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Research Paper

Are There Benefits In Early Diagnosis Of Prosthetic Joint Infection With Multiplex Polymerase Chain Reaction?

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Abstract

Purpose Identification of bacteria and susceptibility are fundamental in periprosthetic joint infection (PJI). Especially in the case of systemic inflammatory response syndrome (SIRS) rapid detection of pathogens is essential for proper therapy. Bacterial cultures are time consuming. The polymerase chain reaction (PCR) is a non-culture molecular method and is able to rapidly identify pathogens and their resistance genes. Multiplex PCR (mPCR) can amplify several different DNA sequences simultaneously. The aim of this study was to show the value of mPCR for early diagnosis of PJI.

Methods 60 patients undergoing total hip or knee revisions were recruited in this prospective single-centre-study. Three groups were created: 26 patients with aseptic loosening (negative control), 26 patients with chronic PJI, and 8 patients with acute PJI/SIRS. We compared the results of joint aspirates obtained intraoperatively investigated by mPCR with the microbiology results of tissue specimens.

Results The overall sensitivity of mPCR was 78.8% (95% CI, 61.1 - 91.0%), the specificity was 100% (95% CI, 87.2 - 100%), the negative predictive value was 79.4% (95% CI, 62.1 – 91.3%), the positive predictive value was 100% (95% CI, 86.8 - 100%), and the overall accuracy was 88.3% (95% CI, 77.4 – 95.2%). The overall accuracy in acute infections/SIRS (87.5%) was greater than in late chronic PJI (76.9%). In PJI the mPCR was able to provide the results within 5 hours whereas the mean time for cultures was 6.4 days.

Conclusions Multiplex PCR is a reliable diagnostic tool in PJI management, especially in acute cases complicated with SIRS. Early diagnosis within several hours is possible, targeted antibiotic treatment can be started promptly.

Key words: prosthetic joint infection; diagnosis; polymerase chain reaction; bacteria; susceptibility; revision arthroplasty.

Introduction

Periprosthetic joint infection (PJI) is a devastating complication of arthroplasty and among the principal etiologies of implant failure [1]. The estimated annual financial burden of PJI on the USA healthcare system is approximated at over 320 million dollars and the number of primary arthroplasties has been projected to increase [2-4]. Several perioperative preventive measures are routinely implemented, most of them cost effective [5]. A high degree of suspicion is required for the early diagnosis and optimal management [6].

Guidelines with several diagnostic criteria based on the current best evidence have been proposed to improve early diagnosis and treatment. Recently, the

International Consensus Meeting on Periprosthetic Joint Infection featured current practices for preventing, diagnosing and managing PJI [7, 8]. Although many of the recommendations are supported by high-quality evidence, others are lacking it. To date Alpha Defensin is the only diagnostic tool with a very high sensitivity and specificity and with the potential to exclude PJI, but without the ability to detect the pathogen [9]. Elevations of inflammatory markers have a low specificity and yield no information about the germ, antibiotic resistance, or appropriate therapy. There are also a small percentage of patients with PJI that may present with normal erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels [10]. The most important method and to date gold standard for diagnosing PJI is the isolation of a pathogen by cultures of joint aspirate or periprosthetic tissue samples [11]. The identification of the specific bacteria and respective susceptibility are fundamental to systemic local antimicrobial therapy. However, some aspirates from PJI patients are culture-negative, for example, due to ongoing empirical antibiotic treatment [12]. Culturing the causative pathogen takes long time and is problematic in less virulent, fastidious and slow growing organism [11, 13]. The time between referral of the patient with an infection and receiving the definitive diagnosis with a positive culture is important, in this period no targeted antibiotic therapy is possible.

