INTRA-OPERATIVE BACTERIAL CONTAMINATION

control and consequences

B.A.S. Knobben

Cover design by Bertram The and Bas Knobben

The front and back cover illustrate the different scenes of this thesis. A biofilm, the operating room, a blood agar plate, and an X-ray of a hip prosthesis.

ISBN: 90-3672568-2

Electronic version ISBN: 90-3672569-0

Rijks*universiteit* Groningen

INTRA-OPERATIVE BACTERIAL CONTAMINATION

control and consequences

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
woensdag 26 april 2006
om 14.45 uur

door

Bas Albertus Stefanus Knobben
geboren op 31 januari 1976
te Raalte

Promotores:	Prof. dr. Ir. H.J. Busscher
	Prof. dr. J.R. van Horn
	Prof. dr. H.C. van der Mei
Beoordelingscommissie:	Prof. dr. S.K. Bulstra
	Prof. dr. J.E. Degener
	Prof. dr. J.A.N. Verhaar

Paranimfen: Drs. B. The

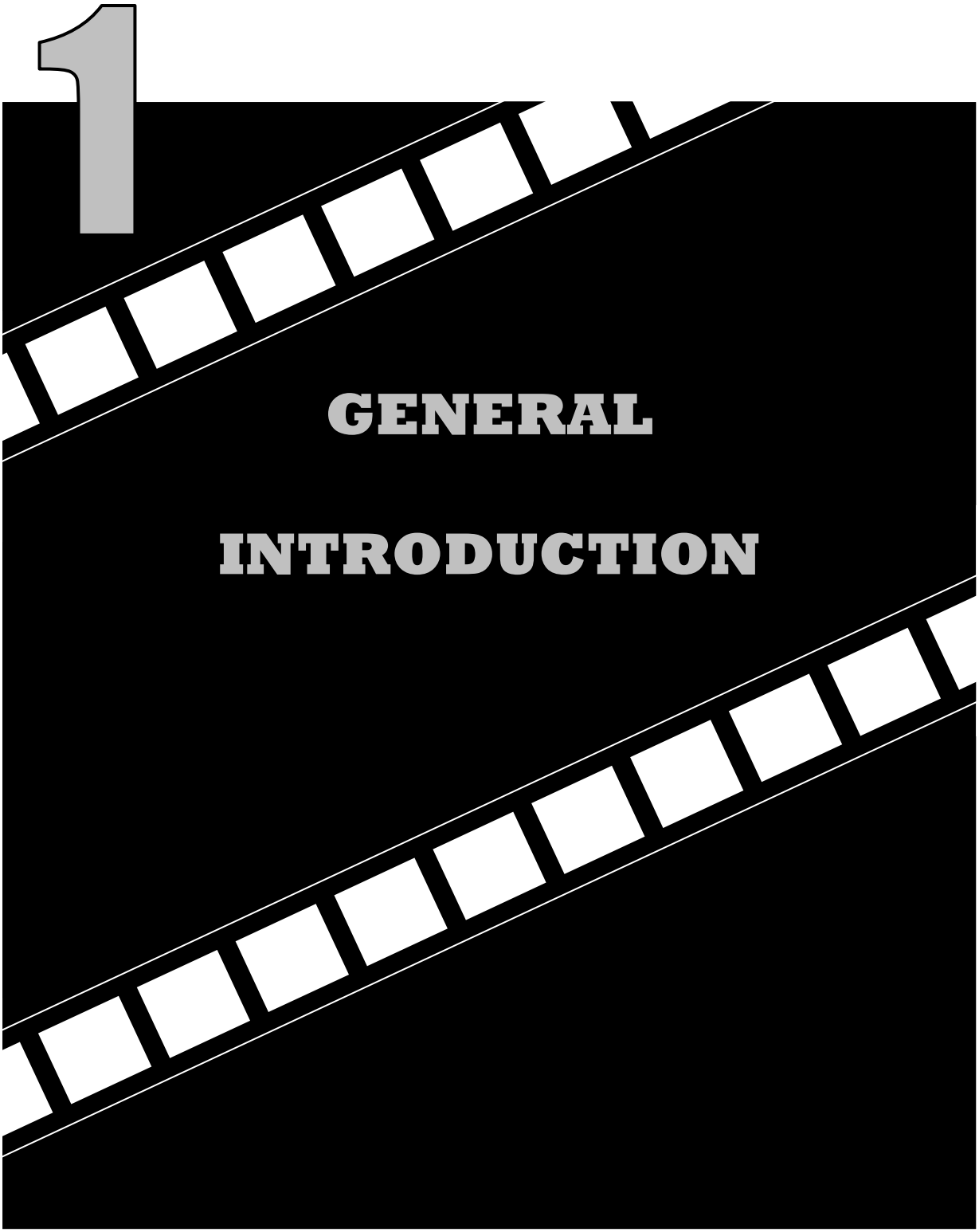
Drs. L. van Rozen

“I’VE GOT YOU UNDER MY SKIN”

(Frank Sinatra, de held van mijn vader,
mijn vader, de held van mij)

Voor paps

Contents


1
GENERAL
INTRODUCTION

Introduction

Total hip and knee arthroplasties are common procedures in orthopaedic surgery and both are routine, effective and successful treatment modalities. A current estimate of the rate of total hip replacement worldwide amounts approximately one million per year, with over 250,000 knee replacements.[1] One of the most devastating complications, however, is deep periprosthetic infection. Conservative estimates of infection rates average 1-2% for hip implants and 2-4% for knee implants.[2-9] In the future, it is expected that the incidence of the prosthetic joint infections will further increase due to (i) better detection methods for prosthetic joint infections, (ii) the growing number of implanted prostheses in an ageing population and (iii) the increasing residence time of prostheses, which are at continuous risk for infection during their implanted lifetime.[10;11] In revision surgery, the incidence of periprosthetic infection is 3.2% for hip implants and 5.6% for knee implants, and can be as high as 40% for failed hip arthroplasties with a positive intra-operative culture.[5;12] Infection remains a serious problem, as it generally requires multiple operations, and not infrequently amputations or mortality remain unavoidable during the treatment of these infections.[13;14]

Biofilm formation

Deep periprosthetic infection belongs to the large group of infections associated with indwelling medical devices, for example prosthetic heart valves, urinary catheters, intra-ocular lenses and breast implants. The major disadvantage of biomaterials implants is the increased risk of attracting infectious micro-organisms when compared to naturally occurring materials.[15] The chance for successful bacterial colonisation is influenced by the prosthetic surface characteristics, presence of dead bone fragments, and it is also dependent on host factors. Implants are covered with blood fractions immediately after their insertion, referred to as a conditioning film.[16] Bacteria are able to adhere by help of a wide range of physical and chemical interactions. Surface characteristics of the biomaterial also seem to be of importance, including hydrophobicity, roughness, and surface charge.[17-26]

Gristina et al. proposed an elegant pathogenetic metaphor for the situation occurring shortly after the insertion of implants: “the race for the surface” between the cells of the body and bacteria which inadvertently are deposited in the surgical wound.[27] The final result

depends primarily on the velocity and configuration of the process of bacterial adhesion and host coverage of the prosthetic surface. If the winners of this race are bacteria, they can display their survival strategy. More virulent pathogens expand through their elaboration of extracellular proteins, which is in contrast to less virulent pathogens producing large amounts of extracellular slime to embed and protect bacterial cells. The biofilm consists of bacterial as well as host parts that are created by fibrin, polymorphonuclear neutrophils, erythrocytes, histiocytes, fibroblasts and many other constituents.[28] A fibrous capsule on the outer surface of the biofilm can be considered as the interface between host and bacterial organisms. Under certain conditions a symbiotic relationship between more than one bacterial species may be advantageous for the development of biofilm colonies. Bacteria in a biofilm do not grow exponentially, but rather exist in a slow-growing or starvation state.[29;30] The extracellular slime enables them to evade the host immune system and antibiotic treatment.[31;32]

Periprosthetic infection

The minimal requirement for the development of deep periprosthetic infection is successful bacterial colonisation of prosthetic and/or bone surfaces around the artificial joint space. Another important aspect is the immune system of the host. Impairment of the immune system (due to prosthesis-related and/or patient-related factors) plays an important role in the pathogenesis and onset of periprosthetic infections. Once the bacteria have reached the artificial joint, they are perceived as a foreign organism in the host body, which will trigger an immune response with inflammation. The character of this response can be modified by a chronically immunoincompetent inflammatory zone surrounding artificial joints,33 probably leading to osteolysis.34-36 Regardless of the mechanism of periprosthetic osteolysis, it is attractive to believe that the same processes that induce osteolysis may maintain immunoincompetency, facilitate expansion of the biofilm community, and may even lead to the development of haematogenous infection.

Infection following total joint arthroplasty remains a serious complication. Virulent pathogens cause an acute form of infection with a consistent clinical picture and laboratory findings. However, the majority of periprosthetic infections are due to human skin saprophytes (from both patient and operating room personnel) of low virulence that are able to provoke only minimal or no symptoms for some time. The cultures obtained from different articular

sites can be negative in spite of evidently infection.[25;37-44] The subsequent incorrect diagnosis may lead to inappropriate surgical procedures associated with a high risk of failure.[37;38;45]

Intra-operative contamination

It is generally believed that intra-operative contamination is common in every operating room.[46-54] The main sources for intra-operative contamination are the skin of the patient and airborne particles from room personnel.[55;56] In 1982, Whyte et al. already stated that bacterial contamination of the wound in the operating room is in 2% of the cases caused by bacteria from the patient and in 98% by bacteria in the air of the operating room. In the latter case, 30% reaches the wound directly via the air and 70% reaches the wound via hands of the surgical personnel or by the instruments used.[54]

Intra-operative contamination is the result of a series of bacterial transfers from the skin of the patient or operating room personnel via instruments and other materials to the wound area.[55;56] Davis et al. identified materials that are frequently contaminated during elective orthopaedic surgery. In 14.5% of the procedures, the light handles were contaminated, in 17% the theatre gowns and in 28.7% the gloves of the operating team.[46] The used sets of instruments were contaminated in 3.2% to 11.4% of the sampled cases.

