MAJOR ARTICLE

Is "Aseptic" Loosening of the Prosthetic Cup after Total Hip Replacement Due to Nonculturable Bacterial Pathogens in Patients with Low-Grade Infection?

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Background. Loosening of the prosthetic cup is the limiting factor in the service life of total hip prostheses (THPs). Despite effective culture methods, the detection of low-grade infection in patients with loose implants still presents a challenge. It is crucial to distinguish between "aseptic" loosening and loosening due to periprosthetic infection, so that appropriate treatment can be administered. We investigated whether aseptic loosening of the acetabular components of THPs is due to unrecognized infection.

Methods. From October through December 2002, a total of 24 patients with acetabular cup loosening were investigated. Only patients without clinical signs of infection and with negative results of bacteriologic culture of synovial fluid (obtained by preoperative aspiration) were included in the study. Intraoperative biopsy samples obtained from the neocapsule and synovia (e.g., the interface membrane) were examined by means of routine culture methods and by polymerase chain reaction (PCR) for detection of 16S ribosomal RNA (rRNA). Control subjects included 9 patients undergoing primary hip arthroplasty.

Results. C-reactive protein levels and erythrocyte sedimentation rates were slightly elevated in the group with loosening, compared with the control group, but the difference was not statistically significant. PCR and routine culture showed no microorganisms in either group, with the exception of 1 patient in the loosening group.

Conclusions. PCR for detection of 16S rRNA in tissue specimens obtained from hip joints is not superior to routine bacteriologic culture techniques for detection of low-grade infections. However, these results demonstrate that the loosening of cups in THPs do not usually result from nonculturable periprosthetic infection, if the microbiological processing is adequate.

Loosening remains the most common problem associated with total hip prostheses (THPs). Approximately 10% of THPs are revised >10 years after the procedure because of loosening [1]. In these cases, the orthopedic surgeon is often faced with the problem of determining whether the implant has been loosened by infection. The etiology of "aseptic" loosening of prosthetic hips is unknown. Periprosthetic osteolysis and particle disease [2], as well as periprosthetic bacterial colonization, have been suspected to be etiological agents [3]. Perdreau-Remington et al. [4] examined 52 patients who underwent surgery for correction of loose hip prostheses. In their prospective study, positive results of bacteriologic culture were recorded for 34 patients (76%) [4]. Cultures of prosthetic shafts and capsular tissue specimens resulted in the highest frequency of positive culture results. Hunter et al. [5] evaluated the results of revision THP in 140 aseptic hips. They found an infection rate of 32% and concluded that every loosened hip arthroplasty must be considered to be the result of infection, if not proven otherwise.

Loosening may be due to the presence of unrecognized infection, at the time of revision surgery, caused by a biofilm of sessile bacteria colonizing the surface of the device. These bacteria may be overlooked if standard culture techniques are used [6]. Antibiotics ad-

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ministered for perioperative prophylaxis, extended transportation time, and inadequate preservation of specimens before processing may lead to negative culture results.

The hypothesis of bacterial infection is supported further by detection of bacterial DNA, which suggests bacterial persistence in the area of loosening in the THP despite sterile culture results. The use of PCR for detection of bacterial rRNA as an indicator of the presence of bacteria is an accepted technique formerly used for detection of environmental and medically important bacteria [7, 8]. PCR that targets highly conserved regions of the bacterial genome (e.g., the 16S rRNA gene) has been used successfully to detect nonculturable bacteria that cause a variety of infections, including septic arthritis [9, 10] and meningitis [11]. The aim of this study was to investigate, by means of PCR amplification of 16S rRNA genes, whether aseptic loosening of the acetabular components of THPs is due to nonculturable bacteria and to compare the efficiency of PCR with that of standard culture techniques in special cases of total hip replacement surgery.

PATIENTS AND METHODS

From October through December 2002, a total of 24 patients underwent revision for loosened THPs. We retrieved 24 tissue samples from the neocapsula and the acetabular synovia–like interface membrane for detection of bacteriologic 16S rRNA by PCR and for bacteriologic culture. Before revision, joint fluid was aspirated from all patients with a loosening cup and investigated for pathogens by bacteriologic culture. All 24 patients received standard preoperative care. Skin was decontaminated with Cutasept G (Bode Chemie). Two grams of cefazolin (Basocef; Curason) was administered for antibiotic prophylaxis. Capsular tissue specimens were obtained for PCR and culture from 9 control patients who underwent primary hip arthroplasty and had no previous history of hip surgery.