The polymerase chain reaction (PCR) is a non-culture molecular method and can be applied to diagnose infection within hours [14, 15]. The technique consists of the amplification of bacterial DNA fragments with the use of synthetic specific primers complementary to the bacterial genome. Several amplification cycles are required to duplicate the target sequence. Next, the specific product can be isolated by electrophoresis or hybridization methods [16-18]. Multiplex PCR (mPCR) assays have been developed to rapidly and simultaneously identify multiple pathogens as well as their resistance genes. Availability of reliable rapid molecular diagnostic tool in the proof of PJI that can provide pathogen detection in hours rather than days might prevent some inappropriate and inadequate therapies [19]. PCR is able to yield results within hours, in contrast to cultures, which may take days and delay the targeted management of infection in the acute scenario. The aim of the current study was to characterize the predictive value of mPCR in the diagnosis of PJI.

Materials and Methods

This study was performed as a prospective single-centre-study. The study was approved by the local ethical committee, and all patients gave written informed consent before starting with the study procedure.

Study population

For this study, we recruited patients undergoing hip or knee revision arthroplasty in our hospital. We included patients with aseptic loosening of a total hip or knee replacement as a negative control group (Group A) as well as patients with chronic deep PJI (Group B) and patients with an acute PJI (Group C) according to MSIS criteria. Group A consisted of 26 patients scheduled for revision arthroplasty due to aseptic loosening, in Group B were 26 patients with chronic PJI scheduled for septic exchange arthroplasty, and Group C was a cohort of 8 patients with clinical signs of acute PJI and systemic inflammatory response syndrome (SIRS). Group C was admitted through the emergency room of the hospital as an acute referral. Patients were recruited and enrolled into the current study in the outpatient clinic on day of referral. All patients underwent total joint replacement in the past; the indication for revision was aseptic loosening in Group A and PJI in Group B and C. Joint aspiration in a designated aspiration theatre (which is a part of the outpatient clinic) was done in all cases prior to surgery, according to a strict diagnostic protocol [13]. When evidence of infection was present (according to the modified MSIS criteria of PJI [20]), the patients were selected for Group B [20]. Patients with negative microbiological results and not fulfilling the MSIS criteria following joint aspiration were recruited for Group A. Patients of Group C with SIRS/acute PJI had a purulent synovial fluid with a high cell count (≥10,000/µL), a positive Leukocyte Esterase (LE) strip test [21] and a high percentage (≥90%) of neutrophils in the aspirate; significantly elevated C-reactive protein (CRP) levels over 100 mg/L in the blood test, with leukocytosis (WBC count ≥12,000/µL) and clinical symptoms as elevated temperature (≥38.0 degrees Celsius), tachycardia (heart rate ≥90 per minute) and tachypnea (breathing rate ≥20 per minute) [22]. These patients underwent acute surgery (irrigation & débridement) on the day of admission.

Study design

We compared the synovial fluid samples obtained from intraoperative joint aspirates as well as the microbiology results from intraoperative tissue specimens with the results of mPCR of joint aspirates. Joint aspiration was performed two times in all patients. The first aspiration was performed in the outpatient clinic for all patients and was used to group the patients in combination with the MSIS

criteria. The second aspiration was carried out in the operation theater at the revision-surgery: during the operation the aspiration was carried out after skin incision and subcutaneous preparation before opening the capsule, in order to obtain a blood-free, non-contaminated amount of synovial fluid. The fluid was filled into two sterile tubes using sterile technique. One tube was used for the mPCR, the other one was sent to the microbiology lab (UKSH, University of Schleswig-Holstein, Kiel, Germany). Intraoperatively obtained tissue samples from the implant-bone interface were sent to the same microbiology lab separately, in order to verify the cultures, considered to be the gold standard for bacteriology diagnostics. For Group A patients, we included specimens from the tissue around the loose implant. In these cases, all preoperative aspirations showed no evidence of pathogens. In patients with a PJI, specific organisms were detected (Table 1). Furthermore, we documented all relevant results, like CRP, the LE test, the organism detected by mPCR, as well as the results of microbiological culture.

