Multiple Polymerase Chain Reaction for Direct Detection of Bloodstream Infection After Cardiac Surgery in a PICU

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OBJECTIVES: Nosocomial infections are a prevalent cause of death and complications in critically ill children. Conventional cultures are able to detect only up to 25% of bacteremia. Several studies have suggested that molecular tests could be a faster and effective tool for detection of bacterial infections. The objective of this study is to compare molecular tests for bacterial detection in whole blood samples, with routine blood culture for the diagnosis of nosocomial bloodstream infections (BSIs).

DESIGN: Prospective cohort study.

SETTING: A PICU of a tertiary center, reference for congenital heart diseases.

PATIENTS: Children, 0–16 years, admitted to PICU between August 2016 and December 2019 after cardiac surgery were prospectively recruited. Demographic, clinical, laboratory, and microbiologic data from patient's medical records, and laboratory and microbiologic results were collected.

INTERVENTIONS: In all patients, blood culture and multiple polymerase chain reaction (PCR) for bacterial detection in a whole blood sample were performed.

MEASUREMENTS AND MAIN RESULTS: Fifty-seven cases (patients with suspected infection) and 36 controls (patients with no suspected infection) were recruited during this period; 51.6% were female. Median age was 6 months (interquartile range [IQR], 0–13 mo), and median weight was 5 kg (IQR, 3.5–9.5 kg).

From the cases, 33% (19/57) had a confirmed BSI with positive blood culture; 52% were Gram-negative bacilli, and 48% were Gram-positive cocci. Thirty-three percentage (19/57) had a positive PCR with only a 26% (five cases) of concordance between PCR result and blood culture (three bacteremias for *Klebsiella pneumoniae*, one for *Serratia marcescens*, and one for *Pseudomonas*).

CONCLUSIONS: Multiple PCRs in whole blood samples did not appear to be more sensitive than blood cultures in this series. Better concordance was found with Gram-negative microorganisms.

KEY WORDS: blood culture; bloodstream infections; cardiac surgery; children; multiple polymerase chain reaction; pediatric intensive care unit

H ealthcare-associated infections are a major cause of morbidity and mortality in PICUs, and bloodstream infection (BSI) is the most frequent infection in this group of patients (1). BSI specially affects the outcome of children undergoing cardiac surgery, increasing complications and mortality (2). For this reason, it is important to improve BSI diagnosis. Blood culture is still the gold standard for bacteremia detection, but results are slow and usually detect less than half of cases with clinical and analytical signs of invasive bacterial infection (3, 4).

Several studies have suggested that molecular tests could be a faster and more effective tool for detection of bacterial infections (5, 6). In this study, we focus on multiplex polymerase chain reaction (Multiplex PCR). It refers to the use of polymerase chain reaction (PCR) to amplify different regions of the bacterial 16S ribosomal ribonucleic acid (rRNA) simultaneously. Some authors have described that multiple PCR is especially useful for patients where systemic antibiotics have been started before blood samples collection (7–9). To detect bacteremia, performing a multiple PCR in blood culture sample that is already growing or in a whole blood sample is possible. In some studies, multiple PCR performed directly in whole blood samples has shown good correlation with blood cultures and higher sensitivity for bacteremia detection (7, 10, 11), and this method would be faster than waiting for some growth in the blood culture sample (12).

This methodology is innovative and exploratory. Considering the limitations of blood cultures, it is imperative to seek new technologies that implement early detection of causative agents to improve early-targeted treatment strategies.

The objective of this study is to evaluate molecular tests for bacterial detection in whole blood samples, testing correlation with conventional blood cultures (gold standard) in diagnosis of nosocomial BSIs, and to evaluate differences in the results in patients with systemic antibiotics prescription.

MATERIALS AND METHODS

Patients, 0–16 years old, admitted to PICU between August 2016 and December 2019 after cardiac surgery with cardiopulmonary bypass (CPB) were prospectively recruited.

Those with suspected infection were considered cases, and those without suspected infection were considered controls.

Local PICU has a rate of proved postoperative bacteremia of 4–7%, and a total of 80–100 cardiac surgery-bypass procedures are performed every year.