Tissue samples from the neocapsula and synovia-like interface membrane of the acetabular component were retrieved and divided into 2 portions. Both specimens were placed into separate sterile tubes without additional substrates. Specimens obtained for PCR were stored at −70°C. PCR was done in the laboratory of J.R., and culturing was done in the laboratory of L.F. These procedures permitted independent examination and interpretation of the results. Histopathologic findings were not recorded because of poor sensitivity, especially in cases of low-grade infection [12].

Bacterial isolation by standard culture methods. Any antimicrobial chemotherapy was stopped 1 week before joint aspiration. Joint aspiration was done according to the standards described in the *Guidelines of Hospital Hygiene and Infectious Disease Prevention* (Robert Koch Institute; Berlin) [13]. No local anesthetics were used. Joint fluid obtained before revision and tissue samples obtained during surgery were transferred into sterile tubes without additional substrate; joint fluid specimens were transported within 4 h to the laboratory, and tissue specimens were stored at −70°C. For all samples, microscopic investigation (i.e., Gram staining) was performed, and then the specimens were incubated in brain-heart infusion broth (bioMérieux), TVLS medium [14], a medium described by Lodenkaemper and Stinen [15], on Columbia blood plates (aerobic 5% CO_2), and then on *Brucella* agar plates (anaerobic) (bioMérieux). The incubation lasted for 14 days. Susceptibility testing was done according to Deutsches Institut für Normung standard 58940 [16].

DNA isolation. Tissue samples were immediately stored at −70°C, and DNA was purified from homogenized specimens after proteinase K digestion and column extraction with the NucleoSpin DNA kit (Macherey-Nagel). All DNA procedures before and after PCR were done in separate designated rooms with separate pipetting devices to avoid contamination of the samples with foreign DNA. Master-mixture water controls were used for every sample that was processed.

PCR amplification. The sequences of the universal primers (16S rRNA gene) and primers for the control gene (glyceraldehyde-3-phosphate dehydrogenase [GAPDH]) are indicated in table 1. Oligonucleotides used in this study were provided by MWG-Biotech. DNA was amplified in a 25-μL reaction mixture consisting of ready-to-go PCR beads (up to 23 μL; Amersham Pharmacia Biotech), 0.5 μL of each primer (100 pmol/mL), and 1 μL of the sample. After amplification, 5 μL of the amplified product was analyzed in a 2% agarose gel. Amplification products were sequenced by SeqLab and were analyzed by use of the National Center for Biotechnology Investigation Blast database [17].

Amplification of the GAPDH control gene was performed using real-time PCR (Light Cycler Detection System; Roche Molecular Biochemicals) with the FastStart DNA Master SYBR kit (Roche Molecular Biochemicals). PCR was performed according to the following protocol: 95°C for 10 min, followed by 40 cycles at 95°C for 10 s, 60°C for 5 s, and 72°C for 10 s. In the dissociation protocol, single peaks were confirmed to exclude nonspecific amplification.

Table 1. Nucleotide sequences of primers used to determine agents responsible for loosening of the acetabular components of total hip prostheses.

Primer	Sequence
16S rRNA	
Forward	5′-AGAGTTTGATCCTGGCTCAG-3′
Reverse	5′-CCCACTGCTGCCCGTAG-3′
GAPDH	
Forward	5′-TCTGCCCCCTCTGCTGATGCCCCC-3′
Reverse	5′-CCATCACGCCACAGTTTCCCGGAG-3′

NOTE. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

RESULTS

The group of case patients who experienced loosening THPs consisted of 19 women and 5 men, with a mean age of 69 years (range, 49–88 years). The mean interval of arthroplasty in situ was 11.9 years (range, 1–31 years). The control group included 3 women and 6 men, with a mean age of 66.2 years (range, 49–89 years).

PCR and routine culture showed no microorganisms in either group, with the exception of 1 of the individuals with a loosening THP. The neocapsular sample obtained from this patient showed *Propionibacterium acnes* in routine culturing. Tissue from the acetabulum yielded negative results by both PCR and culturing. Medical examination and laboratory-based clinical pathological records gave no hint of infectious diseases.