Table 1. Number and distribution of bacteria detected with the Curetis Unyvero® multiplex PCR (polymerase chain reaction) system in patients with chronic or acute prosthetic joint infection (study groups B and C)

Pathogen detected	Number	Distribution %
Coagulase negative Staphylococci	14	41.2
Staphylococcus aureus	5	14.7
Propionibacterium acnes	2	5.9
Enterobacter cloacae	2	5.9
Enterococcus faecalis	2	5.9
Streptococcus agalactiae	1	2.9
Negative	8	23.5
TOTAL	34	100

The aspirate obtained intraoperatively was examined the same day in the Unyvero® Curetis multiplex-PCR testing system (Curetis N.V., Holzgerlingen, Germany). The tests were finished in all cases within 5 hours. We documented the detected organism(s) along with the antibiotic susceptibility, and compared these results later with the bacteriology cultures as well as the antibiotic resistance profile found by the microbiology lab. Furthermore, the time interval between the PCR-result and the microbiology result in case of SIRS was documented. Preoperative antibiotic prophylaxis or therapy was also recorded.

Multiplex PCR cartridge system

For analysis we used the Unyvero® Implant and Tissue Infection cartridge application (U-ITI) (Curetis N.V., Holzgerlingen, Germany). This is a semi-quantitative DNA test which is able to perform eight different mPCR reactions in parallel to detect 114 pathogen-associated nucleic acids and resistance markers in solid, fluid and highly viscous samples [23]. Samples of joint fluid (max. 180 µl) were transferred to a sample tube. The sample was then lysed in 30 minutes via mechanical, thermal, chemical and enzymatic methods according to the user's manual of the manufacturer. Next, the lysed sample of joint fluid was processed in the Unyvero® Cartridge along with the Master Mix including the temperature-stabilized DNA-polymerase, primers, PCR-buffer and nucleotides. The cartridge was then transferred into the Unyvero® A50 Analyzer. Within 4.5 hours the system performed sample lysis, DNA purification, and multiplex nucleic acid amplification by end-point-PCR using fluorescence-labelled primers in eight independent PCR chambers with individual detection array and qualitative amplicon detection by hybridization on a porous array membrane. The software provides the results of the mPCR and shows the indicated microorganism of the joint aspirate as well as the genetic antibiotic resistance markers, representing the susceptibility of the germ. If at least one sample reached the threshold of positivity, the result was documented as valid. Figure 1 shows the coverage of the clinically relevant pathogens that can be detected by the Unyvero® Multiplex PCR cartridge system, which complies with the most relevant pathogens reported in PJI studies [24].

Patients

We included patients older than 18 years with a painful artificial joint after total hip or knee arthroplasty, where a revision arthroplasty was scheduled. Joint aspiration of the target joint (hip or knee) was performed in our outpatient department in all cases before surgery. A negative aspiration result as well as a normal CRP identified the patients for the negative control group (Group A). If the result of the preoperative aspiration was culture positive and the CRP was elevated or LE stripe test was positive (++) patients were considered having a PJI of the target joint (hip or knee). Chronic deep infection was considered (Group B), if the serum CRP level was between 10 and 100 mg/L, the cell count in synovial fluid was between 3,000 and 10,000/µL and granulocyte percentage was not higher than 90%, and clinical signs of PJI revealed at least 3 months after last surgery [25]. An acute PJI was considered if symptoms existed for less than 3 months, local signs of infection were seen (redness, swelling, local hyperthermia or pain) and serum CRP was greater than 100 mg/L, synovial cell count was higher than 10,000/µL and granulocyte percentage was over 90% (Group C). Existence of at least two of the following

criteria allowed for diagnosis of SIRS: body temperature over 38.5° or under 36.0° Celsius, tachycardia (heart rate > 90 bpm), tachypnea (lung rate >20/min) or hyperventilation with pCO2 <32 mmHg, or leukocytosis (WBC >12,000/µL) [22].

Patients under 18 years of age and pregnant patients were excluded from this study. Patients with severe systemic diseases, tumors, and patients from foreign countries were excluded as well.

