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ORIGINAL ARTICLE

Molecular Identification of Bacteria from Aseptically Loose Implants

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Abstract Polymerase chain reaction (PCR) assays have been used to detect bacteria adherent to failed orthopaedic implants, but some PCR assays have had problems with probable false-positive results. We used a combination of a Staphylococcus species-specific PCR and a universal PCR followed by DNA sequencing to identify bacteria on implants retrieved from 52 patients (92 implants) at revision arthroplasty. We addressed two questions in this study: (1) Is this method able to show the existence of bacterial DNA on presumed aseptic loosed implants?; and (2) What proportion of presumed aseptic or culture-negative implants was positive for bacterial DNA by PCR? Fourteen implants (15%) were believed infected, whereas 74 implants (85%) were believed aseptic. Each implant was sonicated and the resulting solution was submitted for dual real-time PCR assay and culture. All implants believed aseptically loose were culture-negative, but nine of the 74 (12%) had bacterial DNA by PCR; two (2.7%) were PCR-positive and also showed histologic findings suggestive of infection. Uniquely developed PCR and bacterial sequencing assays showed bacterial DNA on 12% of implants removed for presumed aseptic loosening. Additional studies are needed to determine the clinical importance of bacterial DNA detected by PCR but not by conventional culture.

Level of Evidence: Level III, diagnostic study. See the Guidelines for Authors for a complete description of levels of evidence.

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Introduction

Total joint arthroplasty provides a high percentage of patients with excellent clinical results, but aseptic loosening and infection remain important complications [5]. Some believe the common mechanism of aseptic loosening is particle-induced bone resorption [9, 23]. However, several reports provide evidence of bacteria associated with implants removed for clinically aseptic loosening [4, 21, 26–28]. Several reports suggest a reduced incidence of aseptic loosening for implants fixed with antibiotic-containing bone cement compared with those fixed with conventional cement [7, 11, 16, 29]. These observations suggest the incidence of occult infections involving loose orthopaedic implants may be underestimated [19].

The most commonly detected bacteria from infected total joint arthroplasties are Staphylococcus aureus and Staphylococcus epidermidis [8], which tend to exist in a biofilm on implant surfaces [1, 20]. If implants have a low concentration of adherent organisms, then it may be necessary to disrupt the biofilm using, for example, an ultrasonication technique to detect them [14, 21, 27, 28]. Furthermore, some type of molecular detection technique

may be useful for identifying small quantities of organisms or slow-growing bacteria that may not be detected with conventional methods [24–26].

The most popular molecular method for detecting a broad range of bacteria is a PCR based on the 16S rRNA gene [30], a gene present in almost all bacteria. However, so-called universal assays of this type may have problems with false-positive results either from contaminating or commensal organisms [3] or from residual Escherichia coli DNA in one of the reagents [6].

We have developed a real-time PCR assay that can detect S. aureus and coagulase-negative Staphylococcus (CNS) with a high degree of specificity and sensitivity [22]. (Real-time means the quantification of amplified DNA in real time without delay at each amplification cycle.) This assay is desirable in cases of orthopaedic infection given the high frequency of the Staphylococcus species as a causative agent of infection [12]. In addition, we developed a broad-range universal PCR combined with pyrosequencing technology that can identify bacterial subgroups [13, 15]. This dual real-time PCR approach has made it possible to improve specificity, which was difficult to achieve using only conventional universal PCR [4, 10, 27].

We addressed two questions in this study: (1) Are two different real-time PCR assays with pyrosequencing after ultrasonication able to detect adherent bacteria on implants removed at revision arthroplasty for presumed aseptic loosening?; and (2) What percent of presumed aseptic or culture-negative implants was positive for bacterial DNA by PCR?

Materials and Methods

To clarify the positive rate of PCR assay for presumed aseptic loosening, we applied two different real-time PCR assays prospectively to retrieved failed joint implants. After brief ultrasonication processing, each sonicate solution was submitted for microbiologic culture and DNA extraction after PCR assays. For discordant samples, histologic findings also were reviewed.

