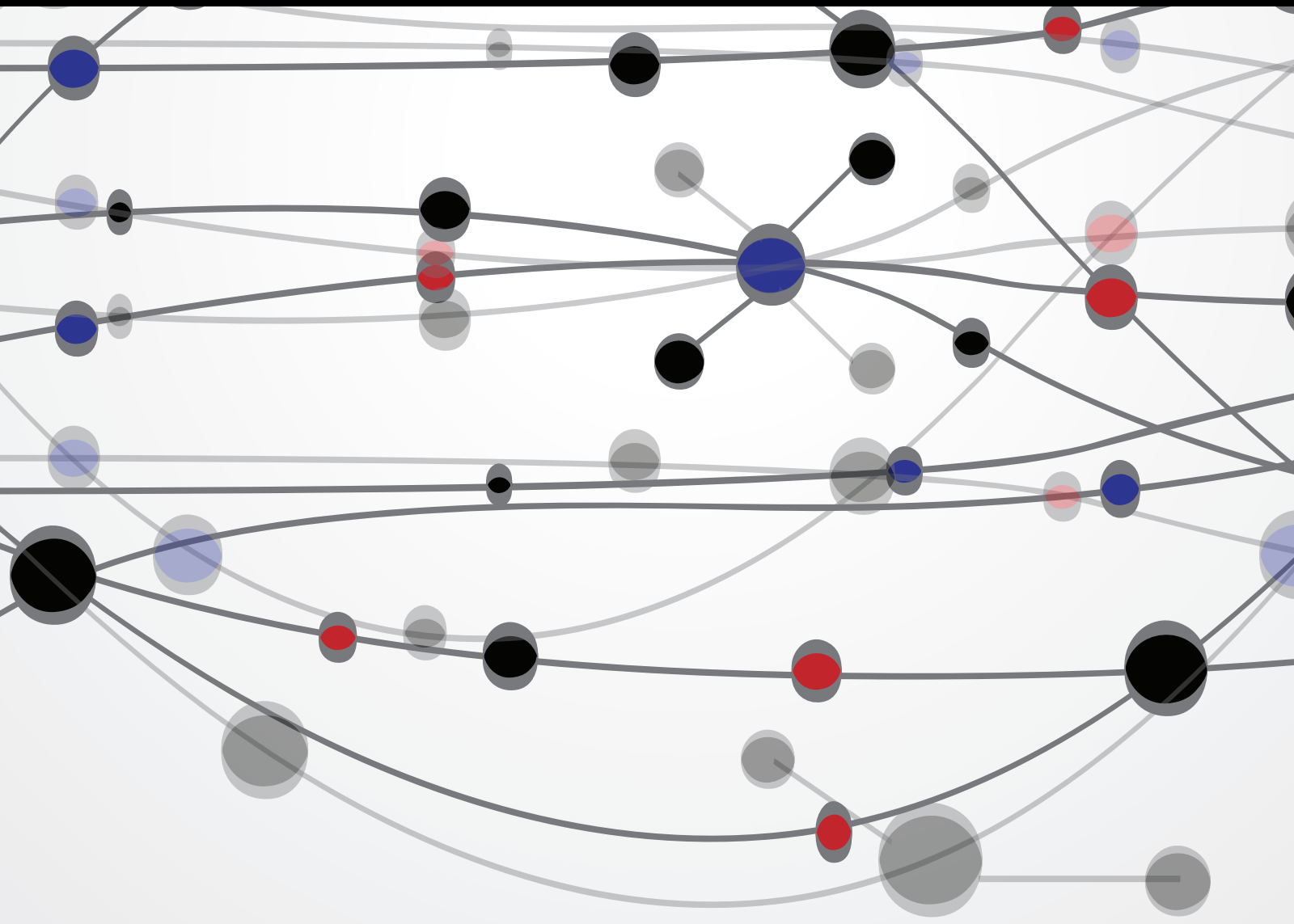
The Scientific World Journal

Periprosthetic Joint Infection: Clinical and Bench

Guest Editors: Mel S. Lee, Andrew Freiberg, Wolfgang Klauser, Christopher S. Mow, and Shin-Yoon Kim



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Editorial

Periprosthetic Joint Infection: Clinical and Bench

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Periprosthetic joint infection (PJI) is a devastating complication for the patient and the health care providers. Its incidence is between 1% and 3% in primary and 4% and 6% in revision total joint arthroplasties. The diagnosis can be straightforward with purulent discharge from the joint but may also be confusing especially when associated with medical morbidities. Often, infection leads to multiple operations, prolonged use of antibiotics, extensive utilization of medical resources, and substantial social, economic, or even psychological impacts on the patients, family, hospitals, physicians, and payers. It is estimated that the direct medical cost for treating a PJI is 3 times to the medical cost without infection in revisions and 10 times to the medical cost with uneventful primary cases. As the demand for total joint arthroplasties and the burden of PJI increase globally, knowledge and technologies for detecting, preventing, and managing PJI need to be shared to provide better care to patients.

In this special issue, we included studies of economic analysis on the treatment of PJI (D. Hernandez-Vaquero et al.), animal models of implant-associated infection (M. Haenle et al. and A. I. Stavrakis et al.), methods to improve the diagnosis (D. S. Evangelopoulos et al. and M. S. Lee et al.), efficacy and the potential use of preformed antibiotic-loaded cement spacer (D. Regis et al. and D. W. Chen et al.), and clinical studies of using antibiotic-loaded cement spacer in hip and knee infections (S. S. Ahmad et al. and K. Uchiyama et al.). The notion to publish the special issue is to bridge the basic studies with the clinical studies. Because PJI is a great mimic that many failed joint arthroplasties initially attributed to aseptic loosening are found to be caused by infection in many occasions. M. Haenle et al. and A. I. Stavrakis et al. highlighted the importance of biofilm formation and the possibility of implant-associated infection with comparatively low dose of bacterial inocula. Sonication of the retrieved prosthesis (D. S. Evangelopoulos et al.) and the use of molecular probes for bacteria-specific genes (M. S. Lee et al.) can improve the accuracy of diagnosis. It therefore can help differentiating easy-to-treat or difficult-to-treat cases (S. S. Ahmad et al.) and the choice of antibiotics in fabricating the cement spacers.

This special issue cannot cover all the issues dealing with PJI. However, by increasing the clinicians and surgeons awareness about PJI, the burdens and challenges could be met by putting more efforts to prevent infection, to design novel bacteria-resistant implants, to improve diagnostic sensitivity and accuracy, to make better antibiotic-loaded spacers, and to develop strategies in treating drug-resistant strains or fungal infections. These works need the collaboration between academic researchers and clinicians to translate basic science to clinical practice.

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Research Article

Molecular Diagnosis of Periprosthetic Joint Infection by Quantitative RT-PCR of Bacterial 16S Ribosomal RNA

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The diagnosis of periprosthetic joint infection is sometimes straightforward with purulent discharge from the fistula tract communicating to the joint prosthesis. However it is often difficult to differentiate septic from aseptic loosening of prosthesis because of the high culture-negative rates in conventional microbiologic culture. This study used quantitative reverse transcription polymerase chain reaction (RT-qPCR) to amplify bacterial 16S ribosomal RNA in vitro and in 11 clinical samples. The in vitro analysis demonstrated that the RT-qPCR method was highly sensitive with the detection limit of bacterial 16S rRNA being 0.148 pg/μl. Clinical specimens were analyzed using the same protocol. The RT-qPCR was positive for bacterial detection in 8 culture-positive cases (including aerobic, anaerobic, and mycobacteria) and 2 culture-negative cases. It was negative in one case that the final diagnosis was confirmed without infection. The molecular diagnosis of bacterial infection using RT-qPCR to detect bacterial 16S rRNA around a prosthesis correlated well with the clinical findings. Based on the promising clinical results, we were attempting to differentiate bacterial species or drug-resistant strains by using species-specific primers and to detect the persistence of bacteria during the interim period before the second stage reimplantation in a larger scale of clinical subjects.

1. Introduction

Periprosthetic infection (PJI) is difficult to treat and sometimes to diagnose. To differentiation septic from aseptic loosening is often challenging because the PJI might be partially suppressed by antibiotics before the loosening of the prosthesis. The utilization of medical resources in treating PJI is 2.8 times higher than that associated with revision surgery because of aseptic loosening [1, 2]. Definite diagnosis of PJI before revision surgery is therefore important because it reduces perioperative risks and medical costs. The American Musculoskeletal Infection Society has recently published new diagnostic criteria for a definite PJI which consists 1 of the 2 major criteria (sinus tract communicating with prosthesis or at least 2 positive tissue culture results) or 4 of the 6 minor criteria [3]. For probable or possible PJI, a consensus has not yet been reached. Because of the high incidence of culture-negative rates in clinical practice, surgeons need to make the decision based on every evidence to determine whether a revision surgery could be performed or an extended period of antibiotics therapy should be commenced.

For those patients with confirmed PJI, a two-stage reimplantation protocol that consists of extensive debridement at the first stage followed by delayed reimplantation is currently the standard of care in many hospitals with the success rate being between 82% and 95% [4–8]. The timing of reimplantation arthroplasty depends on the complete eradication of infection to avoid devastating complications

[9]. Diagnostic methods such as the serum CRP, interleukin-6, culture of joint aspirates, bone scans, frozen sections, and other molecular markers are the most commonly used surrogate parameters to determine the complete eradication of infection [10–17]. However, these tests have limitations such as being time consuming or nonspecific for the diagnosis of infection persistence. Previous studies used bacterial ribosomal RNA (rRNA) as a target for the diagnosis of infection [18]. The rRNAs are highly conserved among bacterial species, abundant in amount, and not present in human. The rRNA can be amplified by RT-PCR. Currently the detection limit of RT-PCR for bacterial rRNA is highly sensitive and avoids the high false-positive rates of amplifying the bacterial DNA [10, 18–21]. It can be served as a cell viability marker to differentiate dead organism from active infection [18, 22]. The purpose of this study was to test the feasibility of using RT-qPCR of bacterial 16S rRNA in the detection of PJI by in vitro and clinical specimens.

2. Materials and Methods

2.1. In Vitro rRNA Detection Limits. Total RNA was isolated from samples for the purposes of detecting rRNA of the assay. The same protocol was used to evaluate clinical samples. Enzymatic bacterial lysis was performed to ensure release of all intracellular RNA species in the samples. One milliliter of each sample was pipetted 2 volumes of RNAprotect Bacteria Reagent (QIAGEN, Valencia, California). The RNeasy Mini Kit (QIAGEN, Valencia, California) was used for column purification of total RNA. Poly(A) RNA (20 ng/5 mL) was used as a carrier species and was added to the specimen before using the RNeasy column to improve RNA yield with dilute samples. DNA contamination was eliminated by means of on-column DNase digestion prior to elution of total RNA from the column with 120 mL of RNase-free water. A 5-mL aliquot of total bacterial RNA was analyzed by the iScript one-step RT-PCR Kit with SYBR Green on an iCycler Thermal Cycler (Bio-Rad, Hercules, California) using universal primer pairs of bacterial 16S rRNA (forward 5′-attagataccctggtagtccacgcc-3′; reverse 5′-cgtcatccccaccttcctcc-3′). The cycling conditions were 50° for 10 minutes and 95° for 5 minutes, followed by 35 cycles of 95° for 10 seconds and 62° for 30 seconds. Limited dilution of standard strains of *E. coli* (dH10β) was used to analyze the detection limits of the assay.

2.2. Clinical Specimen Analysis. Joint fluids from patients who were suspected to have PJI were collected during operation. With informed consent and IRB approval (IRB no. 101-3480A3), demographic data, medical history, laboratory data, and culture results were recorded. The joint fluid was aliquot and subjected to enzymatic bacterial lysis after treating it with RNAprotect Bacteria Reagent (QIAGEN, Valencia, California) as described above. All RT-qPCR protocol was identical to the in vitro analysis except one of the samples was spiked with the standard strain of *E. coli* to serve as a positive control.

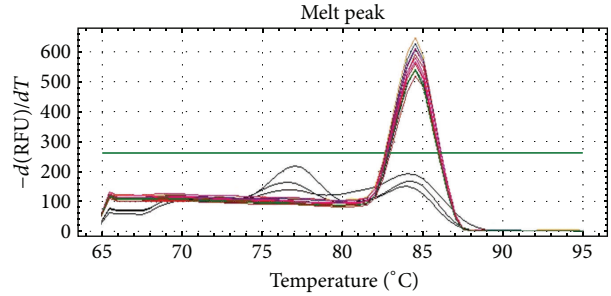

Figure 1: Melting temperature of all amplicons with serial dilution of total RNA.

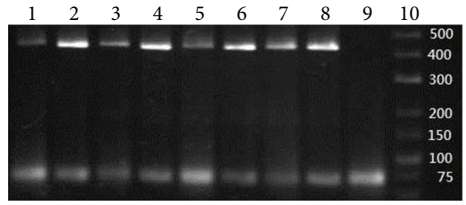

Figure 2: Gel electrophoresis of clinical specimens. Lanes 1, 3, 5, and 7: clinical specimen. Lanes 2, 4, 6, and 8: spiked specimen loaded with RNA of the standard strain of *E. coli* as positive control. Lane 9: negative control. Lane 10: DNA ladder.

3. Results

3.1. Detection Limit. After an overnight culture of the standard strain of *E. coli*, an aliquot of 5 mL culture medium was subjected to total RNA extraction. The average yield of RNA was 1.48 ng/μL. RT-qPCR was performed by a serial dilution of tenfold of the total RNA and analyzed on the iCycler Thermal Cycler (Bio-Rad, Hercules, California). It was found at the detection limit of the system which was at the total RNA concentration of 0.148 pg/μL. The melting temperature of all amplicons with serial dilution was similar between groups (Figure 1). Results were further checked with gel electrophoresis and showed consistent results as the RT-qPCR (Figure 2).

3.2. Clinical Specimens. In the study period, there were 11 patients referred for the diagnosis and treatment of PJI. Of the 11 patients, 10 were definite PJI based on the clinical presentations, laboratory data, and pathologic diagnosis (Table 1). One indeterminate case (case 11) was a staged reimplantation THA case and experienced swelling around the joint. Exploration of the hip joint revealed clear joint fluid and no evidence of infection by pathologic diagnosis. The culture was negative and the RT-qPCR result was negative for infection.

In the 10 confirmed PJI cases, the RT-qPCR results were all positive for infection. Among them, the culture result was no growth of bacteria in 2 cases. One case (case 4) had multiple organisms infection associated with a THA. One case (case 5) had mycobacterium infection. One case (case 6) had anaerobic bacteria infection. The RT-qPCR using the universal primers for 16S rRNA detection could

Table 1: Clinical data on patients with suspected infection status.

Case	Age	Sex	Diagnosis of PJI	ESR (mm/hr)*	CRP (mg/L)	Fistula	Culture†	qPCR result
1	46	F	THA infection	109	15.78	Present	NG	Positive
2	79	M	TKA infection	55	194.49	Present	*Pseudomonas aeruginosa*	Positive
3	68	M	THA infection	48	41.66	Present	NG	Positive
4	48	F	THA infection	NA	76.76	Present	CONS, Staph epi, and MRSA	Positive
5	63	M	TKA infection	124	51.54	Absent	M. chelonae	Positive
6	66	M	THA infection	73	96.76	Absent	*Peptostreptococcus*	Positive
7	73	F	TKA infection	74	18	Absent	*Staphylococcus aureus*	Positive
8	70	F	TKA infection	NA	273.83	Present	MRSA	Positive
9	63	M	THA infection	64	104.76	Absent	MSSA	Positive
10	70	F	Revision of TKA infection	NA	111.01	Absent	MRSA	Positive
11	32	M	Reactive synovitis	NA	12.39	Absent	NG	Negative

*NA: not available.
†NG: no growth; CONS: coagulase negative *staphylococcus*; Staph epi: *Staphylococcus epidermidis*; MRSA: methicillin-resistant *Staphylococcus aureus*; M. chelonae: *Mycobacterium chelonae*.

identify bacterial infection including aerobic, anaerobic, and mycobacteria and those 2 culture-negative cases. In the 11 clinical specimens, the RT-qPCR test was found to be highly accurate in the diagnosis of PJI.

4. Discussion

PJI is a devastating complication for the patient and the health care providers. Its incidence is between 1% and 3% in primary and 4% and 6% in revision total joint arthroplasties [1, 2]. The diagnosis can be straightforward with purulent discharge from the joint but may also be confusing in indeterminate cases. Often infection leads to multiple operations, prolonged use of antibiotics, extensive utilization of medical resources, and substantial social, economic, or even psychological impacts on the patients, family, hospitals, physicians, and payers [2]. An accurate diagnosis of PJI remains a challenging clinical problem and is essential for the success of treatment.

For a two-stage protocol, the existence of living bacteria in the joint is contraindicated for the reimplantation procedure. Usually the decision is made by assessing the wound condition, checking ESR and CRP levels, joint aspiration for analysis and culture, intraoperative frozen sections, or with the help of radioisotope scintigraphy [14, 15, 23]. Unfortunately, these tests are limited in the diagnostic power. A false negative result might lead to repeated surgery and devastating complications.

Bacterial ribosomal RNA (rRNA) has been used as a target for the diagnosis of infection [18]. The 16S rRNA is unique in bacterial species and is highly sensitive as a cell viability marker to differentiate dead organism from active infection [18, 22]. In a study of 64 patients who were suspected of having infection around the total knee arthroplasty, the overall accuracy by using RT-qPCR to detect PJI was 94% [18]. In this study, we were able to detect the 16S rRNA at the picogram levels in vitro. In the clinical specimens, we successfully identified bacterial infection in 10 definite PJI cases including those 2 culture-negative cases. Although the results were still preliminary, the RT-qPCR method using universal primer pairs targeting the 16S rRNA was found to be feasible to detect common bacterial (both aerobic and anaerobic) and mycobacterial infection. The result could help the clinical decision making especially in those cases with negative bacterial culture results. Jacovides et al. used a PCR-based mass spectrometry in 87 arthroplasty procedures and detected bacterial infection in 4 of 5 culture-negative cases and 50 of 57 presumably noninfectious cases [24]. They concluded that the molecular diagnosis of PJI could not only be effective at detecting organisms in culture-negative cases but also identify many of the revision cases that may have subclinical infection components.

In this study, we used universal primers for the 16S rRNA detection. Although the bacterial 16S rRNA is highly conserved, it does mark evolutionary distance and relatedness of organisms [25]. Universal primers that are complimentary to the conserved regions of the 16S rRNA gene could result in some variations in the end products detected by quantitative PCR. However it is difficult to differentiate bacterial species by using the universal primers. We could only differentiate the presence or absence of bacterial infection in our clinical specimens in this study. The choice of antibiotics or the drug-resistant strain detection could not be attained by the current method. These limitations could potentially be addressed by using species-specific primers or targeting on the drug-resistant genes [26, 27].

In conclusion, we found the molecular diagnosis of bacterial infection using RT-qPCR to detect bacterial 16S rRNA which was highly accurate in the diagnosis of PJI. Further studies to detect the persistence of bacteria during the interim period before the second stage reimplantation and to differentiate bacterial species or drug-resistant strains should be done to improve the diagnosis and treatment of the PJI.

Acknowledgment

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References

[1] K. J. Bozic and M. D. Ries, "The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization," *The Journal of Bone and Joint Surgery. American*, vol. 87, no. 8, pp. 1746–1751, 2005.

[2] S. M. Kurtz, E. Lau, J. Schmier, K. L. Ong, K. Zhao, and J. Parvizi, "Infection burden for hip and knee arthroplasty in the United States," *Journal of Arthroplasty*, vol. 23, no. 7, pp. 984–991, 2008.

[3] J. Parvizi, B. Zmistowski, E. F. Berbari et al., "New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society," *Clinical Orthopaedics and Related Research*, vol. 469, no. 11, pp. 2992–2994, 2011.

[4] J. N. Insall, F. M. Thompson, and B. D. Brause, "Two-stage reimplantation for the salvage of infected total knee arthroplasty," *The Journal of Bone and Joint Surgery. American*, vol. 65, no. 8, pp. 1087–1098, 1983.

[5] K. L. Garvin and A. D. Hanssen, "Infection after total hip arthroplasty: past, present, and future," *The Journal of Bone and Joint Surgery. American*, vol. 77, no. 10, pp. 1576–1588, 1995.

[6] A. D. Hanssen and M. J. Spangehl, "Treatment of the infected hip replacement," *Clinical Orthopaedics and Related Research*, no. 420, pp. 63–71, 2004.

[7] A. A. Hofmann, T. D. Goldberg, A. M. Tanner, and T. M. Cook, "Ten-year experience using an articulating antibiotic cement hip spacer for the treatment of chronically infected total hip," *Journal of Arthroplasty*, vol. 20, no. 7, pp. 874–879, 2005.

[8] P.-H. Hsieh, L.-H. Chen, C.-H. Chen, M. S. Lee, W.-E. Yang, and C.-H. Shih, "Two-stage revision hip arthroplasty for infection with a custom-made, antibiotic-loaded, cement prosthesis as an interim spacer," *Journal of Trauma*, vol. 56, no. 6, pp. 1247–1252, 2004.

[9] R. L. Barrack, "Rush pin technique for temporary antibiotic-impregnated cement prosthesis for infected total hip arthroplasty," *Journal of Arthroplasty*, vol. 17, no. 5, pp. 600–603, 2002.

[10] M. F. Schinsky, C. J. della Valle, S. M. Sporer, and W. G. Paprosky, "Perioperative testing for joint infection in patients undergoing revision total hip arthroplasty," *The Journal of Bone and Joint Surgery. American*, vol. 90, no. 9, pp. 1869–1875, 2008.

[11] T. W. Bauer, J. Parvizi, N. Kobayashi, and V. Krebs, "Diagnosis of periprosthetic infection," *The Journal of Bone and Joint Surgery. American*, vol. 88, no. 4, pp. 869–882, 2006.

[12] P. E. di Cesare, E. Chang, C. F. Preston, and C.-J. Liu, "Serum interleukin-6 as a marker of periprosthetic infection following total hip and knee arthroplasty," *The Journal of Bone and Joint Surgery. American*, vol. 87, no. 9, pp. 1921–1927, 2005.

[13] C. L. Jacovides, J. Parvizi, B. Adeli, and K. A. Jung, "Molecular markers for diagnosis of periprosthetic joint infection," *Journal of Arthroplasty*, vol. 26, no. 6, supplement, pp. 99–103, 2011.

[14] W. J. Kraemer, R. Saplys, J. P. Waddell, and J. Morton, "Bone scan, gallium scan, and hip aspiration in the diagnosis of infected total hip arthroplasty," *Journal of Arthroplasty*, vol. 8, no. 6, pp. 611–615, 1993.

[15] C. Love, M. B. Tomas, S. E. Marwin, P. V. Pugliese, and C. J. Palestro, "Role of nuclear medicine in diagnosis of the infected joint replacement," *Radiographics*, vol. 21, no. 5, pp. 1229–1238, 2001.

[16] G. Meermans and F. S. Haddad, "Is there a role for tissue biopsy in the diagnosis of periprosthetic infection?" *Clinical Orthopaedics and Related Research*, vol. 468, no. 5, pp. 1410–1417, 2010.

[17] J. Parvizi, E. Ghanem, S. Menashe, R. L. Barrack, and T. W. Bauer, "Periprosthetic infection: what are the diagnostic challenges?" *The Journal of Bone and Joint Surgery. American*, vol. 88, no. 4, pp. 138–147, 2006.

[18] P. F. Bergin, J. D. Doppelt, W. G. Hamilton et al., "Detection of periprosthetic infections with use of ribosomal RNA-based polymerase chain reaction," *The Journal of Bone and Joint Surgery. American*, vol. 92, no. 3, pp. 654–663, 2010.

[19] M. M. Tunney, S. Patrick, M. D. Curran et al., "Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene," *Journal of Clinical Microbiology*, vol. 37, no. 10, pp. 3281–3290, 1999.

[20] C. E. Corless, M. Guiver, R. Borrow, V. Edwards-Jones, E. B. Kaczmarski, and A. J. Fox, "Contamination and sensitivity issues with a real-time universal 16s rRNA PCR," *Journal of Clinical Microbiology*, vol. 38, no. 5, pp. 1747–1752, 2000.

[21] T. Newsome, B.-J. Li, N. Zou, and S.-C. Lo, "Presence of bacterial phage-like DNA sequences in commercial Taq DNA polymerase reagents," *Journal of Clinical Microbiology*, vol. 42, no. 5, pp. 2264–2267, 2004.

[22] S. Aellen, Y.-A. Que, B. Guignard, M. Haenni, and P. Moreillon, "Detection of live and antibiotic-killed bacteria by quantitative real-time PCR of specific fragments of rRNA," *Antimicrobial Agents and Chemotherapy*, vol. 50, no. 6, pp. 1913–1920, 2006.

[23] M.-J. Huang, P.-H. Hsieh, S. W. N. Ueng, K.-C. Ho, T.-C. Yen, and M. S. Lee, "Use of positron emission tomography to detect infection around antibiotic-loaded cement spacers in patients with high C-reactive protein levels," *Orthopedics*, vol. 34, no. 10, pp. e605–e609, 2011.

[24] C. L. Jacovides, R. Kreft, B. Adeli, B. Hozack, G. D. Ehrlich, and J. Parvizi, "Successful identification of pathogens by polymerase chain reaction (PCR)-based electron spray ionization time-of-flight mass spectrometry (ESI-TOF-MS) in culture-negative periprosthetic joint infection," *The Journal of Bone and Joint Surgery. American*, vol. 94, pp. 2247–2254, 2012.

[25] J. E. Clarridge III, "Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases," *Clinical Microbiology Reviews*, vol. 17, no. 4, pp. 840–862, 2004.

[26] K. Greisen, M. Loeffelholz, A. Purohit, and D. Leong, "PCR primers and probes for the 16S rRNA gene of most species of pathogenic bacteria, including bacteria found in cerebrospinal fluid," *Journal of Clinical Microbiology*, vol. 32, no. 2, pp. 335–351, 1994.

[27] D. C. Oliveira and H. de Lencastre, "Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant Staphylococcus aureus," *Antimicrobial Agents and Chemotherapy*, vol. 46, no. 7, pp. 2155–2161, 2002.

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Research Article

A Model of Implant-Associated Infection in the Tibial Metaphysis of Rats

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Objective. Implant-associated infections remain serious complications in orthopaedic and trauma surgery. A main scientific focus has thus been drawn to the development of anti-infective implant coatings. Animal models of implant-associated infections are considered helpful in the *in vivo* testing of new anti-infective implant coatings. The aim of the present study was to evaluate a novel animal model for generation of implant-associated infections in the tibial metaphysis of rats. *Materials and Methods*. A custom-made conical implant made of Ti6Al4V was inserted bilaterally at the medial proximal tibia of 26 female Sprague-Dawley rats. *Staphylococcus aureus* in amounts spanning four orders of magnitude and each suspended in 15 μl phosphate buffered saline (PBS) was inoculated into the inner cavity of the implant after the implantation into the defined position. Controls were treated accordingly with PBS alone. Animals were then followed for six weeks until sacrifice. Implant-associated infection was evaluated by microbiological investigation using swabs and determination of viable bacteria in the bone around the implant and the biofilm on the implants after sonification. *Results*. Irrespective of the initial inoculum, all animals in the various groups harbored viable bacteria in the intraoperative swabs as well as the sonication fluid of the implant and the bone samples. No correlation could be established between initially inoculated CFU and population sizes on implant surfaces at sacrifice. However, a significantly higher viable count was observed from peri-implant bone samples for animals inoculated with 10^6 CFU. Macroscopic signs of animal infection (pus and abscess formation) were only observed for implants inoculated with at least 10^5 CFU *S. aureus*. *Discussion/Conclusion*. The results demonstrate the feasibility of this novel animal model to induce an implant-associated infection in the metaphysis of rats, even with comparatively low bacterial inocula. The specific design of the implant allows an application of bacteria in reproducible numbers at well-defined contact sites to the animal bone.

1. Introduction

Implant-associated infections remain feared and severe complications in orthopaedic and trauma surgery. Beside the vast pathological and psychosocial significance for the patient, an enormous economic impact can be observed for the hospital in charge and consequently the healthcare system [1–4]. Despite strict specific hygiene measures such as perioperative antibiotic prophylaxis, laminar-air flow operation theatres and the use of sterishields, implant-associated infection rates remained more or less consistent throughout the past two decades [4]. The frequency of infection however is increasing with an increasing number of total joint arthroplasties [4, 5].

Treatment of implant-associated infections is complicated and often of small success because of the special biofilm growth characteristics of the responsible bacteria. First, biofilm growth protects bacteria from host defense mechanisms. Second, biofilm forms a diffusion barrier against systemically applied antibiotics [6–8], leading to a decreased susceptibility towards such antibiotics [9–11]. The problem could be aggravated when multiresistant pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA),

Table 1: Bacteria counts inoculated during surgery.

	Ideal CFU	Experimentally determined CFU
Group I	0	0
Group II	10^3	1,880 ± 219
Group III	10^4	10,546 ± 7,986
Group IV	10^5	124,881 ± 21,946
Group V	10^6	670,000 ± 124,900

vancomycin-resistant *Enterococci* (VRE), or multi-resistant Gram-negative rods cause the infections [12–15].

In this context, anti-infective implant coatings could display dual advantage both in prevention and treatment of implant-associated infections. Therefore, the development of such anti-infective implant coatings has become a major scientific focus. Yet, despite many promising approaches no final breakthrough has been achieved in clinical practice [16, 17].

Animal models of implant-associated infections are considered helpful for *in vivo* testing of potential anti-infective implant coatings and antibiotics. In addition, these models could improve the understanding of the specific pathogenesis as well as could support the optimization of surgical techniques. Consequently, a number of different models of implant-associated infections have been developed [18–22]. The models themselves and individual adjuvants such as soft tissue manipulation are still under discussion [19, 21]. Among other crucial parameters the amount of seeding bacteria is subject to controversial debates. To promote signs of infection, bacterial counts ranging from as little as 10^2 CFU of *S. aureus* [21] to 10^6 CFU of *S. aureus* have been used [20]. Another moot point is the seeding time point in respect to implant insertion, that is, should the implants be covered by a preformed biofilm or should the bacteria be applied during surgery, which would closely mimic the natural situation.

Hence the aim of the present study was to evaluate a novel animal model for generation of implant-associated infections in the tibia metaphysis, in which the amount of bacterial inocula necessary for generating an infection was tested as an independent parameter.

2. Materials and Methods

2.1. Bacteria Strains and Preparation of Inocula. *Staphylococcus aureus* (ATCC 25923) was used for this study as a pathogen of implant-associated infection. The strain was grown in Caso-Bouillon (CB) (Carl Roth, Karlsruhe, Germany) in an overnight culture at 37°C under a 5% CO_2–20% O_2 atmosphere. Bacterial counts as displayed in Table 1 were obtained by washing and suspending these cultures in PBS (2 g KCl, 2.4 g KH_2PO_4, 80 g NaCl, and 14.4 g Na_2HPO_4 per 1000 ml; pH 7.4) and spectrophotometric control (Smart Spec 3000, Bio-Rad Laboratories GmbH, München, Germany).