For all case patients, clinical pathological data—especially C-reactive protein levels, erythrocyte sedimentation rates, and WBC counts—showed no significant increases. Only the C-reactive protein levels were slightly elevated, but these increases were not statistically significant ($P = .69$).

In the group with loosening, the mean C-reactive protein level was 5.5 mg/L (reference, <5 mg/L), whereas in the control group, it was 4.2 mg/L. The mean erythrocyte sedimentation rate in the group with loosening was 11.96 mm/h (reference, <20 mm/h), whereas in the control group it was 9.56 mm/h. The mean WBC count in the group with loosening was 6.56×10^3 cells/μL (normal range, 3.9×10^3–10.0×10^3 cells/μL), whereas in the control group it was 8.96×10^3 cells/μL. Follow-up examinations of all patients (≥19 months after revision) revealed no hint of infection, and no additional revisions were necessary.

DISCUSSION

Loosening and infection in patients who have undergone total hip replacement surgery, especially after revision, remains a challenging problem in orthopedics. Clinical signs, laboratory investigation of infection parameters, and microbiological findings are often insufficient to detect infection of orthopedic devices. Distinguishing between infection and aseptic loosening is essential for determining the proper clinical course of action [18]. The standard analyses for detecting microorganisms—Gram staining (for microscopic investigation) and culturing of joint fluid aspirates and tissue biopsy specimens obtained during surgical revision—are reported to have poor sensitivity. Approximately 15%–20% of these tests failed to detect infection in a recent study by Mariani et al. [19]. Detection of bacteria depends on various factors, including specimen transportation conditions, perioperative antibiotic application, specimen type (swab or tissue), incubation and culture methods, diagnostic tools, and species type [20].

Additional diagnostic analyses, such as measurement of WBC count and erythrocyte sedimentation rate, C-reactive protein assays, and bone scans, cannot provide definitive information. Pre- and perioperative antibiotic administration may prevent bacterial growth in cultures using conventional techniques [11], because failed eradication often leads to additional growth requirements in the culture medium.

It is also possible that viable bacteria are highly adapted to the environment of the in vivo biofilm and that the nutrient media and isolation procedures do not provide the requisite conditions for recovering such bacteria in culture. Another explanation for the difficulty in isolating these bacteria could be the dilution of bacterial signaling molecules, of which a critical concentration may be necessary to trigger bacterial growth; *N*-acyl homoserine lactones in gram-negative bacteria [21] and peptide pheromone in gram-positive bacteria [22] are well known for this property. However, bacteria in a biofilm—representing a relatively static environment that is largely protected from the immune system—may become less adaptable [23].

Therefore, if analysis is limited to standard diagnostic tests (culture and histopathological analysis) that are designed for examining planktonic bacteria and those that are adequate for detecting of sepsis-related pathogens without involvement of foreign material, a significant number of infections of orthopedic devices may remain undetected. Protocols for treatment of conditions due to such pathogens will be ineffective in a number of cases [19].

Amplification of the 16S rRNA gene, a highly conserved region within the bacterial genome, for the detection of bacterial pathogens in infections of orthopedic devices has been described elsewhere [23, 24, 25]. The use of universal broad-range primers facilitates the recognition of most bacterial species, showing high sensitivity for clinical specimens in the presence of low levels of background human DNA. Thus, Mariani et al. [26] showed a detection limit of 10 genome equivalents of *Escherichia coli* or *Staphylococcus aureus* per 100 μL of synovial fluid. The merit of this method is the rapid and highly specific detection of bacterial monocultures in clinical samples, even in the presence of antibiotic treatment, although determination of antimicrobial susceptibility is not feasible. However, detection of bacterial DNA in homogenized specimens of neocapsula and acetabular synovia, which were used in our study, might be limited because of the high levels of background human DNA. Thus, we were able to show that the detection limit dramatically increased to 10^4 genome equivalents of *Staphylococcus epidermidis* when the cultures were stirred with fibrous tissues obtained from the hip joint (data not shown). Further studies are needed to compare the diagnostic sensitivity of tests used to detect bacterial DNA in different materials collected from the hip joint (e.g., capsule and synovial fluid) with the sensitivity of conventional culture methods for aerobic and anaerobic bacteria.