We retrieved failed implants in 52 consecutive patients (92 implants) undergoing revision arthroplasty for failed hip (n = 24) or knee (n = 28) arthroplasty between April 2003 and December 2005. Fourteen of the 92 implants (15%) were believed infected based on preoperative diagnosis made by a surgeon (VK) as described below; 74 implants (85%) were believed aseptic. Twenty-nine of these patients were reported in a previous study in which the molecular Gram-stain characteristics of the PCR were validated [15]. For the purposes of this study, a Staphylococcus genus-specific PCR [22] was used in addition to the molecular Gram-stain characteristics, and PCR was defined as positive if at least one assay showed a positive result. Institutional Review Board approval was obtained for the study.

Before implant retrieval, unimplanted, company-packaged sterile acetabular components (SECUR-FIT-HA PSL; Stryker Orthopaedics, Mahwah, NJ) were submitted to the same sonication processing and DNA extraction and served as a negative control. After sterilization, the negative control test was repeated twice. Three negative control tests revealed all negative results for both PCR assays.

Each retrieved implant was packaged in the bioclean operating room with a laminar air-flow system in an individual sterile container and then immediately transported to our microbiology department. Preoperative diagnoses were made by the surgeon (VK) based on clinical symptoms, local clinical findings, physical findings, and serologic testing. Briefly, we believed patients had aseptic loosening if symptoms of pain or instability were accompanied by radiographic features suggestive of loosening but other serologic tests that usually suggest infection were negative. Patients were believed to have infection if they had local pain, swelling, or redness with evidence of positive serologic testing such as C-reactive protein and sedimentation rate and/or a positive culture of aspirated joint fluid. Preoperative diagnoses for all patients are summarized in Fig. 1. All patients believed not to have infection were considered to be aseptic and were culture-negative, although intraoperative smears in two samples were interpreted as showing Gram-positive cocci. Because of the intraoperative Gram-stain results, the two patients were treated as having infection and underwent two-stage revisions.

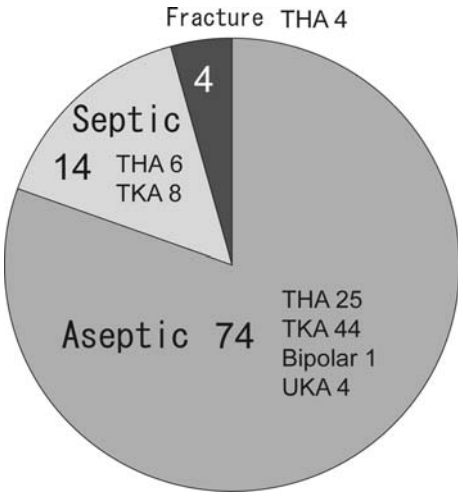

Fig. 1 A diagram shows the preoperative diagnoses. Of the 92 tested implants, 74 (80.4%) were clinically identified as having aseptic loosening, 14 (15%) were clinically infected, and four (4%) were associated with periprosthetic fractures and believed aseptic. UKA = unilateral knee arthroplasty.

In all cases, antibiotics were withheld until fluid and tissue cultures had been taken, then the patients were given 1 mg cephazolin intravenous drip infusion, or if truly allergic to PCN/cephalosporins, they received 1 mg vancomycin hydrochloride.

We placed the retrieved implants in a water-bath sonicator (Branson Ultrasonic Cleaner; Branson Ultrasonics, Danbury, CT) and sonicated for 5 minutes. Twenty-five milliliters of sonicate solution was centrifuged (10,000 × g 20 minutes), the supernatant was discarded, and DNA was extracted using a Qiagen DNA mini kit (Qiagen Inc, Valencia, CA) according to the manufacturer's instructions.

For each implant, we submitted 1 mL of sonicates to standard microbiologic culture to detect aerobic and anaerobic bacteria without subculturing. The microbiologic culture was performed and the bacteria were allowed to grow for up to 2 weeks.