In 1972, Charnley already recognised intra-operative contamination as a major threat in the success of total joint replacements. Others stated that the role of intra-operative contamination as a cause of deep infection was highly overrated.[46;48-50] Hansis et al. stated that the operative wound is contaminated to some extent in all procedures, but every wound is able to tolerate some local host damage and some bacterial inoculum without manifestation of infection.[57]

Purpose of the study

Within the department of Orthopaedic Surgery at the University Medical Centre Groningen, the control of postoperative wound infection with and without subsequent periprosthetic infection was a serious problem. In cooporation with the department of BioMedical Engineering and the department of Medical Microbiology, a project was started to

create a better understanding of this problem, and eventually its control. The ultimate goal of the study was to assess the predictive value of microbiological analyses of the used set of instruments and removed bone chips during primary arthroplasty and of the removed prosthesis during revision surgery. Eventually, this will lead to the identification of patients with a higher risk of deep periprosthetic infection, so these patients could receive early, appropriate treatment with antibiotics.

Starting point for this project was the (predictive) value of intra-operative culturing. During every primary placement and every revision knee or hip arthroplasty intra-operative cultures were taken. Firstly the level and implications of intra-operative culturing had to be assessed. In **Chapter 2** an association was to be found between intra-operative bacterial contamination during primary arthroplasty of hip joints and the occurrence of postoperative infectious complications related to the prosthesis site. As the incidence of deep periprosthetic infection after primary arthroplasty is relatively low, it was being investigated whether a positive intra-operative culture was associated with the occurrence of prolonged wound discharge in the postoperative period. The main reason is that the incidence of prolonged wound discharge, the latter being a proven predictor for postoperative wound infection[3;58;59] and periprosthetic infection[60] seemed to occur with a much higher frequency. Another aim of this study was to identify patient-related risk factors for prolonged wound discharge. If this could be done, patients with a higher risk could be identified in a very early stage and treated accordingly.

Preliminary results led to new questions and interventions. In **Chapter 3** measures were evaluated that could be taken to reduce intra-operative bacterial contamination in primary arthroplasty. Both behavioural and systemic measures were evaluated. New rules involving operating room discipline were introduced and a new laminar airflow system was installed. Secondly, it was being assessed whether intra-operative contamination was of any importance in the development of periprosthetic infection, as some conflicting conclusions on this relationship had been reported in literature.[46;48-51]

In **Chapter 4** the extent and the importance of intra-operative culturing during revision arthroplasty was under investigation. Besides evaluating systemic and behavioural measures, it was also being investigated whether intra-operative bacterial contamination plays a role in the development of infection after revision surgery.

In order to decrease bacterial contamination of the operating wound during surgery even more, a model was developed to investigate the transfer of bacteria from one operating

room material to another. The aim of **Chapter 5** was to quantify this transfer, while accounting for surface hydrophobicity and roughness, moistness and application of friction during transfer. This was done for microorganisms known to cause deep periprosthetic infection: *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionibacterium acnes*.[46;53;54;61-65] As a possible clinical intervention method to prevent transfer, it was investigated whether dipping the gloves in a chlorhexidine splash-basin affected the viability of the transferred bacteria.

Many hospitals dealing with difficulties to control infectious complications after surgery are reluctant to (re)build an operating room because of the high costs involved. In **Chapter 6** the economic implications of intra-operative bacterial contamination during both primary and revision arthroplasty are investigated, in order to show that it is cost-effective to take drastic hygienic measures.

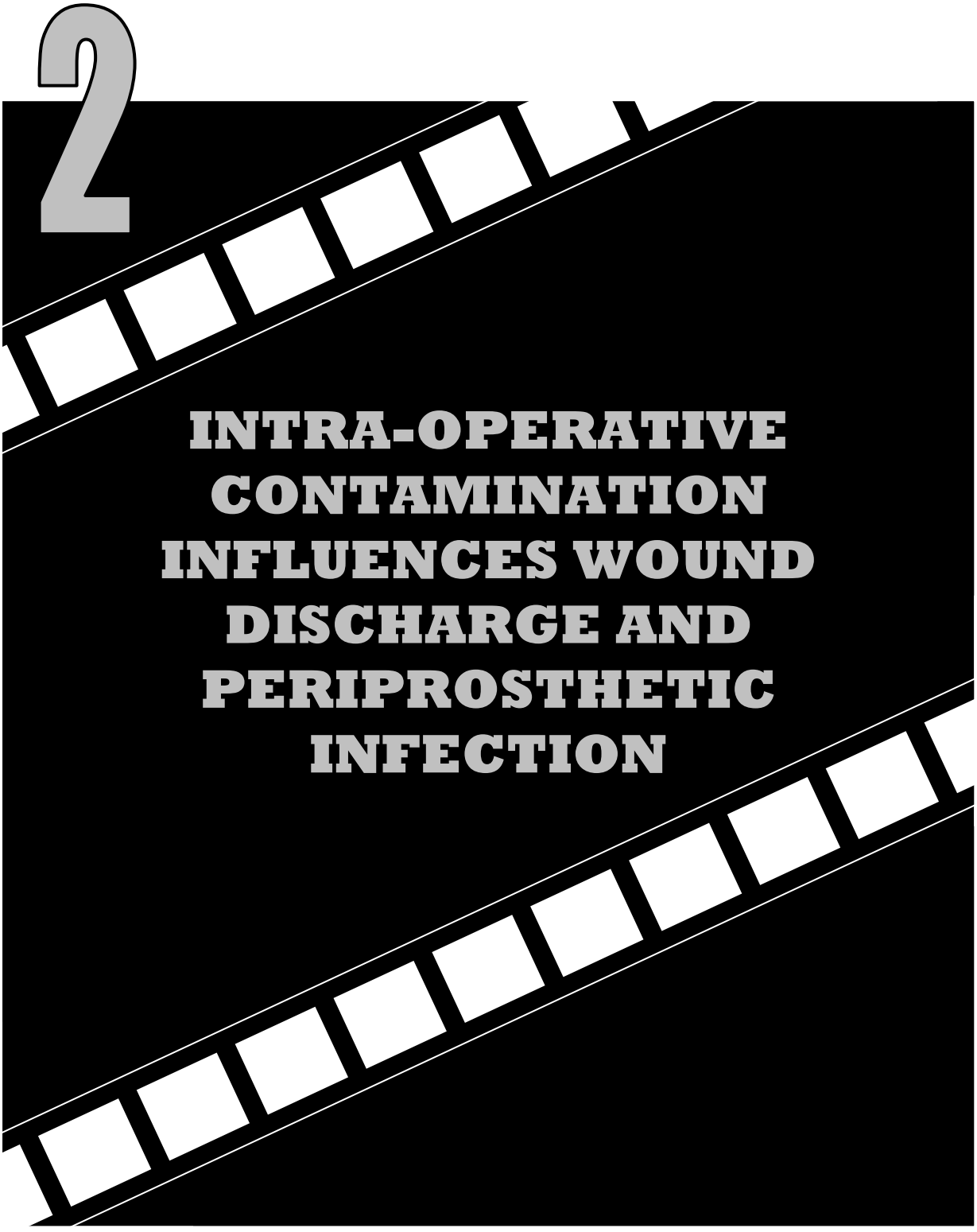
Chapter 7 eventually, gives a summery of findings, general discussion and closing remarks.

References

1. Schierholz JM, Beuth J. Implant infections: a haven for opportunistic bacteria. J Hosp Infect 2001;49:87-93.
2. An YH, Friedman RJ. Prevention of sepsis in total joint arthroplasty. J Hosp Infect 1996;33:93-108.
3. Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS, Osmon DR. Risk factors for prosthetic joint infection: case-control study. Clin Infect Dis 1998;27:1247-1254.
4. Harris WH, Sledge CB. Total hip and total knee replacement (1). N Engl J Med 1990;323:725-731.
5. Hanssen AD, Rand JA. Evaluation and treatment of infection at the site of a total hip or knee arthroplasty. Instr Course Lect 1999;48:111-122.
6. Garvin KL, Hanssen AD. Infection after total hip arthroplasty. Past, present and future. J Bone Joint Surg 1995;77:1578-88.
7. Lidwell OM, Elson RA, Lowbury EJ, Whyte W, Blowers R, Stanley SJ, Lowe D. Ultraclean air and antibiotics for prevention of postoperative infection. A multicenter study of 8,052 joint replacement operations. Acta Orthop Scand 1987;58:4-13.
8. Rand JA, Bryan RS, Morrey BF, Westholm F. Management of infected total knee arthroplasty. Clin Orthop Relat Res 1986;75-85.
9. Sculco TP. The economic impact of infected joint arthroplasty. Orthopedics 1995; 18:871-873.
10. National CFHS. American Academy and American Association of Orthopaedic Surgeons-Bulletin. AAOS 47[3], 14. 1999.
11. Trampuz A, Zimmerli W. Prosthetic joint infections: update in diagnosis and treatment. Swiss Med Wkly 2005;135:243-251.
12. Dupont JA. Significance of operative cultures in total hip arthroplasty. Clin Orthop Relat Res 1986;122-127.
13. Powers KA, Terpenning MS, Voice RA, Kauffman CA. Prosthetic joint infections in the elderly. Am J Med 1990;88:9N-13N.
14. Wang CJ, Huang TW, Wang JW, Chen HS. The often poor clinical outcome of infected total knee arthroplasty. J Arthroplasty 2002;17:608-14.
15. Gristina AG. Biomaterial-centered infection: microbial adhesion versus tissue integration. Science 1987;237:1588-95.
16. van Loosdrecht MC, Lyklema J, Norde W, Zehnder AJ. Influence of interfaces on microbial activity. Microbial Rev 1990;54:75-87.
17. Baker AS, greenham LW. Release of gentamycin from acrylic bone cement. Elution and diffusion studies. J Bone Joint Surg Am 1988;70:1551-7.
18. An YH, Friedman RJ. Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. J Biomed Mater Res 1998;43:338-48.
19. Faille C, Jullien C, Fontaine F, Bellon-Fontaine MN, Slomianny C, Benezech T. Adhesion of Bacillus spores and Escherichia coli cells to inert surfaces: role of surface hydrophobicity. Can J Microbiol 2002;48:728-38.
20. Hogt AH, Donkert J, Feijen J. Adhesion of coagulase-negative staphylococci to methacrylate polymers and copolymers. J Biomed Mater Res 1986;20:533-45.
21. McAllister EV, Carey LC, Brady PG, Heller R, Kovacs SG. The role of polymeric surface smoothness of biliary stents in bacterial adherence, biofilm deposition, and stent occlusion. Gastrointest Endosc 1993;39:422-5.
22. Quirynen M, van der Mei HC, Bollen MC, Schotte A, Marechal M, Doornbusch GI, Naert I, Busscher HJ, van Steenberghe D. An in vivo study of the influence of the surface roughness of implants on the microbiology of supra- and subgingival plaque. J Dent Res 1993;72:1304-9.
23. Satou N, Satou J, Shintani H, okuda K. Adherence of streptococci to surface-modified glass. J Gen Microbiol 1988;13:1299-305.
24. Sattar SA, Springthorpe S, Mani S, Gallant M, Nair RC, Scott E, Kain J. Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using Staphylococcus aureus as a model. J Appl Microbiol 2001;90:962-70.
25. Ramage G, Tunney MM, Patrick S, Gorman SP, Nixon JR. Formation of Propionibacterium acnes biofilms on orthopaedic biomaterials and their susceptibility to antimicrobials. Biomaterials 2003;24:3221-7.
26. van Loosdrecht MC, Lyklema J, Norde W, Schraa G, Zehnder AJ. The role of bacterial cell wall hydrophobicity in adhesion. J Appl Microbiol 2001;90:962-70.
27. Gristina AG, Naylor PT, Webb LX. Molecular mechanisms in musculoskeletal sepsis: the race for the surface. Instr Course Lect 1990;39:471-82.