Statistical Analysis

In order to statistically assess the performance of the mPCR test, the overall accuracy, the specificity, sensitivity, positive predictive value and negative predictive value were evaluated. In particular, specificity (SP) indicates the percentage of subjects without the disease who get a negative test result; sensitivity (SE) indicates the percentage of subjects with the disease who get a positive test result; positive predictive value (PPV) is the probability that the disease is present in case of a positive test; negative predictive value (NPV) is the probability that the disease is not present in case of a negative test. The 95% confidence interval (95% CI) has been calculated for each of the previous statistical measures. The statistical analyses were performed with the SAS® 9.3 for Windows® (SAS Institute Inc., Cary, NC, USA) software.

Results

We investigated 60 patients in total. 26 patients were included in Group A, 26 patients were included in Group B, and eight patients were in Group C. We included 32 male (53.3%) and 28 female (46.7%) patients with a mean age of 70.5 years (range, 41-87 years). 30 (50 %) patients had a revision of the hip and 30 (50 %) patients were revised on the knee. The overall SE of mPCR was 78.8% (95% CI, 61.1 - 91.0%), SP was 100% (95% CI, 87.2 - 100%), the NPV was 79.4% (95% CI, 62.1 – 91.3%), the PPV was 100% (95% CI, 86.8 - 100%), and the overall accuracy was 88.3% (95% CI, 77.4 - 95.2%).

In Group A no organism via both methods was detected, there were no false positive results. The specificity, negative predictive value and overall accuracy of the mPCR were 100% (95% CI, 86.8 - 100%) in the negative control group. 17 patients with aseptic knee and nine patients with aseptic hip revisions were included. The CRP was normal (<5 mg/L) in 23 cases, in three cases it was slightly elevated (< 10 mg/L), the LE tests were negative (negative or +) and the joint fluid was macroscopically clear in 25 aseptic cases, just in one case the synovial fluid was hemorrhagic so the LE test could not be performed. The mean age in this group was 71.6 years (range, 54-87 years). We had a 100% matching result in our negative control patients with aseptic loosening with no false positive controls.

Gram-positive bacteria	*Staphylococcus aureus*		**Enterobacteriaceae**	*Escheria coli*
	Staphylococcus epidermidis			*Enterobacter cloacae* complex
	Coagulase negative staphylococci			*Enterobacter aerogenes*
	Streptococcus mitis group			*Proteus* spp.
	Streptococcus anginosus group			*Klebsiella pneumoniae*
	Streptococcus salivarius group			
	Streptococcus pyogenes		**Non-fermenting bacteria**	*Pseudomonas aeruginosa*
	Enterococcus faecalis			*Acinetobacter baumannii*
	Enterococcus spp.			
			Anerobic bacteria	*Propionibacterium acnes*
Nutritionally variant Streptococci	*Granulicatella adiacens*			*Propionibacterium avidum/granulosum*
	Abiotrophia defectiva			*Finegoldia magna*
				Bacteroides fragilis group
Corynebacteriaceae	*Corynebacterium* spp.			
			Fungi	*Candida* spp.
				Candida parapsilosis
				Candida albicans

Figure 1. Coverage of the clinically relevant pathogens detected by the Unyvero® Multiplex PCR cartridge system. The most relevant pathogens and their sub-groups which have been reported in studies are shown. Modified account based on Curetis Unyvero®.

For Group B 26 patients with chronic PJI were included, with a mean age of 68.4 years (range, 41-83 years). The LE test was positive (++ or +++) for all patients. In six cases (23.1%) mPCR was not able to detect a pathogen, while tissue samples revealed a positive culture. For 20 cases (76.9% of Group B), the mPCR could detect an organism which was confirmed by the tissue cultures, in 19 cases (73.1%) both methods provided the same organism, and in one case (3.8%) entirely different organisms were found. The mPCR detected *Streptococcus agalactiae;* the culture of the peri-implant tissue detected *Staphylococcus lugdunensis* and *Staphylococcus epidermidis;* and the culture from the preoperative aspiration revealed again *Staphylococcus epidermidis*. The sensitivity and overall accuracy in this group were therefore 76.9% (95% CI, 56.4 - 91.0%), the positive predictive value was 100% (95% CI, 83.2 - 100%). All organisms found by the mPCR are shown in Table 1. Tissue cultures identified pathogens in all 26 cases.