To recruit cases, in patients admitted to PICU after cardiac surgery for more than 48 hours with clinical suspicion of BSI (fever, clinical, or laboratory signs of infection), a whole blood sample was collected aseptically through a previously inserted catheter (arterial line and central venous line). Samples

were processed for blood culture (BD BACTEC Peds Plus/F Medium; BD Biosciences, San Jose, CA) and multiple bacterial PCR designed ad hoc. In addition, urine culture, endotracheal tube secretions, and surgical wound swab were collected in several cases. For controls, patients admitted to PICU after cardiac surgery for more than 48 hours, without suspected infection, were randomly selected.

Sample size was calculated based on proportions. Considering that blood cultures are positive in around 20% of bacteremia and with the hypothesis that PCR would be positive in 35%, with 95% confidence level (α error 5%), 80% power (β error 20%), necessary sample size should be around 108 patients with suspected infection. One control for every two cases was recruited to study the possibility of having false positives with molecular methods.

Once a case or control was selected, a blood sample for conventional blood culture (1 mL in BACTEC Peds PLUS/F) and a whole blood sample for multiple PCR detection (1 mL in EDTA tube) were collected. The sample for blood culture was sent and incubated for 7 days at local microbiology unit, and the blood samples for multiple PCR detection were stored in a local freezer (–20°C) for posterior shipping and analysis in a specialized rapid molecular diagnosis laboratory in London (Micropathology, London, United Kingdom).

Specific manual extraction process (including protease, lysozyme, and lysostaphin) to lyse bacteria and minimize the effects of inhibitors and contaminating DNA was performed. An in-house assay to amplify a short, conserved region of the 16S rRNA gene was used. Any product amplified from the unknown sample was sequenced and compared with known sequences available in Genbank (Bethesda, MD) (13), usually using the basic local alignment search tool tool (14). The following bacterial pathogens, the most frequent causing bacteremia in PICU, were selected for multiple PCR analysis: coagulase negative staphylococcus (CoNS) *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus viridans*, *Serratia marcescens*, *Klebsiella pneumoniae/oxytoca*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii*, *Escherichia coli*, and *Enterobacter cloacae*. PCR results were analyzed blinded for blood culture results. A sample was regarded as negative when it was noninhibited, and all pathogen PCRs were negative. The sample was considered noninhibited when

the quantification cycle value of the extraction and amplification control (EAC) signal of the sample was within ± 2 SD of the EAC signal in the negative control samples.

Demographic, clinical, laboratory, and microbiologic data from patient's medical records and laboratory and microbiologic results were collected.

All the procedures developed in this study followed the ethical standards on human experimentation of our clinical research ethics committee and the standards of Helsinki Declaration of 1975. This study received the approval of the 12 de Octubre Hospital's clinical research ethics committee (number 16/362). Written informed consent was presented and signed by parents.

IBM SPSS Statistics Version 22.0 software (IBM, Armonk, NY) was used in statistical analyses. All continuous variables were expressed as mean ± SD or median (interquartile range [IQR]). Comparison of groups was performed using Student t test for continuous variables and the chi-square test or Fisher exact test for categorical variables.

RESULTS

Fifty-seven cases (patients with suspected infection) and 36 controls (patients with no suspected infection) were recruited during this period; 51.6% were female. Median age was 6 months (IQR, 0–13 mo), and median weight was 5 kg (IQR, 3.5–9.5 kg). Twenty-seven percentage (25/93) had some comorbidities (neurologic or respiratory more frequently). For surgical complexity, the mean risk adjustment for congenital heart surgery score was

3.3 (SD, ±1.2). Median CPB time was 157 minutes (IQR, 122–217 min). No differences according demographic or clinical features were found between cases and controls, except in CPB time (**Table 1**).

For cases, median time of mechanical ventilation was 5 days (IQR, 210 d) and time of central line insertion until suspected infection 8 days (IQR, 3–19 d). The median time when blood culture was drawn was 7 days after surgery (IQR, 3–14 d). The median length of PICU stay was 27 days (IQR, 11–45 d) and of hospitalization was 41 days (IQR, 24–75 d). The mortality rate was 12% (5/57 patients), with no mortality among controls ($p = 0.045$).

Thirty-three percentage (19/57) had a confirmed BSI with positive blood culture (four *K. pneumoniae*, three *S. marcescens*, two *A. baumannii*, one *P. aeruginosa*, six CoNS, two *Streptococcus viridians*, and one *S. aureus*). Fifty-two percentage (10) were Gram-negative bacilli (GNB), and 48% (9) were Gram-positive cocci (GPC).

