coli	5	4 (80%)	2 (40%)	1 (20%)	3 (60%)	1 (20%)
P. aeruginosa	5	5 (100%)	2 (40%)	2 (40%)	3 (60%)	0 (0%)
E. cloacea	8	8 (100%)	1 (13%)	1 (13%)	7 (88%)	0 (0%)
K. pneumonia	9	7 (78%)	3 (33%)	1 (11%)	6 (67%)	2 (22%)
S. maltophilia	1	1 (100%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
Corynebacterium spp	0	--	0	---	---	0
M. morganii	1	--	1 (100%)	---	---	1(100%)
S. marcensens	6	3 (50%)	3 (50%)	0 (0%)	3 (50%)	3 (50%)
Fungi	**18**	**12 (67%)**	**9 (50%)**	**3 (17%)**	**9 (50%)**	**6 (17%)**
Aspergillus fumigatus	3	3 (100%)	0 (0%)	0 (0%)	3 (100%)	0 (0%)
Candida albicans	15	9 (60%)	9 (60%)	3 (20%)	6 (40%)	6 (40%)
Total	86	47 (55%)	49 (57%)	11 (13%)	36 (42%)	39 (45%)

Table 3: Characteristics of patients with positive SF/BC result compared to those with negative SF/BC results.

	PCR (Septifast)			Blood culture		
	Negative *(n=237)*	Positive *(n=42)*	P-value	Negative *(n=232)*	Positive *(n=47)*	P-value
Age [years]	68.0 [64.3-70.0]	57 [51.7-68.0]	***0.010***	***67 [62.0-69.0]***	***69 [59-69]***	n.s.
Male Gender [n,%]	161 (68)	30 (71)	n.s.	163 (70)	28 (59)	n.s.
Surgical Procedure [n, %]						
CABG	47 (20)	9 (21)	n.s.	46 (20)	10 (21)	n.s.
Isolated AVS	8 (3)	1 (2)	n.s.	9 (4)	0 (0)	n.s.
Isolated MVS	5 (2)	1 (2)	n.s.	4 (2)	2 (4)	n.s.
Combined procedures	101 (43)	14 (33)	n.s.	99 (43)	16 (34)	n.s.
Aortic surgery	25 (11)	4 (10)	n.s.	24 (10)	5 (11)	n.s.
Thoracic transplant	13 (5)	4 (10)	n.s.	11 (5)	6 (13)	n.s.
Others	38 (16)	9 (21)	n.s.	37 (16)	8 (17)	n.s.
CPB time [min.]	177.0 [147.6-186.4]	149.0 [116.6-207.8]	n.s.	174.5 [147.2-185.0]	161.0 [139.7-198.9]	n.s.
Euro-Score II [%]	7.0 [5.2-10.1]	8.8 [2.9-21.0]	n.s.		10.5 [5.4-21.6]	n.s.
TISS-28 on day of SF/BC	19 [17-21]	21 [15-22]	n.s.	19 [17-21]	18 [14-21]	n.s.
SAPS on day of SF/BC	32 [31-34]	30 [27-35]	n.s.	32 [31-33]	31 [25-33]	n.s.
Oxygenation [mmHg/FiO_2]	216.0 [196.3-230.4]	240.0 [210.2-269.5]	n.s.	220 [210.8-235.0]	214.5 [195.7-282.7]	n.s.
Heart rate [min^{-1}]	80.0 [80.0-90.0]	90.0 [83.4-106.6]	n.s.	90.0 [80.0-90.0]	90 [90.0-100.0]	n.s.
Body temperature [°C]	37.6 [37.4-37.8]	37.6 [37.1-37.9]	n.s.	37.6 [37.4-37.8]	37.6 [37.1-38.0]	n.s.
RRT [n,%]	125 (53)	31 (74)	***0.01***	129 (56)	27 (57)	n.s.
Laboratory values						
Serum lactate [mg/dl]	1.5 [1.4-1.8]	1.4 [1.1-1.9]	n.s.	1.5 [1.4-1.7]	1.4 [1.1-2.5]	n.s.
Billirubine [mg/dl]	0.9 [0.7-1.0]	1.0 [0.8-1.6]	n.s.	0.9 [0.7-1.0]	0.9 [0.5-1.2]	n.s.
Leucocytes [/nl]	14.0 [13.0-15.0]	12 [10.3-15.0]	***0.014***	14.0 [13.0-14.0]	14.0 [10.5-16.0]	n.s.
Fibrinogen [mg/dl]	464.0 [430.0-496.7]	525.0 [389.9-594.2]	n.s.	472 [433.8-503.9]	504 [438.5-544.9]	n.s.
CRP [mg/dl]	14.4 [13.3-15.4]	14.5 [13.3-15.4]	***0.009***	14.8 [13.7-15.9]	15.2 [13.2-19.7]	n.s.
PCT [ng/ml]	3.1 [2.3-4.7]	6.6 [2.7-16.4]	***0.015***	3.4 [2.5-4.9]	2.2 [1.3-3.8]	n.s.
IL-6 [pg/ml]	72.3 [46.5-104.7]	235.0 [83.5-1582.2]	***0.010***	90.9 [61.7-144.3]	141.0 [46.6-240.2]	n.s.
ICU stay [days]	16 [15-19]	22 [16-33]	***0.019***	**16 [15-19]**	**18.5 [14.0-26.2]**	***0.044***
Hospital stay [days]	23 [19-29]	38 [25-59]	n.s.	27 [21-30]	28 [20.7-42.7]	n.s.