In Group C we included 8 patients with a mean age of 73.5 years (range, 57-85 years, p-value 0.12). The LE tests of patients in this group were positive in all cases. In seven out of eight samples there were matching results from the mPCR and the microbiological culture. In six cases the organism identified by mPCR matched with the bacterial culture from the joint aspirate. In one aspirate, neither the mPCR nor the culture result matched to an organism; this patient had ongoing antibiotic therapy with cefuroxim for two weeks prior to surgery. In another case the mPCR and the microbial investigation of the joint fluid showed no evidence of infection, whereas the conventional microbial cultures from synovial samples obtained at the time of revision, showed evidence of PJI caused by *Staphylococcus epidermidis*. In this case the mPCR was also considered as false negative. In two patients of group C with proof of germs a preoperative antibiotic therapy was initiated with vancomycin and meropenem. Furthermore, in two of these eight patients with SIRS the mPCR could detect organisms whereas the aspiration was culture negative, the tissue culturing however confirmed these germs. The sensitivity of the mPCR in the patients with acute PJI and SIRS was 85.7% (95% CI, 42.1-99.6%) and the specificity was 100% (95% CI, 2.5 - 100%), the positive predictive value was 100% (95% CI, 54.1 - 100%), the negative predictive value was 50% (95% CI, 1.3 - 98.7%). Therefore the overall accuracy in this group was 87.5% (95% CI, 47.4 - 99.7%). The mean time for receiving the conventional culture result was 153.6 hours (range, 48-552 hours) whereas the results for mPCR required exact 5 hours in every case.

All patients' results are summarized in Table 2.

Discussion

The early diagnosis of PJI is a substantial challenge in clinicians' daily life. Especially in early infection with SIRS is time a very important factor. Although laboratory tests for PJI are useful screening tools, none of them has the capacity to make a diagnosis independently; a synthesis of several parameters makes it possible to achieve the proper diagnosis. Therefore, cultures are still the gold standard and the most important method for making the diagnosis of infection and for pathogen identification, determining also the proper surgical and antibiotic therapy. Disadvantages of these cultures include the need for a well-equipped microbiology lab, a complex culturing process and long time to achieve final results.

Poor sensitivity of standard cultures, longer incubation time to identify slow growing organisms, and an incidence of 20% of culture-negative PJI pose major issues for early diagnosis, particularly in cases with systemic compromise and SIRS [26]. Polymerase chain reaction (PCR) is reported to be a rapid molecular method for the identification of pathogens [16, 27]. This method has the advantages of providing fast results, being unaffected by previous administration of antibiotics, and showing higher sensitivity rates than cultures in such cases with PJI [28]. We investigated the diagnostic value of multiplex PCR in patients in which late chronic PJI was already diagnosed, and in patients with an acute PJI accompanied by SIRS, where proper identification of causative microorganism and its susceptibility are essential for a prompt and effective targeted treatment. A group of negative control patients with aseptic implant loosening after THA or TKA were also enrolled.

According to our results, mPCR is a useful tool in the diagnosis of PJI, nevertheless the overall accuracy of the method is less than 100%. Table 3 shows the statistical results of the whole collective of our study. The overall accuracy in acute infections with SIRS (87.5%) was greater than in late chronic PJI (76.9%). Similar findings have been shown by Villa et al. [29].

In the presence of SIRS, the mPCR test results were available rapidly within 5 hours compared to the microbial results which showed a positive culture after a mean time of 153.6 hours (6.5 days in average). In acute PJI with SIRS the mPCR had a quite high sensitivity of 85.7%, a specificity of 100%, and a positive predictive value of 100%, showing that this diagnostic tool has a role in the early detection of pathogens in acute scenario.