We obtained all oligonucleotide primers and probes from BioChem (Salt Lake City, UT). The entire tuf gene sequences of Staphylococcus species available from public databases were analyzed with the ClustalW multiple sequence alignment program provided by the European Bioinformatics Institute (www.ebi.ac.uk/clustalw/). Based on multiple sequence alignment, we chose regions of the tuf gene, highly conserved among Staphylococcus species. The selection of PCR primers and probes from these regions was enhanced using LightCycler Probe Design software (version 1.0; Idaho Technology Inc, Salt Lake City, UT). The primers and probes used for this test have been described [22]. Briefly, the sequence of the forward primer was 5′-CAATGCCACAAACTCG-3′ (position 33–48), whereas the sequence of the reverse primer was 5′-GCTTCAGCGTAGTCTA-3′ (position 510–494). The broad-range Staphylococcus genus-specific FRET hybridization probes were 5′-ACGGCCTGTAGCAACAGTAC-FITC-3′ (position 372–391) and 5′-LCRed640-CGACCAGTGA TTGAGAATACGTCC-phosphate-3′ (position 369–346), whereas the S. aureus species-specific FRET hybridization probes were 5′-GGCGATGCTCAATACGAAGAAAAAA TC-FITC-3′ (position 239–265) and 5′-LCRed705-AGA ATCAATGGAAGCTGTAGATAC-phosphate-3′ (position 268–291).

The primers and probes used for the universal PCR and pyrosequencing have been described [13]. Briefly, the selection of these primers was enhanced with the use of primer design software (LC Probe Design Software, Roche, IN). The forward primer was biotinylated for amplicon capture, which is necessary for pyrosequencing. The forward and reverse primer sequences were 5′-biotin-GGATT AGATACCCTGGTAGT-3′ and 5′-GGTAAGGTTCTTC GCG-3′, respectively. The selection of sequencing primer was enhanced using the SNP Primer Design software (Pyrosequencing AB version 1.0.1, Biotage, MA). The sequencing primer was 5′-CGTACTCCCCAGGC-3′.

Polymerase chain reaction mixtures consisted of 3.0 mmol/L $MgCl_2$, 1.0 μmol/L concentration of each Staphylococcus generic primer, 0.2 μmol/L concentration of each Staphylococcus generic probe, and 2 μL of 10× LightCycler FastStart DNA Master Hybridization Probes mixture (Roche, IN). We added 2 μL of template DNA extract to obtain a reaction volume of 20 μL for each capillary tube. The cycling parameters consisted of one 95°C-incubation for 10 minutes for enzyme activation and DNA denaturation followed by 45 PCR amplification cycles. Each cycle consisted of three different incubation temperatures and times: 95°C for 10 seconds, 56°C for 10 seconds, and 72°C for 23 seconds. We performed fluorescence readings after annealing at 56°C for 1 second. Polymerase chain reaction cycling was followed by melting curve analysis of 40°C to 75°C (temperature transition rate of 0.5°C/second) with continuous fluorescence readings.

The presence of an amplification or quantification curve for the LC640 signal captured in the F2 channel of the LightCycler in conjunction with a melt curve with a temperature greater than 58°C was considered a positive result for CNS. The absence of a quantification curve or the presence of a quantification curve in the absence of a corresponding melting curve greater than 58°C was considered a negative result for a member of the CNS. We considered the presence of a melt curve for the LC705 signal captured in the F3 channel of the LightCycler a positive result for S. aureus. The specimen was considered negative for S. aureus if this melt curve was absent.

The extracted DNA was submitted for real-time PCR using the RotorGene 3000 (Corbett Research, Sydney, Australia). The PCR mixture consisted of 2.0 μmol/L $MgCl_2$, 0.2 μmol/L of each primer, and 2 μL of 10× LightCycler FastStart DNA Master SYBR green I (Roche Diagnostics, Indianapolis, IN) for a volume of 15 μL master mix per reaction. We added 5 μL of template DNA extract to the reaction mixture for a final reaction volume of 20 μL for each tube. After incubation of the reaction mixture at 95°C for 10 minutes, 45 cycles of PCR amplification were performed using the following parameters: 95°C for 5 seconds, 57°C for 5 seconds, and 72°C for 15 seconds.