CABG = coronary artery bypass grafting; AVR = Aortic valve replacement, MVS = Mitral valve surgery; TVS = tricuspid valve surgery, LTX = lung transplant; POD = postoperative day; CBP = Cardiopulmonary bypass; , ICU =

intensive care unit; RRT = renal replacement therapy; TISS = Therapeutic Intervention Scoring System, SAPS= Simplified Acute Physiology Score

Table 4: Impact of SF on antimicrobial therapy

No.	Age range, gender	POD, Surgery	Identified pathogen in SF	Identified pathogen in BC	Initial antimicrobial therapy	Adjustment of therapy	Outcome after adjustment of therapy
1	50-59, 1	53, CABG, AVR, MVS	*A. fumigatus*	---	Meropenem, Vancomycin	Escalation with voriconazole	survived
2	50-59, 1	38, LTX	*A. fumigatus*	---	Meropenem, Vancomycin, Fluconazole	Escalation with voriconazole, discontinuation of fluconazole	died
3	70-79, 2	CABG, AVR, MVS, TVS	*A. fumigatus*	---	Piperacilline/ Tazobactam	Escalation with voriconazole	died
4	50-59, 1	18, Aortic surgery	*C. albicans*	C. albicans	Ciprofloxacine, Vancomycin, Colistin	Escalation with caspofungin	died
5	60-69, 2	33, AVR, MVS	*C. albicans*	---	Meropenem, Vancomycin	Escalation with fluconazole	survived
6	60-69, 1	47, AVR	*C. albicans*	---	Linezolid, Imipenem	Escalation with caspofungin	survived
7	70-79, 1	21. CABG, MVS, TVS	*E. faecium*	CoNS	Piperacilline/ Tazobactam	Escalation with vancomycin	died
8	20-29, 1	49. LTX	*P. aeruginosa*	---	Vancomycin, Ceftazidime	Escalation with colistin	survived

POD = postoperative day; CABG = coronary artery bypass grafting; AVR = Aortic valve replacement, MVS = Mitral valve surgery; TVS = tricuspid valve surgery, LTX = lung transplant. To grantee patient's anonymity, gender was discriminated in 1 and 2.

