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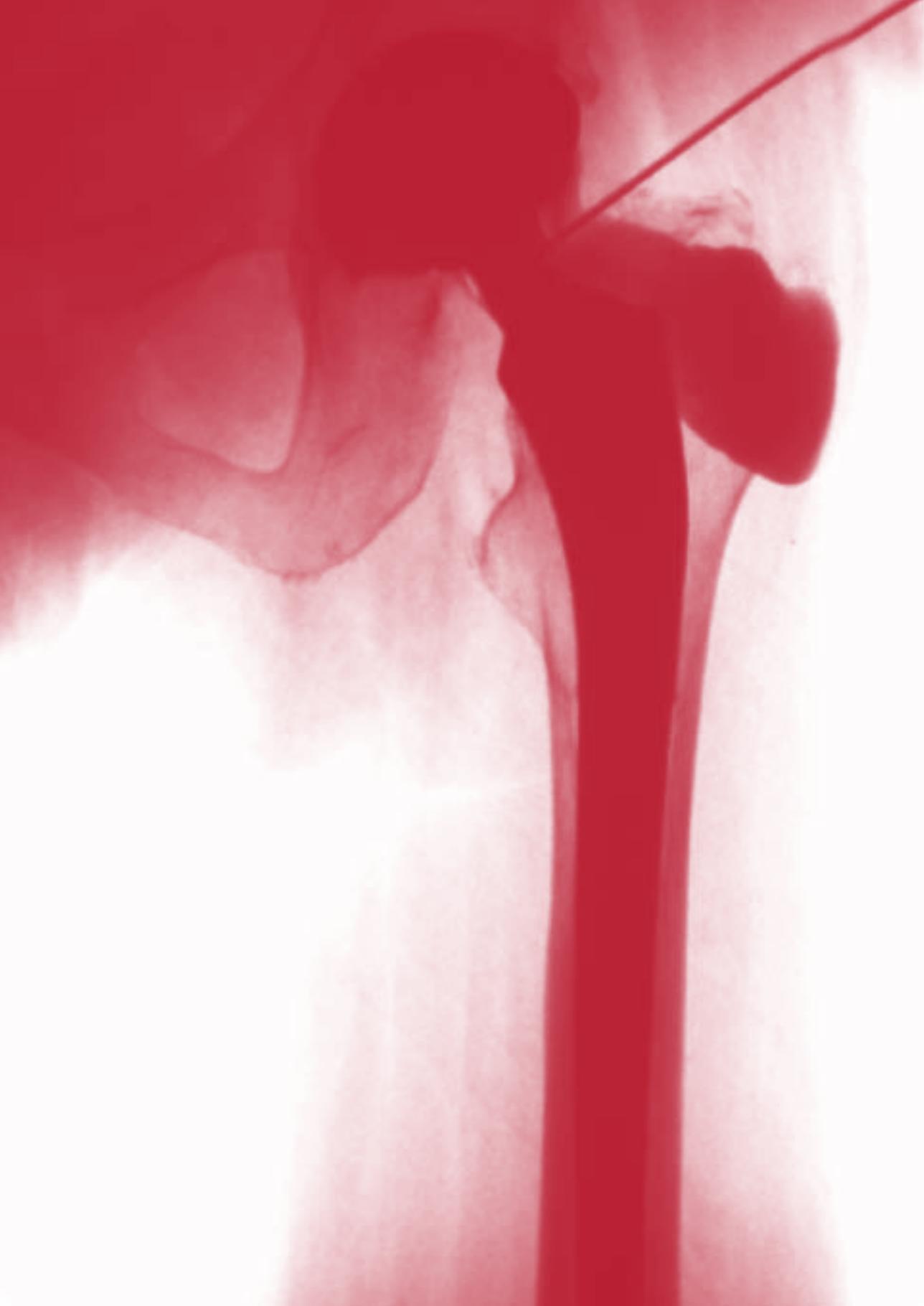
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Chapter 12

## **Broad-range PCR in selected episodes of prosthetic joint infection**

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## Abstract

Prosthetic joint infection (PJI) is associated with high morbidity and costs. For the diagnosis of PJI, the results of microbiological cultures are frequently used as the reference standard, but culture-independent molecular methods have been developed to improve the detection of the causative pathogen. Polymerase chain reaction (PCR) has yielded good results in detecting soft tissue infections. This study reviewed patient characteristics and the diagnostic value of PCR in 26 patients with 29 episodes of suspected PJI and their complex history: infection and/or multiple interventions for the same arthroplasty and/or antimicrobial treatment prior to presentation were evident in the majority of patients' histories. The specificity and sensitivity for the bacterial culture were 71% and 58%; for PCR, 94% and 50%, respectively. The cumulative sensitivity of the combination of both methods was 67%. In this study, PCR showed a limited diagnostic value but helped to identify false-positive culture results in few episodes. The high expense and the limited diagnostic value of broad-range PCR in PJI argue against its routine use in addition to bacterial culture.