Samples were interpreted as positive for bacterial DNA if amplification occurred before the cycle in which the nuclease-free water (ie, the negative control) showed amplification.

We submitted the samples identified as positive by universal PCR for pyrosequencing assay (Pyrosequencing, Biotage, MA). The details of this procedure have been

described [13]. Briefly, 15 μL of biotinylated PCR products was mixed in streptavidin sepharose beads and binding buffer and incubated at 40°C for 20 minutes. The immobilized products were transferred to a 96-well filter plate and processed according to the manufacturer's protocol.

As described [13], if the first three nucleotides obtained during pyrosequencing were GGA or GGG, then the bacteria was most likely Gram-positive, whereas if GGT was obtained, then the bacteria was most likely Gram-negative. The reliability of this definition has been validated using a large number of bacterial strains [13].

The PCR result was defined as positive when either the Staphylococcus PCR or universal PCR was positive.

For culture-positive or for patients in whom there was preoperative suspicion of infection, we also reviewed microscope slides of tissue that had been submitted routinely during surgery, and histologic findings were used to help establish a diagnosis. The microscope slides were considered suggestive of infection if a minimum of five high-power fields (x400) contained five or more neutrophils [18].

Results

Of 82 culture-negative implants, 10 were PCR-positive (12%) (Fig. 2). Of the 74 implants retrieved from a patient believed to have aseptic failure, nine were PCR-positive (12%) (Fig. 3) and two of these (3%) also showed histologic features of infection. Sixty-three of the 82 (77%) culture-negative implants were negative using all test methods (Fig. 3).

The dual PCR assay (Staphylococcus PCR and universal PCR) showed 90% sensitivity and 87.8% specificity compared with conventional microbiologic culture. The positive predictive value was 0.47, accuracy was 0.88, and likelihood ratio for a positive result was 7.38. Interpreted from the opposite perspective, microbiologic cultures showed 47.4% sensitivity and 98.6% specificity compared with the dual PCR assay results.

For nine of the 10 culture-positive implants, melting curve analysis differentiated CNS from S. aureus correctly (compared with culture results). Our molecular Gram-stain using PCR and pyrosequencing matched the conventional Gram-stain results in six implants. Four patients with misidentified molecular Gram stains include two with Gram-negative bacilli by tissue culture (the sonicate cultures grew CNS) and two with CNS for which the universal PCR was negative (therefore pyrosequencing was not performed). One patient with a positive culture result but negative by both PCR assays had acute inflammation in the tissue and we believed this was a false-negative PCR result (Table 1).

Among the eight patients with culture-negative, PCR-positive results, three were treated as having infection and underwent two-stage revision surgery; two (Patients 8, 33)