A MEDLINE search identified 2 studies dealing with PCR and infection associated with total joint replacement. Tunney et al. [25] examined 118 femoral components from loosened THPs by PCR and immunofluorescence. Lack of clinical follow-up of the patients limited the value of this study, because the fractions of false-positive and false-negative results could not be estimated. However, in 72% of samples obtained by sonication of the femoral component, bacterial genes were detected by PCR [25]. Only 4% of tissue cultures yielded bacteria. Remarkably, *P. acnes* alone, or in association with *Staphylococcus* species, was detected in 67% of the patients. However, Steinbrink and Frommelt [12] reported that *Propionibacterium* species were found in only 8.6% of cases (93 of 1077) of infected THPs. Tunney et al. [25] concluded that the incidence of prosthetic joint infection is grossly underestimated.

Mariani et al. [19] reported that 32 of 50 synovial fluid aspirates obtained from patients who had undergone total knee arthroplasty had bacterial genome identified by PCR. Culture of specimens obtained before surgical revision yielded bacteria for only 6 of these patients. Of specimens obtained during revision, standard microbiological techniques showed bacterial growth for 9 patients.

We examined 24 patients who underwent revision hip arthroplasty and 9 patients with a primary hip arthroplasty. In all patients with loosened THPs, no bacteria were detected from specimens of preoperative synovial fluid. No bacterial DNA from the 16S rRNA gene was detected in samples of neocapsules or the acetabular synovia–like interface membrane by PCR. Tissue was used in both methods (PCR and culturing) to provide comparable conditions for examination. Microscopic investigation of the tissue specimen and culture revealed that, with the exception of 1 case, there was no evidence for the presence of bacteria. In this special case, *P. acnes* was identified by culture in 1 of the 2 samples. Results of PCR remained negative. Clinical data, including results of clinical pathological analysis, gave no hint of infectious diseases, so we considered bacterial contamination of the specimen to be the most plausible explanation of these findings. In the group with primary arthroplasty, culture and PCR methods produced no evidence for the presence of bacteria. The methods were performed in 2 different laboratories to provide independent culture and PCR tests and interpretation.

At first glance, our results seem to contradict the conclusions in the above-mentioned literature [19, 25]. However, the design of our study differs from that of the other studies with respect to 2 critical details: we included only patients for whom joint aspirates obtained before surgery did not yield bacteria. At our institution (ENDO-Klinik, Hamburg), culturing of preoperative joint aspirates is done according to a protocol, which includes a prolonged processing period of 14 days, that is specially designed for detection of foreign body infections. Neut et al. [20] reported that meticulously performed and extended processing of bacteriologic culture improves the number and relevance of the microbial findings. This supports our own experience that up to 90% of pathogens found prior to surgery could be confirmed by culture of biopsy samples obtained during revision surgery [12]. We believe that preoperative joint aspiration is essential in the diagnostic procedure for loosened THPs, and further studies are in progress to compare detection of bacterial DNA in preoperative joint aspirates with that in hip tissue specimens obtained during surgical interventions. The preselection of the patients included in the study and the accurate performance of the culture procedure may explain our results.

In our opinion, bacterial culture, when adapted to the special conditions of bacteria causing foreign body infection, is a necessary tool in the diagnostic procedure for loosened THPs. PCR can provide valid data in concert with results from standard culture methods. Unfortunately, this expensive diagnostic tool (PCR) is not yet available for routine use.

Our results from 24 cases of loosened acetabular cups indicate that PCR for 16S rRNA in tissue specimens obtained from hip joints is not superior to routine bacterial culture techniques for detection of low-grade infections. However, these results demonstrate that, in a majority of THPs, the loosening of cups does not result from nonculturable periprosthetic infection, if the microbiological processing is adequate.

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Potential conflicts of interest. All authors: no conflicts.