Thirty-three percentage (19/57) had a positive PCR (three *K. pneumoniae*, one *S. marcescens*, two *S. maltophilia*, two *P. aeruginosa*, three *A. baumannii*, two *Enterobacter*, one *E. coli*, four CoNS, and one *E. faecalis*). Seventy-three percentage (14) were GNB, and 27% (5) were GPC.

Among the 19 microorganisms identified by multiple PCR, only five (26%) could be confirmed by blood culture (three bacteremias for *K. pneumoniae*, one for *S. marcescens*, and one for *Pseudomonas*) (**Tables 2** and **3**).

Sensitivity of multiple PCR compared with blood culture was 26.4% (5/19), and specificity was 76% (29/38).

Both tests were negative in 29 (51%), any positive result was found in 28 (49%), including five cases (8.5%) where both tests were positive. The blood culture and multiple PCR results agreed in 34 of 57 cases (60%) (**Table 4**).

TABLE 1.
Comparison Between Cases and Controls

Demographic and Clinical Variables	Cases	Controls	p
Total number	57	36	
Sex (female/male)	56%	44%	0.27
Mean age (mo)	27	25	0.88
Mean weight (kg)	6	3.8	0.81
Comorbidities	30%	22%	0.41
Surgical complexity (median risk adjustment for congenital heart surgery 1 score 1−6)	3	3.1	0.82
Cardiopulmonary bypass time (min)	206	151	0.028

TABLE 2.
Blood Cultures and Multiple Polymerase Chain Reaction Results

Test Results	Cases (% of 57)	Controls (% of 36)
BC+/PCR+	10 (17.5)	1 (2.7)
BC+/PCR−	9 (15.7)	1 (2.7)
BC−/PCR+	9 (15.7)	7 (20)
BC−/PCR−	29 (51)	27 (75)

BC = blood culture, PCR = polymerase chain reaction.

TABLE 3.
Distribution of Detected Microorganisms in Cases

Microorganisms	Total+	BC+	PCR+	BC+/PCR+	BC+/PCR−	BC−/PCR+
Staphylococcus aureus	1	1	0	0	1	0
Streptococcus viridans	2	2	0	0	2	0
Coagulase negative staphylococcus	10	6	4	0	6	4
Enterococcus faecalis	1	0	1	0	0	1
Klebsiella pneumoniae	4	4	3	3	1	0
Serratia marcescens	3	3	1	1	2	0
Acinetobacter baumannii	5	2	3	0	2	3
Pseudomonas aeruginosa	2	1	2	1	0	1
Stenotrophomonas maltophilia	2	0	2	0	0	2
Enterobacter cloacae	2	0	2	0	0	2
Escherichia coli	1	0	1	0	0	1
Total	33	19	19	5	8	14

BC = blood culture, PCR = polymerase chain reaction.

From 57 cases, 26 patients were receiving systemic antibiotics when samples for blood culture and for multiple PCR were collected. Nine patients (34.6%) had positive blood culture, and nine patients (34.6%) had positive PCR. The microorganism was the same in three cases (33%) (all gram negatives).

We analyzed different outcomes (time of mechanical ventilation, time of central line insertion, length of PICU stay and hospitalization, and mortality) between cases with positive and negative test results (blood culture or PCR), finding no differences between the groups.

From controls, there were two positive blood cultures (5.5%) (one CoNS and one *S. viridans*) considered contaminated, and there were eight positive PCR (22.2%) (five *Enterobacter*, one *Pseudomonas*, one *Acinetobacter*, and one *E. faecalis*), all considered contaminated.

TABLE 4.
Polymerase Chain Reaction Compared With Blood Culture

Test Results	Blood Culture		Total
Polymerase chain reaction	+	−	
+	5	9	14
−	14	29	43
Total	19	38	57

DISCUSSION

In this study, we decided to perform a sequential multiple PCR in whole blood samples because this technique had shown good results in previous reports. We focused on the diagnosis of BSIs after cardiac surgery, because our unit is a reference PICU for these patients, that are prone to suffer bacteremia, and there are no previous studies that evaluate molecular tests compared with blood cultures in children after cardiac surgery.