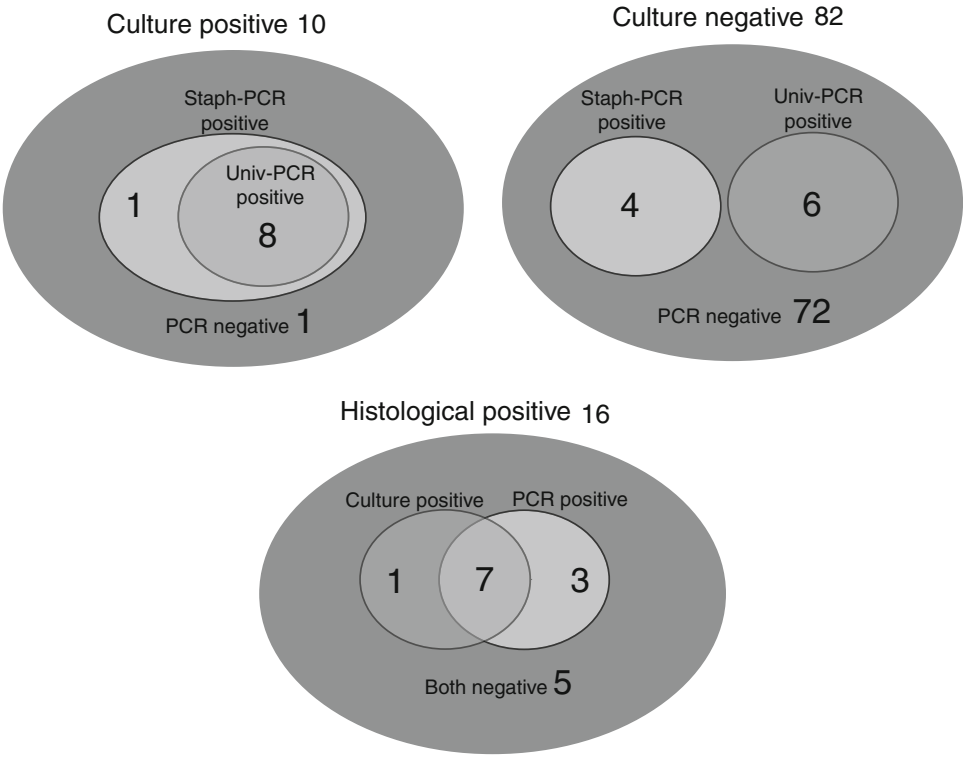

Fig. 2 A diagram shows PCR and culture results for the culture-positive, culture-negative, and histology-positive implants. In 82 culture-negative implants, 10 were PCR-positive.

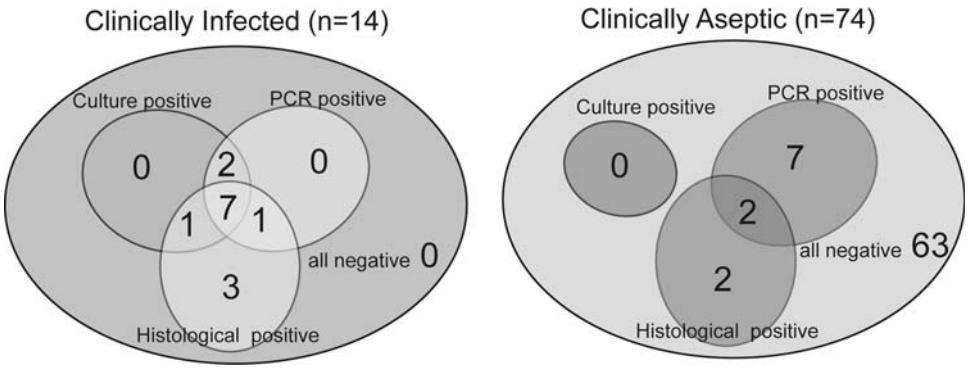

Fig. 3 A diagram shows PCR, culture, and histologic results for each preoperative diagnosis. Of the 74 implants believed to have undergone aseptic failure, nine were PCR-positive.

had histologically positive results by frozen section, and one (Patient 16) was believed to have infection based on the intraoperative fast smear Gram-stain findings (Table 2). These three patients (Patients 8, 16, 33) who underwent two-stage revisions currently are without clinical evidence of recurrent infection.

Discussion

Numerous observations suggest the incidence of occult infections involving loose orthopaedic implants may be underestimated. The research questions of this study were (1) Are two different real-time PCR assays after ultrasonication able to detect the adherent bacteria on implants removed at revision arthroplasty for presumed aseptic loosening?; and (2) What percent of presumed aseptic or culture-negative implants was positive for bacterial DNA by PCR?

The major limitation of this study is the lack of a gold standard for diagnosis of infection, especially with respect to the culture-negative/PCR-positive results. We are not able to determine the viability of bacteria in culture-negative/PCR-positive cases. In this situation, other information can help define infection status, including histologic analysis of periimplant tissues. Several, but not all, of our patients with culture-negative, PCR-positive results showed histologic features of infection. Polymerase chain reaction does not determine bacterial viability, therefore the clinical importance of organisms detected with this method needs to be clarified with long-term prospective studies and with molecular techniques intended to distinguish viable from necrotic organisms.