For preparation of 1 ml deep frozen stocks, 10% glycerol was added to the defined concentrations. The stock suspensions were kept at −70°C until the day of surgery. To quantify a possible loss of viable bacteria during deep-freezing, bacterial CFU were counted from 15 μl aliquots 24 hours after deep freezing and in parallel to each animal experiment. Viability counts were performed by serial tenfold dilution of the initial 15 μl aliquots in PBS. From each dilution step, a 100 μl aliquot was transferred onto Columbia sheep blood agar (Becton-Dickinson, Heidelberg, Germany). The strain identity was determined to species level in each experiment by judging colony morphology and performing catalase and coagulase tests. For identification to strain level, the *spa* gene of the isolate was amplified by PCR and the resulting PCR product was sequenced. The obtained *spa* gene sequence was compared to a commercial database. Strains belonging to the *spa* type of *S. aureus* ATCC25923 were regarded as identical with the inoculum strain.

2.2. Animals and Surgical Procedure. Female Sprague-Dawley rats (Charles River Laboratories, Germany GmbH, Sulzfeld, Germany) were used for this study. Animal experiments have been approved by the local review committee of the Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei M-V (LALLF MV, Reference number 7221.3-1.1-031/09). For acclimatization, the animals were delivered to the animal facility at least one week prior to first treatment. Animals were housed in cages at normal room temperature and daylight illumination with free access to food and water. They were treated according to current guidelines on animal well-being as previously approved by the Local Committee for Animal Experimentation (Reference number 7221.3-1.1-031/09).

Animals were randomly selected for each group. Surgery was performed under general anesthesia. This was induced by an intramuscular injection of 150 μg/kg medetomidine (Dorbene vet, Fort Dodge, Würselen, Germany), 200 μg/kg Midazolam (Ratiopharm, Ulm, Germany), and 5 μg/kg Fentanyl (Ratiopharm). The medial metaphysis of both tibiae was chosen as surgical site.

The skin at the surgical site was shaved and disinfected with octenidine hydrochloride plus phenoxyethanol (Octenisept, Schülke & Mayr, Norderstedt, Germany) before sterile draping. A medial incision of skin and fascia was performed in sterile surgical technique. The preparation of the implant bed was performed using a circular drill (2.8 mm diameter). A custom-made conical implant (3 mm maximum outer diameter and 3 mm length) made of Ti6Al4V was implanted bilaterally into the rats' medial tibia metaphysis (Figures 1 and 2) followed by the injection of 15 μl PBS containing *Staphylococcus aureus* into the implant cavity using a 25 μl microsyringe (Hamilton, Reno, NV). Four different bacterial counts (10^6 CFU, 10^5 CFU, 10^4 CFU, and 10^3 CFU) of *Staphylococcus aureus* were used in the different experiments for seeding of the peri-implant bone via the hollow implant. Controls received identical amounts of sterile PBS into identical types of implants. The outer opening of the implant cavity was finally sealed with bone wax.

Surgical sites were closed using Vicryl sutures (Ethicon, Somerville, NJ, USA). After the operation anesthesia was antagonized by an intramuscular injection of 750 μg/kg atipamezole (Alzane, Pfizer, Berlin, Germany), 200 μg/kg

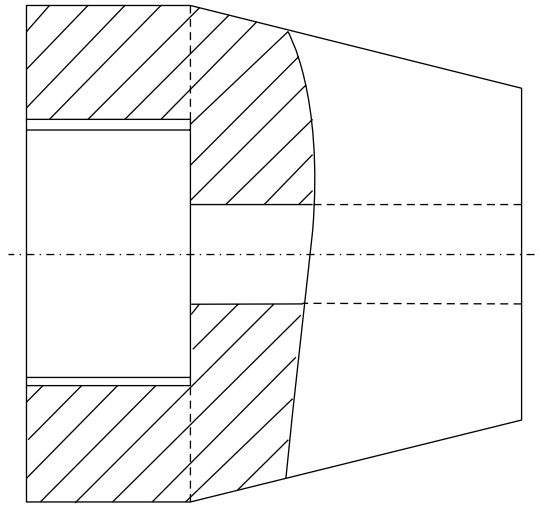

Figure 1: Schematic drawing of the canulated implant (diameter = 3 mm; length = 3 mm).

Flumazenil (Flumazenil-ratiopharm, Ratiopharm, Ulm, Germany), and 120 μg/kg naloxone (Naloxon-Ratiopharm, Ratiopharm, Ulm, Germany). When necessary, 50 μg/kg buprenorphine (Temgesic, Essex Pharma, München, Germany) was injected intramuscularly as postoperative analgesic.

A total of 26 animals were then followed for six weeks until sacrifice. Body temperature and weight were weekly determined. Blood samples were collected prior to sacrifice in order to analyze red blood cell count, white blood cell count, and C-reactive protein levels. Radiographs of the tibial bone were prepared immediately after surgery, three weeks after surgery, and prior to the explantation.

The animals were sacrificed after 6 weeks under general anesthesia, induced as previously described with an overdose of pentobarbital 80 mg/kg. Surgical sites were again shaved and disinfected with octenidine hydrochloride plus phenoxyethanol combination (Octenisept, Schülke & Mayr) before sterile draping. Employing sterile surgery techniques, skin incision within the old scar was performed. Initially, swabs (AMIES W/O CH, Sarstedt, Nümbrecht, Germany) were premoistened with sterile saline and streaked into the wounds of each animal once tibia and implant became visible. During the procedure, direct skin contact was carefully avoided to minimize the risk of contamination. Tibiae were then excised under sterile conditions. Bones and implants were finally separated using surgical instruments and stored in sterile PBS (10 ml for tibia specimens; 1 ml for implants).

In total, 6 specimens from Group I (Control), 10 specimens from Group II, 16 specimens from Group III, 16 specimens from Group IV, and finally 4 specimen from Group V were assigned to microbiological examination. Two specimens of Group I were histologically examined.

2.3. Examination of Bacteriology Swabs. Bacteriology swabs obtained as described above were moistened with one drop of sterile PBS, then evenly streaked onto a plate each of Columbia blood agar, Schaedler agar, and MacConkey agar, and thereafter immersed in nutrient broth and brain-heart infusion. The solid and liquid media were incubated at the below mentioned conditions:

Columbia blood agar (BD): 37°C + 5% CO_2; 24 h,

MacConkey agar (BD): 37°C + 5% CO_2; 48 h,

Schaedler agar (BD): 37°C under anaerobic conditions; 24 h,

Nutrient Broth 1 (Neogen, Lansing MI, USA): 37°C + 5% CO_2; 24 h,

BHI medium (BD): 37°C under anaerobic conditions; 24 h.

After 24 h or 48 h, respectively, the solid and liquid media were analyzed by conventional bacteriology techniques. The identity of potential *S. aureus* isolates was determined to the species level by mass spectrometry (Vitek Mass Spectrometer, BioMerieux) and to strain level by *spa* typing.

2.4. Microbiological Examination of the Implant. Implant-adhering bacteria were detached from the implant immersed in 1 ml PBS using low frequency ultrasound treatment (Sonorex digital 10P, Bandelin, Berlin, Germany: 5 min. at 80% intensity) [23, 24]. After the treatment, tubes were centrifuged at 4000 rpm for 10 min. at 4°C temperature (Heraeus Varifuge 3.OR; Kendro Laboratory Products, Osterode, Germany). The supernatant was resuspended in 300 μl PBS. Viable bacteria were determined as described above.

2.5. Microbiological Examination of Peri-Implant Bone. Cleaned tibia specimens in 10 ml PBS were exposed for 10 sec. to vigorous shaking (Vortex Genie 1, Scientific Industries, USA). Then the tibiae were removed from the resulting suspension and separately analyzed at a later stage.

The suspension was centrifuged at 4000 rpm for 10 min at 4°C temperature (Heraeus Varifuge 3.OR; Kendro Laboratory Products, Osterode, Germany). The sediment was resuspended in 3 ml PBS. For quantitative assessment of viable bacteria, 1 ml aliquots were serially diluted. One hundred μl aliquots from each dilution step were plated on Columbia blood agar plates and incubated for 24 h at 37°C under a 5% CO_2/20% O_2 atmosphere. The bone was weighed in a sterile petri dish, crushed, and prepared for DNA isolation and real-time polymerase chain reaction (PCR) as described above.

2.6. Statistics. Quantitative data is displayed as mean ± standard deviation (SD). Initially the Kruskal-Wallis test as a one-sided analysis of variance was applied. Where applicable, the Mann-Whitney test for independent samples and the Wilcoxon test for dependent samples were used for the statistical analysis. For all tests, the level of significance was set to $P < 0.05$.

3. Results

3.1. X-Ray Investigation. Neither in one of the *S. aureus*-exposed Groups II–V nor in the sterile control Group I were

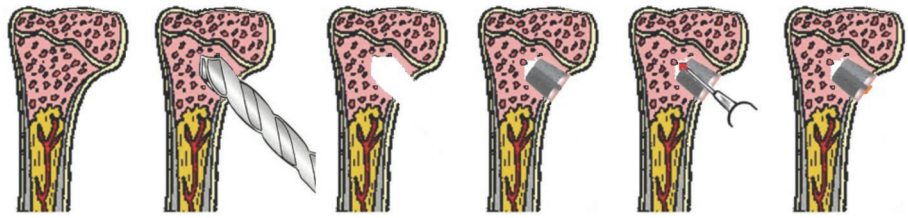

Figure 2: Schematic presentation of the surgical procedure. Cartoons 1 to 3: preparation of the implant bed, cartoon 4: implantation, cartoon 5: inoculation of bacteria into the implant cavity, and cartoon 6: wax sealing of the implant cavity.

radiographic signs of infection present three weeks postoperatively and prior to sacrifice (Figure 3).

3.2. Body Weight and Temperature. Animals from Groups I–V showed an initial decrease in body weight postoperatively without any statistical significance within or between the groups ($P > 0.05$). Furthermore, a gain in body weight was observed in animals from Groups I–V prior to sacrifice. This again was without any statistical significance within the groups ($P > 0.05$).

No obvious differences in rectal temperature were found between Groups I–V at any time of animal examination.

3.3. Blood Cell Count and CRP. Blood samples taken at the time of sacrifice revealed no differences of red blood cell count (RBC) or white blood cell count (WBC) within Groups I–V ($P > 0.05$). Furthermore, no statistical differences were found regarding the CRP values between the groups ($P > 0.05$) (Table 2).

3.4. Macroscopic Evaluation. In 3 animals of Group V (10^6 CFU) and 1 animal of Group IV (10^5 CFU) macroscopic pus and abscess formation was observed. Implant dislocation was observed in one animal from Group V as well as in one animal from Group II. Neither signs of implant dislocation nor pus and abscess formation was found for the remaining animals of all Groups I–V.

3.5. Microbiological Investigation. Average viable counts of bacteria recovered from samples of the *S. aureus*-exposed animal groups are summarized in Table 1 and Figure 4.

After sacrifice of animals of Groups II to V, *S. aureus* was isolated from all bacteriology swabs, as well as from the corresponding sonification fluid of the implant and the peri-implant bone samples. The isolates were identified to species level as *S. aureus* and to strain level as ATCC 25923 as described in Section 2. In all cases, the animal isolates corresponded to the *S. aureus* strain inoculated during initial surgery. In contrary, specimens taken from the PBS controls (Group I) remained sterile.

When analyzing the quantitative data from microbiological investigation of the sonication fluid and the peri-implant bone specimens, the *S. aureus*-exposed Groups II–V displayed numbers of viable bacteria well above the baseline defined by the control Group I treated with sterile fluids (Figure 5; Table 3). Furthermore, comparison of the bacterial quantities recovered from the implant-derived sonication fluid revealed no statistical significant ($P > 0.05$) differences between Groups II–V (Figure 5; Table 3). Moreover, quantitative microbiological investigation from the bone revealed no statistical difference in CFU/ml between Groups II–IV ($P > 0.05$) (Figure 5; Table 3), whereas a significant difference of

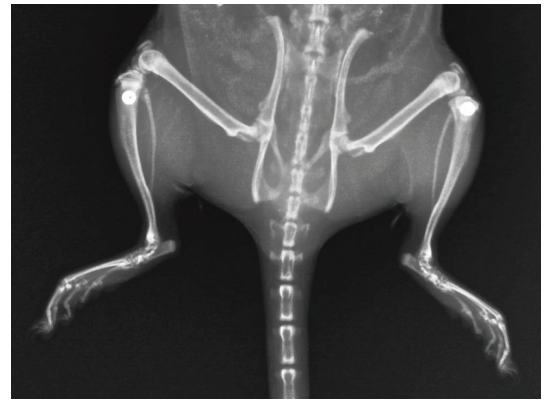

Figure 3: X-ray examination after surgery with the implant properly positioned in the proximal tibial metaphysis.

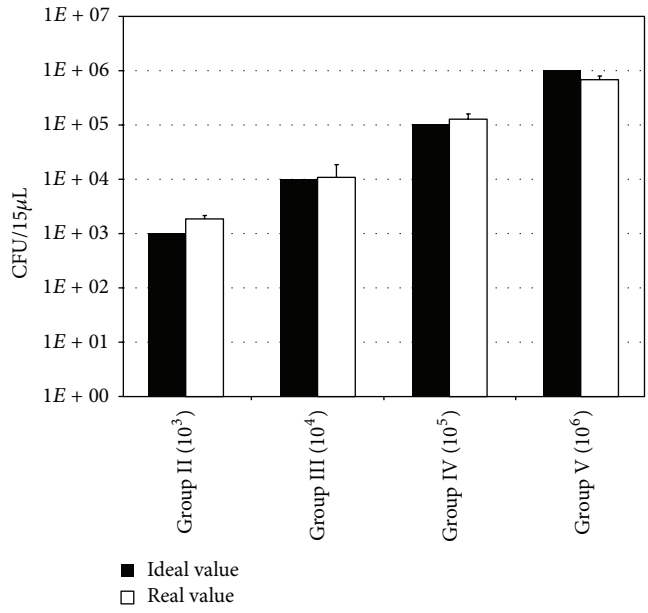

Figure 4: Ideal and experimentally determined CFU values of *S. aureus* bacteria prior to inoculation.

Table 2: Number of leukocytes (WBC), erythrocytes (RBC), and value of C-reactive protein (CRP) in blood samples.

CFU	Group I (control)	Group II (10^3)	Group III (10^4)	Group IV (10^5)	Group V (10^6)
WBC (×10 E9/l)—mean	7,81	5,63	7,54	6,7	11,66
WBC (×10 E9/l)—SD	3,43	1,27	3,7	1,73	6,55
RBC (×10 E12/l)—mean	7,65	7,08	7,12	7,28	7,68
RBC (×10 E12/l)—SD	0,29	0,22	0,73	0,84	0,41
CRP (mg/l)	<1	<1	<1	<1	<1

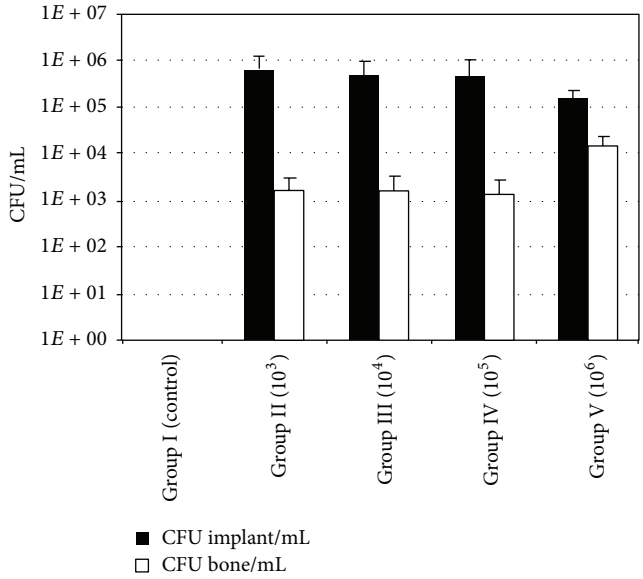

Figure 5: Bacterial counts cultured from the implant sonication fluids and suspensions prepared from periprosthetic tibial metaphysis.

CFU/ml from the peri-implant bone was observed between Group V and Groups II–IV ($P < 0.05$) (Figure 5; Table 3).

4. Discussion

With a constantly rising demand for orthopaedic and trauma surgery, the frequency of associated infections is bound to increase. Studies on implant-associated infections and potential anti-infective implant coating involving animal models are therefore urgently needed.

Most models of implant-associated infection in rats, mice, and rabbits are using intramedullary implants [20–22, 25] or plates [18, 26] and therefore mimic situations from trauma surgery. To our best knowledge, no animal model of implant-related-associated infection of the metaphyseal bone has so far been established.

Therefore, we intended to develop a more precise model for the generation of an implant-associated infection for such implants which also includes the option of extended implantation periods covering the complete "early infection" period up to two months after implantation [27]. Consistently, we chose a comparatively long observation period of six weeks in the present study.

With this study, we also addressed a crucial but moot point: the amount of bacteria to be used in animal models of infected implants is still controversially discussed. Monzón et al. [20] demonstrated that only 25% of animals with sterile tibia implants revealed signs of infection when exposed to a suspension of 10^5 CFU *S. aureus*. Usage of implants precolonized by a bacterial biofilm and additionally the administration of a suspension with 10^6 CFU *S. aureus* was therefore regarded as a reliable model to produce implant-related infections [20].

On the other hand, Lucke et al. [21] developed a model of implant-associated osteomyelitis in rats, displaying histological, microbiological, and radiological signs of infection with as little as 10^3 CFU of *S. aureus* [21]. In the present study, we were able to establish constant bacterial presence on the implant and in its environment and could demonstrate growth to common steady state value irrespective of the initial inocula, which differed by 3 orders of magnitude. However, only with large numbers of bacteria classical signs of local inflammation could be induced. From all bacteriology swabs, implant sonication fluids, and peri-implant bone samples, *S. aureus* (ATCC 25923) was detectable. Thus, we were able to support the findings of Lucke et al. [21] who induced an implant-associated infection with as little as 10^3 CFU of *S. aureus*. Thus we were able to abdicate on further histological investigation of the implants' as microbiological proof of viable bacteria was evident in all septic samples. Histological investigation was therefore mainly performed in a descriptive manor in order to rule out any adverse reaction.

It has previously been argued that the choice of tested implants requiring large bacterial inocula to generate implant-related osteomyelitis [20] could lead to the discrepancies observed between former studies [21]. Hollow implants were thereby hypothesized to provide a contaminated space not accessible for host defence mechanisms [21]. On the other hand it is an accepted statement in clinical practice that implant-associated infection in orthopaedic and trauma surgery could be related to low bacterial inocula even for species of low virulence [27–29]. Another controversial point is bone quality, since rat bone structure and metabolism are different from that of human bones [30], which for itself could be a reason for diverse minimum bacterial inocula necessary in different test settings.

In our novel model of implant-associated infection the implant bears a relatively small, cannulated space. With respect to a "dead space" hypothesis, this space however is considered negligible since the distal part of the cannula is in constant contact with the bone marrow. Hence

Table 3: Statistical significance (expressed in P values) of bacterial counts derived from test Groups I to V as determined by the concerning Mann-Whitney test between groups I to V from the periprosthetic bone (a) and the implant sonification fluid (b).

(a)

	Number of initially seeded bacteria				
	PBS (neg. control)	10^3	10^4	10^5	10^6
Number of initially seeded bacteria					
PBS (neg. control)	—	0.000	0.000	0.000	0.024
10^3	0.000	—	0.815	0.770	0.009
10^4	0.000	0.815	—	0.806	0.002
10^5	0.000	0.770	0.806	—	0.002
10^6	0.024	0.009	0.002	0.002	—

(b)

	Number of initially seeded bacteria				
	PBS (neg. control)	10^3	10^4	10^5	10^6
Number of initially seeded bacteria					
PBS (neg. control)	—	0.000	0.000	0.000	0.024
10^3	0.000	—	0.187	0.123	0.064
10^4	0.000	0.187	—	0.379	0.958
10^5	0.000	0.123	0.379	—	0.824
10^6	0.024	0.064	0.958	0.824	—

the space is at least partially accessible to host defence mechanisms. Histological cross-sectional cuts of the control tibia furthermore proofed tissue in growth, at least into the threaded part of the implant (Figure 6). Moreover, this model design mimics the thread of many well-established total joint systems of numerous manufacturers. Nevertheless, for certain study protocols, such as the investigation of potential anti-infectious implant coatings, this might be considered a limitation. This is due to the fact that certain implant coatings may not be applied to all implant shapes and cavities with small diameter. Furthermore, despite introducing an implant-related infection in the tibial metaphysis of rats, the knee joint was not opened.

In contrast to previous studies [21], we found no quantitative correlation between the amount of bacteria initially inoculated during surgery and viable bacteria retrieved from peri-implant bone (Figure 5; Table 3). Only in the bone material from Group V (initial inoculum 10^6 bacteria) a significantly ($P < 0.05$) higher count of viable bacteria was found compared to the bone material from Groups II to IV (initial inocula 10^3 to 10^5 bacteria). Moreover, no statistical differences are found between the viable bacterial counts from the sonication fluids of all *S. aureus*-challenged groups ($P > 0.05$). These findings could be explained by a consistent environmental situation in all cases, meaning comparable nutritional supply and amount of host defence mechanisms. The bacterial population would reach a similar steady state in all *S. aureus*-exposed groups, where the similar population size depended on the equal environmental conditions [31, 32]. The results of the present study therefore suggest that a stationary phase of bacterial population is reached during implant infection. This ultimate population size appears to be earlier reached on the implant surface than the peri-implant bone structures.

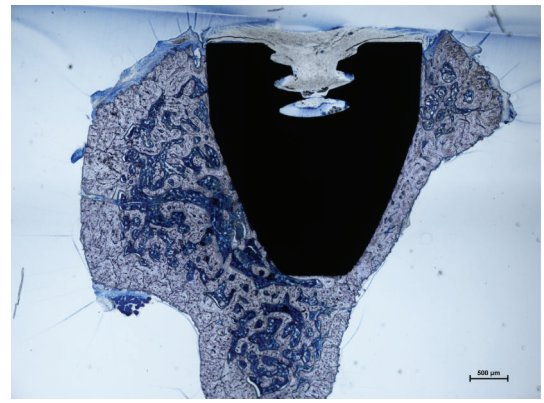

Figure 6: Histological cross-section of the tibial metaphysis including the inserted implant from an animal of Group I (uninfected control). The animal was sacrificed 42 d after the surgical implantation. The cross section shows no microscopic sings of infection and tissue ingrowth into the cavitated part of the implant.

Consistent with publications on examination of human material the sonication of the implants used in this study to detach adhering bacteria was found to work reliably and to lead to consistent results. It has previously been described as precise, sensitive, and with a wide applicability [23]. Furthermore, complete and reproducible detachment of bacteria has been described for sonification [24] and as such it is currently gaining significance in the diagnosis of periprosthetic infection.

Probably related to the phenomenon of bacterial colonization rather than overt infection and also most likely due to way of implant insertion into the bone, that is, the introduction into the metaphysis while leaving the bone

marrow intact, the radiological examination was uneventful for any of the tested animals. This finding is opposed to earlier findings on radiological signs of implant-related osteomyelitis induced by intramedullary implants [21]. Yet, results from conventional X-ray examinations are regarded as being variable and unspecific [33] and as such are inferior to microbiological and histological evaluation of implant-associated infections.

5. Conclusion

The results of the present study demonstrate the applicability of this novel animal model to reliably induce an implant-associated colonization and/or infection in the metaphysis of rats. The design of the conical hollow implant material allows for a deposition of defined bacterial loads without subsequent dilution or surgery technique-associated loss of substantial shares of the inoculum. We were able to confirm the findings of previous studies that a strong infection can be induced by comparatively small bacterial inocula since irrespective of the initial inoculum size a relatively constant population size is reached on the implant surface and in the neighbouring bone structures. Sonication of the implants is considered as a precise, sensitive, and widely applicable technique to suspend the bacterial biofilm from the implant surface. This novel animal model of implant-associated infection has the potential to become a standard for investigation of newly developed coated implant materials.

Authors' Contribution

Rainer Bader and Andreas Podbielski contributed equally to this study.

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References

[1] M. Haenle, C. Skripitz, W. Mittelmeier, and R. Skripitz, "Economic impact of infected total hip arthroplasty in the German diagnosis-related groups system," *Orthopade*, vol. 41, 6, pp. 467–476, 2012.

[2] M. Haenle, C. Skripitz, W. Mittelmeier, and R. Skripitz, "Economic impact of infected total knee arthroplasty," *The Scientific World Journal*, vol. 2012, Article ID 196515, 6 pages, 2012.

[3] S. M. Kurtz, E. Lau, H. Watson, J. K. Schmier, and J. Parvizi, "Economic burden of periprosthetic joint infection in the United States," *Journal of Arthroplasty*, vol. 27, no. 8, pp. 61.e1–65.e1, 2012.

[4] S. M. Kurtz, E. Lau, J. Schmier, K. L. Ong, K. Zhao, and J. Parvizi, "Infection burden for hip and knee arthroplasty in the United States," *Journal of Arthroplasty*, vol. 23, no. 7, pp. 984–991, 2008.

[5] J. L. Del Pozo and R. Patel, "Infection associated with prosthetic joints," *The New England Journal of Medicine*, vol. 361, no. 8, pp. 787–794, 2009.

[6] A. G. Gristina and J. W. Costerton, "Bacterial adherence to biomaterials and tissue. The significance of its role in clinical sepsis," *Journal of Bone and Joint Surgery A*, vol. 67, no. 2, pp. 264–273, 1985.

[7] A. G. Gristina, M. Oga, L. X. Webb, and C. D. Hobgood, "Adherent bacterial colonization in the pathogenesis of ostomyelitis," *Science*, vol. 228, no. 4702, pp. 990–993, 1985.

[8] M. Habash and G. Reid, "Microbial biofilms: their development and significance for medical device-related infections," *Journal of Clinical Pharmacology*, vol. 39, no. 9, pp. 887–898, 1999.

[9] J. W. Costerton, Z. Lewandowski, D. E. Caldwell, D. R. Korber, and H. M. Lappin-Scott, "Microbial biofilms," *Annual Review of Microbiology*, vol. 49, pp. 711–745, 1995.

[10] D. G. Davies, M. R. Parsek, J. P. Pearson, B. H. Iglewski, J. W. Costerton, and E. P. Greenberg, "The involvement of cell-to-cell signals in the development of a bacterial biofilm," *Science*, vol. 280, no. 5361, pp. 295–298, 1998.

[11] D. J. Evans, M. R. W. Brown, D. G. Allison, and P. Gilbert, "Susceptibility of bacterial biofilms to tobramycin: role of specific growth rate and phase in the division cycle," *Journal of Antimicrobial Chemotherapy*, vol. 25, no. 4, pp. 585–591, 1990.

[12] J. Parvizi, B. Bender, K. J. Saleh, T. E. Brown, T. P. Schmalzried, and W. M. Mihalko, "Resistant organisms in infected total knee arthroplasty: occurrence, prevention, and treatment regimens," *Instructional Course Lectures*, vol. 58, pp. 271–278, 2009.

[13] D. Ip, S. K. Yam, and C. K. Chen, "Implications of the changing pattern of bacterial infections following total joint replacements," *Journal of Orthopaedic Surgery*, vol. 13, no. 2, pp. 125–130, 2005.

[14] M. Haenle, A. Podbielski, M. Ellenrieder et al., "Periprosthetic infections following total hip replacement with ESBL-forming bacteria: importance for clinical practice," *Orthopade*, vol. 40, no. 6, pp. 528–534, 2011.

[15] J. C. Martínez-Pastor, F. Vilchez, C. Pitart, J. M. Sierra, and A. Soriano, "Antibiotic resistance in orthopaedic surgery: acute knee prosthetic joint infections due to extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 29, no. 8, pp. 1039–1041, 2010.

[16] M. Haenle, A. Fritsche, C. Zietz et al., "An extended spectrum bactericidal titanium dioxide (TiO_2) coating for metallic implants: in vitro effectiveness against MRSA and mechanical properties," *Journal of Materials Science: Materials in Medicine*, vol. 22, no. 2, pp. 381–387, 2011.

[17] G. Schmidmaier, M. Lucke, B. Wildemann, N. P. Haas, and M. Raschke, "Prophylaxis and treatment of implant-related infections by antibiotic-coated implants: a review," *Injury*, vol. 37, supplement 2, pp. S105–S112, 2006.

[18] Å. Johansson, J. U. Lindgren, C. E. Nord, and O. Svensson, "Local plate infections in a rabbit model," *Injury*, vol. 30, no. 9, pp. 587–590, 1999.

[19] T. Kälicke, U. Schlegel, C. Kraft, C. Wingenfeld, G. Muhr, and S. Arens, "Animal models of osteomyelitis," *Orthopade*, vol. 33, no. 3, pp. 260–266, 2004.

[20] M. Monzón, F. García-Álvarez, A. Laclériga et al., "A simple infection model using pre-colonized implants to reproduce rat chronic *Staphylococcus aureus* osteomyelitis and study antibiotic treatment," *Journal of Orthopaedic Research*, vol. 19, no. 5, pp. 820–826, 2001.

[21] M. Lucke, G. Schmidmaier, S. Sadoni et al., "A new model of implant-related osteomyelitis in rats," *Journal of Biomedical Materials Research B*, vol. 67, no. 1, pp. 593–602, 2003.

[22] N. M. Bernthal, A. I. Stavrakis, F. Billi et al., "A mouse model of post-arthroplasty *Staphylococcus aureus* joint infection to evaluate in vivo the efficacy of antimicrobial implant coatings," *PLoS ONE*, vol. 5, no. 9, Article ID e12580, 2010.

[23] G. D. Christensen, L. Baldassarri, and W. A. Simpson, "Methods for studying microbial colonization of plastics," *Methods in Enzymology*, vol. 253, pp. 477–500, 1995.

[24] H. Gollwitzer, K. Ibrahim, H. Meyer, W. Mittelmeier, R. Busch, and A. Stemberger, "Antibacterial poly(D,L-lactic acid) coating of medical implants using a biodegradable drug delivery technology," *Journal of Antimicrobial Chemotherapy*, vol. 51, no. 3, pp. 585–591, 2003.

[25] V. Alt, A. Bitschnau, J. Österling et al., "The effects of combined gentamicin-hydroxyapatite coating for cementless joint prostheses on the reduction of infection rates in a rabbit infection prophylaxis model," *Biomaterials*, vol. 27, no. 26, pp. 4627–4634, 2006.

[26] S. Arens, C. Kraft, U. Schlegel, G. Printzen, S. M. Perren, and M. Hansis, "Susceptibility to local infection in biological internal fixation. Experimental study of open vs minimally invasive plate osteosynthesis in rabbits," *Archives of Orthopaedic and Trauma Surgery*, vol. 119, no. 1-2, pp. 82–85, 1999.

[27] U. Geipel and M. Herrmann, "The infected implant—part 1: bacteriology," *Orthopade*, vol. 33, no. 12, pp. 1411–1428, 2004.

[28] C. Perka and N. Haas, "Periprosthetic infection," *Der Chirurg*, vol. 82, no. 3, pp. 218–226, 2011.