Table 2. Results of all study patients (n=60): the most important parameters of negative control patients (Group A), of patients with chronic PJI (Group B) and of individuals with acute PJI complicated with SIRS (Group C) are shown. Mismatch between results of mPCR and traditional cultures are highlighted with grey color. [CRP = C-reactive protein, LE = leukocyte esterase, mPCR = multiplex polymerase chain reaction, TKA = total knee arthroplasty, THA = total hip arthroplasty, Neg. = negative result, Hem. = hemorrhagic, CNS = coagulase negative Staphylococci]

Group	Age (ys.)	Joint	CRP (mg/L)	LE test	mPCR	Culture aspiration	Culture tissue sample	Preoperative antibiotics
A	75	TKA	1.3	Neg.	Neg.	Neg.	Neg.	none
n=26	69	TKA	5.9	Neg.	Neg.	Neg.	Neg.	none
	72	THA	1.4	Neg.	Neg.	Neg.	Neg.	none
	60	THA	1.8	Neg.	Neg.	Neg.	Neg.	none
	66	TKA	1.4	Neg.	Neg.	Neg.	Neg.	none
	73	THA	4.9	Neg.	Neg.	Neg.	Neg.	none
	86	THA	0.5	Neg.	Neg.	Neg.	Neg.	none
	77	TKA	1.3	Neg.	Neg.	Neg.	Neg.	none
	80	TKA	1.8	Neg.	Neg.	Neg.	Neg.	none
	71	TKA	2.6	Neg.	Neg.	Neg.	Neg.	none
	75	THA	2.2	Neg.	Neg.	Neg.	Neg.	none
	67	TKA	3.3	Neg.	Neg.	Neg.	Neg.	none
	65	THA	4.9	Neg.	Neg.	Neg.	Neg.	none
	54	TKA	4.8	Neg.	Neg.	Neg.	Neg.	none
	65	TKA	2.1	Neg.	Neg.	Neg.	Neg.	none
	81	TKA	5.6	Neg.	Neg.	Neg.	Neg.	none
	55	TKA	0.6	Hem.	Neg.	Neg.	Neg.	none
	57	TKA	2.1	Neg.	Neg.	Neg.	Neg.	none
	79	THA	0.4	+	Neg.	Neg.	Neg.	none
	80	TKA	0.3	Neg.	Neg.	Neg.	Neg.	none
	76	TKA	1.4	Neg.	Neg.	Neg.	Neg.	none
	74	TKA	1.0	Neg.	Neg.	Neg.	Neg.	none
	76	TKA	0.8	Neg.	Neg.	Neg.	Neg.	none
	87	THA	1.0	Neg.	Neg.	Neg.	Neg.	none
	75	TKA	7.8	Neg.	Neg.	Neg.	Neg.	none
	66	THA	1.2	Neg.	Neg.	Neg.	Neg.	none
B	56	THA	13.5	+++	P. acnes	P. acnes	P. acnes	Vancomycin / Meropenem
n=26	76	THA	23.7	+++	CNS	S. epidermidis	S. epidermidis	none
	73	TKA	65.3	+++	CNS	S. epidermidis	S. epidermidis	none
	74	THA	47.5	+++	CNS	Neg.	S. epidermidis	none
	71	THA	36.3	++	Neg.	P. acnes	P. acnes	none
	69	TKA	58.2	++	Neg.	S. aureus	S. aureus	none
	75	TKA	19.6	+++	E. faecalis	E. faecalis	E. faecalis	none
	68	TKA	79.4	+++	CNS	S. epidermidis	S. epidermidis	none
	54	THA	1.9	+++	CNS	S. capitis	S. capitis	none
	41	THA	18.2	+++	S. agalactiae	S. epidermidis	S. epidermidis S. lugdunensis	none
	74	THA	66.9	+++	Neg.	S. anginosus	S. anginosus	none
	57	THA	45.1	+++	CNS	S. capitis	S. capitis	none
	78	TKA	27.7	+++	CNS	S. epidermidis	S. epidermidis	none
	80	TKA	28.4	+++	S. aureus	S. aureus	S. aureus	none
	70	THA	8.5	+++	S. aureus	S. aureus	S. aureus	none
	52	TKA	33.5	+++	Neg.	Neg.	S. epidermidis	none
	72	TKA	9.3	+++	Neg.	Neg.	S. epidermidis	none
	62	TKA	20.7	+++	CNS	Neg.	S. epidermidis	none
	65	THA	41.8	+++	CNS	S. epidermidis	S. epidermidis	none
	59	THA	6.0	+++	P. acnes	P. acnes	P. acnes	none
	63	THA	8.0	+++	Neg.	Neg.	S. epidermidis	none
	74	TKA	14.4	++	E. faecalis	E. faecalis	E. faecalis	none
	83	THA	88.7	+++	S. aureus	S. aureus	S. aureus	none
	62	THA	16.9	+++	CNS	S. lugdunensis	S. lugdunensis	none
	57	THA	5.9	+++	CNS	S. capitis	S. capitis	none
	76	THA	40.8	+++	CNS	S. capitis	S. capitis	none
C	57	THA	106.0	+++	CNS	Neg.	S. epidermidis	Vancomycin / Meropenem
n=8	57	TKA	100.0	+++	Neg.	Neg.	S. epidermidis	none
	82	TKA	284.0	+++	S. aureus	S. aureus	S. aureus	Vancomycin / Meropenem
	69	TKA	164.0	+++	Neg.	Neg.	Neg.	Cefuroxim
	77	THA	75.5	+++	E. cloacae	Neg.	E. cloacae	none
	84	THA	122.0	+++	S. aureus	S. aureus	S. aureus	none
	79	THA	358.5	+++	CNS	S. epidermidis	S. epidermidis	none
	80	THA	258.8	+++	E. cloacae	E. cloacae	E. cloacae	none