28. Buret A, Ward KH, Olson ME, Costerton JW. An in vivo model to study the pathology of infectious biofilms on biomaterial surfaces. J Biomed Mater Res 1991;25:865-74.
29. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999;284:1318-22.
30. Khoury AE, Lam K, Ellis B, Costerton JW. Prevention and control of bacterial infections associated with medical devices. ASAIO J 1992;38:M174-M178.
31. Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis 2002;8:881-890.
32. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet 2001;358:135-8.
33. Gristina AG. Implant failure and the immuno-incompetent fibro-inflammatory zone. Clin Orthop 1994;106-118.
34. Bi Y, Seabold JM, Kaar SG, Ragab AA, Goldberg VM, Anderson JM, Greenfield EM. Adherent endotoxin on orthopedic wear particles stimulates cytokine production and osteoclast differentiation. J Bone Miner Res. 2001 Nov;16(11):2082-91.
35. Brooks RA, Wimhurst JA, Rushton N. Endotoxin contamination of particles producs misleading inflammatory cytokine responses from macrophages in vitro. J Bone Joint Surg Br 2002;84:295-9.
36. Cho DR, Shanbhag AS, Hong CY, Baran GR, Goldring SR. The role of adsorbed endotosin in particle-induced stimulation of cytokine release. J Orthop Res 2002;20:704-13.
37. Hamblen DL. Diagnosis of infection and the role of permanent excicion arthroplasty. Orthop Clin North Am 1993;24:743-9.
38. Morscher E, Babst R, Jenny H. Treatment of infected joint arthroplasty. Int Orthop 1990;14:161-5.
39. Pandey R, Berendt AR, Athanasou NA. Histological and microbiological findings in non-infected and infected revision arthroplasty tissues. The OSIRIS Collaborative Study Group. Oxford Skeletal Infection Research and Intervention Service. Arch Orthop Trauma Surg. 2000;120(10):570-4.
40. Spangehl MJ, Masri BA, O'Connell JX, Duncan CP. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. J Bone Joint Surg Am 1999;81:672-683.
41. Tunney MM, Patrick S, Curran MD, Ramage G, Hanna D, Nixon JR, Gorman SP, Davis RI, Anderson N. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J Clin Microbiol 1999;37:3281-3290.
42. Virolainen P, Lahteenmaki H, Hiltunen A, Sipola E, Meurman O, Nelimarkka O. The reliability of diagnosis of infection during revision arthroplasties. Scand J Surg 2002;91:178-181.
43. Widmer AF. New developments in diagnosis and treatment of infection in orthopedic implants. Clin Infect Dis 2001;33 Suppl 2:S94-106.
44. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. JAMA 1998;279:1537-1541.
45. Masterson EL, Masri BA, Duncan CP. Treatment of infection at the site of total hip replacement. Instr Course Lect 1998;47:297-306.
46. Davis N, Curry A, Gambhir AK, Panigrahi H, Walker CR, Wilkins EG, Worsley MA, Kay PR. Intraoperative bacterial contamination in operations for joint replacement. J Bone Joint Surg Br 1999;81:886-889.
47. Hughes SP, Anderson FM. Infection in the operating room. J Bone Joint Surg Br 1999;81:754-755.
48. Lawal OO, Adejuyigbe O, Oluwole SF. The predictive value of bacterial contamination at operation in post-operative wound sepsis. Afr J Med Med Sci 1990;19:173-179.
49. Lidwell OM, Lowbury EJ, Whyte W, Blowers R, Stanley SJ, Lowe D. Airborne contamination of wounds in joint replacement operations: the relationship to sepsis rates. J Hosp Infect 1983;4:111-131.
50. Robinson AH, Drew S, Anderson J, Bentley G, Ridgway GL. Suction tip contamination in the ultraclean-air operating theatre. Ann R Coll Surg Engl 1993;75:254-256.
51. Taylor GJ, Leeming JP, Bannister GC. Assessment of airborne bacterial contamination of clean wounds: results in a tissue model. J Hosp Infect 1992;22:241-249.
52. Clarke MT, Lee PT, Roberts CP, Gray J, Keene GS, Rushton N. Contamination of primary total hip replacements in standard and ultra-clean operating theaters detected by the polymerase chain reaction. Acta Orthop Scand 2004; 75:544-548.
53. Maathuis PG, Neut D, Busscher HJ, van der Mei HC, van Horn JR. Perioperative contamination in primary total hip arthroplasty. Clin Orthop Relat Res 2005;136-9.
54. Whyte W, Hodgson R, Tinkler J. The importance of airborne bacterial contamination of wounds. J Hosp Infect 1982;3:123-135.
55. Ha'eri GB, Wiley AM. Total hip replacement in a laminar flow environment with special reference to deep infections. Clin Orthop 1980;163-168.
56. Howorth FH. Prevention of airborne infection during surgery. Lancet 1985;1:386-388.

57. Hansis M. Pathophysiology of infection: a theoretical approach. Injury 1996;27 Suppl 3:SC5-SC8.
58. Abudu A, Sivardeen KA, Grimer RJ, Pynsent PB, Noy M. The outcome of perioperative wound infection after total hip and knee arthroplasty. Int Orthop 2002;26:40-43.
59. Surin VV, Sundholm K, Backman L. Infection after total hip replacement. With special reference to a discharge from the wound. J Bone Joint Surg Br 1983;65:412-418.
60. Saleh K, Olson M, Resig S, Bershadsky B, Kuskowski M, Gioe T, Robinson H, Schmidt R, McElfresh E. Predictors of wound infection in hip and knee joint replacement: results from a 20 year surveillance program. J Orthop Res 2002;20:506-515.
61. Al Maiyah M, Bajwa A, Mackenney P, Port A, Gregg PJ, Hill D, Finn P. Glove perforation and contamination in primary total hip arthroplasty. J Bone Joint Surg Br 2005;87:556-9.
62. Heal JS, Blom AW, Titcomb D, Taylor A, Bowker K, Hardy JR. Bacterial contamination of surgical gloves by water droplets spilt after scrubbing. J Hosp Infect 2003;53:136-9.
63. Neut D, van Horn JR, van Kooten TG, van der Mei HC, Busscher HJ. Detection of biomaterial-associated infections in orthopaedic joint implants. Clin Orthop Relat Res 2003;261-8.
64. Rutala WA, Gergen MF, Jones JF, Weber DJ. Levels of microbial contamination on surgical instruments. Am J Infect Control 1998;26:143-5.
65. Tunney MM, Patrick S, Gorman SP et al. Improved detection of infection in hip replacements. A currently underestimated problem. J Bone Joint Surg Br 1998;80:568-72.



