Culture and PCR analysis of joint fluid in the diagnosis of prosthetic joint infection

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SUMMARY

This prospective study compared PCR and culture techniques in the diagnosis of prosthetic joint infection (PJI). We obtained joint fluid samples (JFS; n=115) from patients who had failed total joint arthroplasty between January 2003 and June 2005; 49 were positive for PJI according to established strict criteria. JFS were analyzed by PCR (n=35; control n=66) or culture (n=46, control n=48). PCR was positive in 71% of PJI cases, resulting in sensitivity, specificity, accuracy, positive predictive value, negative predictive value, and likelihood ratio for positive results as follows: 0.71; 0.97; 0.88; 0.93; 0.87 and 23.6, respectively. Culture was positive in 44% of PJI samples. Corresponding statistics were 0.44; 0.94; 0.69; 0.87; 0.63 and 7.0, respectively. Significantly higher sensitivity, accuracy and negative predictive values were calculated for PCR versus culture, and there was 83% concordance between the results of intraoperative culture and PCR detection of causative bacteria. Therefore, we conclude that PCR analysis of synovial fluid increases the utility of pre-operative aspiration for patients who require revision total joint surgery.

KEY WORDS: Total joint arthroplasty, Prosthetic joint infection, Deep sepsis, PCR, Culture, Diagnostics

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INTRODUCTION

Prosthetic joint infection (PJI) is a serious complication of total joint arthroplasty (TJA). Based on the data from Swedish hip arthroplasty register, PJI is the second to third most common complication of total hip arthroplasty, behind aseptic loosening (Herberts *et al.,* 2004). Ideally, the diagnosis of PJI should be established prior to surgery because the recommended therapeutic strategy for revision due to PJI differs from that for revision due to aseptic reasons. In most cases, the diagnosis of PJI relies on a combination of clinical, laboratory, and imaging studies (Bauer *et al.,* 2006). Serological tests are indicated on the basis of clinical suspicion; if the results are negative, the probability of PJI is low, except cases with inflammatory diseases. However, if serological screening tests are positive, the probability of PJI is high and joint fluid aspiration is indicated. Differential leukocyte count (Trampuz *et al.,* 2004) and culture-based analysis are routinely performed. Both of these assays can strongly support the diagnosis of PJI, but culture can identify the causative bacteria, which is important for further therapy.

However, the utility of joint fluid culture has been repeatedly questioned based on high false negative rates (Bernard *et al.,* 2004), which may be

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due to bacteria-related factors such as their paucity in the joint fluid, highly fastidious growth, the biofilm nature of PJI, or the impact of previous antibiotic therapy. Moreover, sampling factors such as the surgeon's experience, use of special transport medium, time delay, anaerobic environment and improper laboratory practice may play some role as well. Therefore, there is a need for a method that will decrease the false-negative rate of culture assays or another method that could target the blind window of the culture technique.

With regard to improving culture performance, new transport and diagnostic sets have been developed and are in use (Ali *et al.,* 2006). Alternatively, polymerase chain reaction (PCR)-based methods provide a theoretically more sensitive means of detecting and identifying infectious bacteria; a few copies of bacterial DNA may be enough to obtain a positive result (Nazzal *et al.,* 2005; Ortu *et al.,* 2006). This method is dependent neither on the occurrence of viable bacteria in samples nor on antibiotic treatment history. The PCR technique, based on detection of highly-conserved 16S rDNA sequences common to all bacteria, was introduced into the diagnosis of PJI in the early 1990s (Levine *et al.,* 1995), and has met the demanding expectations of the orthopedic community.

Previously we reported on the performance of two different PCR protocols in the diagnosis of PJI (Sauer *et al.,* 2005; Gallo *et al.,* 2006). In this work, we report the results of a prospective study comparing culture- and PCR-based analysis of intra-operatively sampled joint fluid.

MATERIALS AND METHODS

Patient selection

This prospective study included 115 patients with total joint replacements who were treated at the Department of Orthopedics, University Hospital, Olomouc, Czech Republic, between January 2003 and June 2005. Forty-nine joints were considered septic (Table 1) according to the PJI criteria described herein. The control group (n=66) included patients with total joint arthroplasties revised for aseptic reasons (Table 2). All revisions were carried out under standard conditions with written informed patient consent, and the study was approved by the local Ethics Committee. In order to reflect true clinical situations, we included all patients who completed sampling in the study. As a result, the study population consisted of a wide spectrum of clinical cases that ranged from patients who required aseptic revisions to patients with highly probable PJI. Eighteen patients (16%) had received antibiotic pretreatment for a median of 18 days (minimum, 2 days) prior to JFS aspiration.