## Introduction

Prosthetic joint infection (PJI) is, after aseptic loosening, the second most common cause of implant failure<sup>1</sup>. PJI is associated with high morbidity and high health care expenditures<sup>2</sup>. Successful outcome requires detection and identification of the causative pathogen(s) in order to select the appropriate antibiotic regimen. Current microbiology laboratory methods to diagnosis PJI depend on the detection of a pathogen by culture. However, the sensitivity of these methods is not ideal due to e.g., prior antimicrobial exposure, an inappropriate culture medium, or a low bacterial load<sup>3</sup>. Furthermore, the specimen could be contaminated, decreasing specificity of tests<sup>3</sup>. Therefore, culture-independent molecular methods have been used to improve the diagnosis of prosthetic joint infection. Broad-range polymerase chain reaction (PCR) is a molecular diagnostic method that enables replication and amplification of genetic (DNA) material<sup>4</sup>. For the diagnosis of infection in the clinical setting, the target is usually the 16S ribosomal RNA gene, present in all bacteria. PCR has been used successfully to detect pathogens that cause organ<sup>5</sup> and native joint infections<sup>6,7</sup>. As an extension of these practices, several investigators have reported the use of PCR to diagnose PJI<sup>8-18</sup>. However, a diagnostic advantage over the bacterial culture has as yet not been clearly established<sup>11,14,15</sup>.

Several drawbacks need to be considered when using the PCR technique, including the lack of susceptibility results (except for MRSA), problems in identifying a mixture of pathogens in a single specimen, and the possibility of contamination<sup>3</sup>. However, this molecular method can detect bacterial RNA in samples when conventional cultures yield negative results due to previous antimicrobial exposure or unfavorable growth conditions. Taking these considerations into account, PCR is only used in our institute if the chance of bacterial growth is low because of previous surgical and antimicrobial treatment or previous negative culture results. In order to (re)assess the diagnostic value of PCR in those selected episodes of possible PJI for which this method had been applied, we performed a retrospective study to define patient characteristics and to estimate the specificity and sensitivity of PCR versus bacterial culture. Our orthopedic surgery clinic is a 48-bed unit that acts as a primary care center for all types of orthopedic surgery on the extremities and as a tertiary care center for patients needing revision arthroplasty.

## Materials and Methods

The study population consisted of patients with a joint prosthesis who were seen at our clinic between 2001 and 2005, and from whom specimens both for bacterial culture and PCR had been obtained, either when there was suspicion of PJI based on (i) clinical signs (joint pain, effusion, erythema and warmth at the implant site), elevated laboratory signs of inflammation, signs of implant loosening or (ii) the presence of a sinus tract or a reported purulence surrounding the component during a previous intervention<sup>19</sup>. Patient history prior to the diagnostic intervention was assessed for (i) antimicrobial treatment, (ii) number of interventions performed on the affected arthroplasty, (iii) a confirmed and treated infection involving the affected joint within the previous 24 months, and (iv) implant loosening  $\leq$  12 months after implantation without other clinical signs of infection. Specimens (synovial fluid and/or biopsies from periprosthetic tissue) were obtained either during aspiration prior to surgery or during arthroscopy or open surgery. Bacterial culture and histopathologic diagnostics were performed as previously described<sup>20</sup>. The decision whether or not to use PCR was made by the physicians in charge after reviewing patient history and prior to the intervention. However, they were not involved in either analyzing or interpreting the data. Specimens were sent to a reference laboratory for analysis<sup>21</sup>. The broad-range PCR technique consisted of amplification of bacterial 16S ribosomal RNA<sup>4,21</sup>. As a reference with which to compare test results, we used well-defined criteria for the diagnosis of definitive

PJI. Infection was considered to be definite in case of the presence of one of the following: (i) clinical signs of infection in combination with laboratory or radiological signs of infection, (ii) a sinus tract communicating with the prosthesis, (iii) purulence surrounding the prosthesis at surgery, or (iv) acute or chronic inflammation consistent with infection on histopathologic examination<sup>19,22</sup>. Infection was excluded when none of the above-mentioned criteria had been fulfilled, no antimicrobial treatment had been administered after the episode, and no relapse had occurred for at least one year<sup>19</sup>.