Table 3. Overview of the statistical evaluation of the entire study group. [mPCR = Multiplex Poymerase Chain Reaction, **-** = negative mPCR result, + = positive mPCR result, CI = Confidence Interval, NPV = negative predictive value, PPV = positive predictive value, OA = overall accuracy]

	mPCR	
	-	+
	95 % CI	
Culture negative	27	0
Culture positive	7	26
Sensitivity	78.8 % [61.1 - 91.0 %]	
Specificity	100 % [87.2 - 100 %]	
NPV	79.4 % [62.1 - 91.3 %]	
PPV	100 % [86.8 - 100 %]	
OA	88.3 % [77.4 - 95.2 %]	

The most remarkable findings were the two acute PJI patients with SIRS and a negative culture of the joint aspirate but a positive identification of the pathogen by mPCR, which were confirmed later by microbial tissue culturing. To best of our knowledge, no published data exist concerning mPCR diagnostic in acute PJI accompanied by SIRS.

There are some clear limitations of the current study. These include the small overall number of cases, and therefore small study groups in comparison. Nevertheless, statistical analysis of the data was possible, demonstrating the clinical value of the multiplex PCR in diagnosing PJI. The diagnostic test with PCR is quite easy to carry out; however, it is necessary to be cautious, and technical procedures must be applied following a strict protocol. It is also necessary to apply a rigorous strategy when interpreting the results. A further limitation is the fact that results are compared with traditional cultures, actually the gold standard for bacterial identification, where contaminations may occur during obtaining the tissues and when handling in the lab. Intraoperative contamination of the study samples could be avoided with sterile aspiration technique during surgical preparation.

Since the first published studies using a molecular method for the diagnosis of PJI, several improvements and modifications have been made to increase effectiveness and decrease drawbacks. The potential of rapidly obtaining results is among the major advantages of PCR. Therefore, mPCR might be considered as a valuable tool in cases of infections with severe systemic compromise, especially because of the need for fast decision making [17]. There are series demonstrating the value of a prompt confirmation of the diagnosis and early infection treatment in different serious conditions, in a non-PJI context [19, 30].