References

1. Mulroy WF, Estok DM, Harris WH. Total hip arthroplasty with use of so-called second-generation cementing techniques: a fifteen year average follow-up study. J Bone Joint Surg Am **1995**; 77:1845–52.
2. Harris WH. Osteolysis and particle disease in hip replacement: a review. Acta Orthop Scand **1994**; 65:113–23.
3. Peters G, Schumacher-Perdreau F, Jansen B, Ludwig C, Rütt R. "Aseptic" prosthetic hip loosening—a chronic polymer associated infection by coagulase-negative staphylococci? Zentralbl Bakteriol Suppl **1991**; 21:329–31.
4. Perdreau-Remington F, Stefanik D, Peters G, et al. A four-year prospective study on microbial ecology of explanted prosthetic hips in 52 patients with "aseptic" prosthetic joint loosening. Eur J Clin Microbiol Infect Dis **1996**; 15:160–5.
5. Hunter GA, Welsh RP, Cameron HU, Bailey WH. The results of revision of total hip arthroplasty. J Bone Joint Surg Br **1979**; 61-B:419–21.
6. Gristina AG, Costerton JW. Bacterial adherence to biomaterials and tissue: the significance of its role in clinical sepsis. J Bone Joint Surg Am **1985**; 67:264–73.
7. Greisen K, Loefelholz M, Purohit A, Leong D. PCR primers and probes for the 16S rRNA gene of most species of pathogenic bacteria, including bacteria found in cerebrospinal fluid. J Clin Microbiol **1994**; 32:335–51.
8. Wilson MJ, Weightmann AJ, Wade WG. Applications of molecular ecology in the characterisation of uncultured microorganisms associated with human disease. Rev Med Microbiol **1997**; 8:91–101.

9. Canvin JM, Goutcher SC, Hagig M, Gemmell CG, Sturrock RD. Persistence of *Staphylococcus aureus* as detected by polymerase chain reaction in the synovial fluid of a patient with septic arthritis. Br J Rheumatol **1997**; 36:203–6.
10. Ince A, Tiemer B, Gille J, Boos C, Russlies M. Total knee arthroplasty infection due to *Abiotrophia defectiva*. J Med Microbiol **2002**; 51: 899–902.
11. Ni H, Knight AI, Cartwright K, Palmer WH, McFadden J. Polymerase chain reaction for diagnosis of meningococcal meningitis. Lancet **1992**; 340:1432–4.
12. Steinbrink K, Frommelt L. Treatment of periprosthetic infection of the hip using one-stage exchange surgery [in German]. Orthopade **1995**; 24:335–43.
13. Robert Koch Institut Berlin. Anlage 5.1. In: Richtlinie für Krankenhaushygiene und Infektionsprävention. Munich: Urban und Fischer Verlag, **2003**:7–8.
14. Caselitz FH, Freitag V. Halbflüssiges Kombinationsmedium (TVLS-Medium) zur Züchtung anaerober Bakterien. Aerztl Lab **1969**; 15: 426–30.
15. Lodenkämper H, Stinen G. Therapie anaerober Infektionen. Dtsch Med Wochenschr **1956**; 81:1226–31.
16. Deutsches Institut für Normung. Empfindlichkeitsprüfung von Krankheitserregern gegen Chemotherapeutika. DIN 58940, Teil 1–5. In: DIN Taschenbuch 222: Medizinische Mikrobiologie und Immunologie. Diagnostische Verfahren. Berlin: Beuth-Verlag, **2000**:153–206.
17. BLAST database. **2003**. Available at: http://www.ncbi.nlm.nih.gov/blast/Blast.cgi. Accessed 27 June 2002.
18. Zimmerli W, Ochsner PE. Management of infection associated with prosthetic joints. Infection **2003**; 31:99–108.
19. Mariani BD, Martin DS, Levine MJ, Booth RE Jr, Tuan RS. The Coventry award: polymerase chain reaction detection of bacterial infection in total knee arthroplasty. Clin Orthop **1996**; 331:11–22.
20. Neut D, van Horn JR, van Kooten TG, van der Mai HC, Buscher HJ. Detection of biomaterial-associated infections in orthopaedic joint implants. Clin Orthop **2003**; 413:261–8.
21. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science **1998**; 280:295–8.
22. Lazazzera BA, Grossmann AD. The ins and outs of peptide signaling. Trends Microbiol **1998**; 6:288–94.
23. Tunney MM, Patrick S, Curran MD, et al. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J Clin Microbiol **1999**; 37:3281–90.
24. Brosius J, Palmer ML, Kennedy PJ, Noller HF. Complete nuclotide sequence of a ribosomal RNA gene from *Escherichia coli*. Proc Natl Acad Sci U S A **1978**; 75:4801–5.
25. Tunney MM, Patrick S, Gorman SP, et al. Improved detection of infection in hip replacements: a currently underestimated problem. J Bone Joint Surg Br **1998**; 80:568–72.
26. Mariani BD, Levine MJ, Booth RE Jr, Tuan RS. Development of a novel, rapid processing protocol for polymerase chain reaction–based detection of bacterial infections in synovial fluids. Mol Biotechnol **1995**; 4:227–37.