## Results

In 29 episodes (26 patients) with a suspicion of PJI, specimens for both PCR and bacterial culture had been obtained. This number accounted for 7.6% (23 episodes) of all revision arthroplasties (n = 301) and 6.8% (6 episodes) of all joint punctures (n = 88) during the study period. Patient characteristics are presented in table 1. Most episodes (48%) included a history of  $\geq 3$  surgical interventions (median 4, range 3-5) on the affected arthroplasty within a median time of 3.25 years (range 0.9-10.5) prior to the diagnostic intervention. The median number of specimens obtained per patient for bacterial culture was 5 (range 3-7) and for PCR 1 (range 1-2).

In 17 (59%) of the 29 episodes, no infection was present, although duration of follow-up was  $\leq 12$  months in 2 cases (4, 11 months) due to non-episode-related death and loss to followup. In all 17 cases with clinically excluded infection, PCR was negative. In contrast, there was bacterial growth in 5 episodes, but interpreted as contamination. In the infection episodes, one inaccurate PCR result was considered as false-positive. Based on these findings, the specificity for bacterial culture was 71% and that for PCR, 94%.

In 12 (41%) of the 29 episodes, criteria for PJI were fulfilled; these 12 episodes accounted for 8.5% of 142 confirmed PJI episodes (including 62% referred cases) during the study period. The results of bacterial culture and PCR are presented in table 2. In 5 episodes, both diagnostic methods identified the same microorganism. However, in 2 of these, PCR revealed only one pathogen, while two distinct microorganisms grew in culture. In 2 further episodes, the pathogen was identified either only by bacterial culture or only by PCR. Hence, the sensitivity for bacterial culture was 58% and that for PCR, 50%.

Characteristic	Number of episodes (%)
<b>Age (years)</b> median 70.5 (range 53-80)	
<b>Gender</b> male 13 female 13	
<b>Arthroplasty affected</b> hip knee	13 (44.8%) 16 (55.2%)
<b>Patient history prior to presentation</b> $\geq 3$ interventions performed on the arthroplasty	14 (48%)
confirmed and treated infection of the arthroplasty $\leq 24$ months prior to presentation	9 (31%)
previous antimicrobial treatment	8 (27.5%)
0 – 2 weeks prior to collection of specimen	3
2 – 4 weeks prior to collection of specimen	1
4 – 8 weeks prior to collection of specimen	4
implant loosening $\leq 12$ months after implantation without other clinical signs of infection	6 (21%)

**Table 1:** Demographic data and history of 26 patients with 29 episodes of suspected PJI from whom specimens for bacterial culture and broad-range PCR had been obtained.

Episode	Bacterial culture number of positive/ total number of specimens pathogen	Broad-range PCR number of positive/ total number of specimens pathogen
1 synovial fluid	2/2 <i>Streptococcus pyogenes</i>	1/1 <i>Streptococcus pyogenes</i>
2* periprosthetic tissue	2/13 <i>Propionibacterium spp.</i>	1/8 <i>Streptococcus infantis</i>
3 synovial fluid	1/1 <i>Staphylococcus aureus</i>	1 negative
4 periprosthetic tissue	1 no growth	1 negative
5 periprosthetic tissue	1 no growth	1 negative
6 synovial fluid	1 no growth	1/1 <i>Streptococcus bovis</i>
7 periprosthetic tissue	5/11 <i>Staphylococcus aureus</i> 1/11 <i>Enterococcus faecalis</i>	1/1 <i>Staphylococcus aureus</i>
8 periprosthetic tissue	6 no growth	2 negative
9 periprosthetic tissue	6/9 <i>Staphylococcus epidermidis</i>	1/1 <i>Staphylococcus epidermidis</i>
10 periprosthetic tissue	5/6 <i>Pseudomonas aeruginosa</i> 1/6 <i>Enterococcus faecalis</i>	2/2 <i>Pseudomonas aeruginosa</i>
11 periprosthetic tissue	1/5+ <i>Staphylococcus epidermidis</i>	1/1 <i>Staphylococcus epidermidis</i>
12 periprosthetic tissue	5 no growth	1 negative

**Table 2:** Results of bacterial culture and broad-range PCR in 12 episodes of confirmed PJI.

\* Identification of the pathogen in this episode was inconclusive, since in both diagnostic methods the ratio of number of positive : total number of specimens was low. However, based on the previous history (duration of symptoms 19 months) and the small fragment for amplification isolated from the biopsy, *Propionibacterium spp.* was interpreted as possible pathogen, and *Streptococcus infantis* as contamination.