[29] L. Frommelt, "Aspiration of joint fluid for detection of the pathogen in periprosthetic infection," *Orthopade*, vol. 37, no. 10, pp. 1027–1036, 2008.

[30] J. Aerssens, S. Boonen, G. Lowet, and J. Dequeker, "Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research," *Endocrinology*, vol. 139, no. 2, pp. 663–670, 1998.

[31] A. Novick, "Growth of bacteria," *Annual Review of Microbiology*, vol. 9, pp. 97–110, 1955.

[32] F. H. Kayser and E. C. Böttger, "Algemeine Bakteriologie," in *Taschenlehrbuch Medizinische Mikrobiologie*, F. H. Kayser, E. C. Böttger, R. M. Zinkernagel, O. Haller, J. Eckert, and P. Deplazes, Eds., pp. 162–244, Thieme, Stuttgart, Germany, 12th edition, 2010.

[33] A. F. Widmer, "New developments in diagnosis and treatment of infection in orthopedic implants," *Clinical Infectious Diseases*, vol. 33, supplement 2, pp. S94–S106, 2001.

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Clinical Study

Two-Stage Revision Total Hip Arthroplasty for Periprosthetic Infections Using Antibiotic-Impregnated Cement Spacers of Various Types and Materials

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Antibiotic-impregnated hip cement spacers of various types and materials have been used in the treatment of periprosthetic hip infections. We developed a handmade spacer by using polymethylmethacrylate (PMMA) and/or α-tricalcium phosphate (α-TCP). In this study, we retrospectively reviewed the surgical outcomes in 36 consecutive patients treated with 2-stage revision total hip arthroplasty by using our antibiotic-impregnated hip cement spacers. We aimed to analyze the infection control and reinfection rates after revision surgery. Moreover, we analyzed the possible predictors of postoperative reinfection. After exclusion of 1 patient who died immediately after the first-stage surgery, infection was controlled in 33 of the 36 hips (success rate, 91.7%). Two of these 33 hips underwent resection arthroplasty. Of the 36 hips that had been treated with the antibiotic-cement spacer, 31 hips (86.1%) were eligible for the second-stage prosthesis re-implantation. The 31 protocol hip joints of patients followed up for >6 months (mean, 48.6 months). Ten of these 31 hips (32.3%) became reinfected. No possible predictor examined differed significantly between the reinfection-positive and reinfection-negative groups. However, spacers consisting of PMMA cement alone were associated with the highest risk of reinfection. Therefore, α-TCP-containing antibiotic-impregnated hip cement spacers might decrease the reinfection rate in patients undergoing re-implantation.

1. Introduction

Periprosthetic infection of the hip is the most serious complication after total hip arthroplasty (THA) and femoral head prosthesis (FHP) replacement. It imposes physical and mental stress and an economic burden on affected patients [1]. Moreover, postoperative infection can damage the trust-based patient-physician relationship. It is therefore most important to prevent postsurgical infection or, if infection has already occurred, to treat it appropriately. In the present study, we treated late stage (≥3 months postoperatively) or early stage (<3 months postoperatively) post-THA infection characterized by repeated recrudescence despite debridement without implant removal. The first stage, we controlled using an antibiotic-impregnated cement spacer with implant removal for infection. In the second stage, we used bone allografts to restore the bone defects in cases of implant loosening and massive bone defects resulting osteolysis of infection and repeated debridement [2, 3].

Although there are various options for treatment of post-THA infection, a 2-stage protocol with insertion of a type of antibiotic spacer has been widely reported [2, 4–8]. In this study, we aimed to analyze the rates of infection control and reinfection after revision surgery for treatment

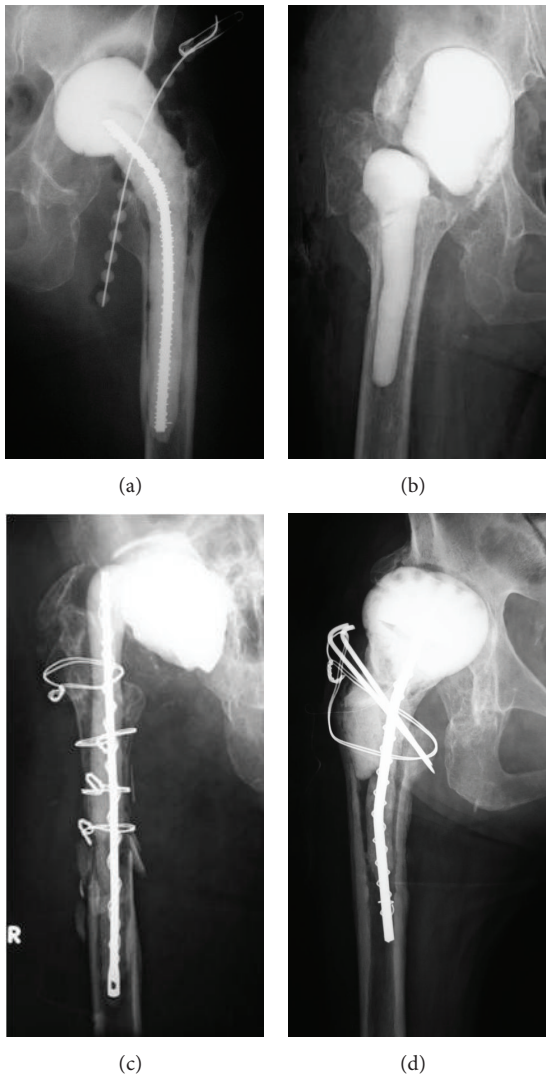

Figure 1: (a) The conventional polymethylmethacrylate (PMMA) cement spacer. (b) The α-tricalcium phosphate (TCP) spacer. (c) The separately prepared PMMA cement spacer (femoral side) and α-TCP spacer (acetabular side). (d) The new type of antibiotic-impregnated spacer.

of periprosthetic infections of the hip at our institution by using antibiotic-impregnated cement spacers of various types and materials. Moreover, we aimed to analyze the prognostic factors that might have influenced the development of post-operative reinfection in the patients in this series.

2. Materials and Methods

The study was approved by our institutional review board. From January 2000 to June 2012, we performed 2-stage revision THA, including FHP replacement, by using an antibiotic-impregnated cement spacer on 37 hips of 36 patients with infected THA. The patients comprised 19 men and 17 women (including both hips of 1 woman) with a mean age of 62.4 years (range, 27–90 years) at the time of the first-stage surgery, and who were followed up for a mean of 48.6 months (range, 6–127 months). The underlying diseases included osteoarthritis in 16 hips, femoral neck fracture in 9 hips, idiopathic osteonecrosis of the femoral head in 5 hips, rheumatoid arthritis in 2 hips, acetabular fracture in 2 hips, septic arthritis in 2 hips, and ankylosis in 1 hip. Twenty-two and 13 hips developed infection after primary THA (FHP replacement) and revision surgery for failed THA (FHP replacement), respectively. One hip developed infection after repeated revision surgery and another after resection of the products of heterotopic ossification after THA.

Table 1: Details of the causative organisms of infection in the 37 hips during the first-stage revision surgery.

Organism (n = 37)	No. of patients, n (%)
Staphylococcus epidermidis	10 (27.0)
Methicillin-resistant *Staphylococcus aureus* (MRSA)	9 (24.3)
Staphylococcus species	5 (13.5)
Methicillin-sensitive *Staphylococcus aureus* (MSSA)	2 (5.4)
Escherichia coli	2 (5.4)
Group B *streptococcus*	2 (5.4)
Klebsiella	1 (2.7)
Polymicrobial organisms	1 (2.7)
Unknown	5 (13.5)

Various materials have been used for spacers throughout the years. A conventional polymethylmethacrylate (PMMA) cement spacer was used in 9 hips (Figure 1(a)). An α-tricalcium phosphate (α-TCP) spacer prepared separately for the femoral and acetabular sides was used in 8 hips (Figure 1(b)). In 6 hips, the same method was used to prepare, separately, a PMMA cement spacer (femoral side) and an α-TCP spacer (acetabular side; Figure 1(c)). Finally, a newly devised spacer with an α-TCP core was used in 14 hips (Figure 1(d)). In addition, all implants were removed during the first surgical stage in 30 hips, whereas a spacer was prepared only for the acetabular side, preserving the stem, in 5 hips (a PMMA spacer in 4 hips and an α-TCP spacer in 1 hip). Bone defect reconstruction by using bone allografts was performed in 19 hips (61.3%) : 5 (16.1%) on the femoral side, 4 (12.9%) on the acetabular side, and 10 (32.3%) on both sides. Table 1 presents the details of the causative organisms of the infections present in the 37 hips during the first reconstruction stage.

One patient was excluded from this study because of death caused by hypovolemic shock on day 3 after the first surgical stage. The remaining 35 patients (36 hips) were included in a survey of the rates of infection control, performance of the second stage of the revision surgery, and reinfection after the second-stage revision surgery. In addition, the patients were divided into 2 groups according to the presence or absence of reinfection at the time of the final follow-up or earlier for statistical comparison to identify the factors likely to be involved in reinfection, including the frequency of previous surgery, type of spacer used in

the first surgical stage, causative bacterium, and use of a bone allograft. The Mann-Whitney U test and Fisher's exact test were used for statistical analysis. We adopted a significance level of $P = 0.05$.

3. Surgical Technique

3.1. The First Surgical Stage. The first stage of the surgical procedure (infection control) involved the following steps.

(1) Prosthesis Removal, Debridement, Cleaning, and Creation of an Antibiotic-Impregnated PMMA Cement Spacer by Using α-TCP (Biopex, Mitsubishi Materials, Tokyo, Japan). All the surgeries were performed with the patient in the lateral position. The approach was preferably made via the previous surgical scar. However, when no old surgical scar was available, a new skin incision was made, with a Gibson skin incision being the most frequently used. The transtrochanteric approach was frequently used to secure a sufficient operative field. In cases of fistula, gentian violet was injected via the fistula to mark the surgical site, the fistula was then resected. Joint fluid samples were collected for bacterial culture. Synovial membrane and periarticular tissue samples were collected for bacterial culture and pathological examination. In cases of a stable stem or difficulty in removing the bone cement, extended trochanteric osteotomy with preservation of the attachments of the gluteus medius and vastus lateralis muscles onto the femur was performed [9]. Contaminated tissues on the acetabulum, around the femoral neck, and in the femoral marrow cavity were thoroughly curetted and sampled for bacterial culture and pathological examination. Granulation tissue that appeared on visual inspection to be caused by infection was curetted completely, whereas bone, except for free sequestra, was preserved as much as possible. After curettage, the lesion was washed with a large volume (more than 10 L) of saline solution by using pulsed irrigation. The spacer was prepared with reference to the shape of the hip prosthesis on a preoperative anteroposterior radiograph. The spacer was prepared by another team either in parallel with the first surgical stage or a day earlier in the same operating room (in the latter case, it was then wrapped in a sterile sheet and drape and refrigerated). After the washing, the gloves, surgical gowns, and surgical equipment used were exchanged for freshly sterilized replacements. The drape used in the operative field was also replaced.

(2) Creation of Handmade Antibiotic-Impregnated PMMA Cement and α-TCP Spacers. Gentamicin (GM) was the antibiotic of choice because it withstands the high temperature generated by cement polymerization, has a broad spectrum, does not lose activity over time, and elutes efficiently from the cement. Because GM powder was difficult to obtain in Japan, liquid GM equivalent to 1200 mg of GM (60 mg/1.5 mL × 20 ampules) was mixed with 40 g of cement, placed in a sterile pack, and dried with hot air before use. In cases of infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA), the spacer was prepared by using 2.0 g of vancomycin (VCM) powder (0.5 g × 4 vials) and 40 g of a cement with a lower polymerization temperature (Cemex RX; Exactech, Gainesville, FL, USA) [10]. To prevent breakage, the spacer was reinforced with an Ender nail wrapped with a soft steel wire. When the causative bacterium was unidentifiable, 2.0 g VCM and 1200 mg GM were mixed with 40 g cement.

Since March 2005, α-TCP has been preferred because it was reported to generate no heat during polymerization and to allow the gradual release of the antibiotic embedded in the spacer [11]. Because FHP-type spacers composed of only α-TCP, which has low strength and frequently collapsed, antibiotic-impregnated α-TCP spacers were developed for separate placement on the femoral and acetabular sides. However, this precluded correction of leg length discrepancy during the waiting period. For this reason, new spacers using a combination of PMMA cement and α-TCP were developed and have been used since February 2008.

(3) Creation of a New Type of Handmade Antibiotic-Impregnated Spacer. The new type of spacer was prepared similarly to the spacer made of a combination of PMMA cement and α-TCP, by winding a soft steel wire around an Ender nail to prevent the nail from breaking, which makes it difficult to remove the distal spacer (Figure 2(a)). The amounts of antibiotics used were 0.5 g of VCM and 60 mg (1.5 mL) of GM to 12 g of α-TCP powder (Figure 2(b)). The core part of the femoral head was formed from 48 g of α-TCP containing 2 g of VCM and 240 mg of GM (Figure 2(c)) and placed at the tip of an Ender nail (Figure 2(d)). The part of femoral head was prepared by wrapping PMMA bone cement containing 2 g of VCM with α-TCP and shaping it by using an appropriately sized ladle (Figures 2(e) and 2(f)). The stem needed to be prepared carefully with PMMA cement so that it did not become too thick (Figure 2(g)), although the neck part should be somewhat thick to prevent fractures (Figure 2(h)). After completion of the spacer, a pore reaching the α-TCP core of the femoral head was made by using an surgical airdrill to create a channel for efficient gradual release of the antibiotics (Figures 2(i) and 2(j)).

To improve the results of spacer placement, advance trial reposition was performed to check whether the femoral head would fit the acetabulum. An excessively large femoral head of the spacer can restrict hip mobility, as well as hip repositioning, postoperatively. To fill the dead space and enhance gradual antibiotic release, antibiotic-impregnated α-TCP was added to the neck part after placement and repositioning of the spacer. The excised portions of the greater trochanter and femoral stem were temporarily fixed by performing tension-band wiring and wiring, respectively, until the next surgery.

(4) Systemic Administration of Antibiotics after the First Surgical Stage. Similarly, as for the conventional surgical technique, a cephem antibiotic was administered systemically for approximately 3 days, including the day of surgery, because the local concentration of antibiotic gradually released from the spacer was sufficient after that time. Since February 2008, antibiotics effective against the causative organisms of infection were chosen and administered until the patient's C-reactive protein (CRP) level returned to within normal limits unless complications such as persistent infection by

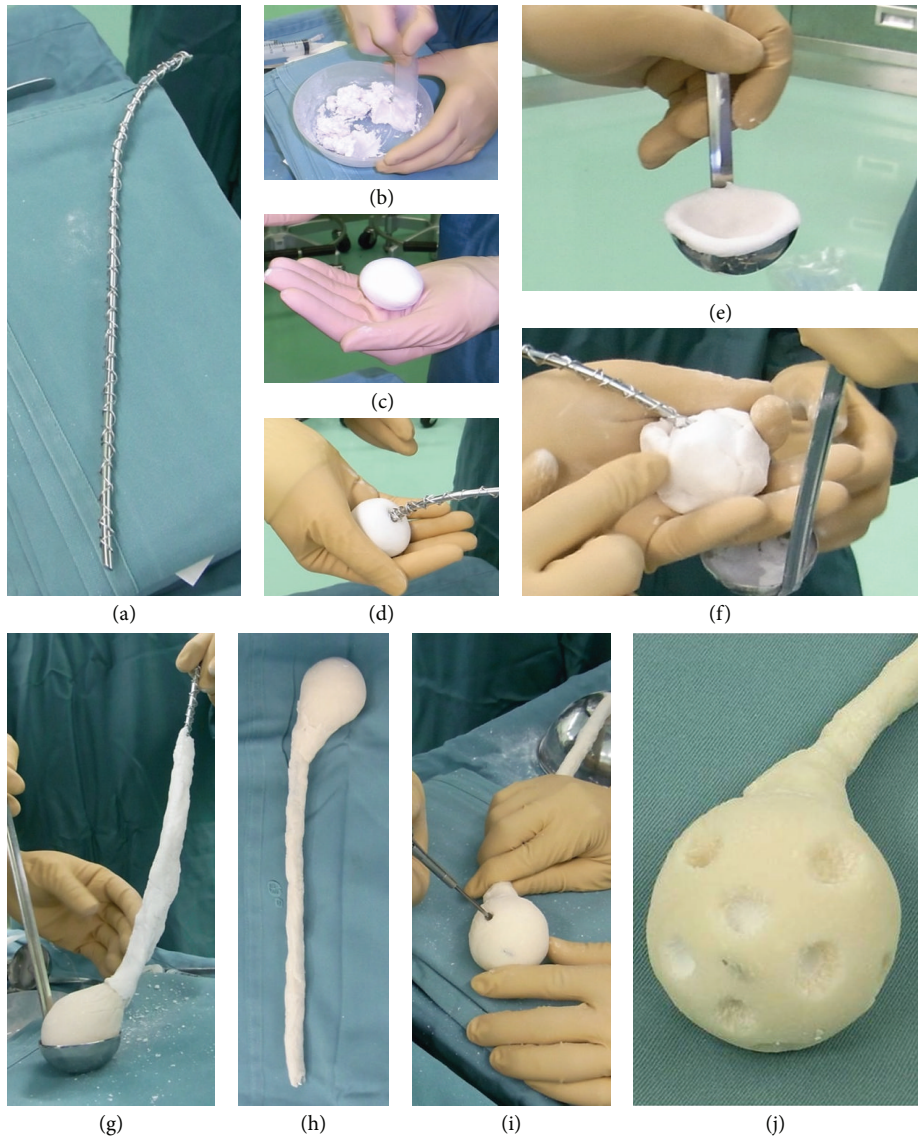

Figure 2: Steps in the making of the new type of handmade antibiotic-impregnated spacer.

the causative organism occurred, in which case the antibiotic susceptibility of the bacteria was examined. In addition, if the causative organism of infection was MRSA, we determined the appropriate antibiotic (including polypharmacy) and the duration of systemic administration by discussion with the infectious control team of our institute. When the infection was effectively controlled, the CRP level was normalized after approximately 3 weeks. When the CRP level remained normal, the second surgical stage was planned after a waiting period of 6–8 weeks. The mean waiting period in the present study was 55 days (range, 16–215 days).

3.2. The Second Surgical Stage (Revision Surgery). In the second surgical stage, the patient was maintained in the lateral position, as in the first surgical stage. After the spacer was removed, the synovial membrane of the pseudosynovial cavity formed around the spacer was curetted and sampled for bacterial culture; a joint fluid sample was also obtained for bacterial culture. Infection control was evaluated based on the presence or absence of bacteria by performing immediate pathological examination of a Gram-stained smear and a polymorphonuclear leukocyte count [12]. The bone defect was reconstructed, with the use of a bone allograft if that had been decided during the preoperative planning, and a hip prosthesis was placed.

4. Results

Excluding 1 patient who died immediately after completion of the first surgical stage, the infection was controlled in 33 of the 36 hips, for a success rate of 91.7%. One hip (2.8%) underwent redebridement and resection arthroplasty because of failure of infection control. Two of the 5 hips in which the stem was preserved underwent reremoval

of the stem followed by debridement and placement of another antibiotic-impregnated cement spacer to control the infection. The second-stage revision surgery could then be performed because the infection had been effectively controlled. Two of 33 hips underwent resection arthroplasty. Of the 36 hips that had been treated with the antibiotic-cement spacer, 31 hips (86.1%) were eligible for the second-stage prosthesis re-implantation.

Ten (32.3%) of the 31 hips became reinfected after second-stage surgery. One patient underwent revision surgery of the acetabular side cup and replacement of the proximal part of the stem to elongate the neck after experiencing repeated dislocation after the second-stage revision surgery. However, reinfection by another bacterium occurred after the surgery, so second-stage revision surgery with a bone allograft was performed again with a good result. One patient underwent second-stage revision surgery with a bone allograft but experienced recrudescence of *E. coli* similar to the causative organism of the initial infection in the early postoperative period. The infection was eventually controlled by replacing the bone allograft with an antibiotic-impregnated α-TCP spacer. One patient developed reinfection by a bacterium different from the causative organism 4 years after the second-stage revision surgery. The patient underwent repeat second-stage revision surgery with a bone allograft, this time with good results. Another patient underwent rereplacement of only the cup during the first stage of the revision surgery because of recrudescence of the same bacterium that caused the initial infection. Two patients underwent repeat second-stage revision surgery because of recrudescence of the same bacterium that caused the infection after the initial second-stage revision surgery. Four patients required additional surgery because of MRSA infection after the second-stage revision surgery.

Comparison between patients with and without reinfection produced the following results. The mean number of previous surgeries was 3.2 (range, 1–7 times) in the groups with reinfection versus 2.8 (range, 1–6) in the group without reinfection. MRSA was the causative bacterium in 4 (40.0%) and 6 hips (28.6%) in the groups with and without reinfection, respectively. Of the 31 hips that underwent the second-stage revision surgery, 5 (50.0%) and 14 (66.7%) in the groups with and without reinfection, respectively, were repaired with bone allografts. A PMMA spacer was used in 4 hips each in the groups with (40.0%) and without (19.0%) reinfection. None of these possible predictors of reinfection differed significantly between the patients in the 2 groups (Table 2).

Table 2: Comparison of the possible prognostic factors of re-infection between the re-infection-positive and re-infection-negative groups after the second-stage revision surgery in 31 hips.

	Re-infection negative (21 hips)	Re-infection positive (10 hips)	*P* value
No. of previous operations	2.8 ± 1.5	3.2 ± 1.7	0.467
Use of an allograft	14/21 (66.7%)	5/10 (50.0%)	0.308
Infection by MRSA	6/21 (28.6%)	4/10 (40.0%)	0.405
PMMA cement spacer	4/21 (19.0%)	4/10 (40.0%)	0.208

MRSA: Methicillin-resistant *Staphylococcus aureus*.
PMMA: Polymethylmethacrylate.

5. Discussion

There are several published reports on the treatment of periprosthetic infections after THA and FHP replacement. Other reported treatment options include 2-stage revision THA [4, 10], 1-stage replacement [13, 14], long-term antibiotic suppression [15], resection arthroplasty, arthrodesis [16], amputation, irrigation, and debridement with liner replacement. Antibiotic-impregnated cement beads have been reported to be effective for preventing infection after 2-stage revision [17]. However, we believe that 2-stage revision surgery comprising debridement, implant resection, implantation of an antibiotic-impregnated cement hip spacer, and delayed re-implantation is the most effective treatment for periprosthetic infections [4, 10] because it allows the maintenance of the patient's leg length and hip function as well as good infection control. In cases of mild periprosthetic infection of the hip, it is difficult to decide whether to remove the entire implant. However, it can be difficult to control periprosthetic infection while preserving the implant. In the present study, periprosthetic infection was not controlled in 2 of the 5 hips in which the stem was preserved, ultimately requiring stem removal followed by redebridement and spacer replacement. Therefore, we consider it difficult to control infection while preserving the prosthesis.

We also previously reported our institution's experience with a 2-stage re-implantation protocol. Takahira et al. [10] reported an infection control rate of 89% with the 2-stage protocol. Hsieh et al. [18] reported an infection control rate of 95.3% by using an antibiotic-impregnated hip cement spacer and beads. In contrast, Fehring et al. [19] reported a failure rate of 63% (54 of 86 patients) for treatment of periprosthetic infection by using irrigation and debridement alone. In addition, we do not currently apply continuous washing because doing so would require patients to undergo bed rest, complicate infection control, and result in lower-limb shortening. The use of an antibiotic-impregnated cement spacer is reported to produce better outcomes than irrigation. Therefore, we consider the spacer, which allows gradual local release of high concentrations of antibiotics, to be highly effective. The present results show control of the infection by using the spacer in 33 (91.6%) of the 36 hips. In addition, the second-stage revision surgery could be performed in 31 (86.1%) of the 36 hips, which is similar to the frequency previously reported [10]. In the treatment of infection, it is important to consider the systemic condition of the patient and to determine during the first-stage revision surgery whether it is necessary and advisable to remove the entire prosthesis and/or perform second-stage reconstruction.

Calcium phosphate cement (CPC) has been used for bone replacement and augmentation because of its good biocompatibility and osteoconductivity. Sasaki et al. [11] reported

that CPC has the advantage of not heating up during cement polymerization. In addition, it allows the maintenance of high antibiotic concentrations within an infected lesion. The authors demonstrated that VCM-impregnated CPC was able to maintain a higher concentration of VCM in focal areas for 2 weeks than was in bone cement and indicated that VCM-impregnated CPC may be more effective than bone cement for treatment of osteomyelitis or prosthesis infections. We also used CPC for our hip spacers. However, we prepared and placed the acetabular and femoral sides of the spacer separately because of the insufficient strength of this material. Nevertheless, we observed unstable hips during the waiting period before the second-stage revision surgery and leg shortening due to insufficient allowance to maintain leg length; we also experienced difficulty removing scattered fragments of fractured CPC during the second-stage revision surgery. For this reason, we recently developed a new type of spacer with a CPC core, which we found to be highly effective for the gradual release of antibiotics and to have strength comparable to that of PMMA cement. In a future study, we will report the therapeutic performance of our new spacer against infectious diseases.

The use of bone allografts to restore bone stock in a previously infected environment is controversial. One of the main concerns of using a bone allograft to treat massive bone loss in revision hip arthroplasty for treatment of infection is the theoretically increased risk for reinfection. Conversely, the use of a bone allograft in second-stage revision surgery for treatment of infection has frequently been reported to produce good results [5, 18, 20, 21]. The present results indicate that the reinfection rate was lower in the patients in whom a bone allograft was used for bone defect in the second-stage revision surgery for treatment of infection than in the patients in whom a bone allograft was not used, although there was no clearly significant difference. Therefore, we do not consider the use of a bone allograft to be a risk factor for reinfection, and this hip reconstruction technique should be implemented actively in the future.

The rate of reinfection after the second-stage revision surgery in the present study, which included recrudescence of infection and reinfection by bacteria different from the initial causative organisms of infection, was 32.3% (10/31 hips), an inferior performance relative to previous reports [22–24]. The causative factors for infection include systemic and local factors. As improving and maintaining the patient's systemic status seems to be necessary for the prevention of reinfection, we consider it important even for orthopedic surgeons to understand the patient's systemic condition (e.g., status of blood glucose control, amount of steroid medication used, presence or absence of urinary tract infection, smoking, nutritional status, and dental health).

Massive hemorrhage and prolonged surgery are expected during hip reconstruction after infection control and impose significant stress on patients, and Berend et al. [4] reported that the mortality rates associated with the treatment of infected THA are substantial. The authors performed a 2-stage protocol in 202 patients (205 hips) with infected primary or revision THA. Fourteen patients (7%; 14 hips) died before re-implantation, and the 90-day mortality rate after first-stage debridement was 4% (8 patients). Of the 186 patients (189 hips) who underwent re-implantation, 157 (83%) achieved infection control. When all of the patients who underwent the first-stage revision surgery were included, the rate of survival and infection control after 2-stage re-implantation was 76%. Berend et al. [4] concluded that successful 2-stage treatment should include not only effective control of infection but also successful second-stage re-implantation. Infection control is not achieved if death occurs before the second-stage operation; therefore, deaths should be excluded when determining the success rate of infection control. We also experienced one case in which 1 patient died of cardiac hypofunction due to hypovolemic shock on day 3 after the first-stage surgery. This case illustrates the importance of fully understanding the patient's systemic condition and likelihood of tolerating surgery and of providing sufficient explanation to the patient and his or her family before obtaining consent to undergo surgery. Moreover, we consider it necessary in the future to provide mental health care to patients who develop infection in order to mitigate their uneasiness about unforeseeable treatment outcomes and the necessity of long-term hospitalization.

6. Conclusion

The examined possible predictors of postoperative reinfection did not differ significantly between the reinfection-positive and reinfection-negative groups; however, the use of only a PMMA cement spacer was associated with the highest risk of reinfection. In the treatment of infection, it is important to determine whether or not the entire prosthesis needs to be removed during the first-stage revision surgery.

References

[1] K. Dairaku, M. Takagi, H. Kawaji, K. Sasaki, M. Ishii, and T. Ogino, "Antibiotics-impregnated cement spacers in the first step of two-stage revision for infected totally replaced hip joints: report of ten trial cases," *Journal of Orthopaedic Science*, vol. 14, no. 6, pp. 704–710, 2009.

[2] K. Uchiyama, N. Takahira, H. Narahara et al., "Revision total hip replacement using a cementless interlocking distal femoral stem with allograft-cemented composite and the application of intramedullary and onlay cortical strut allografts: two case reports," *Journal of Orthopaedic Science*, vol. 17, no. 3, pp. 323–327, 2012.

[3] K. Uchiyama, N. Takahira, K. Fukushima, T. Yamamoto, M. Moriya, and M. Itoman, "Radiological evaluation of allograft reconstruction in acetabulum with Ganz reinforcement ring in revision total hip replacement," *Journal of Orthopaedic Science*, vol. 15, no. 6, pp. 764–771, 2010.

[4] K. R. Berend, A. V. Lombardi Jr., and M. J. Morris, "Two-stage treatment of hip periprosthetic joint infection is associated with a high rate of infection control but high mortality," *Clinical Orthopaedics and Related Research*, vol. 471, no. 2, pp. 510–518, 2013.

[5] P. T. H. Lee, R. A. Clayton, O. A. Safir, D. J. Backstein, and A. E. Gross, "Structural allograft as an option for treating infected hip arthroplasty with massive bone loss," *Clinical Orthopaedics and Related Research*, vol. 469, no. 4, pp. 1016–1023, 2011.

[6] F. Leung, C. J. Richards, D. S. Garbuz, B. A. Masri, and C. P. Duncan, "Two-stage total hip arthroplasty: how often does it control methicillin-resistant infection?" *Clinical Orthopaedics and Related Research*, vol. 469, no. 4, pp. 1009–1015, 2011.

[7] K. P. Kalra, K. K. Lin, K. J. Bozic, and M. D. Ries, "Repeat 2-stage revision for recurrent infection of total hip arthroplasty," *Journal of Arthroplasty*, vol. 25, no. 6, pp. 880–884, 2010.

[8] A. C. Disch, G. Matziolis, and C. Perka, "Two-stage operative strategy without local antibiotic treatment for infected hip arthroplasty: clinical and radiological outcome," *Archives of Orthopaedic and Trauma Surgery*, vol. 127, no. 8, pp. 691–697, 2007.

[9] S.-J. Lim, Y.-W. Moon, and Y.-S. Park, "Is extended trochanteric osteotomy safe for use in 2-stage revision of periprosthetic hip infection?" *Journal of Arthroplasty*, vol. 26, no. 7, pp. 1067–1071, 2011.

[10] N. Takahira, M. Itoman, K. Higashi, K. Uchiyama, M. Miyabe, and K. Naruse, "Treatment outcome of two-stage revision total hip arthroplasty for infected hip arthroplasty using antibiotic-impregnated cement spacer," *Journal of Orthopaedic Science*, vol. 8, no. 1, pp. 26–31, 2003.