Knobben BAS, Engelsma Y, Neut D, Van der Mei HC, Busscher HJ, Van Horn JR

Clinical Orthopaedics and Related Research 2006, accepted

Introduction

A current estimate of the rate of total hip replacement worldwide amounts approximately one million per year, with over 250,000 knee replacements.[1] This number is expected to double between 1999 and 2025 as a result of an ageing society and because hip and knee arthroplasties are implanted at an increasingly early age.[2] One of the major complications in hip and knee arthroplasty is infection. Infection percentages total to about 1-2% for hip implants and 2-4% for knee implants.[3-6] Once such a periprosthetic infection exists, it is associated with a substantial increase in morbidity, which increases hospital admittance time and hence adds significant costs to the health care system. Treating an infected prosthesis can cost up to $ 80,000, 4.1 times the costs for a primary prosthesis, and periprosthetic infections prolong total hospital stay by more than 6 weeks.[7] Moreover, patients with postoperative orthopaedic infections have substantially greater physical limitations and significant reductions in their health-related quality of life.[8;9]

The presence of a superficial wound infection has been identified as a significant risk factor for development of periprosthetic infection, but the exact extent of the risk is unknown.[10-13] Postoperative superficial wound infections occur far more often than periprosthetic infection and reportedly occur in 1.2% to 17.3% of all cases.[10;14-16] The discrepancy in percentages is in part due to the use of definitions. There are two commonly used definitions of superficial wound infection. The Surgical Infection Study Group defines superficial wound infection solely on the basis of clinical observations without microbiological confirmation.[17] The Center for Disease Control and Prevention requires microbiological confirmation before the diagnosis “superficial wound infection” is made.[18] Both groups further state that drain sites should be included and that there should be purulent discharge or a painful spreading erythema. Despite these definitions diagnosing a superficial wound infection, based on the assessment of the individual surgeon, is subject to serious personal variations and as such must be considered to be unreliable.[19] Therefore, it has been suggested to monitor the duration of wound discharge, taking 5 days as a cut-off point. Patients with wound discharge of 5 days or longer were reported to have 12.7 times a higher risk of getting late periprosthetic infection compared to patients with a shorter wound discharge.[20]

Intra-operative contamination is common in every operating room.[21;22] The main sources for intra-operative contamination are the skin of the patient and airborne particles from theatre personnel.[23;24] In 1982, Whyte et al. suggested bacterial contamination of the wound in

the operating room occurs in 2% of the cases caused by bacteria from the patient and in 98% by bacteria in the air of the operating room. In the latter case, 30% reaches the wound directly via the air and 70% reaches the wound via hands of the surgical personnel or by the instruments used.[25]

We asked whether bacterial contamination of the instruments used and of removed bone during primary insertion of hip prostheses can predict the occurrence of prolonged wound discharge. First, we developed a logistic regression model to investigate the unbiased association between intra-operative culturing and prolonged wound discharge. Secondly, it was investigated what combination of intra-operative cultures were the most predictive. Finally, it was calculated how often periprosthetic infection occurred depending on the occurrence of intra-operative contamination and prolonged wound discharge.

Materials and methods

Patients

We prospectively analyzed primary hip arthroplasties in the period from August 2001 to August 2003 in the University of Groningen Medical Center, Groningen, The Netherlands with written permission of the hospital Ethical Committee. In order to obtain a representative sample over the predefined inclusion period of two year (thus minimizing periodic effects), we used a list of random numbers, generated by computer, which determined whether the protocol would or would not be used for the particular patient. A restriction to the amount of patients was applied to minimize the burden for the personnel involved, since the protocol was not yet part of standard practice at the time the study was conducted. We decided to include 100 patients since we observed approximately one-third of our patients to have prolonged wound leakage, which would allow us to use 5 covariates in multivariate modeling (which was arbitrarily judged to be desirable) without great risk of overfitting.

All 100 patients included received antimicrobial prophylaxis (cefazoline, 1000 mg intravenously) twenty minutes before the operation and postoperative anticoagulation (nadroparine, 0.3 mL subcutaneously combined with acenocoumarol orally). Surgery took place in an operating theatre where conventional air flow was used, and the operating team wore disposable impervious drapes. At the end of surgery, drains were placed at the operation site in all patients. General pre-operative parameters, believed to influence postoperative

wound discharge, were collected; these included age and gender, the existence of any immunocompromising disease (i.e. rheumatoid arthritis) or diabetes, and body mass index. Intra-operatively, blood loss more than 400 mL, operating time exceeding 100 minutes and the use of cement (Simplex without antibiotics, Stryker Orthopaedics, Mahwah, New Jersey) were also recorded. The total group consisted of 33 males and 67 females, with a mean age of 61.3 years (28-87, standard deviation 12.8). 13/100 patients suffered from rheumatoid arthritis and 4/100 had diabetes. The mean body mass index of the entire group was 27.0 (18.5-37.2, standard deviation 3.7). The mean operating time was 106 minutes (50-180 minutes, standard deviation 24.8), and in 48 (48%) the duration was more than 100 min. The mean amount of blood loss was 424 mL (40-2000 mL, standard deviation 269) and exceeded 400 mL in 56 (56%) of the cases. Cement was used in 54 of the 100 (54%) cases.

Culture technique

Intra-operatively, samples were being taken at different stages of the procedure, two from the instruments used, two from the instruments not used and two from removed bone. The first sample (culture 1) represents the swab of the smallest unused acetabular broach. After sampling the reaming procedure was started with this broach. The second sample (culture 2) represents the swab of the largest unused acetabular broach after the reaming procedure. This broach was never used at the direct site of the prosthesis. The third sample (culture 3) represents the swab of the smallest unused femoral broach. After sampling the reaming procedure was started with this broach. The fourth sample (culture 4) represents the swab of the largest unused femoral broach after the reaming procedure. This broach was never used at the direct site of the prosthesis.

Removed bone chips were sampled for contamination as well. Culture I represents the acetabulum, culture II represents the femur. During all procedures, a clean swab was shortly taken out of the charcoal medium in the operating room after which it was immediately put back, in order to make sure no contamination occurred during transport and culturing of the samples.

The cotton swabs (cultures 1-4 and the control swab) were transported in a transport medium called Transwab, Charcoal medium (Medical Wire & Equipment Co, Bath, United Kingdom). Removed bone material (cultures I-II) was put into sterile cups filled with Tryptone Soya Broth (TSB, Oxoid, United Kingdom). Within 2 to 4 hours after sampling, the cotton swabs (1-4) were smeared over blood agar and incubated, together with the cups containing

bone cultures denoted I and II, for 7 days at 37ºC, both aerobically and anaerobically. After 7 days the content of the cups was also smeared over blood agar and again incubated for 5 days. Instrumentation or bone material was considered contaminated, when bacterial growth was observed, regardless of the amount of growth. The control swab was negative at all times. The study was performed blind, without informing the orthopaedic surgeon on the test result, in order to ensure that all patients were treated regardless of the evaluation.

Postoperative wound discharge

Wound discharge was recorded postoperatively by a specialized nurse from the local hospital infection committee, monitoring both the wound and the drain site, while taking the fifth day after surgery as the cut-off point. Patients with a leakage time of five days or more formed the case group. Patients with a wound and drain site that closed within four days after surgery served as the control group. Postoperatively, the drain was removed after two days in all patients. The mean duration of wound discharge was 4.2 days (1-28, standard deviation 3.5 days). In 28/100 cases (28%) the wound discharge extended to 5 days or longer, while in all other cases the wound and drain site had closed within 4 days.

Periprosthetic infection

To determine whether periprosthetic infection occurred in patients with and without intra-operative contamination and prolonged wound discharge, patients were followed-up at standard postoperative controls at 6 weeks, 3 months, 6 months, 1 year and 2 years after index surgery, or if a patient came to the emergency room. At follow-up patients symptoms along with C-reactive protein, erythrocyte sedimentation rate and a white blood cell count were evaluated. A prosthesis was considered infected in case of an increase of infection parameters caused by the prosthesis site, as substantiated by culturing of aspirated joint fluid and/or culturing during revision of the prosthesis.

Statistical analysis

To assess the associations between the different variables and prolonged wound discharge, we performed univariate analyses. A Student t-test was used for independent samples for the continuous variable body mass index, while the Pearson Chi square test was used for all categorical variables when all cells of the contingency table contained at least 5 persons. Otherwise the Fisher’s exact test was used. Comparisons were made between the

group with and without prolonged wound discharge. The initial model was based on the results of the univariate analysis and covariates which were clinically judged to be possible confounders. Subsequently, a parsimonious model was created by deletion of the most poorly associated covariates. The odds ratios (OR) were transformed to relative risks (RR) with the following formula:

$$RR = OR/((1-Prev)+(Prev \times OR))$$

Prev meaning prevalence of the risk factor.[26] The associations between the different types of cultures and periprosthetic infection were investigated with the Pearson Chi square or Fisher's exact test. Additionally, the positive predictive values were calculated. The same was done to investigate the associations between intra-operative contamination, prolonged wound discharge and periprosthetic infection. All statistical procedures were performed with use of the software package SPSS version 12.0 (SPSS, Chicago, Illinois).

Results

The univariate analysis indicated that age, rheumatoid arthritis, use of cement, increased blood loss and a positive intra-operative culture were associated with ($p < 0.05$) prolonged wound discharge (Table I). These parameters were entered in the logistic regression model, showing that only rheumatoid arthritis, increased blood loss and a positive intra-operative culture remained as significant factors (step 1 in Table I).

Because the p value of the variable "age" was larger than the p value of the variable "cement", it was decided to delete the variable "age" from the model. This resulted in the variable "cement" now also being a significant factor. The RR of intra-operative bacterial contamination was estimated to be 6.4. The RR of rheumatoid arthritis is 6.4, of the use of cement 1.6, and of increased blood loss 1.5.

Table I. Preoperative and intra-operative risk factors for prolonged wound discharge in patients after primary total hip arthroplasty. Univariate analysis shows candidate variables for prolonged wound discharge ($p < 0.05$), and subsequently the logistic regression model shows the significant variables after deletion of the most poorly associated covariates in two steps. The relative risks (RR) were obtained from the odds ratios.