Figures

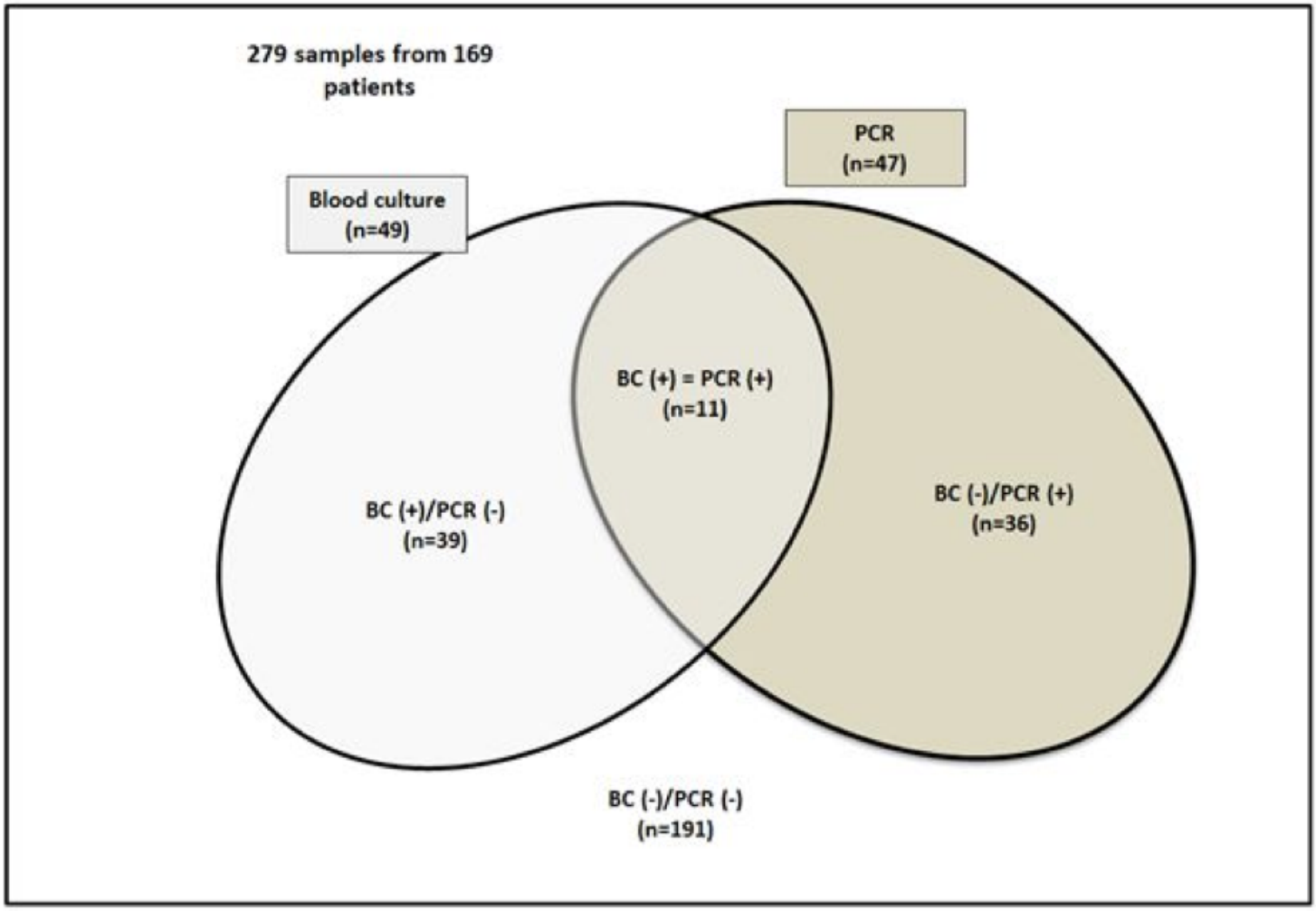

Figure 1

Number of detected microorganisms classified as infection in PCR, blood culture and combina-tion of both methods.