[11] T. Sasaki, Y. Ishibashi, H. Katano, A. Nagumo, and S. Toh, "In vitro elution of vancomycin from calcium phosphate cement," *Journal of Arthroplasty*, vol. 20, no. 8, pp. 1055–1059, 2005.

[12] M. J. Spangehl, B. A. Masri, J. X. O'Connell, and C. P. Duncan, "Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties," *Journal of Bone and Joint Surgery A*, vol. 81, no. 5, pp. 672–683, 1999.

[13] B. M. Wroblewski, "One-stage revision of infected cemented total hip arthroplasty," *Clinical Orthopaedics and Related Research*, vol. 211, pp. 103–107, 1986.

[14] J. J. Callaghan, R. P. Katz, and R. C. Johnston, "One-stage revision surgery of the infected hip: a minimum 10-year followup study," *Clinical Orthopaedics and Related Research*, no. 369, pp. 139–143, 1999.

[15] J. R. Crockarell, A. D. Hanssen, D. R. Osmon, and B. F. Morrey, "Treatment of infection with debridement and retention of the components following hip arthroplasty," *Journal of Bone and Joint Surgery A*, vol. 80, no. 9, pp. 1306–1313, 1998.

[16] J. Kostuik and D. Alexander, "Arthrodesis for failed arthroplasty of the hip," *Clinical Orthopaedics and Related Research*, vol. 188, pp. 173–182, 1984.

[17] L. Hovelius and G. Josefsson, "An alternative method for exchange operation of infected arthroplasty," *Acta Orthopaedica Scandinavica*, vol. 50, no. 1, pp. 93–96, 1979.

[18] P.-H. Hsieh, C.-H. Shih, Y.-H. Chang, M. S. Lee, W.-E. Yang, and H.-N. Shih, "Treatment of deep infection of the hip associated with massive bone loss," *Journal of Bone and Joint Surgery B*, vol. 87, no. 6, pp. 770–775, 2005.

[19] T. K. Fehring, S. M. Odum, K. R. Berend et al., "Failure of irrigation and debridement for early postoperative periprosthetic infection," *Clinical Orthopaedics and Related Research*, vol. 471, no. 1, pp. 250–257, 2012.

[20] I. Nusem and D. A. F. Morgan, "Structural allografts for bone stock reconstruction in two-stage revision for infected total hip arthroplasty: good outcome in 16 of 18 patients followed for 5–14 years," *Acta Orthopaedica*, vol. 77, no. 1, pp. 92–97, 2006.

[21] P. Ammon and I. Stockley, "Allograft bone in two-stage revision of the hip for infection?" *Journal of Bone and Joint Surgery B*, vol. 86, no. 7, pp. 962–965, 2004.

[22] A. S. E. Younger, C. P. Duncan, B. A. Masri, and R. W. McGraw, "The outcome of two-stage arthroplasty using a custom-made interval spacer to treat the infected hip," *Journal of Arthroplasty*, vol. 12, no. 6, pp. 615–623, 1997.

[23] P.-H. Hsieh, C.-H. Shih, Y.-H. Chang, M. S. Lee, H.-N. Shih, and W.-E. Yang, "Two-stage revision hip arthroplasty for infection: comparison between the interim use of antibiotic-loaded cement beads and a spacer prosthesis," *Journal of Bone and Joint Surgery A*, vol. 86, no. 9, pp. 1989–1997, 2004.

[24] S. M. Durbhakula, J. Czajka, M. D. Fuchs, and R. L. Uhl, "Spacer endoprosthesis for the treatment of infected total hip arthroplasty," *Journal of Arthroplasty*, vol. 19, no. 6, pp. 760–767, 2004.

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Clinical Study

Sonication: A Valuable Technique for Diagnosis and Treatment of Periprosthetic Joint Infections

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Background. Periprosthetic joint infection (PJI) is the most severe complication, following joint arthroplasty. Identification of the causal microbial factor is of paramount importance for the successful treatment. *Purpose*. The aim of this study is to compare the sonication fluid cultures derived from joint prosthetic components with the respective periprosthetic tissue cultures. *Methods*. Explanted prosthesis components for suspected infection were placed into a tank containing sterile Ringer's solution and sonicated for 1 minute at 40 kHz. Sonication fluid cultures were examined for 10 days, and the number and identity of any colony morphology was recorded. In addition, periprosthetic tissue specimens (>5) were collected and cultured according to standard practice. The duration of antimicrobial interruption interval before culture sampling was recorded. *Results*. Thirty-four patients composed the study group. Sonication fluid cultures were positive in 24 patients (70.5%). Sixteen of thirty four periprosthetic tissue cultures (47.1%) were considered positive, all revealing the same microbial species with the respective sonication fluid cultures: 3 tissue samples showed polymicrobial infection. All tissue cultures were also found positive by the sonication fluid culture. *Conclusions*. Sonication fluid cultures represent a cheap, easy, accurate, and sensitive diagnostic modality demonstrating increased sensitivity compared to periprosthetic tissue cultures (70.5 versus 47.1%).

1. Introduction

Over the last decades the number of total joint replacement procedures has been explosively increased with nearly 800.000 primary THR and TKR performed in the United States and 130.000 in England in 2006 [1, 2]. Their number is expected to rise by 174% and 673%, respectively, by year 2030 [3].

Periprosthetic joint infection (PJI) is the most severe complication, occurring in 0.3 to 1.7% of THR and 0.8 to 1.9% of TKR [4–8], while these rates rise up to 40% after revision surgery [9]. Their associated mortality is estimated to be between 1.0 and 2.7 percent [10–12]. Although they are still considered as an uncommon problem, PJIs pose a heavy social and economic burden with an estimated cost of up to $50.000 per patient and $250 million per year [10, 13], while others estimate that hospital costs per patient requiring revision THR due to infection reach nearly 5 times that of a primary THR [14].

Identification of the causal microbial factor is of paramount importance for successful treatment. Staphylococci (*aureus* and coagulase-negative species) account for more than half of the cases of PJI [11, 15], but in up to 20% of the cases more than one microorganism is identified (usually involving methicillin-resistant staphylococci *aureus* or anaerobes) [16]. Existing conventional methods for the detection of the underlying pathogen include microbiological cultures of synovial fluid and intraoperative soft tissue samples. However, 7 to 39% of the cases demonstrate negative cultures, attributed mainly to prior use of antibiotics, formation of protective biofilm at the surface of the implant (which allows proliferation of microorganisms on the prosthesis with no

presence at the surrounding soft tissue), and ability of the bacteria to change to a dormant metabolic form with small-colony variants [17–23].

The use of low-intensity ultrasound for the disintegration of biofilm (sonication) on removed implants and the subsequent culture of the sonication fluid is an alternative method for the diagnosis of PJI that has been proved to be more sensitive than conventional periprosthetic tissue cultures [14]. The purpose of the present study was to evaluate the sensitivity of a sonication protocol based on the method of Trampuz et al. [15] in comparison to traditional culture methods for the identification of causal pathogens in PJI following total joint arthroplasties.

2. Materials and Methods

Between October 2011 and June 2012, a prospective cohort study was conducted at the authors institution, a University level A Trauma Centre. The study protocol had been approved by the hospital scientific review board. The patients with periprosthetic joint infections composed the study group. Diagnosis of infection was confirmed on the basis of positive laboratory markers, cultures of preoperative aspirates, technetium-methylene diphosphonate (MDP) bone scintigraphy, and intraoperative tissue cultures. Prosthesis or its components (metal fixed or polyethylene mobile components) were removed for diagnosis of infection as a part of a two-stage revision protocol [11]. The first step of the surgical protocol included the explanation of the prosthetic components: an extensive debridement of the infected joint and the implantation of a temporary spacer. The second surgery was performed at a minimum of twelve weeks after first stage operation.

The explanted prosthesis was sent for sonication to detect microorganisms of the biofilm. The patients who had received intravenous antibiotic for at least 24 h in the 10 days before surgery or perioperative antimicrobial prophylaxis before surgery were excluded. Subjects were also excluded if obvious contamination of a removed component occurred in the operating room or fewer than three periprosthetic tissue samples were collected for culture.

Medical records including demographic characteristics; clinical, radiographic, laboratory, histopathological, and microbiological data; type of surgical management; information about the primary arthroplasty and subsequent revisions (if any) and antimicrobial therapy were reviewed and analyzed.

2.1. Study Definitions. PJI was considered if one of the following criteria was present: (i) visible purulence of a preoperative aspirate or intraoperative periprosthetic tissue (as determined by the surgeon), (ii) presence of a sinus tract communicating with the prosthesis, (iii) acute inflammation in intraoperative permanent periprosthetic tissue sections by histopathology (as determined by the pathologist), (iv) increased synovial fluid leukocyte count with 1700 leukocytes and/or 65% granulocytes, or (v) microbial growth in intraoperative periprosthetic tissue or sonication fluid of the removed implant. Low-virulence microorganisms, such as coagulase-negative staphylococci or *Propionibacterium acnes*, were considered pathogens if at least one additional (culture-independent) criterion for PJI was fulfilled.

2.2. Periprosthetic Cultures. For all patients, at least two intraoperative periprosthetic tissue specimens were retrieved from the bone-cement/bone-prosthesis interface, from sights with obvious inflammatory changes. Tissue specimens were collected in sterile vials and individually homogenized in 3 mL trypticase soy broth for 1 min using mortar and pestle. Tissue homogenate samples were inoculated in 0.1 mL aliquots into aerobic (SBA) and anaerobic sheep blood agar (ASBA) plates and in 1 mL aliquots into thioglycolate broth. The cultures were incubated at 35°C for 10 days. A terminal subculture was performed from all thioglycolate broth specimens on blood agar plates and incubated at 35°C for 5 more days. Each unique colony of isolated microorganisms was identified, and their antimicrobial susceptibility was tested using standard microbiological techniques. Positive tissue cultures were considered those with the same microorganism isolation of at least two periprosthetic tissue samples.

2.3. Sonication Fluid Cultures. The explanted prosthesis (or its components) was aseptically removed in the operating room and transported to the microbiology laboratory in sterile solid air-tight containers (Lock & Lock; Vertrag AG, Stafa, Switzerland) (Figure 1). Sonication of the implant was performed according to the Trampuz et al. technique [22]. Briefly, sterile Ringer solution (solution volume ranged from 50 to 200 mL depending on the size of implant) was added to the container in a laminar airflow biosafety cabinet to cover 85–90% of the volume of a big sized prosthesis or the entire volume of small sized components. The container with the implant was vortexed for 30 s, followed by sonication for 1 min (at a frequency of 40 kHz and power density of 0.22 W/cm^2), as determined by a calibrated hydrophone (type 8103; Bruel and Kjær, Naerum, Denmark). For sonication, ultrasound bath BactoSonic (Bandelin GmbH, Berlin, Germany) was used according to the manufacturer's instructions (http://www.bactosonic.info/) (Figure 2). No differences in frequency or power density were observed at various locations within the ultrasound bath during the study period. The container was subsequently vortexed for an additional 30 s to remove any residual microorganisms and to homogeneously distribute them in the sonication fluid. Aliquots of 0.1 mL sonicate fluid were inoculated into sheep blood agar (SBA) and anaerobic sheep blood agar (ASBA) plates. Additionally, 1 mL of the remaining of sonication fluid was added in 10 mL thioglycollate broth (TGB). The SBA plates and TSB were incubated at 37°C aerobically and the ASBA plates and TGB at 37°C anaerobically and inspected daily for bacterial growth. Every distinct morphotype colony of microorganisms on plates was enumerated (i.e., number of CFU/mL sonication fluid), identified, and subjected to susceptibility testing by means of routine microbiological techniques.

2.4. Negative Controls. Ten consecutive explanted prostheses, revised due to aseptic loosening from patients with no history

Table 1: Sensitivity and ESR-CRP values prior to and six weeks after prosthesis explantation.

	Tissue culture	Sonication fluid culture		ESR (mean ± SD)	CRP (mg/L) (mean ± SD)
No. of pts	16/34	24/34	Prior to explantation	56.4 ± 36.2	160.8 ± 45.7
Sensitivity	47.1%	70.5%	6 w after explantation	29.1 ± 6.3	8.8 ± 4.8

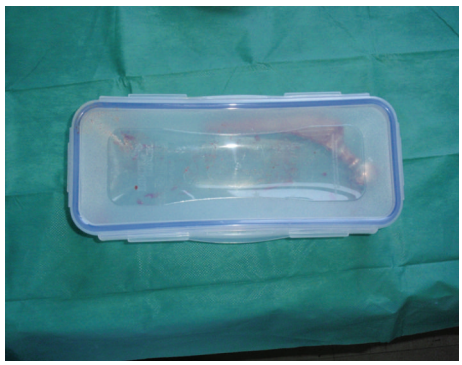

Figure 1

Figure 2

of previous infection, were included as controls. Following removal, the prosthesis was subjected initially to sonication and then to culture similarly to the prosthesis of the study group.

2.5. Statistical Analysis. Comparisons of individual diagnostic tests were performed using the McNemar test. For mixed infections, the test was considered positive if all infecting organisms were detected. Differences were considered significant when P values were ≤ 0.05. All calculations were performed using the statistical software package SPSS (version 13, NC).

3. Results

Thirty-four patients undergoing joint prosthesis removal composed the study group. Mean patients' age was 73.1 years (range 54–89 yrs). Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) values prior to and six weeks after prosthesis explantation are shown in Table 1.

Sonication fluid cultures were positive in 24 patients (70.5%): 12 coagulase-negative staphylococci (8 methicillin-resistant), 7 *Escherichia coli*, 4 *Staphylococcus aureus* (1 methicillin-resistant), 3 *Proteus spp*., 2 *Pseudomonas spp*., and 1 *Candida albicans* were identified. In 5 of 24 infected implants (25.2%), mixed infections were found. Different susceptibility testing was received for the same microbial species, especially for CNS ($n = 4$) and *E. coli* ($n = 2$). For periprosthetic tissue cultures, 16 of 34 samples (47.1%) were considered positive, all revealing the same microbial species with the respective sonication fluid cultures: three tissue samples demonstrated polymicrobial infection. All positive periprosthetic tissue cultures were also confirmed by the sonication fluid cultures. In 8 out of 34 patients (23.5%) in whom sonication fluid cultures were negative, the drug interruption interval before culture sampling was less than 7 days.

4. Discussion

Diagnosis of PJI is often challenging since many of the typical symptoms of infection can be missing. Several diagnostic modalities such as laboratory tests (white blood cell count, ESR, CRP, Il-6, TNF-α, and procalcitonin C), synovial fluid characteristics, histopathological studies of intraoperative samples of periprosthetic tissue, microbiological studies (conventional cultures of five to six intraoperative specimens of periprosthetic tissue), and radiological studies (predominately technetium-methylene diphosphonate (MDP) bone scintigraphy) can be applied to identify the pathogen [23]; however differential diagnosis of low-grade infections can be extremely challenging. Sensitivity and specificity of the aforementioned methods are currently not optimal for single use for the diagnosis of PJI, although several studies have shown that histopathology has a superior sensitivity compared to the microbiological and laboratory exams [24–26]. However, histological diagnosis has the disadvantage that the causative pathogen cannot be identified and so the optimal antibiotic treatment cannot be administered. On the contrary, cultures of periprosthetic tissue and the subsequent antibiogram can provide this essential information. Unfortunately, there is a high rate of negative cultures, a fact that often misleads the clinical decision.

The ability of the bacteria to form biofilms at the surface of implants is a major factor for chronic PJI and one of the main causes for the lack of positive cultures of periprosthetic soft tissue samples obtained intraoperatively [16, 17]. Bacteria can exist in two main forms: the planktonic form characterized by rapid cellular division and the sessile form characterized by slower cellular division, thus being more difficult to grow in cultures [27]. Biofilms are structured consortiums of bacteria in sessile form embedded in a self-produced biopolymer

matrix consisting of polysaccharide, protein, and DNA that originate from the microbes. Quite often these consortiums consist of more than one species living in a harmonic way. The matrix provides structural support to the bacteria, facilitating the communication and protecting them from the host's immune system and antibiotics [27, 28].

Utilization of ultrasound to dislodge biofilms from the surface of removed implants (sonication) has been effective in increasing the sensitivity of microbiological studies to identify the underlying pathogen. In a study of 331 patients with THR and TKR comparing sonication to standard tissue culture, the sensitivities of periprosthetic tissue and sonication fluid cultures were 60.8% and 78.5% ($P < 0.001$), respectively, and the specificities were 99.2% and 98.8%, respectively. There were 14 cases of PJI detected by sonication fluid cultures but not by conventional cultures. Of note, in patients receiving antimicrobial therapy within 14 days prior to surgery, the sensitivity of sonication fluid cultures was significantly superior to that of periprosthetic tissue cultures (75.0% versus 45.0%, $P < 0.001$) [14]. In another similar study with 136 patients undergoing shoulder revision arthroplasty (33 with PJI), sonication fluid cultures were more sensitive than periprosthetic tissue cultures (66.7% versus 54.5%, $P = 0.046$) while specificities were similar to the previous study [29].

The results of our study show that sonication fluid cultures of microorganisms from removed orthopedic implants are more sensitive than tissue cultures (70.5% and 47.1%, resp., $P < 0.005$). The technique is simple and can be performed in most microbiology laboratories. Additionally, as also shown in this study, it demonstrates a higher sensitivity for polymicrobial prosthetic-joint infections compared to intraoperative tissue cultures [15].

Nowadays, even for aseptic loosening, several studies suggest a possible role for bacteria and bacterial biofilm in implant failure [30, 31]. However, since sonication typically yields high numbers of organisms, one has to be aware of false positive results due to explanted prostheses contamination at the operating room or the microbiology laboratory. Further studies are required to quantify the number of microorganisms in sonicate fluid and assess the boundaries between PJI and contamination of explanted prosthesis following aseptic loosening [15].

5. Conclusions

Sonication of removed arthroplasty components using low-frequency ultrasound (35–40 kHz) was shown to improve microbiologic diagnosis of periprosthetic infections. Sonication fluid culture represents a cheap, easy, accurate, and sensitive diagnostic modality compared to periprosthetic tissue cultures. Staphylococci (especially coagulase-negative staphylococci) were the predominant pathogen, followed by *E. coli*.

References

[1] J. L. del Pozo and R. Patel, "Infection associated with prosthetic joints," *The New England Journal of Medicine*, vol. 361, no. 8, pp. 787–794, 2009.

[2] M. A. Cataldo, N. Petrosillo, M. Cipriani, R. Cauda, and E. Tacconelli, "Prosthetic joint infection: recent developments in diagnosis and management," *Journal of Infection*, vol. 61, no. 6, pp. 443–448, 2010.

[3] S. Kurtz, K. Ong, E. Lau, F. Mowat, and M. Halpern, "Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030," *Journal of Bone and Joint Surgery A*, vol. 89, no. 4, pp. 780–785, 2007.

[4] L. Pulido, E. Ghanem, A. Joshi, J. J. Purtill, and J. Parvizi, "Periprosthetic joint infection: the incidence, timing, and predisposing factors," *Clinical Orthopaedics and Related Research*, vol. 466, no. 7, pp. 1710–1715, 2008.

[5] P. F. M. Choong, M. M. Dowsey, D. Carr, J. Daffy, and P. Stanley, "Risk factors associated with acute hip prosthetic joint infections and outcome of treatment with a rifampinbased regimen," *Acta Orthopaedica*, vol. 78, no. 6, pp. 755–765, 2007.

[6] J. E. Phillips, T. P. Crane, M. Noy, T. S. J. Elliott, and R. J. Grimer, "The incidence of deep prosthetic infections in a specialist orthopaedic hospital. A 15-year prospective survey," *Journal of Bone and Joint Surgery B*, vol. 88, no. 7, pp. 943–948, 2006.

[7] E. Jämsen, H. Huhtala, T. Puolakka, and T. Moilanen, "Risk factors for infection after knee arthroplasty a register-based analysis of 43,149 cases," *Journal of Bone and Joint Surgery A*, vol. 91, no. 1, pp. 38–47, 2009.

[8] G. Peersman, R. Laskin, J. Davis, and M. Peterson, "The insall award paper: infection in total knee replacement: a retrospective review of 6489 total knee replacements," *Clinical Orthopaedics and Related Research*, no. 392, pp. 15–23, 2001.

[9] A. F. Widmer, "New developments in diagnosis and treatment of infection in orthopedic implants," *Clinical Infectious Diseases*, vol. 33, supplement 2, pp. S94–S106, 2001.

[10] W. Zimmerli, "Prosthetic-joint-associated infections," *Best Practice and Research: Clinical Rheumatology*, vol. 20, no. 6, pp. 1045–1063, 2006.

[11] W. Zimmerli, A. Trampuz, and P. E. Ochsner, "Prosthetic-joint infections," *The New England Journal of Medicine*, vol. 351, no. 16, pp. 1645–1654, 2004.

[12] L. Ahnfelt, P. Herberts, H. Malchau, and G. B. J. Andersson, "Prognosis of total hip replacement. A Swedisch multicenter study of 4,664 revisions," *Acta Orthopaedica Scandinavica, Supplementum*, vol. 61, no. 238, pp. 1–26, 1990.

[13] T. P. Sculco, "The economic impact of infected joint arthroplasty," *Orthopedics*, vol. 18, no. 9, pp. 871–873, 1995.

[14] K. J. Bozic and M. D. Ries, "The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization," *Journal of Bone and Joint Surgery A*, vol. 87, no. 8, pp. 1746–1751, 2005.

[15] A. Trampuz, K. E. Piper, M. J. Jacobson et al., "Sonication of removed hip and knee prostheses for diagnosis of infection," *The New England Journal of Medicine*, vol. 357, no. 7, pp. 654–663, 2007.

[16] C. E. Marculescu and J. R. Cantey, "Polymicrobial prosthetic joint infections: risk factors and outcome," *Clinical Orthopaedics and Related Research*, vol. 466, no. 6, pp. 1397–1404, 2008.

[17] E. F. Berbari, C. Marculescu, I. Sia et al., "Culture-negative prosthetic joint infection," *Clinical Infectious Diseases*, vol. 45, no. 9, pp. 1113–1119, 2007.

[18] J. L. del Pozo and R. Patel, "The challenge of treating biofilm-associated bacterial infections," *Clinical Pharmacology and Therapeutics*, vol. 82, no. 2, pp. 204–209, 2007.

[19] D. Malekzadeh, D. R. Osmon, B. D. Lahr, A. D. Hanssen, and E. F. Berbari, "Prior use of antimicrobial therapy is a risk factor for culture-negative prosthetic joint infection," *Clinical Orthopaedics and Related Research*, vol. 468, no. 8, pp. 2039–2045, 2010.

[20] J. Holinka, L. Bauer, A. M. Hirschl, W. Graninger, R. Windhager, and E. Presterl, "Sonication cultures of explanted components as an add-on test to routinely conducted microbiological diagnostics improve pathogen detection," *Journal of Orthopaedic Research*, vol. 29, no. 4, pp. 617–622, 2011.

[21] D. Neut, H. C. van der Mei, S. K. Bulstra, and H. J. Busscher, "The role of small-colony variants in failure to diagnose and treat biofilm infections in orthopedics," *Acta Orthopaedica*, vol. 78, no. 3, pp. 299–308, 2007.

[22] R. M. Donlan, "New approaches for the characterization of prosthetic joint biofilms," *Clinical Orthopaedics and Related Research*, no. 437, pp. 12–19, 2005.

[23] D. R. Osmon, E. F. Berbari, A. R. Berendt et al., "Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the infectious diseases society of America," *Clinical Infectious Diseases*, vol. 56, no. 1, pp. 1–10, 2013.

[24] B. Fink, C. Makowiak, M. Fuerst, I. Berger, P. Schäfer, and L. Frommelt, "The value of synovial biopsy, joint aspiration and C-reactive protein in the diagnosis of late peri-prosthetic infection of total knee replacements," *Journal of Bone and Joint Surgery B*, vol. 90, no. 7, pp. 874–878, 2008.

[25] M. Müller, L. Morawietz, O. Hasart, P. Strube, C. Perka, and S. Tohtz, "Diagnosis of periprosthetic infection following total hip arthroplasty—evaluation of the diagnostic values of pre- and intraoperative parameters and the associated strategy to preoperatively select patients with a high probability of joint infection," *Journal of Orthopaedic Surgery and Research*, vol. 3, no. 1, article 31, 2008.

[26] S. W. Tohtz, M. Müller, L. Morawietz, T. Winkler, and C. Perka, "Validity of frozen sections for analysis of periprosthetic loosening membranes," *Clinical Orthopaedics and Related Research*, vol. 468, no. 3, pp. 762–768, 2010.

[27] J. W. Costerton, P. S. Stewart, and E. P. Greenberg, "Bacterial biofilms: a common cause of persistent infections," *Science*, vol. 284, no. 5418, pp. 1318–1322, 1999.

[28] N. Høiby, T. Bjarnsholt, M. Givskov, S. Molin, and O. Ciofu, "Antibiotic resistance of bacterial biofilms," *International Journal of Antimicrobial Agents*, vol. 35, no. 4, pp. 322–332, 2010.

[29] K. E. Piper, M. J. Jacobson, R. H. Cofield et al., "Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication," *Journal of Clinical Microbiology*, vol. 47, no. 6, pp. 1878–1884, 2009.

[30] C. S. M. Hoenders, M. C. Harmsen, and M. J. A. van Luyn, "The local inflammatory environment and microorganisms in "aseptic" loosening of hip prostheses," *Journal of Biomedical Materials Research B*, vol. 86, no. 1, pp. 291–301, 2008.

[31] Y. Tamaki, Y. Takakubo, K. Goto et al., "Increased expression of toll-like receptors in aseptic loose periprosthetic tissues and septic synovial membranes around total hip implants," *Journal of Rheumatology*, vol. 36, no. 3, pp. 598–608, 2009.

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Clinical Study

The Cement Prosthesis-Like Spacer: An Intermediate Halt on the Road to Healing

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Background. Periprosthetic infections remain a devastating problem in the field of joint arthroplasty. In the following study, the results of a two-stage treatment protocol for chronic periprosthetic infections using an intraoperatively molded cement prosthesis-like spacer (CPLS) are presented. *Methods*. Seventy-five patients with chronically infected knee prosthesis received a two-stage revision procedure with the newly developed CPLS between June 2006 and June 2011. Based on the microorganism involved, patients were grouped into either easy to treat (ETT) or difficult to treat (DTT) and treated accordingly. Range of motion (ROM) and the knee society score (KSS) were utilized for functional assessment. *Results*. Mean duration of the CPLS implant in the DTT group was 3.6 months (range 3–5 months) and in the ETT group 1.3 months (range 0.7–2.5 months). Reinfection rates of the final prosthesis were 9.6% in the ETT and 8.3% in the DTT group with no significant difference between both groups regarding ROM or KSS ($P = 0.87$, 0.64, resp.). *Conclusion*. The results show that ETT patients do not necessitate the same treatment protocol as DTT patients to achieve the same goal, emphasizing the need to differentiate between therapeutic regimes. We also highlight the feasibility of CLPS in two-stage protocols.

1. Introduction

Osteoarthritis is nowadays a major cause of disability in adults with a growing trend. Alone knee osteoarthritis has a prevalence of over 30% amongst a population aged ≥60 years; this expresses the dimensions of the problem [1, 2].

Total knee arthroplasty (TKA) maintains its position as a major treatment option for knee osteoarthritis [3–5]. A feared complication of KA is infection of the prosthetic implant [6–8]. The reason for such concern is the substantial increase in morbidity and health care expenditure [9].

At least one of the following criteria has to be fulfilled to set the diagnosis of a prosthetic infection: growth of one microorganism species in two or more cultures of synovial fluid or periprosthetic tissue, purulence of the synovial fluid or macroscopic changes at the site of the implant, acute inflammation on histopathological examination of periprosthetic tissue, or presence of a sinus tract communicating with the prosthesis [10–12].

The gold standard for treating chronic periprosthetic infection is based on a two-stage protocol, including initial explantation of the infected components, adequate debridement, and antibiotic cement spacer prostheses implantation with systemic antibiotic therapy followed by secondary TKA once the optimal condition is achieved [13, 14]. The antimicrobial-impregnated spacer utilized in this process allows for maintenance of limb length, partial mobility during the recovery process, and infection control rates of 91% to 100% [15, 16]. Initially, cement spacers were static, therefore not providing sufficient range of motion (ROM); bone loss, soft tissue contracture, and increased scar tissue formation as a result have been mentioned [17–19]. The attempt to achieve a degree of ROM using dynamic cement on cement spacers with a joint geometry gained interest as a possible solution for the problems associated with static spacers [18, 19].

The use of either premolded spacers, intraoperative handcrafting by the surgeon, or intraoperative molding using

standard predesigned moulds has been described in the literature [20–22].

In this paper we verify the safety and efficacy of an intraoperatively produced custom made polymethyl methacrylate (PMMA) cement prostheses like spacer (CPLS) for a two-stage revision protocol of infected total KA.

2. Materials and Methods

Two molds were produced using a computerized numerical-control sinking machine (DMU 70eV-process) based on the design of the balanSys knee system (size B; D) (Mathys AG, Bettlach, Switzerland). The molds for the femoral spacer consisted of 3 components and the tibial of 2 components made of 100% Teflon). These molds were utilized intraoperatively to produce the spacer in its wanted shape.

The first surgical step involved explantation the prosthetic components and extensive debridement of the infected region, and biopsies were taken during the process for microbiological culture and histological examination. CPLS was finally performed. Initially, the components' appropriate sizes were assessed by means of conventional anterior posterior and lateral knee radiographs. The parts of the femoral mould were mounted, and the mould was filled with cement by hand. Due to cement expansion, the increase in pressure inside the closed mould created a smooth surface on the final cement spacer. After polymerization, the screws which interlink the mould were opened, and the femoral component was easily removed. The femoral component was implanted first with a small portion of additional cement. The distance between the tibia and the femur was measured in neutral position and the tibial component has filled with PMMA cement according to the distance measured. After polymerization, the tibial part of the spacer was removed from the mould, and the mounting cement on the posterior and lateral side of the spacer was removed with a Luer pincer. The tibial component was then implanted with a small portion of cement. The stability and range of motion were tested, and the wound was closed.

For all PMMA spacers, an antibiotic loaded cement was applied: PALACOSR + G 40 (Heraeus Medical GmbH, Wehrheim, Germany), containing 0.5 g of Gentamycin. The system permits the incorporation of different antibiotics into the PMMA spacer according to the antibiogram obtained by the initial puncture. Figure 2 shows the molds used intraoperatively, Figure 3 shows X-ray view of the implanted CPLS and Figure 4 shows an intraoperative view of an implanted CPLS immediately before revision TKA.

Seventy-five patients with chronically infected TKA received a two-stage revision procedure with the newly developed CPLS between June 2006 and June 2011 (mean age 67.5 years, range 57–85 years). However, two different protocols were considered according to the microorganism involved and treatment response (Figure 5). Patients infected with multidrug-resistant microorganisms, gram negative microorganisms, enterococcus species, or polymicrobial infections were considered *difficult to treat (DTT) (8)*, whereas patients infected with other microorganisms were considered *easy to treat (ETT)*. All patients underwent joint aspiration for microbiological examination prior to surgery.