Parameters	Wound Discharge		Univariate Analysis	Logistic Regression Model		
	≥ 5 days (n = 28)	< 5 days (n = 72)	p value	Step 1 p value	Step 2 p value	RR
Preoperative parameters						
- Gender (women)	22 (79%)	45 (63%)	0.125			
- Age (> 60 years)	22 (79%)	34 (47%)	0.005	0.581		
- Rheumatoid arthritis	9 (32%)	4 (6%)	0.001*	0.001	0.001	6.4
- Diabetes mellitus	1 (4%)	3 (4%)	1*			
- Body mass index (mean ± SD)	26.6 (± 3.8)	27.9 (± 3.6)	0.118†			
Intra-operative parameters						
- Cement	22 (79%)	32 (44%)	0.002	0.352	0.005	1.6
- Blood loss (> 400 mL)	21 (75%)	35 (49%)	0.017	0.036	0.035	1.5
- Operating time (> 100 minutes)	16 (57%)	32 (44%)	0.254			
- Intraoperative contamination	20 (71%)	16 (22%)	0	0	0	2.5

SD = standard deviation; RR = relative risk; *Fisher's exact test; †Student's two tailed t test

The positive predictive values of the instrument swabs for predicting prolonged wound discharge are fairly low (17-67%), while the positive predictive values for the bone chip cultures are much higher (81-90%). The association between positive bone chip cultures and the occurrence of prolonged wound discharge is significant (Table II). In the group, where bacterial contamination was demonstrated, chances to develop wound discharge are 56% (PPV), while in the absence of bacterial contamination of instruments and bone, the chances to not develop prolonged wound discharge are 87%. Bacterial growth was demonstrated in at least one of the intra-operative cultures in 36/100 cases (36%). In one patient, four cultures were positive, in 11 cases two were positive and in 24 cases one culture was positive.

Table II. The description of intraoperative swabs and bone chips and their positive predictive value (PPV) for the occurrence of prolonged wound discharge. The Pearson chi square test was used to calculate the significance of the association (the Fisher's exact test was used if one of the cells of the contingency table contained less than 5 persons).

Sample	Description	PPV (%)	Chi square test
Instrument swab 1	Of smallest acetabulum broach before reaming	30	1.000*
Instrument swab 2	Of unused acetabulum broach after reaming	67	0.189*
Instrument swab 3	Of smallest femur broach before reaming	60	0.132*
Instrument swab 4	Of unused femur broach after reaming	17	1.000*
Bone chips I	Removed acetabular bone chips	90	0.000*
Bone chips II	Removed femoral bone chips	81	0.000*
Total	One or more of the cultures showed growth	56	0.000

* Fisher's exact test

The association between intra-operative contamination and the occurrence of a periprosthetic infection is highly significant (0.008), as is the association between prolonged wound discharge and periprosthetic infection (0.002). The PPV of both intra-operative contamination and prolonged wound discharge for the occurrence of periprosthetic infection is 25%, while its NPV is 98% (p = 0.003), as can be seen in Table III.

Table III. The incidence of periprosthetic infection if intra-operative contamination and/or prolonged wound discharge are present. The positive and negative predictive value (PPV and NPV, respectively) and the p value of the Fisher's exact test are shown.

Variable	PPV (%)	NPV (%)	Fisher's Exact Test
Intra-operative contamination	14	98	0.008
Prolonged wound discharge	21	99	0.002
Both	25	98	0.003

Periprosthetic infection occurred in six of the 36 cases where intra-operative contamination was measured. In 20 of the 36 patients with intra-operative contamination, prolonged wound discharge was monitored in the postoperative period. Five of these 20 patients subsequently developed periprosthetic infection (Figure 1).

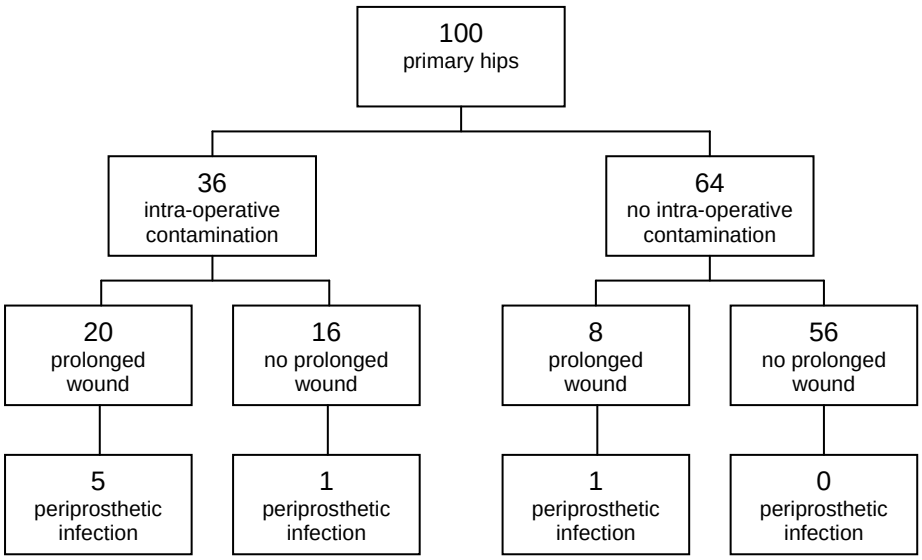


Figure 1. A diagram shows the numbers of patients with intraoperative contamination, postoperative prolonged wound discharge, and periprosthetic infection after primary hip replacement.

One of them, the patient with four positive cultures, developed a periprosthetic infection within one month after the primary surgery. Of the other 16 patients with intra-operative contamination in the absence of prolonged wound discharge, one patient developed an infection. In the group of 64 hips without intra-operative contamination, only one hip (1.6%) became infected and in this patient prolonged wound discharge was monitored in the postoperative period. In the 56 patients without both intra-operative contamination and prolonged wound discharge, periprosthetic infection was never diagnosed during the first two years of follow-up.

Discussion

Several studies on intra-operative culturing of equipment and bacterial analysis of air samples have been performed, yielding conflicting conclusions on relationships with postoperative infections.[27-31] The relations between prolonged wound discharge and postoperative wound infection and between postoperative wound infection and periprosthetic infection were already found.[10;32-34] This study describes significant associations between intra-operative contamination of the operating site itself (instruments used and bone chips), the occurrence of prolonged wound discharge and the development of periprosthetic infection. To our knowledge this study is the first to provide evidence for the association between intra-operative contamination and prolonged period of postoperative wound discharge, with a positive predicting value going up to 80 to 90%.

Although in this study, we associate prolonged wound-discharge with intra-operative contamination, strictly speaking it remains uncertain whether a discharging wound is infected during surgery or in the post-operative period, or just discharging because of a limited ability of the local skin tissue to heal, the latter creating a risk for cross-infection. As another possible limitation, out of all possibilities to sample an operating room,35-38 we choose to take swabs from the used set of instruments and collected bone chips, as these are most likely to represent possible contamination of the wound itself as confirmed in our study. Also the selection or removal of covariates in our model deserves some further debate, as this does not imply that covariates are (un)important from an etiological or causal point of view. “Age” was deleted, despite being clinically important, because of the strong correlation between “age” and “the use of cement” in this series of patients and despite the fact that “the use of cement” increases

the immunocompromising zone surrounding prostheses or further decreases the immune system in general.39 Since “age” in itself is not as directly linked to infection risk as “the use of cement”, “age” as a covariate was removed from the model. Covariates which were confounders of other relations in this dataset were not deleted from the model.

Binary logistic regression also showed that rheumatoid arthritis, the amount of intra-operative blood loss and the use of cement are significant predictors for prolonged wound discharge after hip prosthetic surgery. Rheumatoid arthritis[5] and extensive intra-operative blood loss[40] have been described before as risk factors for postoperative wound infection and periprosthetic infection, as found here. In addition to age, our model also demonstrates that body mass index and operating time drop out as risk factors for prolonged wound discharge, when accounting for the multifactorial nature of wound discharge. Operating time did not predict prolonged wound discharge, although in the literature, this parameter often is considered a risk factor for wound infection.[5;41-44]

The identification of cement as a risk factor in our study might have excluded operating time as a risk factor, because these factors are interrelated (just like age and cement) and our study takes into account this multifactorial nature. Inserting an uncemented prosthesis requires less time than needed for a cemented prosthesis, decreasing exposure to airborne bacteria in the operating room. It could be hypothesized that the use of cement alone is a more important risk factor than the increase in operating time. Similarly, because the patients with a high body mass index were the ones suffering from rheumatoid arthritis and the ones receiving a cemented prosthesis, body mass index dropped out as a risk factor too.

Prolonged wound discharge is important, because it can be a risk factor on its own, as well as a potential marker for periprosthetic infection. If prolonged wound discharge is monitored together with intra-operative bacterial contamination as measured in this study, a periprosthetic infection is likely to occur (Figure 2). Alternatively, if prolonged wound discharge is monitored in the absence of intra-operative bacterial contamination, it is important to identify whether one of the other risk factors for prolonged wound discharge exist. In this group, prolonged wound discharge in patients suffering from rheumatoid arthritis, with a cemented prosthesis or with more than normal blood loss, does not require immediate additional antibiotic therapy. In this study no periprosthetic infection occurred when both prolonged wound discharge and intra-operative contamination were absent (N=56).

Since current treatment modalities usually include culturing of wound discharge on the fifth day postoperatively, prior to administration of antibiotics, the authors recommend that

intra-operative cultures be routinely conducted to yield an indication on whether it is appropriate to initiate immediate antibiotic treatment after prolonged wound discharge, without waiting for culture results of the wound.