Given the limited results of blood cultures, especially in pediatric patients, where blood samples are scarce, it is essential to find new strategies for the early diagnosis of the causative bacteria. The novel approach using multiple PCR attempts to improve the standard methodology of simply relying on the results of blood culture, which often are time-consuming and may result in falsely negative.

Both tests, blood culture, and multiple PCR detected a high percentage of Gram-negative organisms (52% by blood culture and 74% by multiple PCR). These results are similar to other published reports. Abou Elella et al (2) described 67% of BSI caused by GNB (mainly *Pseudomonas* and *Enterobacter*). In addition, Murray et al (15) reported 48% of BSI caused by GNB (mainly *Klebsiella*). These findings could be explained because risk factors for Gram-negative organisms are common in pediatric cardiac surgery and may also be due to the common practice of using prophylactic postoperative

antibiotics that cover primarily Gram-positive organisms (16).

In our series, blood culture was positive in 33% of patients (19/57) with suspected infection, a higher percentage than previously reported in other studies, ranging between 15% and 25% (3, 4, 7). Multiple PCR was positive also in 19 cases, but with low concordance in results: only a small percentage (5/19, 26%) of microorganisms identified by multiple PCR could be confirmed by blood culture. These results are similar to the ones described by Pilarczyk et al (17). They performed SeptiFast (Roche, Rotkreuz, Switzerland) (multiple PCR performed in whole blood samples) and blood cultures in suspected BSIs after cardiothoracic surgery in adult patients and did not find differences in percentage of positive SeptiFast tests (14.7%) compared with positive blood cultures (17.2%). In addition, only 23.4% of microorganisms identified by SeptiFast could be confirmed by blood culture.

All concordant results between multiple PCR and blood culture results were found with Gram-negative microorganisms. Pilarczyk et al (17) pointed out in their study that multiple PCR applies for individuals with suspected Gram-negative BSI due to the low performance in detecting Gram-positive pathogens.

We found that multiple PCR and blood culture results agreed in 60% (five positive culture cases and 29 both negative tests). Previous studies obtained higher sensitivity for molecular tests and better concordance with blood cultures. The study done by Korber et al (7) described that SeptiFast and blood cultures results agreed in 85.5% cases, and SeptiFast detected 81.7% of relevant microorganisms identified compared with 52.5% detected by blood cultures. Overall, SeptiFast was positive in 26.6% and blood culture in 15.1%. In other study by Denina et al (10) performed in cases of BSIs in oncohematological patients, they described that Magicplex Sepsis Real-Time test (Seegene, Seoul, South Korea) (multiple PCR performed in whole blood samples) allowed a 143% increase in the diagnostic value of blood culture. Magicplex was positive in 36.6% of blood samples (55/150), considering significant pathogen in 78% (43/55). Although from confirmed/suspected BSI, multiple PCR and blood culture results agreed in only 12.2% of cases (11/90), more similar to our results.

Van den Brand et al (12) compared a multiplex PCR in whole blood samples with blood cultures performed in the cases of suspected sepsis in the neonatal intensive care, obtaining 58% (53/91) of positive PCR versus 66% (60/91) of positive blood cultures, yielding no significant difference in detection rate, as we found in our series. Nonetheless, they found a higher concordance between PCR and blood culture results (both agreed in 72% of cases, 66/91). They also highlighted that PCR results were available in 4 hours, faster than blood culture results.

Considering patients receiving systemic antibiotics when samples for blood culture and multiple PCR were collected, the same proportion (34.6%) had positive blood culture and positive PCR. These results contrast with other previous reports. For example, in the study of Rampini et al (8), they described 56 patients with clinical and analytic signs of infection, all with negative cultures and 24 with a positive PCR (all with previous systemic antibiotics). In the report by Korber et al (7), they found that for treated patients, the number of positive PCRs with negative blood culture was significantly higher than the number of patients with negative PCR and positive blood cultures. In addition, the ratio of positive and negative PCR did not differ considerably between patients with or without antibiotics. In the study of Gies et al (9), patients with systemic inflammatory response syndrome under empiric antibiotic treatment had a 35% of multiple PCR (SeptiFast) positive tests compared with a 10% of positive blood cultures. They also found that patients with higher procalcitonin level were more likely to have a positive SeptiFast test and that patients with positive SeptiFast test had a significantly higher risk to be deceased on day 30. We analyzed our data, and we did not find differences on procalcitonin levels or mortality rate between patients with positive and negative multiple PCRs.