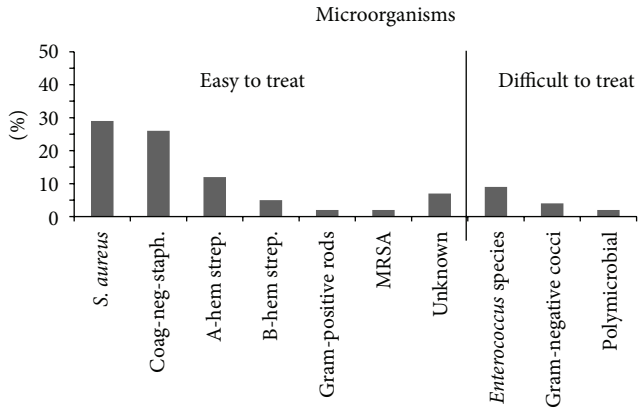


Figure 1: The percentage abundance of the encountered organisms in the cohort of this study. Coag-neg-staph: coagulase-negative staphylococci; A-hem strep: alpha-hemolytic streptococci. B-hem strep. Beta-hemolytic streptococci.

In the DTT ($n = 13$) group, a CPLS was implanted for 12 weeks, during which systemic antibiotics were administered, an open biopsy performed after a two-week antibiotic-free interval to confirm absence of microorganism growth before performing the revision TKA and finally continuing the systemic antibiotic therapy for 3 months.

In the ETT ($n = 62$) group, a CPLS was implanted for 4–6 weeks, during which systemic antibiotics were administered, and finally the revision TKA was performed after normalization of inflammatory markers and optimization of the soft tissue condition. Otherwise the patient was considered DTT and treated according to protocol. Antibiotic treatment was discontinued after the second stage TKA in the ETT group.

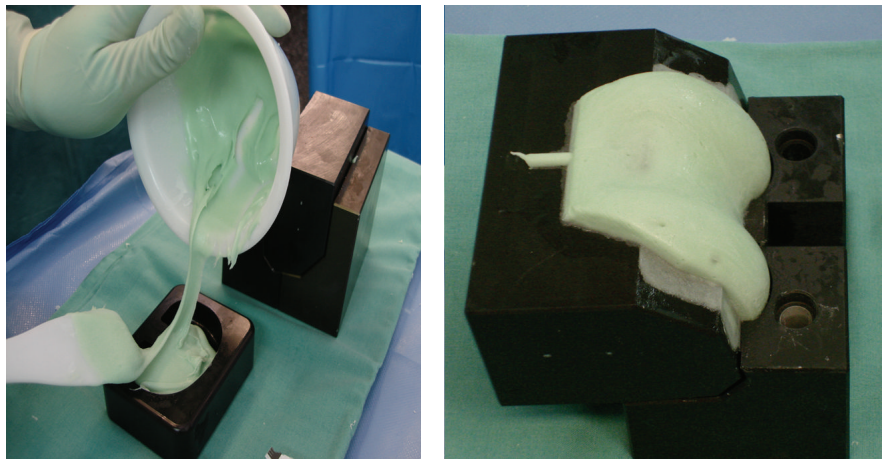
Knee society score (KSS) [23] and range of motion (ROM) were used for functional assessment at the time of spacer implantation and at one-year followup after TKA reimplantation. Results were compared using the students t-test, and $P \leq 0.05$ was considered statistically significant.

3. Results

Twenty-one ETT patients (27.9%) were infected with *Staphylococcus aureus*, nineteen (25.6%) with *coagulase-negative staphylococci*, nine (11.6%) with *alpha-hemolytic streptococci*, four (4.7%) with *beta-hemolytic streptococci*, two (2.3%) with *gram-positive rods*, two (2.3%) with *methicilin resistant staphylococcus aureus,* and five (7%) with an unknown microorganism.

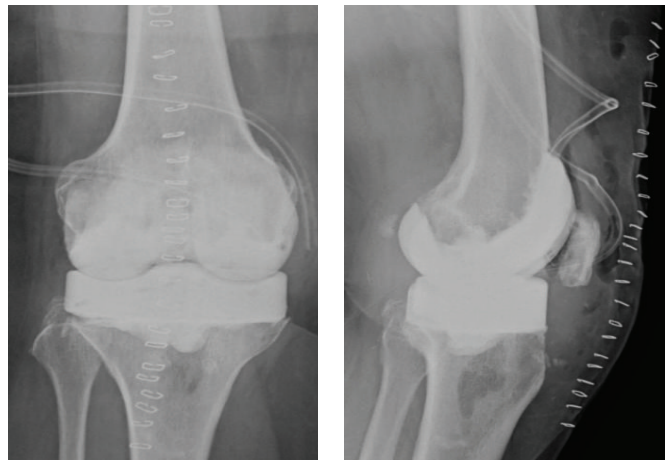
Seven DTT patients (9.3%) were infected with *enterococcus* species, three (4%) were infected with *gram-negative cocci,* and two (2.3%) had a polymicrobial infection (Figure 1).

The knee patient database of our department was utilized for retrieval of data. The mean duration of the CPLS implant in the DTT group was 3.6 months (range 3–5 months) and in the ETT group was 1.3 months (range 0.7–2.5 months). With the CPLS *in situ*, all patients were mobilized on crutches till final revision, and with a maximum weight of 15–20 kg, full range of motion was allowed. Squeaking was reported by all patients during the first 2 weeks of CPLS implantation, and



(a) (b)

Figure 2: (a) Tibial mold, (b) femoral mold used intra-operatively.



(a) (b)

Figure 3: *X-ray images showing* (a) anterior posterior view with the CLPS implanted and (b) lateral view with the CLPS implanted.

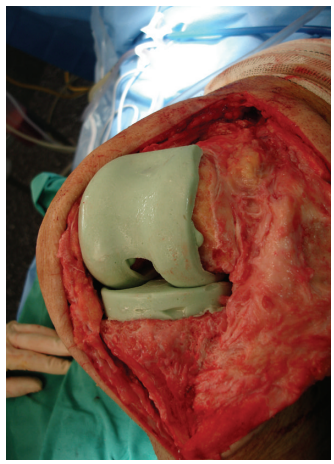


Figure 4: An intraoperative view of an implanted CPLS immediately before revision TKA.

no pain was associated. CPLS components were stable upon X-ray followup 2 months after implantation in all patients. The mean range of ROM with CPLS generally was 103° (range 75°–130°): DTT group was 104° (range 77°–130°) was ETT 102° (range 75°–130°), and no significant difference between both groups ($P = 0.87$). The mean KSS was 84.4 (range 71–93): DTT group was 85.1 (range 71–91), ETT group 84.2 (range 73–93), with no significant difference between both groups ($P = 0.64$).

Initial mean C-reactive protein (CRP) value immediately before explanation of the prosthesis was 150.1 mg/L (range 98 mg/L–235 mg/L): DTT group, mean 144.2 mg/L (range 114–235 mg/L), ETT group, mean 153.2 mg/L (range 98–222 mg/L), with no significant difference between both groups ($P = 0.471$). The mean CRP value immediately before performing the second stage TKA procedure was 8.87 mg/L (range 3–18 mg/L): DTT group, mean 9.4 mg/L

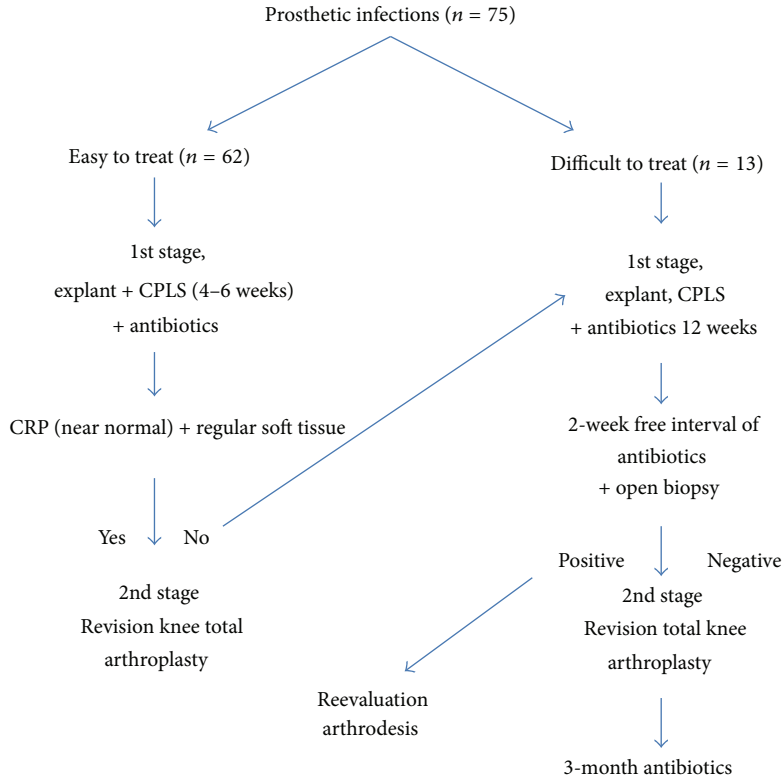


Figure 5: Protocol upon which the treatment strategy was based.

(range 5–18 mg/L), ETT group, mean 8.1 mg/L (range 3–16 mg/L), with no significant difference between both groups ($P = 0.152$).

The mean follow-up interval after the final revision arthroplasty was 4.3 years (range 2–7 years).

The mean ROM with the final prosthesis generally was 115° (range 90°–125°): DTT group, mean 112° (range 90°–125°) and ETT group, mean 117° (range 82°–130°), with no significant difference between both groups ($P = 0.76$). The mean KSS was generally 89.5 (range 74–95): DTT group, mean 88.4 (range 76–93), ETT group, mean 90.2 (range 74–95), with no significant difference between both groups ($P = 0.354$).

In the ETT group, 6 reinfections (9.6%) of the final prosthesis occurred, 3 *Staphylococcus aureus, 2 Streptococci* and *1 coagulase-negative staphylococcus during the first 6 months*. Two of these were persistent infections during the first 6 weeks requiring a divert of treatment protocol from ETT to DTT, one was a reinfection within the first 6 months requiring a divert to a DTT treatment protocol and three required a divert during the remaining follow-up interval.

In the DTT group one reinfection (8.3%) with *gram negative cocci* occurred 3.5 years after revision ending up in arthrodesis.

4. Discussion

Due to the devastating problem of chronic joint infections, work on the development of new strategies and material to tackle the problem is necessary.

The one-stage revision arthroplasty is widely spread and maintains its place as treatment standard in many centers [24–26]. However, a review article published recently by Romano et al. showed that two-stage procedures provide benefit over one-stage procedures regarding reinfection rates, and that far more two-stage procedures are being reported in the literature showing the increasing popularity of two-stage procedures [27]. The eradication rate of 90.7% achieved in our 75 patient of two-stage series was higher than the literature average of 81.9 for one-stage procedures and close to the literature average of 91.2 for two-stage procedures using articulating spacers [27].

In this study, we present a therapeutic plan for periprosthetic infections based on the microorganism involved (Figure 4). Zimmerli et al. first described the term difficult to treat DTT prosthetic infections in association with the microorganisms mentioned above [8]. According to Zimmerli, we differentiated between ETT and DTT patients and used two different therapeutic protocols (Figure 4). The duration of treatment for ETT patients was significantly less than ETT patients (1.3 months versus 3.6 moths, resp.). Same for the antibiotic therapy that was discontinued immediately after revision TKA in ETT patients and continued for three months in DTT patients. The results did not show any significant difference in outcome regarding reinfection rates of the final prosthesis, ROM, or KSS scores between both groups.

The insertion of an intraoperative moulded PMMA articulating spacer presents surgical advantages and apparently is associated with less reinfections than static spacers [27].

We noted that the application of such a spacer resulted in considerably less scar tissue formation, thus facilitating surgical exposure during the second intervention. The fact that less scar tissue removal had to be performed facilitated joint exposure avoiding complex soft tissue procedures, thus resulting in shorter operation time and easier postoperative rehabilitation.

In conclusion, the results show that ETT patients do not necsseate the same treatment protocol as DTT patients to achieve the same goal, emphasizing the need to differentiate between therapeutic regimes. We also highlight the feasibility of CLPS in two-stage protocols.

Conflict of Interests

The authors declare no competing interests in any means or any conflicting financial interests.

References

[1] D. T. Felson, A. Naimark, and J. Anderson, "The prevalence of knee osteoarthritis in the elderly: the Framingham Osteoarthritis Study," *Arthritis and Rheumatism*, vol. 30, no. 8, pp. 914–918, 1987.

[2] K. Ronn, N. Reischl, E. Gautier, and M. Jacobi, "Current surgical treatment of knee osteoarthritis," *Arthritis*, vol. 2011, Article ID 454873, 9 pages, 2011.

[3] W. Zhang, R. W. Moskowitz, G. Nuki et al., "OARSI recommendations for the management of hip and knee osteoarthritis, Part I: critical appraisal of existing treatment guidelines and systematic review of current research evidence," *Osteoarthritis and Cartilage*, vol. 15, no. 9, pp. 981–1000, 2007.

[4] W. Zhang, R. W. Moskowitz, G. Nuki et al., "OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines," *Osteoarthritis and Cartilage*, vol. 16, no. 2, pp. 137–162, 2008.

[5] W. Zhang, G. Nuki, R. W. Moskowitz et al., "OARSI recommendations for the management of hip and knee osteoarthritis. Part III: changes in evidence following systematic cumulative update of research published through January 2009," *Osteoarthritis and Cartilage*, vol. 18, no. 4, pp. 476–499, 2010.

[6] L. A. Whiteside, "Treatment of infected total knee arthroplasty," *Clinical Orthopaedics and Related Research*, no. 299, pp. 169–172, 1994.

[7] G. Peersman, R. Laskin, J. Davis, and M. Peterson, "The Insall award paper: Infection in total knee replacement: a retrospective review of 6489 total knee replacements," *Clinical Orthopaedics and Related Research*, no. 392, pp. 15–23, 2001.

[8] W. Zimmerli, A. Trampuz, and P. E. Ochsner, "Current concepts: prosthetic-joint infections," *The New England Journal of Medicine*, vol. 351, no. 16, pp. 1645–1654, 2004.

[9] R. O. Darouiche, "Treatment of infections associated with surgical implants," *The New England Journal of Medicine*, vol. 350, no. 14, pp. 1422–1429, 2004.

[10] E. F. Berbari, A. D. Hanssen, M. C. Duffy et al., "Risk factors for prosthetic joint infection: case-control study," *Clinical Infectious Diseases*, vol. 27, no. 5, pp. 1247–1254, 1998.

[11] A. M. Meehan, D. R. Osmon, M. C. T. Ouffy, A. D. Hanssen, and M. R. Keating, "Outcome of penicillin-susceptible streptococcal prosthetic joint infection treated with debridement and retention of the prosthesis," *Clinical Infectious Diseases*, vol. 36, no. 7, pp. 845–849, 2003.

[12] P. Tattevin, A.-C. Crémieux, P. Pottier, D. Huten, and C. Carbon, "Prosthetic joint infection: when can prosthesis salvage be considered?" *Clinical Infectious Diseases*, vol. 29, no. 2, pp. 292–295, 1999.

[13] A. H. Wilde and J. T. Ruth, "Two-stage reimplantation in infected total knee arthroplasty," *Clinical Orthopaedics and Related Research*, no. 236, pp. 23–35, 1988.

[14] R. P. Pitto and I. A. Spika, "Antibiotic-loaded bone cement spacers in two-stage management of infected total knee arthroplasty," *International Orthopaedics*, vol. 28, no. 3, pp. 129–133, 2004.

[15] B. Fink, A. Rechtenbach, H. Büchner, S. Vogt, and M. Hahn, "Articulating spacers used in two-stage revision of infected hip and knee prostheses abrade with time," *Clinical Orthopaedics and Related Research*, vol. 469, no. 4, pp. 1095–1102, 2011.

[16] G. S. Van Thiel, K. R. Berend, G. R. Klein, A. C. Gordon, A. V. Lombardi, and C. J. Della Valle, "Intraoperative molds to create an articulating spacer for the infected knee arthroplasty," *Clinical Orthopaedics and Related Research*, vol. 469, no. 4, pp. 994–1001, 2011.

[17] T. F. Calton, T. K. Fehring, and W. L. Griffin, "Bone loss associated with the use of spacer blocks in infected total knee arthroplasty," *Clinical Orthopaedics and Related Research*, no. 345, pp. 148–154, 1997.

[18] T. K. Fehring, S. Odum, T. F. Calton, and J. B. Mason, "Articulating versus static spacers in revision total knee arthroplasty for sepsis," *Clinical Orthopaedics and Related Research*, no. 380, pp. 9–16, 2000.

[19] A. A. Hofmann, K. R. Kane, T. K. Tkach, R. L. Plaster, and M. P. Camargo, "Treatment of infected total knee arthroplasty using an articulating spacer," *Clinical Orthopaedics and Related Research*, no. 321, pp. 45–54, 1995.

[20] F. S. Haddad, B. A. Masri, D. Campbell, R. W. McGraw, C. P. Beauchamp, and C. P. Duncan, "The PROSTALAC functional spacer in two-stage revision for infected knee replacements. Prosthesis of antibiotic-loaded acrylic cement," *Journal of Bone and Joint Surgery*, vol. 82, no. 6, pp. 807–812, 2000.

[21] S. Kohl, A. Krueger, C. Roeder et al., "An aluminium mold for intraoperative production of antibiotic-loaded PMMA knee prostheses," *Acta orthopaedica*, vol. 80, no. 3, pp. 389–391, 2009.

[22] S. Kohl, D. S. Evangelopoulos, H. Kohlhof et al., "An intraoperatively moulded PMMA prostheses like spacer for two-stage revision of infected total knee arthroplasty," *Knee*, vol. 18, no. 6, pp. 464–469, 2011.

[23] J. N. Insall, L. D. Dorr, R. D. Scott, and W. N. Scott, "Rationale of the knee society clinical rating system," *Clinical Orthopaedics and Related Research*, no. 248, pp. 13–14, 1989.

[24] S. B. Goksan and M. A. R. Freeman, "One-stage reimplantation for infected total knee arthroplasty," *Journal of Bone and Joint Surgery*, vol. 74, no. 1, pp. 78–82, 1992.

[25] L. S. Borden and P. F. Gearen, "Infected total knee arthroplasty: a protocol for management," *Journal of Arthroplasty*, vol. 2, no. 1, pp. 27–36, 1987.

[26] M. A. R. Freeman, R. A. Sudlow, M. W. Casewell, and S. S. Radcliff, "The management of infected total knee replacements," *Journal of Bone and Joint Surgery*, vol. 67, no. 5, pp. 764–768, 1985.

[27] C. L. Romano, L. Gala, N. Logoluso, D. Romano, and L. Drago, "Two-stage revision of septic knee prosthesis with articulating knee spacers yields better infection eradication rate than one-stage or two-stage revision with static spacers," *Knee Surgery, Sports Traumatology, Arthroscopy*, vol. 20, pp. 2445–2453, 2012.

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Review Article

Understanding Infection: A Primer on Animal Models of Periprosthetic Joint Infection

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Periprosthetic joint infections are devastating complications for patients and for our health system. With growing demand for arthroplasty, the incidence of these infections is projected to increase exponentially. This paper is a review of existing animal models to study periprosthetic infection aimed at providing scientists with a succinct presentation of strengths and weaknesses of available in vivo systems. These systems represent the tools available to investigate novel antimicrobial therapies and reduce the clinical and economic impact of implant infections.

1. Introduction

Periprosthetic joint infection is a devastating complication of total joint arthroplasty. Despite advances in perioperative antibiotics and aseptic surgical technique, periprosthetic joint infection is currently the most common indication for revision total knee arthroplasty and the third most common indication for revision total hip arthroplasty in the United States [1]. Postarthroplasty infections occur in approximately 1% of primary arthroplasties and 3%–5% of revisions [2–6] and the incidence of infections continues to rise with the increasing demand for arthroplasty surgery. The annual number of total knee arthroplasties performed in the United States is estimated to reach 3.48 million by 2030, while the number of total hip arthroplasties is projected to increase to 572,000. With this projection of roughly 4 million arthroplasty surgeries performed each year by 2030, the annual incidence of arthroplasty infection is projected to rise from its 2005 estimate of 17,000 to a projected 266,000 by 2030 [3, 7, 8].

Arthroplasty infections are clinically devastating, often leading to multiple operations, prolonged hospitalization, and worse clinical outcomes. Severe infections often lead to amputation and can even result in death [9, 10]. These infections also pose a significant economic burden through direct medical costs and lost wages and productivity [7]. Medical costs alone average $144,514 per patient (compared with $30,173 for an uncomplicated arthroplasty) [9], which corresponds to a projected annual national healthcare burden of $8.63 billion by 2015 [8].

When arthroplasty implants become infected, they are exceedingly difficult to treat, especially when such an infection presents in a chronic setting. The bacteria produce a biofilm, a polysaccharide layer that blocks the penetration of antibiotics and cells of the host immune system [2, 11, 12]. The majority of such infections, approximately 70%, are caused by staphylococcal species. Over the past decade, hospitals have seen an increase in arthroplasty infection by antibiotic-resistant strains, the most common being methicillin-resistant *Staphylococcus aureus* [13–15].

Two fundamental criteria are used to classify periprosthetic joint infections: mechanism of infection and the timing of diagnosis. The mechanism of infection can be either direct seeding of the implant at the time of surgery or hematogenous spread of infection from elsewhere in the body. Surgeons attempt to prevent direct inoculation through sterile technique, perioperative antibiotics, and limiting duration of the operation. Once an implant is in place, there is concern for hematogenous spread any time the patient has an infection or

transient bacteremia. Hematogenous spread of bacteria can be minimized by aggressively treating infections elsewhere in the body as well as prescribing prophylactic antibiotics for small procedures that lead to postoperative bacteremia (i.e., dental procedures).

In terms of timing, infections that are identified in the first 4 weeks after surgery or within 4 weeks of another identifiable source of seeding (i.e., dental work or another surgical procedure) are categorized as "acute" infections. Infections greater than 4 weeks after index surgery and with no identifiable precipitating event are classified as "chronic" infections. This distinction is admittedly opaque but is based on our concept of a biofilm "establishing itself" on the implant over some period of time after seeding.

Current treatment protocols are driven by this timing-based classification. Acute infections are most often treated with irrigation and debridement, polyethylene liner exchange, and retention of the metallic components. Conceptually (although with little scientific validation), acute infections can be treated with irrigation and debridement because a mature biofilm has not yet been established. Good results have been reported with this technique [16]. Despite prompt management, irrigation and debridement of acute arthroplasty infections can result in recurrent infection [17]. In one study of acutely infected total knee arthroplasties treated with debridement, component retention, and intravenous antibiotics, only 35% of patients successfully retained their components at a four-year follow-up period. When a subset analysis was performed, only 8% of patients who were infected with *S. aureus* in comparison to 56% with *S. epidermidis* or streptococcal species were successfully treated via this method [18]. In addition to the virulence of the bacteria, other important prognostic factors also need to be considered prior to attempting component retention, such as the immune status and past medical history of the patient.

The current standard of care for treatment of chronic infections involves a multiple stage process beginning with surgical removal of all prosthetic components, debridement of the surrounding tissue, and placement of an antibiotic impregnated cement spacer. Patients are then placed on a 6-week course of intravenous antibiotics tailored to susceptibilities of the bacteria cultured from the surgery. Once this infection clears (as supported by a benign appearing wound, normal C-reactive protein, normal erythrocyte sedimentation rate, and negative joint aspiration cultures), a second-stage revision arthroplasty may be attempted [3]. In a prospective series, Mortazavi et al. reported that at an average of 3.4-year follow-up, 28% of patients who had a two-stage revision arthroplasty for an infected total knee arthroplasty required reoperation for infection [19]. In severe or persistent infections, long-term suppressive antibiotic therapy, arthrodesis, or even amputation is sometimes necessary [20, 21].

2. A History of Animal Models

There are a lot of interest and research on the prevention and treatment of implant infections since it is the single most common cause of arthroplasty failure. As in many areas of medicine, animal models have been used to better understand the pathophysiology of post-arthroplasty infection. Animal models are also an essential intermediary between *in vitro* laboratory work and clinical trials.

The first joint infection animal model was established in rabbits and was published in the Journal of Bone and Joint Surgery in 1975 [22]. In this model, infection was tested in both a native knee and a knee with metal "implants" present. The "implant" arm of the study was made up of mice who had sterile stainless steel particles (<3 μm in diameter) suspended in normal saline injected into the knee via the suprapatellar pouch. Infection was then produced via the inoculation of serial tenfold dilutions of a culture of either *Staphylococcus aureus* or Micrococcus species into the suprapatellar pouch. On postprocedure day 6 and at weekly intervals thereafter, cultures of the joint fluid were obtained via placement of a needle into the suprapatellar pouch, irrigation of the joint with 1.5 mL sterile saline, followed by aspiration and culture of the aspirate. In this study, stainless steel particles in the knee did not appear to increase susceptibility to infection from injected micrococci but did make established micrococcal infections more persistent [22]. However, because the metal was suspended in normal saline rather than implanted into the bone, there was question as to how appropriately this modeled the arthroplasty situation from both a bone-implant interface perspective as well as an opportunity for bacterial adherence.

The first canine model was described by Petty et al. in 1985 [23]. Using a sterile technique, an incision was made over the tip of the greater trochanter and the bone was exposed subperiosteally. A hand drill and bone awl were used to penetrate the cortex and a 5 mm drill bit was used to ream the medullary cavity of the femur. The canal was then inoculated with the desired bacterial suspension (*Staphylococcus epidermidis*, *Staphylococcus aureus*, or *Escherichia coli*) and a 4 by 6 cm cylinder was introduced into the canal (stainless steel alloy, cobalt-chromium alloy, high-density polyethylene, or polymethylmethacrylate). The wound was then closed using a Dexon suture. At postoperative day 15, all the animals were euthanized and tissue was retrieved and cultured. The effects of the different implant materials on the susceptibility to infection were then compared. This model was later used to compare the effect of intraoperative irrigation and postoperative antibiotic treatment on infection rate [24]. One significant advantage of this animal model, in comparison to the rabbit model described previously, is that the metal implant used in this model (4 by 6 cm cylinder placed into the proximal femur) more closely represents an arthroplasty, in comparison to the stainless steel particles injected into the knee joint space in the prior model. Weaknesses of the model include the single, static data time point, postoperative day 15, and as a questionable surgical representation. Inoculating the bone and then placing an implant is perhaps a better model of introducing an implant into an existing osteomyelitis. The site of bacterial seeding is intraosseous, rather than intra-articular.

This concern of intra-articular bacterial seeding was addressed by a novel arthroplasty infection rabbit model published by Craig et al. in 2005 [25]. A stainless-steel screw

with an ultrahigh molecular weight polyethylene washer was cemented using polymethyl methacrylate in a defect created in an intra-articular, nonarticulating portion of the lateral femoral condyle of each knee. This was followed by inoculation of various concentrations of methicillin resistant staphylococcus aureus (MRSA). The animals were euthanized at postoperative day 7, at which time joint aspirate, tissues, and biomaterial samples were cultured. This model was also used to compare the infection rate of various biomaterials (i.e., polymethyl methacrylate (PMMA), bone cement, ultra high molecular weight polyethylene (UHMWPE), and stainless steel) [25]. One advantage of this model was that bacteria were introduced directly into the knee joint following wound closure whereas older models inserted bacteria directly into the femoral canal prior to implant placement or immersed biomaterials in a bacterial suspension prior to intra-articular placement. This method of inoculation more closely modeled an arthroplasty infection. A second advantage of this model was that it included the major biomaterials used in total knee arthroplasty, with use of PMMA, UHMWPE, and metal.

Another modification addressed by recent work in animal modeling has been based on the observation that a significant number of hardware infections may be the result of inoculation by mature bacterial biofilms rather than independent bacteria [26–32]. Williams et al. explored this observation and hypothesize that using a biofilm as initial inocula, rather than native bacteria, may provide more clinically relevant information for the prevention and treatment of hardware infections. In this model, a clinical isolate of methicillin-resistant *S. aureus* was used and was grown on the surface of membranes composed of polyetheretherketone (PEEK) for 48 hours. The biofilms were then isolated and used as an inoculum in a Gustilo type IIIB open tibia fracture model in sheep. An anterior midline sagittal incision was made from the tibial tuberosity extending distally along the anterior aspect of the tibia. In order to mimic a type IIIB Gustilo open fracture with significant periosteal stripping, bone exposure, and massive contamination, a section of periosteum was removed from the proximal anteromedial aspect of the tibia. A construct consisting of a stainless steel plate with a membrane containing the biofilm was placed against the tibial surface with the biofilm membrane between the plate and cortical bone. Each plate was then secured with cortical bone screws and the incision was finally closed with suture (Figures 1 and 2). Postoperatively, the wound was observed for signs of infection such as erythema, warmth, and dehiscence. At 12 weeks postoperatively, the sheep were euthanized and several samples were cultured, including the incision site, the subcutaneous tissue, the plate, bone, and the biofilm membrane. Radiographic and histological analyses were also performed from these samples. All sheep in the group inoculated with the biofilm membrane showed signs of infection, specifically osteomyelitis, at the 12-week postoperative period in comparison with no infection in any of the sheep treated without the biofilm. These findings strongly support the hypothesis that biofilms can cause infection. Although this model examines infection in an open fracture model rather than an arthroplasty model, it provides a key modification in animal modeling of arthroplasty with this concept of biofilm inoculation as the inciting event [33].

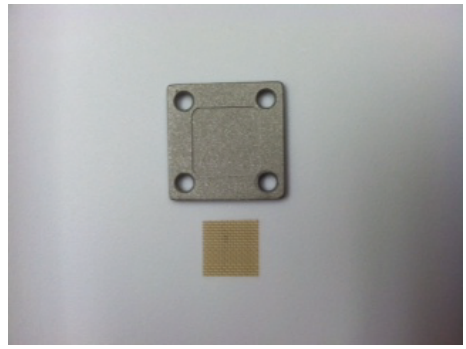

Figure 1: Photograph of a stainless steel plate and the PEEK membrane used for preimplantation formation of biofilm used in the Williams sheep model. *Courtesy of Dr. D. Williams*.

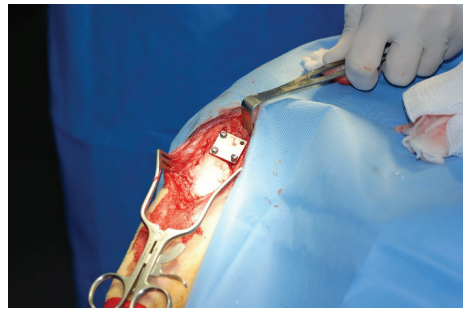

Figure 2: Intraoperative photographs of stainless steel plates placed in the proximal tibia used in the Williams et al. sheep model. *Courtesy of Dr. D. Williams*.