Another advantage of PCR is the identification of specific bacteria in cases of culture-negative PJI, mainly as a possible consequence of previous antibiotic administration [6, 31]. The most common cause for negative cultures in case of suspicion of PJI is administration of an oral antibiotic prior to joint aspiration and cultures, a practice that should be avoided. In such scenario of culture negative PJI, PCR was demonstrated to be useful [32, 33]. A study that compared cultures obtained from sonication fluid and PCR for PJI determined that in patients with prior antibiotic therapy mPCR was positive in 100%, whereas cultures from sonication only in 42% [32]. In further studies could be shown another advantage that PCR of prosthesis sonication samples is more sensitive than tissue culture for the microbiologic diagnosis of PJI and provides same-day diagnosis with definition of microbiology [34, 35]. In a recent study was stated, that PCR and microarray-based platform provide the attractive possibility of faster bacterial diagnosis than with routine culture, and the molecular methods were most helpful in PJI diagnostics during ongoing antimicrobial treatment [36]. In our present study four patients received antibiotic therapy prior to surgery. The cultures of the joint aspiration were negative in two of those patients, and the PCR was positive in three of them. In one case neither the PCR nor the cultures of the aspiration or of the intraoperatively obtained tissue was positive. This patient underwent antibiotic therapy with cefuroxim before surgery and was admitted to our hospital as an emergency referral from abroad.

Regarding the SE and SP of PCR for the diagnosis of PJI, there are several series demonstrating superiority or at least similar results compared to traditional cultures according to the MSIS and ICM [14, 28, 37]. A recent meta-analysis determined a SE and SP for the PCR technique of 86% and 91% respectively [14]. The results of this meta-analysis were then modified by other authors; in their meta-analysis were more studies involved, and a narrower confidence interval could be achieved. This study revealed a SE of 79% and a SP of 86% for the PCR-based diagnostic in PJI, nevertheless authors suggest an adequate diagnostic value for PCR, especially in cases of low grade infections [38]. Only few studies showed lower sensitivity of PCR and suggested no benefits over cultures [39, 40]. In a prospective cross-sectional multicentre study a relatively low SE of 73.3%, but a high SP of 95.5% was demonstrated [39]. In another study 92 prosthetic joint revisions were evaluated with the use of a 16S rRNA gene PCR and showed a high SE (92%) but a low SP (74%), with a very low PPV of 34% [41]. 12 cases were diagnosed as infected according to the surgeon's opinion and laboratory tests. The PCR was positive in 32 cases and the authors attributed this false positive

result to contamination during the collection of the sample and also in the laboratory process. Contamination is one of the most important disadvantages. This issue has been broadly evaluated and there have also been solutions proposed [27, 42]. There are several steps that are crucial for avoiding contamination. The sample collection may be by fluid aspiration or periprosthetic tissue and should be performed under sterile OR conditions and the sample should also be processed immediately after obtaining. We acquired the sample by intraoperative aspiration, without skin contact or any manipulation of the sample. The second critical step and also a possibility for contamination is the manipulation at the DNA extraction. Our multiplex PCR is an automated system that allows these steps to take place in a controlled environment preventing contamination [42]. Another disadvantage of PCR is low availability as well as high costs, the equipment is for the most revision centres not affordable.

Multiplex PCR is able to detect specific organisms depending on the targeted primers used, unlike broad-range PCR that identifies nucleic acid sequences conserved in many bacterial species, but limited to use in the detection of polymicrobial infection. In another study a multiplex PCR testing with sonication cultures were compared, and showed positive results in 17 cases (100%) versus 29 cases (59%) respectively, but only after exclusion of 8 cases of PJI caused by *Propionibacterium acnes* and *Corynebacterium sp.* [32]. One limitation of multiplex PCR is that if it does not include the specific primers for the organisms, the result will be false negative. However, recent multiplex PCR systems have incorporated more primers, demonstrated by the inclusion of *P. acnes* and others in this study (Figure 1). The spectrum of organisms identifiable should always be taken into consideration.

Declarations

Each author certifies that his or her institution approved the human protocol for this investigation that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained.

This work was performed at Helios ENDO Klinik, Hamburg, Germany.

Competing Interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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