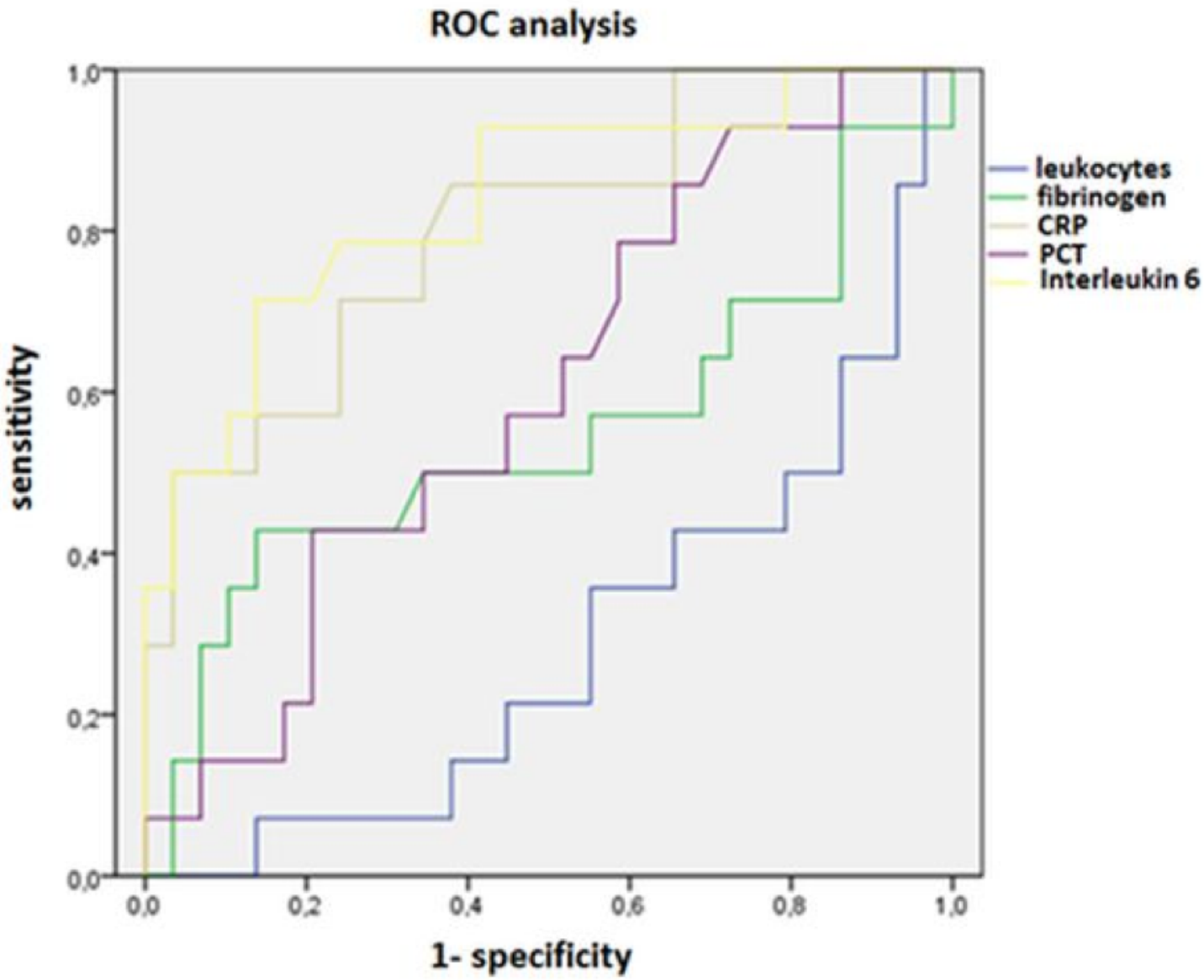

Figure 2

Receiver operator characteristic (ROC) curve for the prediction of SF positivity.