Definition of PJI

PJI was diagnosed according to the following criteria:

1) presence of sinus tract communicating with a joint and/or intra-articular pus;

TABLE 1 - *Basic characteristics of the group of patients revised due to PJI (n=49).*

Variables	*Report*
Males/Females	17/32 (35% 65%)
Age at revision surgery	69.8 years (39-80, SD 9.7)*
Time to prosthetic failure	21.7 months (0.6-152, SD 35.2)
Site of PJI	Hip - 19 (39%) Knee - 29 (59%) Elbow - 1 (2%)
PJI modes	Early - 12 (24%) Delayed - 19 (39%) Late - 11 (22%) Relapsing infection - 2 (4 %) Joint sepsis from the surroundings - 5 (10%)
CRP	Positive - 41 (84%)
ESR	56 mm/h (6-180, SD 39.5)
Scintigraphy (n=24)	Positive - 18 (18/24, 75%)
Histological examination (n=46)	Positive - 36 (36/46, 78%)

CRP = C-reactive protein, ESR erythrocyte sedimentation rate. *Values are presented as the median (minimum-maximum value, SD = Standard Deviation).

TABLE 2 - *List of variables describing the control group (n=66).*

Variables	*Report*
Males/Females	25/41 (38%/62%)
Age at revision surgery	62 years (35-80, SD 10.9)*
Time to prosthetic failure	88 months (12-240, SD 39.5)
Site of revision	Hip - 52 (79%) Knee - 14 (21%)
Reasons for revision	Aseptic loosening - 25 (38%) Periprosthetic osteolysis - 38 (58%) Other reason - 3 (5%)
CRP	Negative - 59 (89%)
ESR	18 mm/h (2-78, SD 14.0)
Histological examination (n=63)	Negative - 62 (62/63, 98%)

*Values are presented as the median (minimum-maximum value, SD = Standard Deviation).

2) coincidentally positive results of histological examination (five or more neutrophils per high power field) and culture of intraoperative samples (Spangehl *et al.,* 1999);
3) if only intraoperative culture or histological results were positive, then at least two of the following signs had to be present: high clinical suspicion of infection (acute onset, fever, erythema, edema, persistent local pain, early prosthetic failure, wound healing disturbances, etc.), erythrocyte sedimentation rate >30 mm/h, C-reactive protein elevated more than 1.5 times above the laboratory reference value, positive 99m Technetium leukocyte scintigraphy. Plain radiography was not included in the diagnostic algorithm because of its low specificity with regard to PJI (Bauer *et al.,* 2006). Aseptic revisions were those that did not fulfill the conditions for PJI.

Three PJI modes were distinguished (Trampuz and Zimmerli, 2005): early postoperative infection (onset before the 4th postoperative month), delayed infection (onset at 4 to 24 months postoperative), and late infection (manifesting more than 2 years after surgery). Relapsing infection was defined as repeated failure of a previously revised joint due to repeat infection by the same bacteria regardless of the time since surgery. Direct spreading from infected surroundings was assumed, such as when erysipelas was followed by PJI of knee arthroplasty.

Culture

In order to minimize the risk of false positive results, synovial joint fluid aspiration was performed prior to surgical joint capsule incision. A minimum of 1 ml per method under study was collected, and aspirated fluids were transported in collection syringes. After opening the joint, granulation tissue was sampled from at least three different sites (one from the capsule and two from host bone beds). Further, hard swabs were taken from the implant surfaces. After that, systemic or local antibiotic administration was performed in all but the 18 patients who had received previous antibiotic treatment. Overall, we collected at least five samples (range, 5-9) per patient. Tissue samples were deposited in sterile tubes, while swabs were kept in transport medium (Amies medium, Dispolab, Czech Republic). All materials were transported to the laboratory immediately after sampling. Specimens were processed by the laboratory within 2 hours of collection. JFS were centrifuged and sediments were inoculated into 5 ml of Brain-Heart Infusion (BHI) broth (Becton Dickinson) and onto aerobic sheep blood agar plates, chocolate agar plates, and anaerobic sheep blood agar plates (Becton Dickinson). Aerobic sheep blood agar plates and chocolate agar plates were incubated in 5-10% CO_2 at 35-37ºC; anaerobic sheep blood agar plates were cultivated anaerobically at 35-37ºC. We examined subcultures for growth at 24, 48, 72, 96, and 120 hours. BHI broth was cultivated for 24 hours and then 1 ml was transferred to each of the above plates. Tissue samples and swabs were homogenized in 5 ml of BHI broth with tissue mill for 1 minute and the homogenate was processed in the same manner as JFS.