References

1. Schierholz JM, Beuth J. Implant infections: a haven for opportunistic bacteria. J Hosp Infect 2001;49:87-93.
2. National CFHS. American Academy and American Association of Orthopaedic Surgeons-Bulletin. AAOS 47[3], 14. 1999.
3. An YH, Friedman RJ. Prevention of sepsis in total joint arthroplasty. J Hosp Infect 1996;33:93-108.
4. Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS, Osmon DR. Risk factors for prosthetic joint infection: case-control study. Clin Infect Dis 1998;27:1247-1254.
5. Hanssen AD, Rand JA. Evaluation and treatment of infection at the site of a total hip or knee arthroplasty. Instr Course Lect 1999;48:111-122.
6. Harris WH, Sledge CB. Total hip and total knee replacement (1). N Engl J Med 1990;323:725-731.
7. Hebert CK, Williams RE, Levy RS, Barrack RL. Cost of treating an infected total knee replacement. Clin Orthop Relat Res 1996;140-145.
8. Kirkland KB, Briggs JP, Trivette SL, Wilkinson WE, Sexton DJ. The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs. Infect Control Hosp Epidemiol 1999;20:725-730.
9. Whitehouse JD, Friedman ND, Kirkland KB, Richardson WJ, Sexton DJ. 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. Infect Control Hosp Epidemiol 2002;23:183-189.
10. Abudu A, Sivardeen KA, Grimer RJ, Pynsent PB, Noy M. The outcome of perioperative wound infection after total hip and knee arthroplasty. Int Orthop 2002;26:40-43.
11. Gaine WJ, Ramamohan NA, Hussein NA, Hullin MG, McCreath SW. Wound infection in hip and knee arthroplasty. J Bone Joint Surg Br 2000;82:561-565.
12. Saleh K, Olson M, Resig S, Bershadsky B, Kuskowski M, Gioe T, Robinson H, Schmidt R, McElfresh E. Predictors of wound infection in hip and knee joint replacement: results from a 20 year surveillance program. J Orthop Res 2002;20:506-515.
13. Surin VV, Sundholm K, Backman L. Infection after total hip replacement. With special reference to a discharge from the wound. J Bone Joint Surg Br 1983;65:412-418.
14. Gaine WJ, Ramamohan NA, Hussein NA, Hullin MG, McCreath SW. Wound infection in hip and knee arthroplasty. J Bone Joint Surg Br 2000;82:561-565.
15. Saleh K, Olson M, Resig S, Bershadsky B, Kuskowski M, Gioe T, Robinson H, Schmidt R, McElfresh E. Predictors of wound infection in hip and knee joint replacement: results from a 20 year surveillance program. J Orthop Res 2002;20:506-515.
16. Taylor GJ, Bannister GC, Calder S. Perioperative wound infection in elective orthopaedic surgery. J Hosp Infect 1990;16:241-247.
17. Peel AL, Taylor EW. Proposed definitions for the audit of postoperative infection: a discussion paper. Surgical Infection Study Group. Ann R Coll Surg Engl 1991;73:385-388.
18. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. Infect Control Hosp Epidemiol 1992;13:606-608.
19. Surin VV, Sundholm K, Backman L. Infection after total hip replacement. With special reference to a discharge from the wound. J Bone Joint Surg Br 1983;65:412-418.
20. Saleh K, Olson M, Resig S, Bershadsky B, Kuskowski M, Gioe T, Robinson H, Schmidt R, McElfresh E. Predictors of wound infection in hip and knee joint replacement: results from a 20 year surveillance program. J Orthop Res 2002;20:506-515.
21. Davis N, Curry A, Gambhir AK, Panigrahi H, Walker CR, Wilkins EG, Worsley MA, Kay PR. Intraoperative bacterial contamination in operations for joint replacement. J Bone Joint Surg Br 1999;81:886-889.
22. Hughes SP, Anderson FM. Infection in the operating room. J Bone Joint Surg Br 1999;81:754-755.
23. Ha'eri GB, Wiley AM. Total hip replacement in a laminar flow environment with special reference to deep infections. Clin Orthop 1980;163-168.
24. Howorth FH. Prevention of airborne infection during surgery. Lancet 1985;1:386-388.
25. Whyte W, Hodgson R, Tinkler J. The importance of airborne bacterial contamination of wounds. J Hosp Infect 1982;3:123-135.
26. Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. JAMA 1998;280:1690-1691.

27. Davis N, Curry A, Gambhir AK, Panigrahi H, Walker CR, Wilkins EG, Worsley MA, Kay PR. Intraoperative bacterial contamination in operations for joint replacement. J Bone Joint Surg Br 1999;81:886-889.
28. Lawal OO, Adejuyigbe O, Oluwole SF. The predictive value of bacterial contamination at operation in post-operative wound sepsis. Afr J Med Med Sci 1990;19:173-179.
29. Lidwell OM, Lowbury EJ, Whyte W, Blowers R, Stanley SJ, Lowe D. Airborne contamination of wounds in joint replacement operations: the relationship to sepsis rates. J Hosp Infect 1983;4:111-131.
30. Robinson AH, Drew S, Anderson J, Bentley G, Ridgway GL. Suction tip contamination in the ultraclean-air operating theatre. Ann R Coll Surg Engl 1993;75:254-256.
31. Taylor GJ, Leeming JP, Bannister GC. Assessment of airborne bacterial contamination of clean wounds: results in a tissue model. J Hosp Infect 1992;22:241-249.
32. Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS, Osmon DR. Risk factors for prosthetic joint infection: case-control study. Clin Infect Dis 1998;27:1247-1254.
33. Saleh K, Olson M, Resig S, Bershadsky B, Kuskowski M, Gioe T, Robinson H, Schmidt R, McElfresh E. Predictors of wound infection in hip and knee joint replacement: results from a 20 year surveillance program. J Orthop Res 2002;20:506-515.
34. Surin VV, Sundholm K, Backman L. Infection after total hip replacement. With special reference to a discharge from the wound. J Bone Joint Surg Br 1983;65:412-418.
35. Davis N, Curry A, Gambhir AK, Panigrahi H, Walker CR, Wilkins EG, Worsley MA, Kay PR. Intraoperative bacterial contamination in operations for joint replacement. J Bone Joint Surg Br 1999;81:886-889.
36. Lawal OO, Adejuyigbe O, Oluwole SF. The predictive value of bacterial contamination at operation in post-operative wound sepsis. Afr J Med Med Sci 1990;19:173-179.
37. Lidwell OM, Lowbury EJ, Whyte W, Blowers R, Stanley SJ, Lowe D. Airborne contamination of wounds in joint replacement operations: the relationship to sepsis rates. J Hosp Infect 1983;4:111-131.
38. Robinson AH, Drew S, Anderson J, Bentley G, Ridgway GL. Suction tip contamination in the ultraclean-air operating theatre. Ann R Coll Surg Engl 1993;75:254-256.
39. Gristina AG. Implant failure and the immuno-incompetent fibro-inflammatory zone. Clin Orthop 1994;106-118.
40. Surin VV, Sundholm K, Backman L. Infection after total hip replacement. With special reference to a discharge from the wound. J Bone Joint Surg Br 1983;65:412-418.
41. Charnley J. Postoperative infection after total hip replacement with special reference to air contamination in the operating room. Clin Orthop 1972;87:167-187.
42. Fitzgerald RH, Jr., Nolan DR, Ilstrup DM, Van Scoy RE, Washington JA, Coventry MB. Deep wound sepsis following total hip arthroplasty. J Bone Joint Surg Am 1977;59:847-855.
43. Ilstrup DM, Nolan DR, Beckenbaugh RD, Coventry MB. Factors influencing the results in 2,012 total hip arthroplasties. Clin Orthop 1973;95:250-262.
44. Saleh K, Olson M, Resig S, Bershadsky B, Kuskowski M, Gioe T, Robinson H, Schmidt R, McElfresh E. Predictors of wound infection in hip and knee joint replacement: results from a 20 year surveillance program. J Orthop Res 2002;20:506-515.

3

EVALUATION OF MEASURES TO DECREASE INTRA-OPERATIVE BACTERIAL CONTAMINATION IN ORTHOPAEDIC IMPLANT SURGERY

Knobben BAS, Van Horn JR, Van der Mei HC, Busscher HJ

Journal of Hospital Infections 2006. Feb;62(2):174-80

Introduction

Infection is one of the most common complications in surgery. In particular deep periprosthetic infections in orthopaedic surgery constitute a disaster for both patient and doctor. Conservative estimates of infection rates average 1-2% for hip implants and 2-4% for knee implants.[1-7] The number of joint replacements is expected to double in the next twenty years and if the infection rate is not reduced, also the incidence of infection will double, yielding increased morbidity, hospital stay and costs for the healthcare system.[8]

Deep prosthetic infections can be subdivided into: (i) early (within three months after surgery); (ii) delayed (within one-and-a-half to two years after surgery); and (iii) late infections. Both early and delayed infections can be caused during surgery by direct contact with the wound, airborne colonisation or cross-infection on the ward. Late infection is mostly caused by bloodborne contamination; for example during insertion of a urinary catheter, infection of an intravenous canula, or skin or dental sepsis.[9] However, haematogenous infection only plays a minor role in orthopaedic surgery, with an incidence of 0.3-7%.[10;11]

This study focused on early and delayed infections caused by intra-operative contamination. It has been suggested that the main sources of contamination are the patient's skin and airborne particles from theatre personnel.[12-15] Whyte et al. found that the source of contamination was the patient's skin in 2% of cases and theatre personnel in 98% of cases. In the latter, 30% of contaminants reach the wound directly via the air and 70% reach the wound via hands of the surgical personnel or the instruments used.[16]

In general, the policy to reduce intra-operative contamination is based on a behavioural and systemic approach. In a behavioural approach, preventive measures focus on reducing the number of airborne particles in the operating room through disciplinary measures. Simple and cheap measures include limiting the number of personnel in the operating room and restricting the movements of personnel in the operating room to a minimum, as it has been shown that increased activity enhances the dispersion of bacteria.[17]

A systemic approach consists of improving the airflow system. The introduction of laminar airflow systems has greatly reduced infection in orthopaedic implant surgery. Laminar flow, as opposed to turbulent flow, allows airborne particles to pass the operating area and prevent them from landing in the wound area. For example, in a downflow laminar system, the unidirectional air enters the operating room in the ceiling above the operating area through filters.

Adjustments to existing operating rooms is presently estimated to cost about € 540,000 for two new airflow systems. This should be compared with the costs of treating a septic joint (estimated to be $50,000 to $62,100).[4;18-20] It should be emphasized that such a comparison only includes direct medical costs.

The aim of this study was to evaluate whether behavioural and systemic measures decrease intra-operative contamination as monitored during 207 total hip or knee replacements. The influence of these measures on subsequent prolonged wound discharge, superficial surgical site infection and deep periprosthetic infection was also investigated during an 18-month follow-up of the patients involved.