Such studies using histology and culture data provide extremely useful preclinical information; however, these studies are costly and labor intensive and require the use of a significant number of animals, as euthanasia is required to determine the bacterial burden at each time point postoperatively. In 2010, Bernthal et al. published a novel mouse model for post-arthroplasty infection that abdicated this need, using *in vivo* imaging of bioluminescent bacteria to replace histologic assessment [3]. Following a medial parapatellar approach to the knee, a metal pin was placed retrograde, from the knee joint into the femoral canal with 1 mm of the pin remaining protruding into the joint space. A bioluminescent strain of bacteria was then used to inoculate the intra-articular portion of the metal pin in the joint space (Figure 3). Postoperatively, the Xenogen *in vivo* imaging system was used to monitor the infection by quantifying bacterial burden in real-time (Figure 4). The *in vivo* bioluminescent signals were confirmed to accurately represent the bacterial burden *in vivo* by performing traditional bacterial counts on the last day of imaging. The initial model was created with use of a stainless steel Kirschner wire and a bioluminescent strain of *S. aureus*. This model was then applied to test a variety of biomaterials and various bacterial strains. This model has unique elements that may complement or provide an alternative to the use of

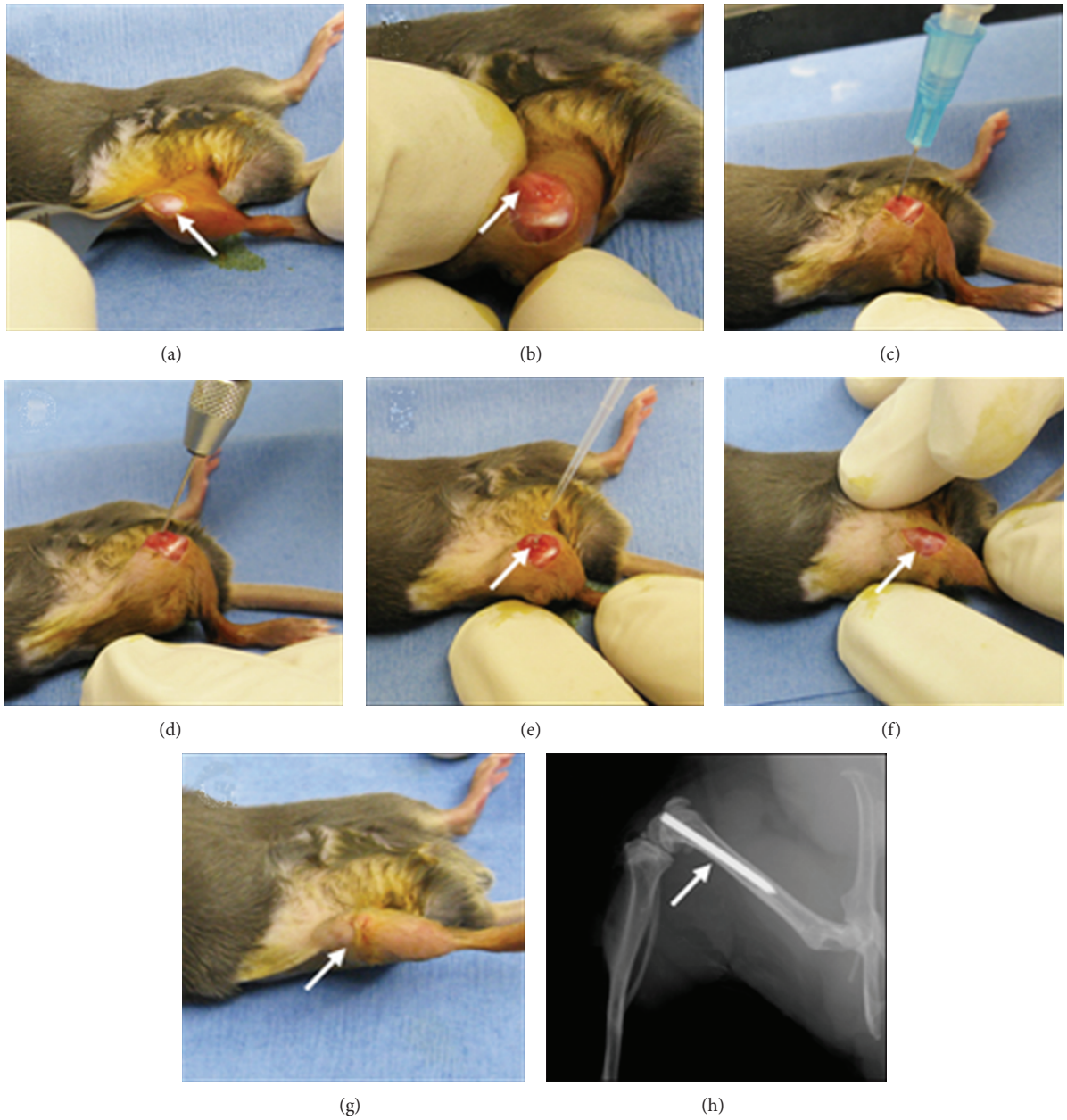

Figure 3: ((a)–(g)) Bernthal et al. surgical approach in a representative mouse. (h) A radiograph demonstrating placement of the implant in the femoral canal with the cut end extending into the knee joint [3].

other previous animal models. One unique characteristic of this model is that it uses advanced techniques of *in vivo* imaging, which provides longitudinal, real-time quantification of bacterial burden. Thus, an infection in a certain animal can be followed over several days or even weeks (may simulate an acute, subacute, or chronic post-arthroplasty infection). This bypasses the need to euthanize a large number of animals at subsequent time points to quantify bacterial burden. Genetically modified mouse lines are readily available, which can also be helpful in studying post-arthopalsty infections. For example, the use of various immunologic knockout mice or mice with fluorescent immune cells may aid in understanding the complex immune response against such infections [34].

3. Conclusions

Researchers have come a long way since the initial animal model of arthroplasty infection in 1975. The development of novel scientific techniques, from biofilm harvesting to *in vivo* imaging has provided opportunities to improve animal models to a more accurate and humane depiction of the human condition. And yet, each iteration along the way has made an important contribution. The ideal model offers the anatomic similarities to human joints that a large animal model offers, the immunogenic modulation available in a mouse model, the longitudinal data collection that bioluminescence offers, and potentially, the use of biofilm inoculation

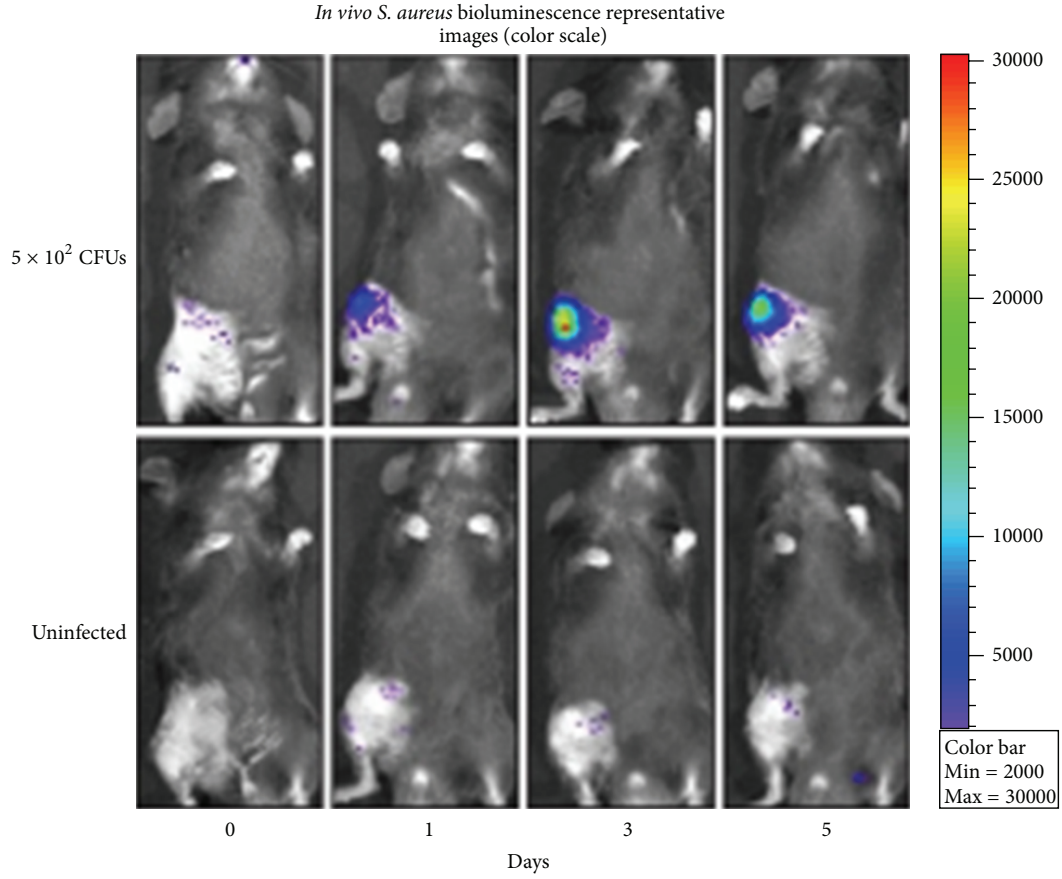

Figure 4: Representative *in vivo* bioluminescent images [3].

that was recently described [31]. While all of these assets may not be available in a single model, one could devise a combination of existing models that utilizes the strengths of small animal modeling as an initial high-throughput screen and large animal modeling as a preclinical test. Additionally, future models would ideally be able to test a representative panel of bacteria, more accurately representing the clinical scenarios that patients and clinicians face.

As the prevalence of periprosthetic infection continues to rise alongside the increasing demand for arthroplasty, there is a great need to identify both preventative and therapeutic options. Such treatment strategies will continue to depend on animal models as an intermediary between bench concepts and clinical care. Thus, developing an appropriate, efficient and accurate animal model or series thereof is of the utmost importance.

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References

[1] J. Parvizi, B. Adeli, B. Zmistowski, C. Restrepo, and A. S. Greenwald, "Management of periprosthetic joint infection," *The Journal of Bone and Joint Surgery*, vol. 94, no. 14, Article ID e104, 2012.

[2] J. L. Del Pozo and R. Patel, "Infection associated with prosthetic joints," *The New England Journal of Medicine*, vol. 361, no. 8, pp. 787–794, 2009.

[3] N. M. Bernthal, A. I. Stavrakis, F. Billi et al., "A mouse model of post-arthroplasty *Staphylococcus aureus* joint infection to evaluate in vivo the efficacy of antimicrobial implant coatings," *PloS ONE*, vol. 5, no. 9, Article ID e12580, 2010.

[4] D. M. Urquhart, F. S. Hanna, S. L. Brennan et al., "Incidence and risk factors for deep surgical site infection after primary total hip arthroplasty: a systematic review," *Journal of Arthroplasty*, vol. 25, no. 8, pp. 1216.e3–1222.e3, 2010.

[5] K. J. Bozic, S. M. Kurtz, E. Lau et al., "The epidemiology of revision total knee arthroplasty in the United States," *Clinical Orthopaedics and Related Research*, vol. 468, no. 1, pp. 45–51, 2010.

[6] K. J. Bozic, S. M. Kurtz, E. Lau, K. Ong, D. T. P. Vail, and D. J. Berry, "The epidemiology of revision total hip arthroplasty in the United States," *Journal of Bone and Joint Surgery A*, vol. 91, no. 1, pp. 128–133, 2009.

[7] S. Kurtz, K. Ong, E. Lau, F. Mowat, and M. Halpern, "Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030," *Journal of Bone and Joint Surgery A*, vol. 89, no. 4, pp. 780–785, 2007.

[8] S. M. Kurtz, K. L. Ong, J. Schmier et al., "Future clinical and economic impact of revision total hip and knee arthroplasty,"

Journal of Bone and Joint Surgery A, vol. 89, no. 3, pp. 144–151, 2007.

[9] K. J. Bozic and M. D. Ries, "The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization," *Journal of Bone and Joint Surgery A*, vol. 87, no. 8, pp. 1746–1751, 2005.

[10] S. M. Kurtz, E. Lau, J. Schmier, K. L. Ong, K. Zhao, and J. Parvizi, "Infection burden for hip and knee arthroplasty in the United States," *Journal of Arthroplasty*, vol. 23, no. 7, pp. 984–991, 2008.

[11] R. O. Darouiche, "Treatment of infections associated with surgical implants," *The New England Journal of Medicine*, vol. 350, no. 14, pp. 1422–1429, 2004.

[12] A. Trampuz and A. F. Widmer, "Infections associated with orthopedic implants," *Current Opinion in Infectious Diseases*, vol. 19, no. 4, pp. 349–356, 2006.

[13] E. Fulkerson, C. J. Della Valle, B. Wise, M. Walsh, C. Preston, and P. E. Di Cesare, "Antibiotic susceptibility of bacteria infecting total joint arthroplasty sites," *Journal of Bone and Joint Surgery A*, vol. 88, no. 6, pp. 1231–1237, 2006.

[14] C. D. Salgado, S. Dash, J. R. Cantey, and C. E. Marculescu, "Higher risk of failure of methicillin-resistant *Staphylococcus aureus* prosthetic joint infections," *Clinical Orthopaedics and Related Research*, no. 461, pp. 48–53, 2007.

[15] R. J. Walls, S. J. Roche, A. O'Rourke, and J. P. McCabe, "Surgical site infection with methicillin-resistant *Staphylococcus aureus* after primary total hip replacement," *Journal of Bone and Joint Surgery B*, vol. 90, no. 3, pp. 292–298, 2008.

[16] M. A. Mont, B. Waldman, C. Banerjee, I. H. Pacheco, and D. S. Hungerford, "Multiple irrigation, debridement, and retention of components in infected total knee arthroplasty," *Journal of Arthroplasty*, vol. 12, no. 4, pp. 426–433, 1997.

[17] T. F. Moyad, T. Thornhill, and D. Estok, "Evaluation and management of the infected total hip and knee," *Orthopedics*, vol. 31, no. 6, pp. 581–588, 2008.

[18] C. Deirmengian, J. Greenbaum, P. A. Lotke, R. E. Booth Jr., and J. H. Lonner, "Limited success with open debridement and retention of components in the treatment of acute *Staphylococcus aureus* infections after total knee arthroplasty," *Journal of Arthroplasty*, vol. 18, no. 7, pp. 22–26, 2003.

[19] S. M. J. Mortazavi, D. Vegari, A. Ho, B. Zmistowski, and J. Parvizi, "Two-stage exchange arthroplasty for infected total knee arthroplasty: predictors of failure," *Clinical Orthopaedics and Related Research*, vol. 469, no. 11, pp. 3049–3054, 2011.

[20] K. Bargiotas, D. Wohlrab, J. J. Sewecke, G. Lavinge, P. J. DeMeo, and N. G. Sotereanos, "Arthrodesis of the knee with a long intramedullary nail following the failure of a total knee arthroplasty as the result of infection. Surgical technique," *The Journal of Bone and Joint Surgery*, vol. 89, pp. 103–110, 2007.

[21] S. A. McGarry, J. J. Engemann, K. Schmader, D. J. Sexton, and K. S. Kaye, "Surgical-site infection due to *Staphylococcus aureus* among elderly patients: mortality, duration of hospitalization, and cost," *Infection Control and Hospital Epidemiology*, vol. 25, no. 6, pp. 461–467, 2004.

[22] D. J. Schurman, B. L. Johnson Jr., and H. C. Amstutz, "Knee joint infections with *Staphylococcus aureus* and micrococcus species. Influence of antibiotics, metal debris, bacteremia, blood, and steroids in a rabbit model," *Journal of Bone and Joint Surgery A*, vol. 57, no. 1, pp. 40–49, 1975.

[23] W. Petty, S. Spanier, J. J. Shuster, and C. Silverthorne, "The influence of skeletal implants on incidence of infection. Experiments in a canine model," *Journal of Bone and Joint Surgery A*, vol. 67, no. 8, pp. 1236–1244, 1985.

[24] W. Petty, S. Spanier, and J. J. Shuster, "Prevention of infection after total joint replacement. Experiments with a canine model," *Journal of Bone and Joint Surgery A*, vol. 70, no. 4, pp. 536–539, 1988.

[25] M. R. Craig, K. A. Poelstra, J. C. Sherrell, M. S. Kwon, E. L. Belzile, and T. E. Brown, "A novel total knee arthroplasty infection model in rabbits," *Journal of Orthopaedic Research*, vol. 23, no. 5, pp. 1100–1104, 2005.

[26] J. R. Lawrence, D. R. Korber, B. D. Hoyle, J. W. Costerton, and D. E. Caldwell, "Optical sectioning of microbial biofilms," *Journal of Bacteriology*, vol. 173, no. 20, pp. 6558–6567, 1991.

[27] G. G. Geesey, W. T. Richardson, and H. G. Yeomans, "Microscopic examination of natural sessile bacterial populations from an alpine stream," *Canadian Journal of Microbiology*, vol. 23, no. 12, pp. 1733–1736, 1977.

[28] G. A. James, E. Swogger, R. Wolcott et al., "Biofilms in chronic wounds," *Wound Repair and Regeneration*, vol. 16, no. 1, pp. 37–44, 2008.

[29] L. M. Feazel, L. K. Baumgartner, K. L. Peterson, D. N. Frank, J. K. Harris, and N. R. Pace, "Opportunistic pathogens enriched in showerhead biofilms," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 38, pp. 16393–16398, 2009.

[30] S. E. Dowd, Y. Sun, P. R. Secor et al., "Survey of bacterial diversity in chronic wounds using Pyrosequencing, DGGE, and full ribosome shotgun sequencing," *BMC Microbiology*, vol. 8, article 43, 2008.

[31] A. G. Gristina and J. W. Costerton, "Bacteria-Laden biofilms: a hazard to orthopedic prostheses," *Surgical Infections*, vol. 3, pp. 655–662, 1984.

[32] T. J. Marrie, J. Nelligan, and J. W. Costerton, "A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead," *Circulation*, vol. 66, no. 6, pp. 1339–1341, 1982.

[33] D. L. Williams, B. S. Haymond, K. L. Woodbury et al., "Experimental model of biofilm implant-related osteomyelitis to test combination biomaterials using biofilms as initial inocula," *Journal of Biomedical Materials Research A*, vol. 100, no. 7, pp. 1888–1900, 2012.

[34] N. M. Bernthal, J. R. Pribaz, A. I. Stavrakis et al., "Protective role of IL-1β against post-arthroplasty *Staphylococcus aureus* infection," *Journal of Orthopaedic Research*, vol. 29, no. 10, pp. 1621–1626, 2011.

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Clinical Study

Release of Gentamicin and Vancomycin from Preformed Spacers in Infected Total Hip Arthroplasties: Measurement of Concentrations and Inhibitory Activity in Patients' Drainage Fluids and Serum

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Gentamicin (G) and vancomycin (V) concentrations in drainage fluids obtained from patients during the first 24 hours after implantation of antibiotic-loaded polymethylmethacrylate (PMMA) spacers in two-stage revision of infected total hip arthroplasty were studied. The inhibitory activity of drainage fluids against different multiresistant clinical isolates was investigated as well. Seven hips were treated by implantation of industrial G-loaded spacers. Vancomycin was added by manually mixing with PMMA bone cement. Serum and drainage fluid samples were collected 1, 4, and 24 hours after spacer implantation. Antibiotics concentrations and drains bactericidal titer of combination were determined against multiresistant staphylococcal strains. The release of G and V from PMMA cement at the site of infection was prompt and effective. Serum levels were below the limit of detection. The local release kinetics of G and V from PMMA cement was similar, exerting a pronounced, combined inhibitory effect in the implant site. The inhibitory activity of drainage fluids showed substantial intersubject variability related to antibiotic concentrations and differed according to the pathogens tested. Gentamicin and vancomycin were released from temporary hip spacers at bactericidal concentrations, and their use in combination exerted strong inhibition against methicillin-resistant *S. aureus* and Coagulase Negative Staphylococci strains.

1. Introduction

Polymethylmethacrylate (PMMA) cements preloaded with antibiotics, mainly gentamicin (G), are used in some cases for prophylaxis but especially for the surgical revision of prosthetic infections [1]. Frequent microorganisms isolated from joint fluid or periprosthetic tissue are the Coagulase Negative Staphylococci (CoNS), *S. aureus* and most commonly *S. epidermidis*, and *Streptococcus haemolyticus* [2]. Currently, the routinely used methods of culturing are likely to detect in most, not all, cases the pathogens possibly involved in infection of a total hip arthroplasty (THA) [3]. Some difficult-to-treat bacteria, such as methicillin-resistant *S. aureus* (MRSA), methicillin-resistant CoNS, enterococci, and *Pseudomonas aeruginosa* present much greater failure risks. In a number of cases *S. aureus* infection is the significant factor associated with treatment failure, along with retained prosthesis and treatment with inappropriate antibiotics [4]. A two-stage revision of an infected arthroplasty with antibiotic-loaded spacer implantation is considered an effective procedure for these infections [2, 5].

Because of the increasing resistance of staphylococci to gentamicin, surgeons commonly add antibiotics to bone cement directly in the operating room according to microorganism susceptibility. Vancomycin (V) is frequently utilized because of its antimicrobial activity against MRSA and other Gram-positive cocci and anaerobes, such as propionibacteria.

This drug delivery system offers the advantage of local release of high antibiotic concentrations, which considerably exceed those obtained after systemic administration.

Combining two antibiotics in bone cement is common in clinical practice. As the effect of mixing on elution characteristics is still debated, only limited data are available on antibiotic release *in vivo* from prosthetic devices after implantation [6–8], as well as after removal [9, 10]. Moreover, the wide variability of the results makes it difficult to compare studies and draw general conclusions. The properties of various bone cements, the preparation, type and concentrations of different antibiotics mixed with PMMA, the pathogens involved, and patient characteristics are all factors contributing to the clinical outcome and should be taken into account in the final evaluation of treatments. We previously observed that gentamicin and vancomycin were still present in explanted spacers after 3 to 9 months of permanence in situ; the residual drug concentrations showed great variability [11].

Open questions are related to the concentrations of antibiotics at the implantation site and to the duration of their effective inhibitory activity.

The aims of the present study were (1) to measure the concentrations of antibiotics present at the infection site in the first few hours after implanting the hip preformed spacer; (2) to evaluate if antibiotics are released in large amounts consistent with the results of *in vitro* experiments; (3) and finally to assess the antimicrobial activity of drainage fluids against multiresistant microorganisms.

To the best of our knowledge, this is the first study which details the antibiotic release from industrially manufactured temporary spacers in infected hip arthroplasties.

2. Materials and Methods

From January 2004 to September 2005, 7 patients who received preformed spacers for two-stage revision of a THA were investigated. There were 4 male and 3 female patients, whose age ranged between 51 and 78 years (average, 65.6 years) at the time of implantation. All THAs were performed for osteoarthritis. Diagnosis was suspected on the basis of clinical findings (persistent pain or recurrence 3–5 years later, presence of a secreting fistula, swelling, erythema, local warmth, and restricted range of motion) and of the ESR and the CRP (which were always elevated) [12]. Standard X-ray and scintigraphy with labeled leukocytes were performed in all the patients [12–16]. Intraoperative biopsy of bone and soft tissue was always carried out, and the Feldman and the Athanasou criteria were used to define infection [17, 18]. The management of infection included removal of the prosthesis and insertion of a preformed antibiotic-loaded PMMA spacer (Tecres S.p.A., Sommacampagna, Verona, Italy).

The hip preformed spacer, Spacer-G, has a structure in stainless steel AISI 316ESR, and it is available in 3 different diameters of the head (46, 54, and 60 mm) and 2 lengths of the stem (153 and 270 mm). The gentamicin concentration is 2.5%. Currently, it is also available with flat rod (Flat Stem Spacer-G) and industrially supplemented with vancomycin at concentration of 2.5% (Vancogenx Hip-Space).

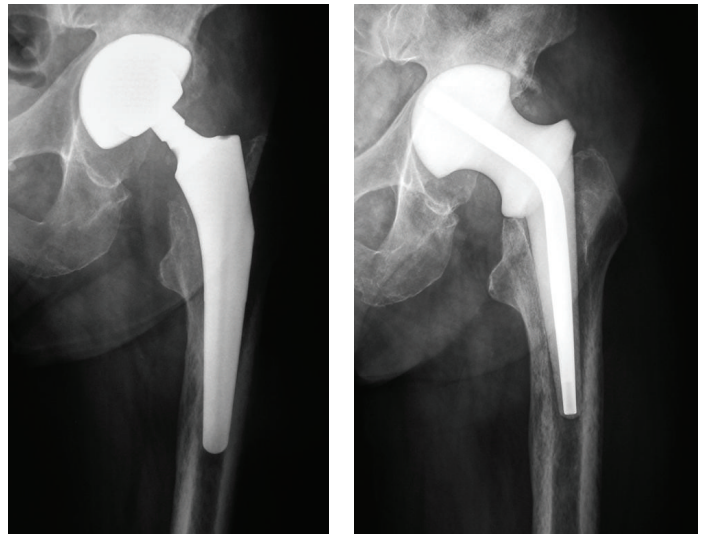
Removal of the septic implant was followed by a thorough periprosthetic debridement and implantation of the spacer (Figures 1(a) and 1(b)). A vancomycin-loaded bone cement was prepared manually by mixing 40 g of powered cement PMMA polymer (Cemex, Tecres S.p.A., Sommacampagna, Verona, Italy) and 1 g of vancomycin (Vancocin, Eli Lilly, Milan, Italy). Finally, 35 mL of liquid MMA monomer was added and carefully mixed with a spatula [11, 19]. Vancomycin addition to hip spacers was obtained by filling with the cement mixture 17-18 holes (10–12 mm diameter, 2-3 mm depth) which were drilled in the surface of the Spacer-G immediately before implantation (Figure 2). Each device received 6-7 g of cement, corresponding to 150–170 mg of vancomycin, respectively. Vancomycin (Vancocin, 1 g, twice daily) was also administered intravenously to 1 patient as control case.

Two- to 3-week standard parenteral antibiotics administration (cefazolin, Cefamezin, Pfizer Italia, Roma, Italy; 1 g four times a day; i.v.) was given to the remaining patients, followed by oral therapy, according to infectious disease consultant, for an overall treatment of 6 weeks. Outpatients clinical evaluation was arranged monthly, including laboratory tests (WBC, ESR, and CRP) and radiographic examination (anteroposterior and lateral views). Due to an immediate pain relief after surgery, a standard physiotherapy regimen including continuous passive motion was carried out. Partial to total weight bearing on the operated leg using two crutches was allowed until reimplantation.

In all the cases, eradication of infection was obtained, and the second surgical step, including the removal of the spacer and the application of a new THA, was performed successfully when patient's laboratory indices became normal and when bone scintigraphy with labeled leukocytes was negative for infection. In the postoperative period, parenteral antibiotic treatment was administered for 6 weeks in all the patients according to the pathogen identification or with broad-spectrum antibiotics in case of lack identification (2 patients) starting from day 3rd with the exception of the patients control case which started preoperatively.

Fluids drainage and serum samples to 1, 4, and 24 hours after the first surgical step were collected in all cases. Concentrations of gentamicin and vancomycin were determined in parallel by Fluorescence Polarisation Immunoassay (TDx, Abbott). The lowest measurable level of drug concentration was defined as that which could be distinguished from 0 with 95% confidence; this was determined as 0.27 mg/L for G and 2.0 mg/L for V [11]. The antibacterial activity determination was also done on different orthopaedic strains isolates with differing degrees of resistance. Bacterial strains were multiresistant clinical isolates obtained from Intensive Care Unit in patients, kindly provided by the Microbiology Department of the local university (Table 1).

The MICs of gentamicin, vancomycin, and their combinations were determined using the broth microdilution technique as recommended by the CLSI (Clinical and Laboratory Standards Institute) guidelines [20]. Resistance of the staphylococcus strains was determined according to international standard methods [21]. Resistance to gentamicin was defined by $MIC_{90} > 32$ mg/L; gentamicin-intermediate resistance by



(a) (b)

Figure 1: Preoperative X-ray of a total hip arthroplasty complicated by chronic infection (a). Radiograph obtained after removal of the infected prosthesis and implantation of an industrially manufactured spacer (b).

Figure 2: Image of the preformed gentamicin-loaded cement spacer after surgical addition of vancomycin using the "surface drill hole" technique.

Table 1: *In vitro* activity of gentamicin and vancomycin in combination against multiresistant clinical isolates.

Strain	Vancomycin + gentamicin		
	MIC (mg/L)		
	Vancomycin	Gentamicin	FICI
S. aureus	2.5	10	0.15
S. epidermidis	2.5	3750	1.00
S. haemolyticus	1.25	3750	1.00
S. haemolyticus Methicillin-resistant	1.25	3750	1.00
S. epidermidis Methicillin-resistant	2.5	58.6	0.50
S. hominis Methicillin-resistant	1.25	15	1.02
E. coli	156.25	5.0	0.25
P. aeruginosa	1250	5.0	0.12

MIC_{90} = 8.0 mg/L, and resistance to vancomycin by MIC_{90} > 4.0 mg/L for the strains tested. Synergy testing was performed in duplicate using the chequerboard method in microtiter plates with Mueller-Hinton Broth (MHB, Difco). Gentamicin and vancomycin were diluted in MHB and tested at different twofold concentrations (from 0.3 to 20.0 mg/L) against all strains (final inoculum 1×10^5 CFU/mL). The fractional inhibitory concentration index (FICI) was calculated and interpreted for each strain [22]: the FICI was defined as synergistic if the values were <0.5, indifferent or additive if the values were from 0.5 to 4.0, and antagonistic if the values were >4.0 [23] (Table 1).

Twofold serial dilutions of patient drainages were prepared in microtiter plates using Mueller-Hinton Broth as diluent. The final volume was 0.1 mL in each well, and 0.01 mL of each strain from overnight cultures was added to each well, including a growth control well, without drainage; an absolute control (MHB only) was also provided. Microplates were incubated for at least 18 h at 37°C. Subcultures for the 99.9% bactericidal endpoints were performed in Brain Heart Agar. The drainage fluid bactericidal titer (DBT) is a measure of the drainage fluid killing capacity against the infecting organism; it was determined as the highest fluid dilution achieving 99.9% bacterial killing. The score 3, corresponding to a 1/8 dilution, was considered the lowest effective titer for orthopaedic infections [24, 25].

Table 2: Bactericidal titer of drainage fluids collected from 7 patients within the first 24 hours of spacer implantation against multiresistant clinical isolates.

Strain	DBT													
	PT 1		PT 2		PT 3		PT 4		PT 5		PT 6		PT 7	
	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
S. aureus	6	9	9	9	6	5	5	5	3	2	3	4	6	4
S. epidermidis	3.3	6	5	3	4	4	4	4	0	1	4	4	5	3
S. haemolyticus	3.3	6	6	7	4	3	4	4	0	1	3	3	4	3
S. haemolyticus Methicillin-resistant	3	5	4	3	4	4	4	3	0	0	4	5	4	3
S. epidermidis Methicillin-resistant	3.3	6	4.3	2	4	3	4	4	0	0	7	7	4	3
S. hominis Methicillin-resistant	5	8	7	5	5	5	4	5	0	1	5	5	6	4
E. coli	6	9	5	6	4	4	4	5	2	1	5	3	6	4
P. aeruginosa	4	6	5	5	4	4	3	4	0	0	5	6	5	3

3. Results

The release of gentamicin from PMMA cement at the site of infection showed high local concentrations (range 15.0–90.0 mg/L) in the first few hours after spacer implantation. Gentamicin serum levels were invariably very low (<0.2–1.0 mg/L). The local administration of vancomycin (2.5%) produced high concentrations (ranging from 13.8 to 40.0 mg/L) at the implant site in the first hour. This behaviour persisted 4 and 24 hours after spacer implantation. The corresponding serum levels were below the threshold for systemic toxicity (<10 mg/L for gentamicin and <40 mg/L for vancomycin); however, vancomycin attained therapeutic concentrations after parenteral administration, but again below systemic toxicity limits.