+ Results from diagnostic arthroscopy. Four weeks later, a one-stage revision of the TKA was performed and *Staphylococcus epidermidis* grew in 8 out of 16 obtained biopsies.

## Discussion

Standard microbiological cultures are frequently used as the reference standard for diagnosing PJI. However, prior antibiotic treatment, low numbers of the organism, or fastidious pathogens may be responsible for false-negative results. The method of broad-range PCR is capable of detecting bacterial DNA, even when cultures are negative, and this technique has proven useful in the diagnosis of native joint infection<sup>7</sup>. As a result, several investigators have addressed the usefulness of PCR in diagnosing PJI<sup>9-12,14,16-18</sup>. Using this approach, while a high specificity (95% to 100%) has been reported<sup>9,11,16</sup>, the sensitivity was often<sup>10,11,16,17</sup> poor ( $\leq 50\%$ ), and the conclusion was that PCR provided no advantage over the standard cultures in diagnosing PJI.

From these studies, however, little information is available to select episodes in clinical practice for which PCR could be superior or complementary to bacterial culture to diagnose PJI. The previous history of the patients included in this study (table 1) highlights the importance of distinguishing PJI from other causes of joint failure. Therefore, it was reasonable to expand diagnostic means by an additional tool in these selected episodes.

Specificity was excellent in our selected episodes, in accordance to earlier studies<sup>9,11,12,14,16-18</sup>. In all episodes with a previous history of multiple revisions or previously treated PJI, PCR remained negative when no infection was present. Also, in only 1 out of 6 episodes with early implant loosening ( $\leq 12$  months after implantation) an infection was present without other clinical signs of PJI, and the PCR results matched accordingly. Importantly, in 5 episodes PCR was useful in identifying bacterial culture false-positives. The sensitivity of PCR in our study was poor (50%). The overall sensitivity in diagnosing PJI increased to 67% when both PCR and bacterial culture were considered together (i.e. one or other or both were positive).

Mariani et.al, performed PCR on synovial fluid of 50 symptomatic TKAs and detected bacterial DNA in 60%, whereas standard tissue cultures revealed pathogen growth in only 30% of the cases<sup>12</sup>. These results suggested that PCR is more sensitive in diagnosing PJI than standard cultures. However, the presence of PJI was not defined by well-formulated standard criteria, as was in our study; therefore it is possible that some PCR results were false-positive.

To our knowledge, there are two studies<sup>15,18</sup> that used well-formulated criteria to diagnose the infection and which found a higher sensitivity of PCR in diagnosing PJI as compared to standard cultures<sup>23</sup>. Panousis et al<sup>15</sup> performed PCR on synovial fluid in 92 episodes of failed arthroplasties (both TKA and THA) and reported a sensitivity of 92%, but specificity was a low 74% which was possibly the result of contamination, and therefore the regular use of PCR was not recommended. Mooijjen et al.<sup>18</sup>, performed PCR on tissue samples. The sensitivity was 97%, but 5 out of 40 PCR positive results did not have an infection, leading to a specificity of 88%, which was lower than the specificity of the standard culturing. Moreover, the characteristics of the included episodes were heterogeneous: 17 episodes out of a total of 76 were not associated with an arthroplasty. Hence, these results might not be completely applicable to a group of patients with possible PJI. Since our study was performed retrospectively, and patients selected for PCR were not recruited according to strictly predefined criteria, the study could be influenced by a selection bias. Therefore, no immediate recommendations about the use of PCR in PJI can be made.

However, in our opinion and that of others<sup>10,11,14,15</sup>, the expense and diagnostic value of PCR in comparison to bacterial culture warrants only selective use of this molecular method; it is not suited for routine use in addition to a bacterial culture. In our center, PCR was performed in less than 10% of all revision arthroplasties (i.e., in patients with a complex history of joint disease); the limited diagnostic value was shown. PCR was helpful in recognizing false-positive culture results in less than 20% of the episodes. For clinical practice, more studies are required in order to identify both a patient population and a diagnostic strategy in which the use of this molecular method would be beneficial in diagnosing or excluding the possibility of PJI.

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