Materials and methods

Interventions

During the two-and-a-half year evaluation period, interventions were carried out on two occasions in order to decrease bacterial contamination in the operating room. Both interventions are described in Table I. The first intervention was implemented in March 2003 and was a behavioural intervention. From that time on, instrumentation and other sterile equipment were only unpacked and used in the area of laminar flow (the so-called ‘plenum’).

The second intervention was introduced in August 2003 and consisted of some major behavioural changes as well as a systemic change. The behavioural changes were new guidelines for patient work up, use of body coverage, and restricting activity in the operating room. In the second intervention, the old conventional airflow system was replaced with a new laminar system, yielding a major increase in airflow from 2700 m^3 to 8100 m^3 per hour by the introduction of large quantities of recirculating air (5400 m^3 per hour). The air inflow speed was increased from 10 to 20 cm per second. Consequently, airflow was diluted rather than mixed, increasing the total number of air changes in the entire operating theatre from 22 to 60 per hour. Better laminar flow was achieved due to the use of new glass panels extending from the ceiling which, in combination with the increase in airflow, resulted in 240 air changes per hour at the operating table. Besides this, the plenum size was increased from 3 m^2 to 10.2 m^2, and the filter and bottom ceiling layer were replaced.

Table I. Behavioural interventions undertaken in the operating room.

<table>
<tr><th colspan="2">Intervention 1 (March 2003)</th></tr>
<tr><td colspan="2">Correct use of plenum
- Instrumentation unpacking only in plenum
- Instrumentation unpacking just before surgery
- Instrumentation never leaves plenum, else considered unsterile
- Head of patient always out of plenum</td></tr>
<tr><th colspan="2">Intervention 2 (August 2003)</th></tr>
<tr><td>Work up in preparation room, not in operating room
- Anaesthetic work up
- Shaving
- Putting on blood bands and blankets
- Positioning patient with leg support

Proper wearing of body coverage
- No hair visible
- No nose visible
- Beard mask and safety glasses for persons working in plenum
- Renew mouth mask after every operation
- Change clothes each time after leaving the operating complex</td><td>Limiting needless activity
- Number of people in operating room kept to minimum
- Opening of doors kept to minimum
- Use only smallest door to washing room
- Movement of people kept to minimum
- No changing of personnel during an operation
- If other equipment necessary, use intercom
- All communication with world outside via intercom
- Only conversation if needed for surgery</td></tr>
</table>

Selection of operations

Between July 2001 and January 2004, intra-operative bacterial cultures were taken during 207 random operations involving placement of primary knee or hip prostheses. Before the first intervention, from July 2001 to March 2003, cultures were taken during 70 operations that were performed under original, control conditions (control group). Sixty-seven operations were monitored after the first intervention (group 1). The second intervention was initiated in August 2003 and 70 operations were evaluated from August 2003 to January 2004 (group 2). All operations involved a total hip or knee arthroplasty in patients with osteoarthritis or rheumatoid arthritis, and took place in the University Medical Centre Groningen, Groningen, The Netherlands. All patients received antimicrobial prophylaxis (cefazoline, 1000 mg intravenously) twenty minutes before the operation and postoperative anticoagulation (nadroparine, 0.3 mL subcutaneously combined with acenocoumarol orally). Patient characteristics were not significantly different between the three groups.

Culture technique

Intra-operatively, samples were taken at different stages during the operation, two from the instruments used, two from the instruments not used and two from removed bone. In the hip procedure, the first sample (culture 1) represents the swab of the smallest acetabular broach before it was used for reaming. The second sample (culture 2) represents the swab of an unused

acetabular broach after the reaming procedure. In the knee procedure, cultures 1 and 2 represent swabs of the adjustable femur sizer before and after sawing the femur. Furthermore, in the hip procedure, the third sample (culture 3) represents the swab of the smallest femoral broach before it was used for reaming. The fourth sample (culture 4) represents the swab of an unused femoral broach after the reaming procedure. In the knee procedure, cultures 3 and 4 represent swabs of the adjustable tibia saw before and after sawing the tibia.

Removed bone was sampled for contamination as well. Culture I represents the acetabular bone in case of the hip joint and the femoral bone in case of the knee joint; culture II represents the femoral bone in case of the hip joint and tibia bone in case of the knee joint. Cultures 1, 2 and I were taken during the early phase of the operation and cultures 3, 4 and II during the late phase.

During all procedures, a clean swab was quickly (10 s) taken out of its transport medium (Transwab Charcoal medium, Medical Wire & Equipment Co, Bath, United Kingdom) into the operating room after which it was immediately put back into the medium in order to make sure no contamination occurred during transport and culturing of the samples (control swab).

Cotton swabs (cultures 1-4 and the control swab) were transported in the Transwab Charcoal medium. Removed bone material (cultures I-II) was put into sterile cups filled with a growth medium, Tryptone Soya Broth (TSB, Oxoid, United Kingdom).

Within 2 to 4 h after sampling, the cotton swabs (1-4) were smeared over blood agar and incubated, together with the cups containing cultures I and II, for 7 days at 37ºC, both aerobically and anaerobically. After 7 days, the content of the cups was also smeared over blood agar and again incubated for 5 days. Instrumentation or bone material was considered contaminated, when bacterial growth was observed, regardless of the amount of growth.

Follow up

In order to investigate whether infectious complications occurred post-operatively in relation with the interventions taken, all patients were followed up for 18 months. Previous studies in our hospital pointed out that nearly all periprosthetic infections became manifest within 18 months after surgery. First, patients were monitored during their stay at the orthopaedic ward to see whether prolonged wound discharge or superficial surgical site infection occurred. Wound discharge was recorded postoperatively by a specialized nurse from the local hospital infection committee, monitoring both the wound and the drain site, taking the

fifth day after surgery as the cut-off point. The diagnosis of a superficial wound infection was made by the orthopaedic surgeon based on the definition of the Surgical Infection Study Group. This definition relies solely on clinical observations in the absence of microbiological confirmation.[21] Deep periprosthetic infection was, eventually, defined by an increase of infection parameters caused by the prosthesis site, as judged by the orthopaedic surgeon.

Statistical analysis

The Pearson's Chi-square test for categorical data was used to test differences between the experimental groups and the control group, when all cells of the contingency table contained at least five people. Otherwise the Fisher's exact test was used. Statistical calculations were performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

Intra-operative bacterial contamination before and after the interventions

In the control group, contamination of one or more of the samples was seen in 23/70 (32.9%) cases. Group 1 showed contamination in 34.3% of the cases (23 out of 67) and group 2 showed contamination in 6/70 cases, equalling 8.6%.

In order to follow the contamination percentage in time, the total number of 207 patients was divided in 9 groups of about 20 patients, consecutively operated upon in time. Figure 1 shows that the contamination percentage in the control period and in the period after the first intervention ranges between 30 and 40%. It was only after the second intervention in August 2003 that the contamination percentage decreased to 15%. After that it further reduced to 5% in the end of 2003. In the first few months of 2004, the contamination percentage amounted 7%.

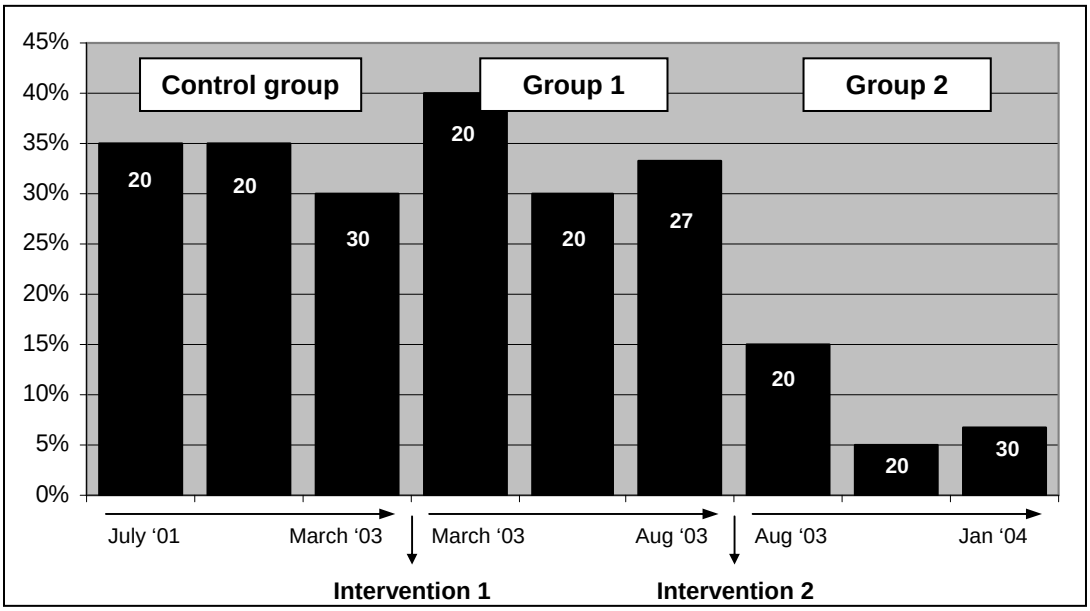


Figure 1. Intra-operative contamination (as percentage) per group of 20-30 patients during the entire period. The control group of 70 was divided into three groups (20, 20 and 30 patients), group 1 was divided into groups of 20, 20 and 27 patients, and group 2 was divided into groups of 20, 20 and 30 patients. The interventions are indicated with arrows.

Early and late intra-operative bacterial contamination during surgery

During all included procedures, four swabs of the instruments used were cultured (1-4), as well as two portions of bone chips (I-II). The control swab did not show bacterial growth at all times. The implantation of a hip or knee prosthesis can be divided in two parts: first, the preparation of the acetabulum (hip) or femur (knee) and secondly the preparation of femur (hip) or tibia (knee). The samples 1, 2 and I were taken during the early phase of the operating procedure and the samples 3, 4 and II during the late phase. In Table II the early samples are compared with the late samples. In all three groups more samples taken in the late phase showed bacterial growth as compared to those taken in the early phase. These differences only reached statistical significance in group 1 ($p=0.022$). In the total group of 207 procedures, growth was found in 40/207 samples taken in the early phase and in 23/207 samples taken in the late phase ($p=0.020$).