The levels of each antibiotic in drainage fluids were all above the concentrations needed to inhibit susceptible bacteria, and their use in combination appears to be capable of exerting pronounced antimicrobial activity and also a synergistic effect against some multiresistant microorganisms.

The DBT score was high (above 3) in the first few hours after drug release (1/8 titer) for all strains tested; an effective titer was maintained for almost 24 hours. The same drainage fluid presented different inhibitory capacities against various multiresistant strains. For example, patient n. 1 exhibited good inhibitory activity (DBT = 6) against *E. coli*, *S. aureus*, and lower inhibitory activity (DBT = 4) against *P. aeruginosa*; patient n. 6 had good inhibitory activity (DBT = 7) against *S. epidermidis* Methicillin-resistant and lower inhibitory activity (DBT = 3-4) against *S. aureus*, *S. haemolyticus* (2 strains), and *S. epidermidis*. Patient n. 7 showed good inhibitory activity (DBT = 6) against *S. hominis*, *S. aureus* and lower inhibitory activity (DBT = 4) against *S. haemolyticus* (2 strains) and *S. epidermidis*. Moreover, the fluid maintained high activity against the Gram-negative strains *E. coli* and *P. aeruginosa* (Table 2).

The fluid collected from patient who also received local and systemic vancomycin (control case) was inhibitory against the majority of tested strains and higher against *S. aureus* during the first 24 hours after implantation.

Depending on the different antibiotic concentrations in the microtiter plates and microorganisms tested, the samples inhibitory activity was variable. DBT scores indicated good inhibitory activity after 24 hours when G and V in combination were present at adequate concentrations (in these conditions, ≥8 mg/L and ≥2 mg/L, resp.) and when the G : V ratio was at least 2 : 1.

Pain relief after application of the spacer was obtained in all cases, and partial weight bearing with crutches was allowed. There were no general or local complications (dislocation, breakage, and loosening of the spacer). No adverse drug reaction (hypersensitivity, erythema, edema, etc.) attributable to gentamicin or vancomycin was reported after local and systemic drug administration.

4. Discussion

Periprosthetic hip infection following THA is a serious problem, and different treatment options related to the type of infection are available. In two-stage revision procedure, temporary spacers made of antibiotic-loaded PMMA represent a viable option for a chronically infected THA, allowing local antibiotic delivery and maintaining soft tissue length, which facilitates reimplantation [10, 26].

Industrially preformed spacer has some advantages such as ease of use, high availability in sizes, and excellent acetabular bone quality at the time of revision [26]. With the use of this specific device, many authors have reported good eradication rate ranging from 80 to 93.3% [26–29]. Industrial production ensures procedure standardization eliminating the time necessary to intraoperative manufacturing [26]. However, spacer-related complications, such as dislocations and fractures, have been described as well, ranging from 3.3 to 17% [26–29].

In this study, the release of gentamicin and vancomycin in the first 24 hours after implantation of hip preformed spacers was evaluated. Gentamicin and vancomycin concentrations were very high and strongly bactericidal in suction drainage fluid samples one hour after spacer implantation and remained high for at least 24 hours. These results confirm the findings of Anagnostakos et al., who firstly reported high concentrations of antibiotics in drainage fluids in the first few days after implantation of beads or spacer [10]. In addition, we

observed different inhibitory capacities exerted by the same drainage fluid against several multiresistant clinical isolates.

Gentamicin and vancomycin concentrations determined singly in drainage fluids in the first 24 hours were very high and stable but not inhibitory against multiresistant strains. However, gentamicin and vancomycin act sinergistically against several multiresistant staphylococcal strains, as shown by the FICI and the DBT scores. The therapeutic rationale for combining G and V depends on the susceptibility of the infecting pathogens, and vancomycin use should be limited to infections likely to be caused by more resistant Gram-positive bacteria, such as *S. epidermidis*, methicillin-resistant staphylococci, CoNS, or enterococci [30, 31]. Cefazolin, not dosed, could contribute to antimicrobial activity of drainage fluids; it is effective mainly against susceptible strains, *S. aureus* (9A28) and *S. aureus* (3A10), and ineffective against the multiresistant strains as confirmed recently [32].

In our patients, gentamicin and vancomycin serum levels were below the threshold for systemic toxicity, and no signs of nephrotoxicity or local cytotoxic effects were observed. These data confirm the safety aspects of local drug delivery and the good tolerability of systemic and local levels. A low frequency of adverse reactions has been reported with antibiotic-containing spacers [10], though damage to the kidney and increased mortality has also been reported. In a systematic review including 10 observational studies, Luu et al. [33] showed an average incidence of acute kidney injury of 4.8% using antibiotic spacer. Berend at al. [34] studied mortality rates associated with two-stage treatment of infected THA in 202 patients undergoing two-stage treatment for infection, including removal of all implants and foreign material with implantation of an antibiotic-loaded cement spacer in the first stage followed by intravenous culture-specific antibiotics for a minimum of 6 weeks. Fourteen patients (7%) died before reimplantation, and two were not candidates because of medical comorbidities. The 90-day mortality rate after the first-stage debridement was 4%.

5. Conclusions

The results of the present investigation provide data on the release of gentamicin and vancomycin from preformed antibiotic cement spacers in the first 24 hours after implantation, supporting the potential clinical efficacy of the gentamicin-vancomycin combination in two-stage management of infected THA. Preformed spacers loaded with G and V are a safe method of delivering high concentration of antibiotics to the infection site with low serum levels, achieving effective release kinetics. The use of industrially preformed spacers should be advantageous in terms of standardization of the device characteristics, uniform cement mix with antibiotics, and reproducible drug release.

Conflict of Interests

The authors declare that they have no potential conflict of interests.

References

[1] M. Diefenbeck, T. Mückley, and G. O. Hofmann, "Prophylaxis and treatment of implant-related infections by local application of antibiotics," *Injury*, vol. 37, supplement 2, pp. S95–S104, 2006.

[2] E. Moran, I. Byren, and B. L. Atkins, "The diagnosis and management of prosthetic joint infections," *Journal of Antimicrobial Chemotherapy*, vol. 65, no. 3, pp. iii45–iii54, 2010.

[3] C. A. Fux, P. Stoodley, L. Hall-Stoodley, and J. W. Costerton, "Bacterial biofilms: a diagnostic and therapeutic challenge," *Expert Review of Anti-Infective Therapy*, vol. 1, no. 4, pp. 667–683, 2003.

[4] J. Lee, C.-I. Kang, J. H. Lee et al., "Risk factors for treatment failure in patients with prosthetic joint infections," *Journal of Hospital Infection*, vol. 75, no. 4, pp. 273–276, 2010.

[5] A. D. Hanssen and J. A. Rand, "Evaluation and treatment of infection at the site of a total hip or knee arthroplasty," *Journal of Bone and Joint Surgery A*, vol. 80, no. 6, pp. 910–922, 1998.

[6] A. González della Valle, M. Bostrom, B. Brause, C. Harney, and E. A. Salvati, "Effective bactericidal activity of tobramycin and vancomycin eluted from acrylic bone cement," *Acta Orthopaedica Scandinavica*, vol. 72, no. 3, pp. 237–240, 2001.

[7] J. Kelm, T. Regitz, E. Schmitt, W. Jung, and K. Anagnostakos, "In vivo and in vitro studies of antibiotic release from and bacterial growth inhibition by antibiotic-impregnated polymethylmethacrylate hip spacers," *Antimicrobial Agents and Chemotherapy*, vol. 50, no. 1, pp. 332–335, 2006.

[8] S. L. Henry and K. P. Galloway, "Local antibacterial therapy for the management of orthopaedic infections: pharmacokinetic considerations," *Clinical Pharmacokinetics*, vol. 29, no. 1, pp. 36–45, 1995.

[9] J. Mutimer, G. Gillespie, A. M. Lovering, and A. J. Porteous, "Measurements of in vivo intra-articular gentamicin levels from antibiotic loaded articulating spacers in revision total knee replacement," *Knee*, vol. 16, no. 1, pp. 39–41, 2009.

[10] K. Anagnostakos, P. Wilmes, E. Schmitt, and J. Kelm, "Elution of gentamicin and vancomycin from polymethylmethacrylate beads and hip spacers in vivo," *Acta Orthopaedica*, vol. 80, no. 2, pp. 193–197, 2009.

[11] E. Bertazzoni Minelli, A. Benini, B. Magnan, and P. Bartolozzi, "Release of gentamicin and vancomycin from temporary human hip spacers in two-stage revision of infected arthroplasty," *Journal of Antimicrobial Chemotherapy*, vol. 53, no. 2, pp. 329–334, 2004.

[12] N. V. Greidanus, B. A. Masri, D. S. Garbuz et al., "Use of erythrocyte sedimentation rate and C-reactive protein level to diagnose infection before revision total knee arthroplasty: a prospective evaluation," *Journal of Bone and Joint Surgery A*, vol. 89, no. 7, pp. 1409–1416, 2007.

[13] W. J. Gillespie, "Prevention and management of infection after total joint replacement," *Clinical Infectious Diseases*, vol. 25, no. 1, pp. 1310–1317, 1997.

[14] K. P. Iyengar and S. Vinjamuri, "Role of 99mTc Sulesomab in the diagnosis of prosthetic joint infections," *Nuclear Medicine Communications*, vol. 26, no. 6, pp. 489–496, 2005.

[15] B. Zicat, D. M. Rahme, K. Swaraj, S. Qurashi, R. Loneragan, and H. Van der Wall, "Septic arthritis of the acromioclavicular joint: Tc-99m leukocyte imaging," *Clinical Nuclear Medicine*, vol. 31, no. 3, pp. 145–146, 2006.

[16] D. Fuster, A. Soriano, S. Garcia et al., "Usefulness of 99mTc-ciprofloxacin scintigraphy in the diagnosis of prosthetic joint

infections," *Nuclear Medicine Communications*, vol. 32, no. 1, pp. 44–51, 2011.

[17] N. A. Athanasou, R. Pandey, R. de Steiger, D. Crook, and P. M. Smith, "Diagnosis of infection by frozen section during revision arthroplasty," *Journal of Bone and Joint Surgery B*, vol. 77, no. 1, pp. 28–33, 1995.

[18] D. S. Feldman, J. H. Lonner, P. Desai, and J. D. Zuckerman, "The role of intraoperative frozen sections in revision total joint arthroplasty," *Journal of Bone and Joint Surgery A*, vol. 77, no. 1, pp. 1807–1813, 1995.

[19] E. Bertazzoni Minelli, C. Caveiari, and A. Benini, "Release of antibiotics from polymethylmethacrylate cement," *Journal of Chemotherapy*, vol. 14, no. 5, pp. 492–500, 2002.

[20] Clinical and Laboratory Standards Institute, "Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically," Approved Standard M7-A8, CLSI, Wayne, Pa, USA, 2009, 8th edition.

[21] Clinical and Laboratory Standards Institute, "Performance standards for antimicrobial susceptibility testing," 19th Informational Supplement M100-S19, CLSI, Wayne, Pa, USA, 2009.

[22] G. M. Eliopoulos and R. C. Moellering, "Antimicrobial combination," in *Antibiotics in Laboratory Medicine*, V. Lorian, Ed., pp. 330–396, Williams & Wilkins, Baltimore, Md, USA, 4th edition, 1996.

[23] I. C. Gyssens, "Vancomycin," in *Kucers' the Use of Antibiotics*, M. L. Grayson, S. M. Crowe, J. S. McCarthy et al., Eds., pp. 569–600, Hodder Arnold, London, UK, 6th edition, 2010.

[24] D. Amsterdam, "Susceptibility testing of antimicrobials in liquid media," in *Antibiotics in Laboratory Medicine*, V. Lorian, Ed., pp. 52–111, Williams & Wilkins, Baltimore, Md, USA, 4th edition, 1996.

[25] C. W. Stratton, "The usefulness of the serum bactericidal test in orthopedic infections," *Orthopedics*, vol. 7, no. 10, pp. 1579–1580, 1984.

[26] D. R. P. Neumann, T. Hofstaedter, C. List, and U. Dorn, "Two-stage cementless revision of late total hip arthroplasty infection using a premanufactured spacer," *Journal of Arthroplasty*, vol. 27, no. 7, pp. 1397–1401, 2012.

[27] B. Magnan, D. Regis, R. Biscaglia, and P. Bartolozzi, "Preformed acrylic bone cement spacer loaded with antibiotics: use of two-stage procedure in 10 patients because of infected hips after total replacement," *Acta Orthopaedica Scandinavica*, vol. 72, no. 6, pp. 591–594, 2001.

[28] F. D'Angelo, L. Negri, T. Binda, G. Zatti, and P. Cherubino, "The use of a preformed spacer in two-stage revision of infected hip arthroplasties," *Musculoskeletal surgery*, vol. 95, no. 2, pp. 115–120, 2011.

[29] R. M. Degen, J. R. Davey, J. R. Davey, J. L. Howard, R. W. McCalden, and D. D. R. Naudie, "Does a prefabricated gentamicin-impregnated, load-bearing spacer control periprosthetic hip infection?" *Clinical Orthopaedics and Related Research*, vol. 470, no. 10, pp. 2724–2729, 2012.

[30] E. Hershberger, J. R. Aeschlimann, T. Moldovan, and M. J. Rybak, "Evaluation of bactericidal activities of LY333328, vancomycin, teicoplanin, ampicillin-sulbactam, trovafloxacin, and RP59500 alone or in combination with rifampin or gentamicin against different strains of vancomycin-intermediate Staphylococcus aureus by time-kill curve methods," *Antimicrobial Agents and Chemotherapy*, vol. 43, no. 3, pp. 717–721, 1999.

[31] H. van de Belt, D. Neut, W. Schenk, J. R. van Horn, H. C. van der Mei, and H. J. Busscher, "Staphylococcus aureus biofilm formation on different gentamicin-loaded polymethylmethacrylate bone cements," *Biomaterials*, vol. 22, no. 12, pp. 1607–1611, 2001.

[32] S. W. Ueng, P. H. Hsieh, H. N. Shih et al., "Antibacterial activity of joint fluid in cemented total-knee arthroplasty: an in vivo comparative study of polymethylmehacrylate with and without antibiotic loading," *Antimicrobial Agents and Chemotherapy*, vol. 56, no. 11, pp. 5541–5546, 2012.

[33] A. Luu, F. Syed, G. Raman et al., "Two-stage arthroplasty for prosthetic joint infection: a systematic review of acute kidney injury, systemic toxicity and infection control," *The Journal of Arthroplasty*, 2013.

[34] K. R. Berend, A. V. Lombardi Jr., M. J. Morris, A. G. Bergeson, J. B. Adams, and M. A. Sneller, "Two-stage treatment of hip periprosthetic joint infection is associated with a high rate of infection control but high mortality," *Clinical Orthopaedic and Related Research*, vol. 471, no. 2, pp. 510–518, 2013.

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Research Article

The Influence of Storage Temperature on the Antibiotic Release of Vancomycin-Loaded Polymethylmethacrylate

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Periprosthetic joint infection is devastating and increases medical expenditure and socioeconomic burden. Antibiotic-loaded cement spacer is useful in the interim period before the reimplantation surgery. Prefabricated antibiotic-loaded cement spacers can decrease operation time but have been limitedly used clinically. In the literature, there is no clear recommendation on the storage temperature for the prefabricated cement spacers. We used an in vitro model to analyze whether the storage temperature at 25°C, 4°C, or −20°C for 2 weeks or 3 months could affect the release of vancomycin from the cement. We found that the storage temperature and time had no significant effects on the pattern and amount of vancomycin release. The patterns of vancomycin release from the cement stored at different temperatures were similar with an abrupt release in the first 3 days and steadily declined in the following period. This study provides a preliminary result to justify the storage of fabricating antibiotic-loaded cement spacer sterilely packed at room temperature. Further studies to examine the effects of storage temperature on the mechanical strength and the release pattern of other antibiotics should be done to provide more evidence to support the clinical use of prefabricated ready-to-use antibiotic-loaded cement spacer.

1. Introduction

Periprosthetic joint infection (PJI) is a devastating condition that increases medical expenditure and patient's economic burden [1, 2]. For established PJI, the most accepted treatment modality is a two-stage reimplantation protocol [2]. During the interim period before reimplantation, antibiotic-loaded cement spacer has been widely adopted as an effective method to deliver high levels of local antibiotics for infection control and to maintain the soft tissue tension before the reimplantation surgery [3]. Antibiotic-loaded cement spacer is usually manufactured by surgeons during operation. This can be time consuming. If the causing bacteria are known preoperatively, the antibiotic-loaded cement spacer can be fabricated in advance. Prefabricated antibiotic-loaded cement spacer is appealing because it not only reduces operation time but also decreases blood loss [4–6]. Hailey et al. reported that the mechanical properties of bone cement stored at 37°C were more brittle than those stored at 21°C [7]. However, in the literature, there is no recommendation for the storage of antibiotic-loaded cement spacer. In our previous study, we found that the antibacterial activity of antibiotics in the bone cement could be maintained at −80°C [8]. But it is impractical to store and ship the cement spacer at −80°C in common clinical settings. We hypothesized that the storage temperatures of antibiotic-loaded cement had no significant influence on the antibiotic release. We tested our hypothesis by choosing room temperature (25°C), refrigerator (4°C), and freezer (−20°C) as the storage conditions by in vitro antibiotic release analysis.

2. Materials and Methods

Vancomycin 8 g (Gentle Pharmaceutical Co, Yunlin, Taiwan) was thoroughly mixed with 40 g of Surgical Simplex bone cement powder (Stryker Orthopaedics, Limerick, Ireland) in a stainless-steel container prior to the addition of the liquid monomer. After mixing with liquid monomer for 2 min with

a doughy consistency, the cement mixture was pressed into plastic molds and cured at room temperature. The vancomycin-loaded cement discs were sterilely packed and divided into 3 groups with the storage temperature at 25°C, 4°C, and −20°C. The specimens were then divided into 2-week storage and 3-month storage.

After the completion of storage time, each cement disc (8 samples in each group) was immersed in polypropylene tube with 5 mL phosphate-buffered saline (PBS; pH 7.3) and agitated in an incubator at 37°C. Daily transfer of the cement disc into a new tube with PBS was continued for 28 days. The elution samples of 2 mL PBS at days 1, 3, 7, 14, and 28 were collected and stored at −80°C until analysis.

The concentration of vancomycin was determined using high-performance liquid chromatography (HPLC, model ALC 717, Waters Associates, Milford, MA, USA) with a stainless-steel column (RP18 column, 10 mm by 4.6 mm, 5 μm particle size). The mobile phase consisted of water-acetonitrile 100 mM ammonium formate (composite ratio, 78/12/10). Accumulated amounts of vancomycin release from the cement discs were calculated.

Statistical analysis of repeated measure analysis of variance was used to determine differences in the vancomycin release between groups of different storage temperatures. A P value less than 0.05 was considered significant.

3. Results

The patterns of vancomycin release from the cement discs stored at different temperatures were similar with an abrupt release in the first 3 days and steadily declined in the following period (Figures 1 and 2). The average weight of each cement disc was 3.94 g (range, 2.86 g to 4.75 g). The amount of vancomycin release from the cement discs was adjusted by their weight. The amount of vancomycin release on the first day was 1575 ± 96 μg/mL/g, 1881 ± 116 μg/mL/g, and 1678 ± 86 μg/mL/g, respectively, with the storage temperatures at 25°C, 4°C, and −20°C for 2 weeks (mean ± standard deviation) (Figure 1). On the 14th day, it was 68 ± 10 μg/mL/g, 85 ± 10 μg/mL/g, and 86 ± 7 μg/mL/g, respectively, at 25°C, 4°C, and −20°C. On the 28th day, it was 68 ± 2 μg/mL/g, 24±2 μg/mL/g, and 24±5 μg/mL/g, respectively, at 25°C, 4°C, and −20°C. When the storage time was 3 months, the vancomycin release on the first day was 1665 ± 469 μg/mL/g, 2014 ± 492 μg/mL/g, and 2057 ± 598 μg/mL/g, respectively, with the storage temperature at 25°C, 4°C, and −20°C (Figure 2). On the 14th day, it was 132 ± 6 μg/mL/g, 160 ± 13 μg/mL/g, and 156 ± 15 μg/mL/g, respectively, at 25°C, 4°C, and −20°C. On the 28th day, it was 18 ± 2 μg/mL/g, 26 ± 3 μg/mL/g, and 20 ± 2 μg/mL/g, respectively, at 25°C, 4°C, and −20°C. No difference could be found between the groups with different storage temperatures with 2 weeks or 3 months storage time.

The accumulated amount of vancomycin release from the each g of cement discs was, 29.17 mg, 28.23 mg, and 27.70 mg, respectively, when stored at 25°C, 4°C, and −20°C for 2 weeks. The antibiotic release ratios were 14.6%, 14.1%, and 13.8%, respectively. It was 30.45 mg (15.2%), 33.78 mg (16.9%), and 32.36 mg (16.2%), respectively, when stored at 25°C, 4°C, and −20°C for 3 months. There were no differences in terms of the accumulated vancomycin release from the cement discs stored at different temperature with 2 weeks or 3 months storage time.

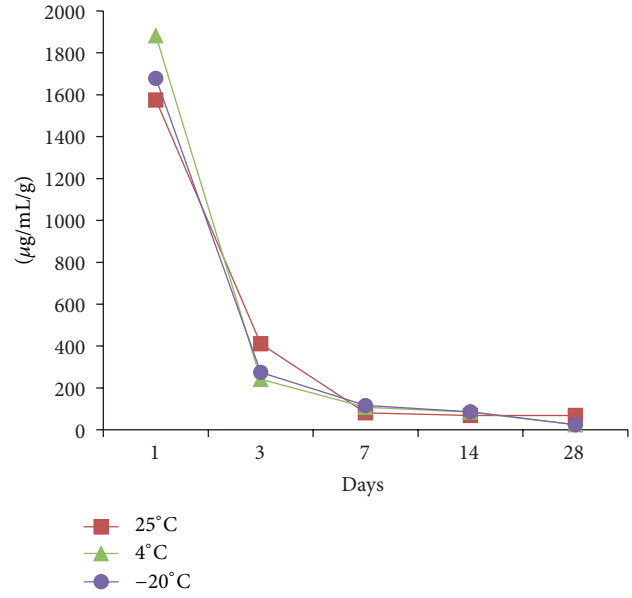

Figure 1: The release of vancomycin from samples stored at different temperatures for 2 weeks.

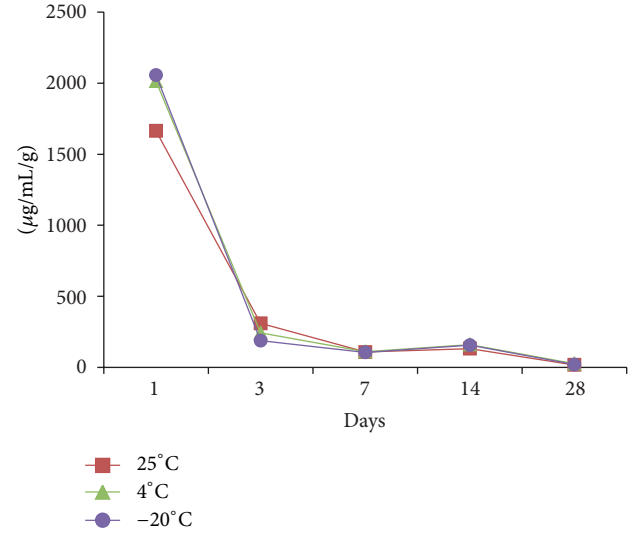

Figure 2: The release of vancomycin from samples stored at different temperatures for 3 months.

4. Discussion

Antibiotic-loaded cement spacer has been used for periprosthetic joint infection in the interim period to deliver local antibiotics while maintaining soft tissue tension and facilitating reimplantation surgery. The cement spacer can be articulating or nonarticulating depending on the surgeon's preference and the patient's condition. A PROSTALAC hip

system is a commercial available spacer which consists of an all-polyethylene cemented acetabular component, a metal head, and a mold to construct antibiotic-loaded cement on a metal endoskeleton [3]. Reusable silicon, metal molds, or nonreusable plastic molds have also been fabricated with a metal endoskeleton for mechanical support. The PROSTALAC knee system has also been introduced with the femoral component incorporating metal runners and the tibial component incorporating inlay polyethylene plateaus [9]. The clinical success rates by using the antibiotic-loaded cement spacer are around 90% in the two-stage protocol [3, 9–11].

In clinical practice, a prefabricated antibiotic-loaded cement spacer is beneficial to patients with periprosthetic joint infection [4–6]. Severe PJI associated with sepsis can induce disseminated intravascular coagulopathy. Less severe PJI can also cause abnormal systemic coagulation problem [12]. In patients who have medical morbidities such as liver cirrhosis or coagulation abnormality, any measure to decrease the operation time and blood loss will be beneficial for the treatment of PJI [12–14]. Unfortunately, a pre-fabricated ready-to-use antibiotic-loaded cement spacer is not popular on the market because it is only available in some countries and the choice of antibiotics needs to be patient-specific according to the culture sensitivity results [4–6]. In chronic PJI, when the causative organisms are known, a prefabricated antibiotic-loaded cement spacer in sterile packing can facilitate surgery and save operation time. However, there is no recommendation or any guideline about the storage condition for antibiotic-loaded cement spacer. In our previous in vitro studies, we stored the antibiotic-cement specimens at −80°C and found that it would not affect the characteristics of antibiotics release from the cement as well as the bacterial killing abilities [8]. In this study, we examined the release of vancomycin from cement stored at 25°C (room temperature), 4°C (refrigerator), and −20°C (freezer). We found that the storage temperature did not affect the antibiotic release pattern and the daily or the accumulated amount of vancomycin released from the cement when the storage time was 2 weeks or 3 months. At 28 days, the concentration of vancomycin in the supernatant was still many folds higher than the minimal inhibitory concentration (MIC) for the bactericidal effects.

This study provides a preliminary result to justify the practice of fabricating in-house antibiotic-loaded cement spacer sterilely packed and stored at room temperature before use. However, this result is limited and should not be translated to all clinical settings since only vancomycin was tested by using the in vitro model. In addition, the cement discs used in the study were not equal to the bulky cement spacer used clinically. Although the commercial available gentamicin-loaded cement beads (Septopal, Merck, Darmstadt, Germany) have been packed and shipped without temperature control since its introduction to the market, this study is the first report to examine the feasibility of storage condition of prefabricated antibiotic-loaded cement in terms of the antibiotic release.

In summary, we found that the storage temperature at room temperature (25°C), refrigerator (4°C), or freezer (−20°C) of the antibiotic-loaded cement had no effect on the vancomycin release up to 3 months of the storage time. Further studies to examine the effects of storage temperature on the mechanical strength and the release pattern of other antibiotics should be done to provide more evidence to support the clinical use of prefabricated ready-to-use antibiotic-loaded cement spacer.

References

[1] K. J. Bozic and M. D. Ries, "The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization," *Journal of Bone and Joint Surgery A*, vol. 87, no. 8, pp. 1746–1751, 2005.

[2] C. Diaz-Ledezma, C. A. Higuera, and J. Parvizi, "Success after treatment of periprosthetic joint infection: a Delphi-based international multidisciplinary consensus," *Clinical Orthopaedics and Related Research*, vol. 471, pp. 2374–2382, 2013.

[3] Q. Cui, W. M. Mihalko, J. S. Shields, M. Ries, and K. J. Saleh, "Antibiotic-impregnated cement spacers for the treatment of infection associated with total hip or knee arthroplasty," *Journal of Bone and Joint Surgery A*, vol. 89, no. 4, pp. 871–882, 2007.

[4] B. Magnan, D. Regis, R. Biscaglia, and P. Bartolozzi, "Preformed acrylic bone cement spacer loaded with antibiotics: use of two-stage procedure in 10 patients because of infected hips after total replacement," *Acta Orthopaedica Scandinavica*, vol. 72, no. 6, pp. 591–594, 2001.

[5] D. J. F. Moojen, B. Hentenaar, H. Charles Vogely, A. J. Verbout, R. M. Castelein, and W. J. A. Dhert, "In Vitro release of antibiotics from commercial PMMA beads and articulating hip spacers," *Journal of Arthroplasty*, vol. 23, no. 8, pp. 1152–1156, 2008.

[6] D. R. P. Neumann, T. Hofstaedter, C. List, and U. Dorn, "Two-stage cementless revision of late total hip arthroplasty infection using a premanufactured spacer," *Journal of Arthroplasty*, vol. 27, pp. 1397–1401, 2011.

[7] J. L. Hailey, I. G. Turner, and A. W. Miles, "An in vitro study of the effect of environment and storage time on the fracture properties of bone cement," *Clinical Materials*, vol. 16, no. 4, pp. 211–216, 1994.

[8] Y. Chang, W.-C. Chen, P.-H. Hsieh et al., "In vitro activities of daptomycin-, vancomycin-, and teicoplanin-loaded polymethylmethacrylate against methicillin-susceptible, methicillin-resistant, and vancomycin-intermediate strains of Staphylococcus aureus," *Antimicrobial Agents and Chemotherapy*, vol. 55, no. 12, pp. 5480–5484, 2011.

[9] J. T. Munro, D. S. Garbuz, B. A. Masri, and C. P. Duncan, "Articulating antibiotic impregnated spacers in two-stage revision of infected total knee arthroplasty," *Journal of Bone and Joint Surgery*, vol. 94, no. 11, pp. 123–125, 2012.

[10] C. R. Gooding, B. A. Masri, C. P. Duncan, N. V. Greidanus, and D. S. Garbuz, "Durable infection control and function with the PROSTALAC spacer in two-stage revision for infected knee arthroplasty," *Clinical Orthopaedics and Related Research*, vol. 469, no. 4, pp. 985–993, 2011.

[11] G. S. Biring, T. Kostamo, D. S. Garbuz, B. A. Masri, and C. P. Duncan, "Two-stage revision arthroplasty of the hip for infection using an interim articulated Prostalac hip spacer: a 10- to 15-year follow-up study," *Journal of Bone and Joint Surgery - Series B*, vol. 91, no. 11, pp. 1431–1437, 2009.

[12] A. Saxena, M. Baratz, M. S. Austin, J. J. Purtill, and J. Parvizi, "Periprosthetic joint infection can cause abnormal systemic

coagulation," *Journal of Arthroplasty*, vol. 26, no. 1, pp. 50–57, 2011.

[13] J. Cordero-Ampuero and M. de Dios, "What are the risk factors for infection in hemiarthroplasties and total hip arthroplasties?" *Clinical Orthopaedics and Related Research*, vol. 468, no. 12, pp. 3268–3277, 2010.