Conventional microbiologic culture has long been the gold standard for diagnosis of periprosthetic infection although several other tests are available, and the generally good clinical results based on those cultures suggest we should apply PCR to routine clinical use only with caution. Some studies report differences in sensitivity and specificity between PCR and culture results such that it is difficult to know the clinical importance of discrepancies (culture-negative/PCR-positive or culture-positive/PCR-negative). For example, Mariani et al. [17] were the first investigators who applied PCR assay for detection of bacterial DNA in knee fluid aspirates and reported its high sensitivity. Tunney et al. [27] used a universal PCR based on the 16S rRNA gene and reported positive results for 72% of implants revised for aseptic loosening. Although universal PCR assays of that type have very high sensitivity, specificity sometimes is compromised because of contamination of some commercially available Taq polymerase reagents with Escherichia coli DNA, because the polymerase is derived from a recombinant E. coli source [6, 8]. The possibility of Propionibacterium acnes (P. acnes) was described in the study by Tunney et al. [27], whereas no specimens were P. acnes-positive by culture in our study. Our molecular Gram stain suggested Gram-negative infection in five patients despite negative culture results possibly related to P. acnes infection. Clarke et al. [4] also investigated the use of a universal PCR to identify evidence of bacteria in patients with presumed aseptic loosening. They reported a 46% positive rate in specimens from revision surgery and a 21.4% positive rate in specimens from primary surgery, again suggesting high sensitivity but possibly low specificity with respect to clinically important organisms. However, Ince et al. [10] reported universal PCR was not superior to routine bacteriologic culture techniques for detecting low-grade infections associated with failed hip arthroplasty. The differences of PCR methods and results among these prior studies and current study are summarized in Table 3, although it is difficult to compare the positive PCR rates in each study. The discrepancy between prior studies and ours might be attributable to several factors such as the primer and probe sequences, other technical factors related to PCR methods, patient criteria, and tissue sampling.

The selection of the PCR assay (universal versus species-specific) is an important and difficult issue for every study based on PCR. The most important concept of the current study is the use of two different real-time PCR assays: a genus-specific PCR that detects the most frequent bacteria associated with orthopaedic infections

Table 1. Clinical data

Implant	Patient number	Implant	Time in vivo (months)	Preoperative diagnosis	Culture	PCR	Histopathology
1	1	TKA femoral	24	I	–	–	Infection
2		TKA tibial	24	I	–	–	Infection
3	2	THA femoral	20	I	S. aureus	+	Infection
4		THA acetabular	20	I	S. aureus	+	Infection
5	3	THA stem	324	F	–	–	N/A
6		THA acetabular	324	F	–	–	N/A
7	4	THA femoral	N/A	A	–	–	N/A
8	5	THA femoral	48	I	CNS	+	N/A
9		THA acetabular	48	I	CNS	+	N/A
10	6	THA femoral	173	A	–	+	Negative
11		THA acetabular	173	A	–	–	N/A
12	7	TKA femoral	120	A	–	–	N/A
13		TKA tibial	120	A	–	–	N/A
14	8	TKA femoral	11	I	–	–	Infection
15		TKA tibial	11	I	–	+	Infection
16	9	Bipolar femoral	108	A	–	+	N/A
17	10	THA femoral	216	F	–	–	N/A
18		THA acetabular	216	F	–	–	N/A
19	11	TKA femoral	88	I	CNS	+	Infection
20		TKA tibial	88	I	CNS	+	Infection
21	12	TKA femoral	10	A	–	–	N/A
22		TKA tibial	10	A	–	–	N/A
23	13	THA acetabular	252	A	–	–	N/A
24	14	THA acetabular	210	A	–	–	N/A
25	15	TKA femoral	22	A	–	–	N/A
26		TKA tibial	22	A	–	–	N/A
27	16	TKA femoral	204	A	- but Smear Gram-positive cocci	+	Negative
28		TKA tibial	204	A	- but Smear Gram-positive cocci	–	Negative
29	17	TKA femoral	180	A	–	+	Negative
30		TKA tibial	180	A	–	–	N/A
31	18	TKA femoral	204	A	–	–	N/A
32		TKA tibial	204	A	–	–	N/A
33	19	THA cup	156	A	–	+	Negative
34	20	THA femoral	276	A	–	–	N/A
35		THA acetabular	276	A	–	–	N/A
36	21	TKA femoral	52	A	–	–	N/A
37		TKA tibial	52	A	–	–	N/A
38	22	THA femoral	N/A	A	–	–	N/A
39		THA acetabular	N/A	A	–	–	N/A
40	23	THA femoral	252	A	–	–	N/A
41		THA acetabular	252	A	–	–	N/A
42	24	TKA femoral	60	A	–	–	N/A
43		TKA tibial	60	A	–	–	N/A
44	25	TKA femoral	180	A	–	+	Negative
45		TKA tibial	180	A	–	+	Negative
46	26	THR femoral	168	A	–	–	N/A
47		THR acetabular	168	A	–	–	N/A