Bacteria were diagnosed using standard microbiology procedures for aerobic and anaerobic culture accompanied by direct sample microscopy (Isenberg, 2004). Susceptibility of the isolates to antimicrobial agents was assessed by the stan-

dard dilution micromethod (Performance standards for antimicrob. susceptibility testing, 2007). Two *Staphylococcus aureus* reference strains, ATCC 29213 and ATCC 25923, were used to meet the protocol-defined quality control.
Interpretation of the cultures: joint fluid aspirations were considered positive if there were any signs of growth, including in enrichment broth, but concordance between these and intraoperative findings was required. Intraoperative samples were considered positive for infection if the same bacterium was cultured from at least two different operative sites (Atkins *et al.,* 1998).

PCR analysis
PCR assay targeting the 16S rDNA gene was carried out in only 101 patients due to financial limitations. Joint fluid aspiration was performed in the same manner as for culture. In rare cases of dry puncture, the joint was injected and re-aspirated with Ringer's solution.
DNA was extracted from joint fluid samples using a commercially available kit (QIAmp DNA Blood Mini Kit, Qiagen, Germany). DNA isolated from a 500 µL sample was eluted with 50 µL of elution buffer. Positive and negative controls were processed in conjunction with the examined sample. The presence of bacterial DNA was based on PCR amplification from primers complementary to phylogenetically conserved segments of the 16S rDNA gene.
The 50 µL PCR volume contained 25 pmol of each primer (forward primer RW01: 5' aac tgg agg aag gtg ggg at 3'; reverse primer DG74: 5' tgc ggt tgg atc acc tcc t 3'); 1× HotStart Taq Master Mix (Qiagen, Germany); 0.7 mM of $MgCl_2$; 100 mM of dUTP (Roche Diagnostics, Germany), and 8-methoxypsoralen (Sigma, USA) at a final concentration of 25 µg/mL. All components were mixed and the PCR mixture was incubated at 4°C and then decontaminated by UV light (265 nm). Following decontamination, PCR MIMIC internal standard and 5 µL of the sample were added (Siebert and Larrick, 1997). The PCR reaction was performed in the PTC-200 thermocycler (MJ Research, USA) under the following amplification conditions: initial denaturation for 15 minutes at 96°C followed by 45 amplification cycles comprising 10-second denaturation at 96°C, 10-second annealing at 58°C and 30-second extension at 72°C, followed by final extension for 2 minutes at 72°C. Amplification products were resolved by electrophoresis on 2% agarose gel containing 5 µg/ml ethidium bromide at 5 V/cm, and visualized by UV illumination.
Bacteria species were identified by restriction endonuclease (HaeIII and NcoI; New England Biolabs, USA) analysis of the amplification products according to standard protocols (Lu *et al.,* 2000).
Interpretation of PCR assays: In negative samples, only the 477-bp amplification product (PCR MIMIC internal standard) was detected, whereas an additional 370-bp amplification product of the 16S rDNA gene was present in positive samples. With regard to bacteria identification, congruency between the restriction enzyme analysis and culture analysis on the species level provided definitive results. When results were discordant, the culture results were considered to be true positives.

Study design, statistical analysis
During the study neither the microbiologists nor the molecular biologists knew the clinical and histological data. The data were collected and continuously entered into electronic spreadsheets (Microsoft Excel, Microsoft Corp., WA, USA). After completion of the study, a consultant orthopedic surgeon and two experienced microbiologists classified the patients into two groups (those with and those without PJI) according to the criteria described above. All statistical calculations (sensitivity, specificity, accuracy, positive and negative predictive values [PPV, NPV], positive and negative likelihood ratios [LR+, -] and confidence intervals) were performed using SPSS v.10.1 (SPSS Inc., Chicago, USA). These values were further evaluated by means of the test of the equality of two ratios. Statistical significance was set at p=0.05.

RESULTS

Diagnostic utility of PCR
The details of the PCR technique are shown in Table 3. Concordance between PCR and intraoperative culture methods for identifying causative pathogens could be evaluated for 23 patients. In 19 cases (82.6%) the same bacteria were identified by both methods at the species-level, where-

TABLE 3 - *Results of PCR assay of joint fluid obtained from total joint arthroplasty.*

	PJI	*Controls*	
PCR positive	25	2	27
PCR negative	10	64	74
	35	66	101
			95% CI
Sensitivity	71.4	61.5	75.5
Specificity	97.0	91.7	99.1
Accuracy	88.1	81.8	94.4
Positive predictive value	92.6	79.8	97.9
Negative predictive value	86.5	81.8	88.4
Likelihood ratio +	23.6	5.90	93.8
Likelihood ratio -	0.29	0.17	0.49

Likelihood ratio +, -: Likelihood ratio for positive, negative result.