[14] S. M. J. Mortazavi, J. T. O'Neil, B. Zmistowski, J. Parvizi, and J. J. Purtill, "Repeat 2-stage exchange for infected total hip arthroplasty: a viable option?" *Journal of Arthroplasty*, vol. 27, pp. 923–926, 2012.

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Review Article

Treatment of Periprosthetic Infections: An Economic Analysis

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This review summarizes the existing economic literature, assesses the value of current data, and presents procedures that are the less costly and more effective options for the treatment of periprosthetic infections of knee and hip. Optimizing antibiotic use in the prevention and treatment of periprosthetic infection, combined with systemic and behavioral changes in the operating room, the detection and treatment of high-risk patient groups, as well as the rational management of the existing infection by using the different procedures according to each particular case, could allow for improved outcomes and lead to the highest quality of life for patients and the lowest economic impact. Nevertheless, the costeffectiveness of different interventions to treat periprosthetic infections remains unclear.

1. Introduction

Infection is the cause of 14.8% of Total Hip Arthroplasty (THA) revisions [1] and the most common cause of Total Knee Arthroplasty (TKA) revisions (25.2%) [2]. At least one rehospitalization due to deep infection during the first year after primary THA or TKA occurs in 1.3% of patients, 26% of them being revised [3]. The economic burden of periprosthetic joint infection (PJI) is expected to exceed 50% of the inpatient resources spent in revisions by 2016 for TKA and by 2025 for THA [4]. This constitutes a substantial economic burden on patients, physicians, hospitals, healthcare systems, and society as a whole.

The optimization of existing resources compels healthcare professionals to analyze in depth—and critique—the value of different therapeutic methods and technologies to provide cost-effective high-quality care. It is very important to correlate outcomes with the expenses incurred to achieve them. The identification and valuing of costs is often an additional step of the decision-making process when evaluating multiple competing strategies. Differential risks and balancing costs and benefits with various potential outcomes, especially when substantial uncertainty exists or when the timing of subsequent events is important, must be taken into account. Economic and decision analyses are evidence-based tools to guide healthcare choices. The guidelines these economic analyses provide have been reported in the literature and must be understood and used in order to adequately compare procedures and choose the best option [5, 6].

While the favorable cost effectiveness of a primary or revision THA/TKA has been demonstrated [7, 8], there is not the same certainty regarding the management of PJI

[9, 10]. Determining the less expensive therapeutic methods that may best control infection and at the same time improve outcomes by minimizing patient morbidity and mortality might yield the highest quality of life for patients and the lowest economic impact on the healthcare systems as on society. This review summarizes the existing economic literature base, assesses the value of available data, and reports the less costly and more effective procedures for the treatment of PJI.

2. Prevention of Periprosthetic Infections

Prevention remains the least expensive approach against periprosthetic infection. The attainment of effective, low-cost, safe, and easy-to-use methods to elude periprosthetic infection is certainly the most logical methodology.

The efficacy and cost effectiveness of antibiotics to prevent PJI depend on the antibiotic in case, the required quantity per dose, and the number of doses. With a similar efficacy, safety, and prices, a prophylactic regimen with cefazolin (1987 US$6.55/g)—a first-generation cephalosporin, one preoperative dose of 1 g followed by 500 mg every eight hours for six doses, saved cost when compared to a regimen with cefamandole (1987 US$6.99/g), a second-generation cephalosporin, 2 g preoperatively and then 1 g every eight hours for six doses (1987 US$26.20 versus US$55.92) [11].

The efficacy of a single-dose or short-term prophylaxis regimen has been estimated as equivalent to that of a long-term regimen, but with an associated reduction of risk of adverse effects and bacterial resistance and with lower costs [12]. In 1986, whether cefazolin was administered as a single 1 g parenteral dose intraoperatively or repeatedly every six hours for 24 hours, 48 hours, or seven days, the cost savings of an intraoperative antibiotic regimen versus a 48-hour regimen would have been US$77 per case. Changing from a seven-day regimen to a one-dose antibiotic, the savings would have been US$297 per patient without any difference in the infection rate [13]. The cost savings with current prices of these antibiotics could be US$31.45 per case of one dose versus a 48-hour regimen and US$110.04 per case using one dose instead of the seven-day regimen.

There is no evidence to suggest that new-generation cephalosporins or the administrations of antibiotics beyond 24 hours postoperatively are more effective at preventing postoperative PJI in THA/TKA surgery than first-generation cephalosporins or single-dose or short-term administration. The use of one-dose first-generation cephalosporin is effective enough, reducing costs, risk of toxicity, and the development of bacterial resistance [12, 14].

The comparison between systemic administration of antibiotics and the use of antibiotic-loaded cement in order to prevent PJI has turned out inconclusive for a long time [12]. A favorable effect of adding antibiotics to the bone cement has been reported in the literature [15]. A cost effectiveness study [16], with a level II of evidence, has reported that antibiotic-impregnated bone cement in primary THA is cost-effective, avoiding revision due to infection, whereas the cost of revision is more than 3.5 times the cost of primary THA and in patients younger than 71 years, among other circumstances. The estimated cost of a 40 g packet of antibiotic-impregnated bone cement at the authors' institution was approximately 2002 US$365, while standard bone cement cost approximately 2002 US$65. Two packets of cement are used on average, resulting in an additional cost of 2002 US$600 per primary THA. The infection rate assumed in the model was 0.7% over ten years using standard bone cement and 0.4% with antibiotic-impregnated bone cement. A higher risk of infection from baseline makes the option of using antibiotic-impregnated bone cement even more cost-effective. A study carried out in 2011 determined the cost-savings of an initiative which aimed to reduce the costs of infection-related revisions by 10%. The savings were over US$70,000 [17].

3. Treatment of Periprosthetic Infections

Two high-level quality studies [19, 20] were analyzed. The treatment of patients with PJI is associated with significantly greater resource utilization compared with patients who underwent a primary or aseptic revision of TJR, which constitutes a substantial economic burden for patients, taxpayers, and hospitals. PJIs often require multiple reoperations, the prolonged use of antibiotics, a longer rehabilitation period, and frequent follow-up visits. Revision procedures for PJI are associated with a significant higher number of hospitalizations, hospital days, and number of operations, as well as a longer operative time, more blood loss, a lengthier antibiotic therapy, a higher number of radiographic examinations, and more total outpatient visits during the twelve-month period following the index procedure. A higher number of complications is also common. In general, in the case of an infected TKA, these parameters and costs were 3 to 4 times that of a primary TKA and more than twice that of an aseptic revision [21]. Sculco estimated an average cost of 1993 US$50,000 to $60,000 per case of infected THA [22].

The cost of hospital stay was the more relevant component of the whole set. In the Durham Regional Hospital (NC, USA), during the 90s, the total direct cost of hospitalization was estimated as an average of US$8206 for infected TJR versus US$5492 for uninfected arthroplasties [23]. In the Kraków Jagielloński University (Poland), in 2005, the direct cost of hospitalization for infected TJR reached US$37,903, and the cost of antibiotic treatment was US$11,067 [24]. In the Hospital of the University of Lund (Sweden), the cost of hospitalization was 1988 US$2530 for primary TKA versus US$33,663 for infected TKA; the cost of operation was US$3684 and US$10,411, respectively, and the cost of antibiotic therapy was US$65 and US$3778, respectively [25].

There were also notable differences between the costs generated by the use of antibiotics during hospitalization and those of antibiotics administered to outpatients. In TKA infections, the average length of hospital treatment was 157 days against the 850 total days of outpatient treatment. The effort to reduce inpatient treatment, even if the outpatient treatment is long, has a notable impact on the total cost. In this sense, the trend of treatment of PJI would be directed

towards the use of oral antibiotic therapy in outpatients, reducing hospital stays essentially to those related to surgical procedures.

4. Options in Surgical Techniques and Cost

Surgical options for treatment include debridement and retention of the prosthesis (DR), one- or two-stage exchange (OSE and TSE), resection arthroplasty, arthrodesis, and amputation. The success rates in eradication of PJI were below 50% with DR in retrospective series, but over 70% in the prospective modern studies with an optimal use of antibiotics [26, 27]. When prosthetic components are mechanically stable, symptoms have lasted three weeks or less, soft tissues are in good condition, and an agent active against the specific germs is available, an adequate implant DR achieving an 82%–100% cure rate of infection after three to six months of systemic therapy with ciprofloxacin and rifampin, as compared with a 58% cure rate with ciprofloxacin and placebo [27]. Control of acute PJI with DR and an adequate antibiotic regimen has been reported in 87%–89% of cases recently, but only when the bacteria is not multi resistant [28, 29].

Exchange arthroplasty is supported by many studies but has a higher rate of surgical morbidity and is more expensive than DR. In a systematic review of longitudinal studies with series of more than 50 patients, the success rate to eradicate PJI in THA was reported to be between 73.6% and 96.7% for OSE and between 87.7% and 95.1% for TSE, depending on the different authors. The random-effects analysis showed the rates of reinfection after one- and two-stage revisions were 10.56% and 8.71%, respectively [9]. In a recent meta-analysis, reinfection occurred with an estimated absolute risk of 13.1% with OSE and 10.4% with TSE [32].

In a decision-analysis, assuming that success of a given procedure was a period greater than 2 years without additional surgery, OSE might be the best solution for an acute THA infection and could lead to the greatest health-related quality of life, whereas the failure rate of DR is greater than 40% and the success rate of OSE is 66% or higher. With less than 38% success of DR and less than 69% success of OSE, TSE might lead to the greatest health-related quality of life [33].

The economic effects of OSE and TSE differ considerably. Although OSE may require a long hospital stay to administrate parenteral antibiotic therapy, the main determinant of cost is the requirement for additional surgery in TSE, with a cost 1.7 times more than OSE [30]. A short interval until reimplantation (two to four weeks) could allow both procedures to be performed during a single hospitalization [34].

The clinical and cost effectiveness of DR outcomes and TSE, with a median time to reimplantation of 2 months (range 1–12 months), in 65-year-old and frail 80-year-old patients with infected THA have been compared [33]. Patients who underwent initial DR were subjected to more additional operations than those who had initial exchange arthroplasty (3.2 versus 2.4 on average). In all cohorts, initial TSE provided a higher rate of infection-free survival than initial DR. However, the quality-adjusted life expectancy associated with DR was greater than with TSE only when old and frail population was considered. Incremental cost effectiveness ratio of DR compared with initial exchange arthroplasty was 1999 US$19,700 per QALY gained for 65-year-old men, US$21,800 per QALY gained for 65-year-old women, US$500 per QALY for 80-year-old men, and US$8200 per QALY for 80-year-old women. Initial DR became a cost-saving strategy relative to exchange arthroplasty when age at initial diagnosis of infection was over 80 years, when indirect and patient time costs were included in the analysis, and when the annual rate of infection recurrence after debridement was less than 19%. Even if the annual relapse rate after exchange arthroplasty was as low as 0.6%, initial DR remained cost-effective for patients over 80 years. The authors conclude that debridement and retention is a reasonable strategy for the treatment of PJI in patients over 80 years, staphylococcal or streptococcal infection, and well-fixed prosthesis.

For TKA, the efficacy of the different approaches to heal PJI is 20% for antibiotic therapy alone, 24% for debridement of soft tissue, 50% for resection arthroplasty, 76% for exchange arthroplasty, 90% for arthrodesis, and 100% for amputation [25]. In a systematic search of the literature about infected TKA, the overall success rate of PJI eradication was 73%–100% after OSE and 82%–100% after TSE, with 12–122 months follow up [35]. The clinical outcome (knee scores and range of motion) of OSE was no different from that of TSE.

From the perspective of hospitals which run their own operating rooms, the net financial impact range from cost savings is between US$5.09 and US$36.15 per case. In the case of surgeons who rent operating room space, the 24 minutes gained in operative time amount to a reduction in fees of approximately US$1794.91 per case, and when the cost of the device is included, net savings may be estimated in 2011 to be US$1294.91 per case [36].

The use of an antibiotic-loaded spacer in the TSE treatment of infected THA provides better infection control with good functional results and is superior a spacer-free two-stage treatment. The recurrence of infection was significantly higher without spacer (33.3% versus 10.5%). The use of a spacer increased the surgical time of the first stage by 40.1 minutes; but reduced the mean duration of the second surgical stage by 1 hour because reimplantation is easier; the surgical planes are found faster, the bone structures are well identified, and the bed for the prosthesis is accurately prepared. The stay in the intensive care unit after the second surgical stage was shorter when using a spacer (average, 1.4 days versus 4.1 days). Patients without a spacer stayed in hospital almost twice as long as patients with a spacer because a period of skeletal traction is mandatory to allow healing of the soft tissues maintaining the length of extremity as much as possible.

Another way to realize cost savings in the treatment of PJI is to use the liquid form of gentamicin mixed with the bone cement fixing the prosthetic components or filling the cement spacers employed in TSE. It is the most widely and readily available antibiotic for mixing bone cement, and much less costly (US$4 for a 480 mg dose) than tobramycin

Table 1: Cost of noninfected TJR and debridement and retention for treatment of infected TJR [18].

	Non-infected TJR	Infected TJR DR	P
Total inpatient	22,688	57,494	0.001
Medical	1732	9117	0.001
Nursing	7830	28,140	0.001
Operating room	11,173	18,977	0.001
Implants	7468	8336	0.3
Intensive care unit	0	0	1.0
Allied health	1562	3707	0.001
Medical imaging	64	278	0.001
Pathology	188	1710	0.001
Pharmacy	331	2388	0.001
Hospital at home	469	1624	0.02
Total outpatient	377	4426	0.001
Medical	23	901	0.001
Nursing	278	442	0.03
Allied health	0	44	0.002
Medical imaging	0	120	0.001
Pathology	0	146	0.001
Pharmacy	0	1846	0.001
Total emergency	0	553	0.001
Total costs	24,073	75,661	0.001

(US$120–310 per 1.2 g dose) and than the powdered form of gentamicin, which is at least as expensive as tobramycin [37, 38]. The limitation imposed by using liquid gentamicin in the bone cement fixing prosthesis is a decrease of mechanical properties of the cement produced, but this is irrelevant for the temporary cement spacers. If tobramycin is replaced by liquid gentamicin in bone cement spacers, an annual antibiotic cost saving of US$7,400,000 could be achieved in the United States alone [38].

From the current literature, a certain consensus emerges regarding complete cost coverage of PJI treatment not being feasible in most healthcare systems. An estimated average loss is of approximately $15,000 per case for the total group of patients as a whole treated for infected TKA, between $30,000 per case per Medicare patient in USA in 1993 [21], and 7745 per case in Germany [39]. Furthermore, inflation decreased the value of estimated mean reimbursement per hospitalization for PJI in USA from 2004 US$9746 in 1997 to US$8719 in 2004 [40]. The lack of incremental reimbursement for these procedures discourages physicians and hospitals from treating patients with PJI [41] (Table 1). Reimbursement to both hospitals and physicians should be more accurate and reflect the actual magnitude of resources consumed by these patients.

Determining the treatment that best controls infection minimizing patient morbidity and mortality with the less cost possible may offer the best solution to the problem. Among the protocols and techniques currently used to reduce the incidence and to treat PJI, there is not a clear option. The resource allocation and financial costs of treating PJI in orthopedic surgery can often rise 3–13 times more than the cost of the index procedure, thus making PJI an ideal target for cost-effective solutions in a value-driven healthcare model [18]. The recommendations set out by different authors [34, 42] and proven useful when choosing the method of treatment of PJI [43] must be tempered in view of results of these economic studies (Table 2).

5. Level of Evidence

There are only a few high-quality studies dealing with the accurate evaluation of cost effectiveness of PJI treatment. The lack of level-I evidence studies regarding interventions in PJI has made it difficult to perform high-quality cost-utility analyses. The difficulties and ethical concerns of performing randomized studies in this field are evident, more so as they are related with treatment procedures. The number of patients needed to carry on correctly such studies is yet another concern. It has been estimated that 7,000–14,000 patients would be needed to demonstrate a 20% reduction in infection rate if the baseline infection rate was 5% [44]. A powered study assuming a 1.5% to 2.0% infection rate would require in the range of 10,000 patients to determine the effect of any one independent variable, with power greater than 80% [45]. In order to show a 50% reduction in an infection rate of 2%, for example, at the 5% significance level and 80% power, over 2,300 patients would be required in each treatment arm [12]. Multiple variables would require at least 70,000 patients [45]. Given the low PJI rates, it may not be cost-effective to carry out mega-trials in this area. It is necessary to analyze risk factors to identify high-risk groups on whom profitable high-quality studies of new or additional prophylactic, diagnosis, or treatment measures could be performed with sufficient power to achieve a statistically significant difference.

Most studies in this field have estimated charges or costs of management of PJI assuming partially the actual price of actions. The direct medical costs, length of hospitalization, and total hospital costs were the most frequently considered parameters as indicators to evaluate resource utilization [21, 39, 46], while outpatient charges, the costs associated with retreatment of a failed treatment, and the indirect costs associated with lost wages and productivity only sometimes were accounted for [19, 25, 46, 47]. The use of the direct costs of hospitalization has been suggested as the best method to estimate the costs related to infection treatment, since they represent the real costs to the hospital for the items and services used by each patient, but this approach probably underestimates the total resource utilization and also misjudges the overall financial and personal impact of PJI on the patients themselves [48].

On the other hand, charges are only a proxy for cost and an inaccurate measure of health-care resource utilization for many reasons, essentially due to the fact that the economic basis of charges differs substantially among healthcare facilities and geographic locations. Impact on functional outcomes, working and daily activities, quality of life, and

Table 2: Different options of treatment of PJI. Estimated average cost in 2012 adjusted currencies and normalized to US$.

Author (date)	Debridement and retention	One-stage revision	Two-stage revision	Resection arthroplasty	Arthrodesis	Amputation
TJR (THA + TKA)						
Peel et al. (2013) [18]*	75,661					
THA						
Fisman et al. (2001) [19]*	74,015		70,634			
Klouche et al. (2010) [30]*		43,586	75,737 × 1.7			
TKA						
Hebert et al. (1996) [21]**			150,984	121,866	101,346	347,789
Lavernia et al. (2006) [31]**		133,970	134,670	113,575		

*Total hospital costs.
**Total hospital costs + total outpatient costs.

well-being should also be considered. Thus, the burden on patients of PJI could far exceed the costs usually evaluated in this kind of studies.

References

[1] K. J. Bozic, S. M. Kurtz, E. Lau, K. Ong, D. T. P. Vail, and D. J. Berry, "The epidemiology of revision total hip arthroplasty in the united states," *Journal of Bone and Joint Surgery A*, vol. 91, no. 1, pp. 128–133, 2009.

[2] K. J. Bozic, S. M. Kurtz, E. Lau et al., "The epidemiology of revision total knee arthroplasty in the united states," *Clinical Orthopaedics and Related Research*, vol. 468, no. 1, pp. 45–51, 2010.

[3] E. R. Bohm, M. J. Dunbar, J. J. Frood, T. M. Johnson, and K. A. Morris, "Rehospitalizations, early revisions, infections, and hospital resource use in the first year after hip and knee arthroplasties," *Journal of Arthroplasty*, vol. 27, no. 2, pp. 232–237, 2012.

[4] S. M. Kurtz, K. L. Ong, J. Schmier et al., "Future clinical and economic impact of revision total hip and knee arthroplasty," *Journal of Bone and Joint Surgery A*, vol. 89, supplement 3, pp. 144–151, 2007.

[5] C. A. Brauer and K. J. Bozic, "Using observational data for decision analysis and economic analysis," *Journal of Bone and Joint Surgery A*, vol. 91, supplement 3, pp. 73–79, 2009.

[6] C. A. Brauer, A. B. Rosen, N. V. Olchanski, and P. J. Neumann, "Cost-utility analyses in orthopaedic surgery," *Journal of Bone and Joint Surgery A*, vol. 87, no. 6, pp. 1253–1259, 2005.

[7] A. W. R. Burns, R. B. Bourne, B. M. Chesworth, S. J. MacDonald, and C. H. Rorabeck, "Cost effectiveness of revision total knee arthroplasty," *Clinical Orthopaedics and Related Research*, vol. 446, pp. 29–33, 2006.

[8] A. Laupacis, R. Bourne, C. Rorabeck et al., "The effect of elective total hip replacement on health-related quality of life," *Journal of Bone and Joint Surgery A*, vol. 75, no. 11, pp. 1619–1626, 1993.

[9] A. D. Beswick, K. T. Elvers, A. J. Smith, R. Gooberman-Hill, A. Lovering, and A. W. Blom, "What is the evidence base to guide surgical treatment of infected hip prostheses? Systematic review of longitudinal studies in unselected patients," *BMC Medicine*, vol. 10, article 18, 2012.

[10] J. L. Cahill, B. Shadbolt, J. M. Scarvell, and P. N. Smith, "Quality of life after infection in total joint replacement," *Journal of Orthopaedic Surgery*, vol. 16, no. 1, pp. 58–65, 2008.

[11] C. S. Bryan, S. L. Morgan, R. J. Caton, and E. M. Lunceford, "Cefazolin versus cefamandole for prophylaxis during total joint arthroplasty," *Clinical Orthopaedics and Related Research*, vol. 228, pp. 117–122, 1988.

[12] A. Glenny and F. Song, "Antimicrobial prophylaxis in total hip replacement: a systematic review," *Health Technology Assessment*, vol. 3, no. 21, pp. 1–57, 1999.

[13] J. S. Heydemann and C. L. Nelson, "Short-term preventive antibiotics," *Clinical Orthopaedics and Related Research*, vol. 205, pp. 184–187, 1986.

[14] B. AlBuhairn, D. Hind, and A. Hutchinson, "Antibiotic prophylaxis for wound infections in total joint arthroplasty: a systematic review," *Journal of Bone and Joint Surgery B*, vol. 90, no. 7, pp. 915–919, 2008.

[15] L. B. Engesæter, S. A. Lie, B. Espehaug, O. Furnes, S. E. Vollset, and L. I. Havelin, "Antibiotic prophylaxis in total hip arthroplasty: effects of antibiotic prophylaxis systemically and in bone cement on the revision rate of 22,170 primary hip replacements followed 0–14 years in the Norwegian Arthroplasty Register," *Acta Orthopaedica Scandinavica*, vol. 74, no. 6, pp. 644–651, 2003.

[16] J. S. Cummins, I. M. Tomek, S. R. Kantor, O. Furnes, L. B. Engesæter, and S. R. G. Finlayson, "Cost-effectiveness of antibiotic-impregnated bone cement used in primary total hip arthroplasty," *Journal of Bone and Joint Surgery A*, vol. 91, no. 3, pp. 634–641, 2009.

[17] J. Slover, J. P. Haas, M. Quirno, M. S. Phillips, and J. A. Bosco, "Cost-effectiveness of a *Staphylococcus aureus* screening and decolonization program for high-risk orthopaedic patients," *Journal of Arthroplasty*, vol. 26, no. 3, pp. 360–365, 2011.

[18] T. N. Peel, M. M. Dowsey, K. L. Buising, D. Liew, and P. F. M. Choong, "Cost analysis of debridement and retention for management of prosthetic joint infection," *Clinical Microbiology and Infection*, vol. 19, no. 2, pp. 181–186, 2013.

[19] D. N. Fisman, D. T. Reilly, A. W. Karchmer, and S. J. Goldie, "Clinical effectiveness and cost-effectiveness of 2 management strategies for infected total hip arthroplasty in the elderly," *Clinical Infectious Diseases*, vol. 32, no. 3, pp. 419–430, 2001.

[20] J. Parvizi, C. Jacovides, V. Antoci, and E. Ghanem, "Diagnosis of periprosthetic joint infection: the utility of a simple yet unappreciated enzyme," *Journal of Bone and Joint Surgery A*, vol. 93, no. 24, pp. 2242–2248, 2011.

[21] C. K. Hebert, R. E. Williams, R. S. Levy, and R. L. Barrack, "Cost of treating an infected total knee replacement," *Clinical Orthopaedics and Related Research*, vol. 331, pp. 140–145, 1996.

[22] T. P. Sculco, "The economic impact of infected total joint arthroplasty," *Instructional Course Lectures*, vol. 42, pp. 349–351, 1993.

[23] K. B. Kirkland, J. P. Briggs, S. L. Trivette, W. E. Wilkinson, and D. J. Sexton, "The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs," *Infection Control and Hospital Epidemiology*, vol. 20, no. 11, pp. 725–730, 1999.

[24] A. Rózańska, J. Wójkowska-Mach, E. Jaje, M. Bulanda, and P. B. Heczko, "Costs of surgical site infections in selected procedures in orthopedic surgery," *Chirurgia Narzadow Ruchu i Ortopedia Polska*, vol. 75, no. 2, pp. 117–120, 2010.

[25] S. Bengtson, L. Borgquist, and L. Lidgren, "Cost analysis of prophylaxis with antibiotics to prevent infected knee arthroplasty," *British Medical Journal*, vol. 299, no. 6701, pp. 719–720, 1989.

[26] J. Ariza, G. Euba, and O. Murillo, "Infecciones relacionadas con las prótesis articulares," *Enfermedades Infecciosas y Microbiología Clínica*, vol. 26, no. 6, pp. 380–390, 2008.

[27] W. Zimmerli, A. F. Widmer, M. Blatter, R. Frei, and P. E. Ochsner, "Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial," *Journal of the American Medical Association*, vol. 279, no. 19, pp. 1537–1541, 1998.

[28] I. Byren, P. Bejon, B. L. Atkins et al., "One hundred and twelve infected arthroplasties treated with "DAIR" (debridement, antibiotics and implant retention): antibiotic duration and outcome," *Journal of Antimicrobial Chemotherapy*, vol. 63, no. 6, pp. 1264–1271, 2009.

[29] F. Vilchez, J. C. Martínez-Pastor, S. García-Ramiro et al., "Outcome and predictors of treatment failure in early post-surgical prosthetic joint infections due to *Staphylococcus aureus* treated with debridement," *Clinical Microbiology and Infection*, vol. 17, no. 3, pp. 439–444, 2011.

[30] S. Klouche, E. Sariali, and P. Mamoudy, "Total hip arthroplasty revision due to infection: a cost analysis approach," *Orthopaedics and Traumatology*, vol. 96, no. 2, pp. 124–132, 2010.

[31] C. Lavernia, D. J. Lee, and V. H. Hernandez, "The increasing financial burden of knee revision surgery in the United States," *Clinical Orthopaedics and Related Research*, vol. 446, pp. 221–226, 2006.

[32] J. Lange, A. Troelsen, R. W. Thomsen, and K. Søballe, "Chronic infections in hip arthroplasties: comparing risk of reinfection following one-stage and two-stage revision: a systematic review and meta-analysis," *Clinical Epidemiology*, vol. 4, no. 1, pp. 57–73, 2012.

[33] H. Bedair, N. Ting, K. J. Bozic, C. J. della Valle, and S. M. Sporer, "Treatment of early postoperative infections after THA: a decision analysis," *Clinical Orthopaedics and Related Research*, vol. 469, no. 12, pp. 3477–3485, 2011.

[34] W. Zimmerli, A. Trampuz, and P. E. Ochsner, "Current concepts: prosthetic-joint infections," *The New England Journal of Medicine*, vol. 351, no. 16, pp. 1645–1654, 2004.

[35] E. Jämsen, I. Stogiannidis, A. Malmivaara, J. Pajamäki, T. Puolakka, and Y. T. Konttinen, "Outcome of prosthesis exchange for infected knee arthroplasty: the effect of treatment approach—a systematic review of the literature," *Acta Orthopaedica*, vol. 80, no. 1, pp. 67–77, 2009.

[36] A. F. Kamath, R. C. Clement, P. B. Derman, J. P. Garino, and G. Lee, "Bipolar sealing in revision total hip arthroplasty for infection: efficacy and cost analysis," *Journal of Arthroplasty*, vol. 27, no. 7, pp. 1376–1381, 2012.

[37] P. Hsieh, C. Tai, P. Lee, and Y. Chang, "Liquid gentamicin and vancomycin in bone cement. A potentially more cost-effective regimen," *Journal of Arthroplasty*, vol. 24, no. 1, pp. 125–130, 2009.

[38] R. M. Seldes, R. Winiarsky, L. C. Jordan et al., "Liquid gentamicin in bone cement: a laboratory study of a potentially more cost-effective cement spacer," *Journal of Bone and Joint Surgery A*, vol. 87, no. 2, pp. 268–272, 2005.

[39] M. Haenle, C. Skripitz, W. Mittelmeier, and R. Skripitz, "Ökonomische Auswirkungeninfizierter Hüfttotalendoprothesenim DRG-System," *Der Orthopäde*, vol. 41, no. 6, pp. 467–476, 2012.

[40] M. Hellmann, S. D. Mehta, D. M. Bishai, S. C. Mears, and J. M. Zenilman, "The estimated magnitude and direct cost hospital costs of prosthetic joint infections in the United States, 1997 to 2004," *Journal of Arthroplasty*, vol. 35, no. 5, pp. 766.el–771.el, 2010.

[41] K. J. Bozic and M. D. Ries, "The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization," *Journal of Bone and Joint Surgery A*, vol. 87, no. 8, pp. 1746–1751, 2005.

[42] A. F. Widmer, "New developments in diagnosis and treatment of infection in orthopedic implants," *Clinical Infectious Diseases*, vol. 33, supplement 2, pp. S94–S106, 2001.

[43] B. Y. Betsch, S. Eggli, K. A. Siebenrock, M. G. Täuber, and K. Mühlemann, "Treatment of joint prosthesis infection in accordance with current recommendations improves outcome," *Clinical Infectious Diseases*, vol. 46, no. 8, pp. 1221–1226, 2008.

[44] A. J. Kallen, C. T. Wilson, and R. J. Larson, "Perioperative intranasal mupirocin for the prevention of surgical-site infections: systematic review of the literature and meta-analysis," *Infection Control and Hospital Epidemiology*, vol. 26, no. 12, pp. 916–922, 2005.

[45] R. P. Evans, "Current concepts for clean air and total joint arthroplasty: laminar airflow and ultraviolet radiation: a systematic review," *Clinical Orthopaedics and Related Research*, vol. 469, no. 4, pp. 945–953, 2011.

[46] K. Dal-Paz, P. R. D. Oliveira, A. P. de Paula, M. C. S. da Emerick, J. R. Pécora, and A. L. L. M. Lima, "Economic impact of treatment for surgical site infections in cases of total knee arthroplasty in a tertiary public hospital in Brazil," *Brazilian Journal of Infectious Diseases*, vol. 14, no. 4, pp. 356–359, 2010.

[47] B. A. S. Knobben, *Intra-operative bacterial contamination: control and consequences [thesis]*, Groningen University, Groningen, The Netherlands, 2006.

[48] J. D. Whitehouse, N. Deborah Friedman, K. B. Kirkland, W. J. Richardson, and D. J. Sexton, "The impact of surgical-site infections following orthopedic surgery at a community hospital and a university hospital: adverse quality of life, excess length of stay, and extra cost," *Infection Control and Hospital Epidemiology*, vol. 23, no. 4, pp. 183–189, 2002.