Table 1. continued

Implant	Patient number	Implant	Time in vivo (months)	Preoperative diagnosis	Culture	PCR	Histopathology
48	27	THR femoral	12	I	CNS	−	Infection
49		THR acetabular	12	I	CNS	+	Infection
50	28	THR acetabular	72	A	–	−	N/A
51	29	TKR femoral	N/A	A	–	−	N/A
52		TKR tibial	N/A	A	–	−	N/A
53	30	THA acetabular	240	A	–	−	N/A
54	31	TKA femoral	72	A	–	−	N/A
55		TKA tibial	72	A	–	−	N/A
56	32	TKA femoral	36	A	–	−	N/A
57		TKA tibial	36	A	–	−	N/A
58	33	THA femoral	16	A	–	+	Infection
59		THA acetabular	120	A	–	+	Infection
60	34	TKA femoral	11	A	–	−	N/A
61		TKA tibial	11	A	–	−	N/A
62	35	TKA femoral	3	A	–	−	N/A
63		TKA tibial	3	A	–	−	N/A
64	36	THA femoral	30	A	–	−	N/A
65	37	THA femoral	N/A	A	–	−	N/A
66	38	THA femoral	13	A	–	−	N/A
67	39	THA femoral	276	A	–	−	N/A
68	40	TKA femoral	48	A	–	−	N/A
69		TKA tibial	48	A	–	−	N/A
70	41	TKA femoral	24	A	–	−	N/A
71		TKA tibial	24	A	–	−	N/A
72	42	UKA femoral	36	A	–	−	N/A
73		UKA tibial	36	A	–	−	N/A
74	43	TKA femoral	144	A	–	−	N/A
75		TKA tibial	144	A	–	−	N/A
76	44	TKA femoral	N/A	I	CNS	+	Infection
77		TKA tibial	N/A	I	CNS	+	Infection
78	45	UKA femoral	24	A	–	−	N/A
79		UKA tibial	24	A	–	−	N/A
80	46	THA femoral	216	A	–	−	N/A
81	47	TKA femoral	N/A	A	–	−	N/A
82		TKA tibial	N/A	A	–	−	N/A
83	48	TKA femoral	30	A	–	−	N/A
84		TKA tibial	30	A	–	−	N/A
85	49	TKA femoral	N/A	A	–	−	N/A
86		TKA tibial	N/A	A	–	−	N/A
87	50	TKA femoral	18	A	–	−	Infection
88		TKA tibial	18	A	–	−	Infection
89	51	TKA femoral	N/A	A	–	−	N/A
90		TKA tibial	N/A	A	–	−	N/A
91	52	THA femoral	72	A	–	−	N/A
92		THA acetabular	72	A	–	−	N/A

PCR = polymerase chain reaction; N/A = not applicable; I = infected; F = fracture; A = aseptic failure; PCR+; PCR-positive with at least one assay; PCR− = negative for both PCR tests; CNS = coagulase-negative staphylococcus; negative results for histology means no significant acute inflammation.