TABLE 4 - *List of characteristics for culture of joint fluid obtained from total joint arthroplasty.*

	PJI	*Controls*	
Culture positive	20	3	23
Culture negative	26	45	71
	46	48	94
			95 % CI
Sensitivity	43.5	35.2	47.7
Specificity	93.8	85.8	97.8
Accuracy	69.1	59.8	78.5
Positive predictive value	87.0	70.5	95.3
Negative predictive value	63.4	58.0	66.1
Likelihood ratio +	7.0	2.2	21.8
Likelihood ratio -	0.60	0.46	0.79

Likelihood ratio +, -: Likelihood ratio for positive, negative result.

as for 4 patients the results from PCR and culture assays differed. In one case, *Staphylococcus aureus* recovered by culture was identified by PCR as *Enterobacteriaceae*; two failures were due to staphylococci and streptococci mismatch, and in the last case *Streptococcus intermedius* was identified by PCR as atypical mycobacterium.

Diagnostic utility of joint fluid cultures

Synovial joint fluid cultures from 94 joints were examined (46 PJIs and 48 controls). Of these, 20 were true positives and 26 were false negatives (Table 4). When considering only the PJI cases who had received antibiotics prior to joint fluid sampling, sensitivity, specificity, accuracy, PPV, NPV, and LR+/- were: 0.46, 0.94, 0.85, 0.63, 0.88, 7.3, and 0.6, respectively. Culture cases with and without pre-aspiration antibiotic administration did not differ significantly in any of the compared parameters.

Comparisons of culture and PCR results

The sensitivity (p=0.014), accuracy (p=0.0014), and NPV (p=0.0016) were significantly greater for the PCR technique than for the culture technique, as determined by the test of the equality of two ratios (Table 5). However, the LR+s for the two techniques were not significantly different.

Intraoperative culture

In 42 cases (85.7%), intraoperative sample culture identified the infectious pathogen; no pathogens were detected in 7 patients (14.3%). Interestingly, in three negative cultures, PCR detected *Staphylococcus* sp., *Streptococcus* sp.-*Enterococcus* sp. group, and "universal DNA of fungus".

Table 6 shows the frequency of PJI bacterial pathogens. Three coagulase-negative staphylococci (15.8%) and one *Staphylococcus aureus* strain (8.3%) were resistant to oxacillin. All staphylococci were susceptible to glycopeptides (vancomycin, teicoplanin) and linezolid, while five strains were resistant to clindamycin (16.1%).

TABLE 5 - *Comparisons of characteristics derived for each method under study by test of the equality of two ratios. The accepted significance level was 0.05.*

	PCR	*Culture*	*Significance*
Sensitivity	0.714	0.435	0.014
Specificity	0.97	0.938	0.410
Accuracy	0.88	0.691	0.0014
PPV	0.926	0.870	0.513
NPV	0.865	0.634	0.0016
LR+	23.6	7.0	NS

PPV = positive predictive value; NPV = negative predictive value; NS = non-significant.

TABLE 6 - *Bacterial pathogens detected in 42 PJI included in the study.*

Pathogens	*Absolute number*	*Percentage*
Staphylococcus sp. (coagulase-negative; *S. epidermidis*, *S. haemolyticus* and *S. hominis*)	19	38
Staphylococcus aureus	12	24
Streptococcus sp. (viridans group)	5	10
Escherichia coli	3	6
Pseudomonas aeruginosa	2	4
Serratia marcescens	2	4
Acinetobacter baumannii	1	2
Corynebacterium sp.	1	2
Enterococcus faecalis	1	2
Haemophilus sp.	1	2
Streptococcus pyogenes	1	2
Peptococcus sacharolyticus	1	2
Peptostreptococcus anaerobius	1	2

DISCUSSION

Based on the literature, PCR should be considered a helpful addition to the diagnostic tools used in cases of bone and joint infection, with particular clinical value in cases with negative culture results (Tarkin *et al.,* 2005; Fenollar *et al.,* 2006). One of the main advantages of PCR is its high sensitivity regardless of cell viability, fastidious growth, or previous antibiotic treatment. On the other hand, the lack of standard protocols for PCR technique and the unequal quality of the reporting has prohibited direct comparison of published results (Gallo *et al.,* 2004).