CONCLUSIONS

Multiple PCR in whole blood samples failed to correlate with blood cultures in this series, with lower agreement than other previous reports, and showed low sensitivity to detect positive blood cultures results. Systemic antibiotics administration did not modify results of both tests differently.

We think that these results may be consequence of a small sample of patients, as we could not manage to achieve the calculated sample size. Results also could have been affected by our choice of the bacterial for sequential PCR analysis instead of a previously validated multiple PCR for whole blood samples (like SeptiFast or Magicplex, having better results published).

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REFERENCES

1. Elward AM, Fraser VJ: Risk factors for nosocomial primary bloodstream infection in pediatric intensive care unit patients: A 2-year prospective cohort study. *Infect Control Hosp Epidemiol* 2006; 27:553–560

2. Abou Elella R, Najm HK, Balkhy H, et al: Impact of bloodstream infection on the outcome of children undergoing cardiac surgery. *Pediatr Cardiol* 2010; 31:483–489

3. Menezes LC, Rocchetti TT, de Castro Bauab K, et al: Diagnosis by real-time polymerase chain reaction of pathogens and antimicrobial resistance genes in bone marrow transplant patients with bloodstream infections. *BMC Infect Dis* 2013; 13:166

4. Reier-Nilsen T, Farstad T, Nakstad B, et al: Comparison of broad range 16S rDNA PCR and conventional blood culture for diagnosis of sepsis in the newborn: A case control study. *BMC Pediatr* 2009; 9:5

5. Harris KA, Hartley JC: Development of broad-range 16S rDNA PCR for use in the routine diagnostic clinical microbiology service. *J Med Microbiol* 2003; 52:685–691

6. Santolaya ME, Farfán MJ, De La Maza V, et al: Diagnosis of bacteremia in febrile neutropenic episodes in children with cancer: Microbiologic and molecular approach. *Pediatr Infect Dis J* 2011; 30:957–961

7. Korber F, Zeller I, Grünstäudl M, et al: Septifast versus blood culture in clinical routine-a report on 3 years experience. *Wien Klin Wochenschr* 2017; 129:427–434

8. Rampini SK, Bloemberg GV, Keller PM, et al: Broad-range 16S rRNA gene polymerase chain reaction for diagnosis of culture-negative bacterial infections. *Clin Infect Dis* 2011; 53:1245–1251

9. Gies F, Tschiedel E, Felderhoff-Müser U, et al: Prospective evaluation of SeptiFast multiplex PCR in children with systemic inflammatory response syndrome under antibiotic treatment. *BMC Infect Dis* 2016; 16:378

10. Denina M, Scolfaro C, Colombo S, et al; Regina Margherita Children's Hospital Bloodstream Infections Study Group participants: Magicplex™ sepsis real-time test to improve bloodstream infection diagnostics in children. *Eur J Pediatr* 2016; 175:1107–1111

11. Gonçalves Quiles M, Carballo Menezes L, De Castro Bauab K, et al: Diagnosis of bacteremia in pediatric oncologic patients by in-house real-time PCR. *BMC Infect Dis* 2015; 15:1–8

12. van den Brand M, van den Dungen FAM, Bos MP, et al: Evaluation of a real-time PCR assay for detection and quantification of bacterial DNA directly in blood of preterm neonates with suspected late-onset sepsis. *Crit Care* 2018; 22:105

13. Benson DA, Karsch-Mizrachi I, Lipman DJ, et al: GenBank. *Nucleic Acids Res* 2009; 37:D26–D31

14. Altschul SF, Gish W, Miller W, et al: Basic local alignment search tool. *J Mol Biol* 1990; 215:403–410

15. Murray MT, Krishnamurthy G, Corda R, et al: Surgical site infections and bloodstream infections in infants after cardiac surgery. *J Thorac Cardiovasc Surg* 2014; 148:259–265

16. Kato Y, Shime N, Hashimoto S, et al: Effects of controlled perioperative antimicrobial prophylaxis on infectious outcomes in pediatric cardiac surgery. *Crit Care Med* 2007; 35:1763–1768

17. Pilarczyk K, Rath PM, Steinmann J, et al: Multiplex polymerase chain reaction to diagnose bloodstream infections in patients after cardiothoracic surgery. *BMC Anesthesiol* 2019; 19:59