Table 2. Culture-negative, PCR-positive data

Patient number/PCR	Preoperative diagnosis	Operative findings/procedure	Molecular subtyping by PCR or sequencing	Histologic findings	Clinical outcome
6/univ PCR	Aseptic loosening THA	Grossly loose femoral component/ revision	Gram-negative species by sequencing	No acute inflammation	Stable up to 31 months
8/univ PCR	Infected TKA	Granulation tissue/débridement, insertion of antibiotic-impregnated spacer	Gram-negative species by sequencing	Acute inflammation	After two-stage revision, stable up to 8 months
9/staph PCR	Aseptic loosening of bipolar hip	Grossly loosed femoral component/revision	CNS by PCR	N/A	Stable up to 5 months
16/univ PCR	Aseptic loosening of TKA	No gross purulence, but fast smear of joint fluid was positive for Gram-positive cocci/ débridement, insertion of antibiotic-impregnated spacer	Gram-positive species by sequencing	No acute inflammation	After two- stage revision, stable up to 12 months
17/staph PCR	Aseptic loosening of TKA	Grossly loose patella component/ revision	CNS by PCR	No acute inflammation	Knee pain after 14 months
19/staph PCR	Aseptic loosening THA	Grossly loose acetabular component	CNS by PCR	No acute inflammation	Stable up to 13 months
25*/univ PCR	Unstable TKA, severe femoral osteolysis	Large cavity at distal femur, tissue culture grew S. aureus after surgery	Gram-negative species by sequencing	No acute inflammation	After antibiotic therapy for 6 weeks, stable up to 5 months
33/univ PCR staph PCR	Aseptic loosening of THA	Severe pelvic bone loss/ débridement, insertion of antibiotic-impregnated spacer	Gram-negative species by sequencing/CNS by PCR	Acute inflammation	After two-stage revision, stable up to 9 months

* Specimen was culture-negative from sonicate solutions, but tissue cultures were positive several days later; frozen sections of periimplant tissue for cases showed acute inflammation and histologic features suggestive of ongoing infection; PCR = polymerase chain reaction; univ = universal; staph = staphylococci; CNS = coagulase-negative staphylococcus; N/A = not available.

Table 3. Comparison of PCR studies

Study	PCR method/Primer design	DNA sequencing	Specimen
Mariani et al. [17]	Conventional/broad-range	None	Joint fluid
Tunney et al. [27]	Conventional/broad-range	None	Implant
Clarke et al. [4]	Conventional/broad-range	None	Tissue around implant/joint fluid
Ince et al. [10]	Conventional/broad-range	None	Tissue around implant
Current study	Real-time/species-specific and broad-range	Done after broad-range PCR	Implant

PCR = polymerase chain reaction.

(ie, Staphylococcus species) and a broad-range universal PCR followed by a pyrosequencing assay to address the issue of false-positive reactions secondary to residual E. coli DNA in the DNA polymerase. We also intentionally reduced the sensitivity of our universal PCR because of our stringent definition of a positive PCR result. Our PCR-positive rate in patients believed to have aseptic loosening was 12.2% (nine of 74 patients) at the maximum, considerably lower than the results reported by Tunney et al. [27] and Clarke et al. [4].

Instead of only comparing PCR and culture results, other possible outcome measures include the histology of peri-implant membranes, preoperative clinical findings, serologic tests, and the ultimate clinical followup [2]. Unfortunately, it still is not possible to define with certainty the existence of bacteria or the clinical importance of a positive test result using only one assay. Interpreting all outcome measures together in our study, two of 74 patients with culture-negative results (2.7%) who were believed to have aseptic loosening now are believed to have had infection based on the combination of positive PCR results and histologic findings. Seven of 74 patients with culture-negative results believed to have experienced aseptic loosening (9.5%) had PCR-positive (only) results. It is unclear if these represent false-positive PCR results or very low-grade infections, but the patients were treated as not having infection and no evidence of infection was seen at least with a short followup.

Detection of bacteria using dual real-time PCR assays provides evidence of bacterial DNA in some patients believed to have experienced aseptic loosening. If all of our PCR results are interpreted as true-positives, then 12% of the patients with aseptic loosening actually were colonized with bacteria, which is lower than the rates reported in many other PCR studies.

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