In this prospective study, we found that PCR analysis of JFS had significantly higher sensitivity, accuracy and NPV than culture of the same material. However, the sensitivity of the PCR protocol used in our study was only 71%, lower than that reported by others (Mariani *et al.,* 1996; Rozkydal *et al.,* 1999; Panousis *et al.,* 2005), although Kordelle *et al.* (2004) reported a much lower sensitivity of PCR for intraoperative PJI diagnosis. Theoretically, a single copy of bacterial DNA is sufficient for successful detection and identification of bacteria. However, the probability that this single copy would be sampled for examination is low, so many more copies are needed in order to reach the "detection threshold". This is particularly true for joint fluid investigations because the amount of bacterial DNA is not a simple function of the extent of bacterial colonization or infection (Gallo *et al.,* 2004), it is instead influenced by numerous factors, including the biofilm staging and phagocytic cells activity. In order to maximize the impact of PCR in PJI diagnostics and to enable results comparison, a standardized PCR assay that can be readily incorporated into routine procedures by diagnostic laboratories is required.

In general, the predictive value of most laboratory tests increases with the increasing rate of infection among patients being studied. In line with this rule, the PPV of the PCR protocol used in our study was 92.6% and the prevalence of PJI was 0.347, suggesting that the PCR assay is better used to confirm the diagnosis than to exclude it. Likewise, at our institution joint fluid aspiration is indicated only for patients with high pre-test probability of PJI and not for screening. Furthermore, the LR for a positive PCR result (LR+ =23.6) indicates that a positive PCR result is almost 24 times more likely to be found in a patient with a PJI than in a patient without it. Surprisingly, both of these parameters were much lower in previous studies (Mariani *et al.,* 1996; Rozkydal *et al.,* 1999; Panousis, *et al.,* 2005) than in this study, despite the fact that the prevalence of PJI in these studies was comparable to the prevalence in our study. The one exception comes from the study by Panousis *et al.* who reported a PJI prevalence of only 13%. Similarly, the detection threshold of the PCR reaction in our study was comparable with the detection thresholds in previous studies, except for that of Panousis *et al.* who did not determine the detection threshold at the outset.

One important therapeutic implication of PCR analysis is the rapid identification of causative agents, enabling targeted antimicrobial therapy

(Trampuz and Zimmerli, 2005). Bacterial identification can be achieved by restriction endonuclease assay (Lu *et al.,* 2000), using a mixture of species-specific primer sets (Yang and Rothman, 2004) or sequencing of amplified DNA products (Xu *et al.,* 2005); the latter two approaches have better potential for diagnosing polymicrobial infections. Fenollar *et al.* (2006) published excellent results for cloning and sequencing of 16S rDNA amplicons. They reported an overall congruency between culture and PCR results in 475 of 525 samples (90.5%), with 89 patients presenting positive results and 386 patients presenting negative ones. They detected discrepancies between culture and PCR identification of causative pathogen in seven cases, all of which were polymicrobial infections. Kordelle *et al.* (2004) sequenced the 16S rDNA amplicons and found 100% concordance between PCR- and culture-based pathogen identification, but they evaluated only seven cases of PJI. In our study, the congruency between PCR- and culture-based pathogen identification in JFS was 83%. We observed four cases of discrepancy between the two methods, with an especially interesting mismatch between *Streptococci* and atypical mycobacterium. The reasons for this discrepancy are not clear because restriction enzyme analysis was not followed by DNA sequencing. In three negative intraoperative cultures, PCR detected *Staphylococcus* sp., *Streptococcus* sp.-*Enterococcus* sp. group, and "universal DNA of fungus".

The sensitivity of joint fluid culture for detecting and identifying the infectious organism was low in our study, despite the rigorous practices of the certified laboratory. However, this phenomenon is not uncommon, and some investigators report even lower sensitivities (Teller *et al.,* 2000). Slightly better results were published by Barrack *et al.* (1997) who investigated knee arthroplasties, but only in cases without antibiotic pretreatment. In another study by Somme *et al.* (2003), JFS culture recovered the causative organisms in only 60.5% of cases with or without previous antibiotic treatment. On the other hand, significantly better sensitivities were reported by other authors who used protocols similar to ours. Our use of syringes as transport medium in place of a specialized device might be questioned. However, Ali *et al.* (2006) utilized a special transport device (BacTec culture bottle) and reported an increased rate of false-positive results, while Lachiewicz *et al.* (1996) and Spangehl *et al.* (1999) reported better results than did Ali *et al.*, despite their lack of a special transport device.

CONCLUSION

In this study, we found a higher PPV and LR+ for PCR than for culture analysis of JFS for diagnosing PJI in patients undergoing revision joint arthroplasty. Therefore, we recommend incorporating PCR analysis of JFS into the preoperative diagnostic algorithm for patients with a high probability of PJI.

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