Table II. Number of intra-operatively acquired swabs and bone chip portions that showed contamination in the early and late phases of the operating procedure. Numbers and percentages are given for each group. P values indicate the significance of the difference between early and late samples (indicates p<0.05).*

Sample		Control group (N = 70)		Group 1 (N = 67)		Group 2 (N = 70)		Total (N = 207)	
Early	Instrument swab 1 Instrument swab 2 Bone chips portion I	16/70	22.9%	20/67	29.9%	4/70	5.7%	40/207	19.3%
Late	Instrument swab 3 Instrument swab 4 Bone chips portion II	11/70	15.7%	9/67	13.4%	3/70	4.3%	23/207	11.1%
	P value	0.284		0.022 *		1.000		0.020 *	

Follow up

During the control period, prolonged wound discharge was found in 16/70 (22.9%) cases, of which 8 were diagnosed with a superficial wound infection (11.4%). After a follow up of 18 months, deep periprosthetic infection became manifest in 5 of these cases (7.1%), all of which needed revision surgery.

After the first intervention, wound discharge was found in 21/67 (31.3%) cases, of which 10 had a significant superficial wound infection (14.9%). In the end, after an 18 month follow up, three of these patients suffered a deep periprosthetic infection (4.5%), two of which underwent revision surgery. The third patient was inoperable because of underlying disease and only received intravenous antibiotic therapy.

After the second intervention, wound discharge was found in only 7/70 (10%) patients, of which one suffered a superficial wound infection (1.4%). This superficial infection later on appeared to be a deep periprosthetic infection, needing revision surgery.

Figure 2 graphically summarizes the parameters contamination, prolonged wound discharge, superficial surgical site infection and deep periprosthetic infection over the different groups. Surprisingly, contamination, prolonged wound discharge and superficial surgical site infection all increased after the first intervention. Only the incidence of deep periprosthetic infection decreased. These changes, however, were not statistically significant. The second intervention established significant decreases in contamination (p=0.001), prolonged wound discharge (p=0.002) and superficial surgical site infection (p=0.004). The decrease in deep periprosthetic infection was not statistically significant (p=0.359).

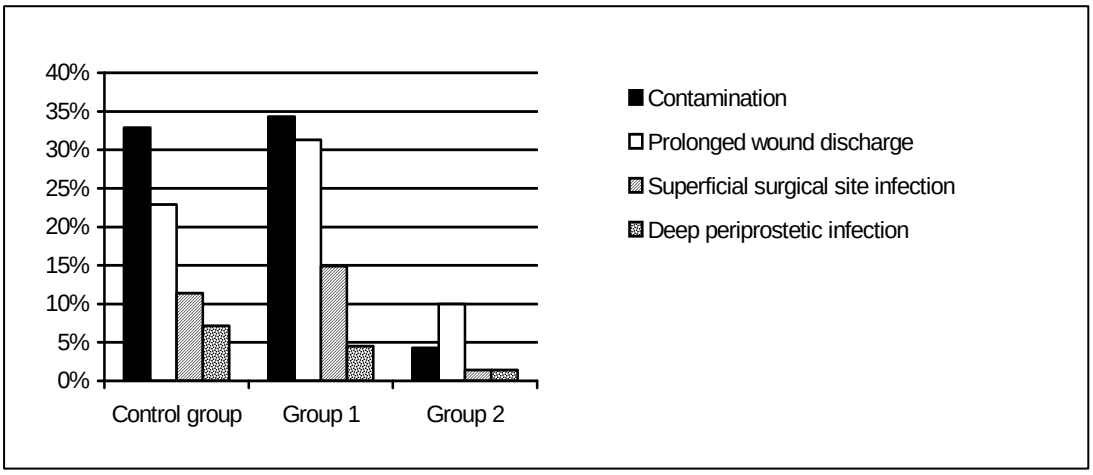


Figure 2. Bacterial contamination, prolonged wound discharge, superficial surgical site infection and deep periprosthetic infection in each of the three periods. Periprosthetic infection was diagnosed during 18 months of follow-up. All data are presented as percentages with respect to the size of the control group and groups 1 and 2.

Discussion

This study found that a combination of systemic and behavioural changes in an operating room significantly decreased the incidence of intra-operative bacterial contamination, and subsequently decreased the incidence of prolonged wound discharge and superficial surgical site infection. After one year of follow up there was also a decrease in deep periprosthetic infection; however, this difference did not reach statistical significance because of the small numbers of patients involved. Most of the individual parameters combined in the interventions have been shown to reduce contamination in the operating room,[1;22-29] but their combined effects have not been determined previously. However, combination of all these parameters evidently creates the most effective weapon against infection. In 1972, Charnley recognised that intra-operative contamination was a major threat to the success of total joint replacements, but others stated that its role as a cause of deep infection was highly overemphasised.[30;31] The major decrease in intra-operative contamination after the second intervention, followed by the decrease in prolonged wound discharge, superficial surgical site infection and subsequent deep periprosthetic infection, suggests that intra-operative contamination does influence postoperative infection.

The first intervention in March 2003, the better use of the plenum, did not yield any significant decrease in the outcome parameters, perhaps because the plenum was too small. In

orthopaedic implant surgery, many baskets of instruments are present in the operating room. Although the baskets were unpacked within the plenum, they were still standing near the edge of it, and hence close to the turbulent zone. Clearly, unpacking of the baskets just before surgery caused a considerable amount of bacterial shedding that could not be handled adequately by the conventional airflow system before the operation commenced.

The decrease in intra-operative contamination after the second intervention in August 2003 occurred in two steps (Figure 1). The first decrease was from 33% to 15%, and the second decrease from 15% to 5%. Air sampling demonstrated that the air flow system, as part of this intervention, worked properly; subsequently, the infection committee of the authors' our hospital enforced the desired behavioural changes more strictly in September. This indicates that the second intervention actually consisted of two parts: a systems part in August 2003 and a behavioural part in October 2003. This correlates with the two steps in the decrease of intra-operative contamination.

One might expect that the longer the duration of an operation, the more bacteria are present in the operating area and thus able to gain access to the wound. In 2004, Clarke et al. stated, after investigating 40 total hip procedures with both polymerase chain reaction and normal culture, that the contamination percentage at the end of surgery was significantly higher than at the start of surgery,[32] with both cultures from early and late stages taken from the posterior joint capsule. This is in contrast to the present results, which showed more contamination during the early phase of a procedure than during the late phase. However, samples from the present study were taken at six different times during surgery and originated from six different sites. It is hypothesized that just prior to an operating procedure, considerable movement is taking place in the operating area in terms of final preparations, covering the patient and entry of the surgeon. After this high initial movement, movement is limited as much as possible during the entire procedure. Consequently, it is not surprising that the initial samples in this study showed a higher contamination rate than the samples taken during the late phase.

In summary, radical alterations in behaviour and airflow system in an operating room can decrease intra-operative contamination. To maintain low bacterial counts, both the airflow system and behaviour have to be monitored consistently. Both the manufacturer of the airflow system and the hospital's infection control officer (e.g. a consultant microbiologist) should advice on the microbiological performance of the airflow system, and therefore have responsibility for the monitoring. An infection committee should monitor the behavioural

changes and report frequently to the people working in the operating room. Both positive and negative feedback help to maintain the reduction in bacterial dispersal. Finally, it is important to emphasize that all personnel working in the operating room, including surgeons, operating room assistants, anaesthesiologists and cleaning personnel, must follow hygiene protocols very strictly.

References

1. Hanssen AD, Rand JA. Evaluation and treatment of infection at the site of a total hip or knee arthroplasty. Instr Course Lect 1999; 48:111-122.
2. Abudu A, Sivardeen KA, Grimer RJ, Pynsent PB, Noy M. The outcome of perioperative wound infection after total hip and knee arthroplasty. Int Orthop 2002; 26:40-43.
3. An YH, Friedman RJ. Prevention of sepsis in total joint arthroplasty. J Hosp Infect 1996; 33:93-108.
4. Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS, Osmon DR. Risk factors for prosthetic joint infection: case-control study. Clin Infect Dis 1998; 27:1247-1254.
5. Fender D, Harper WM, Gregg PJ. Outcome of Charnley total hip replacement across a single health region in England: the results at five years from a regional hip register. J Bone Joint Surg Br 1999; 81:577-581.
6. Grogan TJ, Dorey F, Rollins J, Amstutz HC. Deep sepsis following total knee arthroplasty. Ten-year experience at the University of California at Los Angeles Medical Center. J Bone Joint Surg Am 1986; 68:226-234.
7. Harris WH, Sledge CB. Total hip and total knee replacement (1). N Engl J Med 1990; 323:725-731.
8. National CFHS. American Academy and American Assoc of Orthopaedic Surgeons-Bulletin.AAOS. 47[3], 14. 1999.
9. Fitzgerald RH HA. Postoperative deep wound infection. In: Morrey F, ed. Joint replacement arthroplasty, First ed. Edinburgh: Churchill-Livingstone, 1991:835-850.
10. Ahnfelt L, Herberts P, Malchau H, Andersson GB. Prognosis of total hip replacement. A Swedish multicenter study of 4,664 revisions. Acta Orthop Scand Suppl 1990; 238:1-26.
11. Ainscow DA, Denham RA. The risk of haematogenous infection in total joint replacements. J Bone Joint Surg Br 1984; 66:580-582.
12. Ha'eri GB, Wiley AM. Total hip replacement in a laminar flow environment with special reference to deep infections. Clin Orthop 1980;163-168.
13. Howorth FH. Prevention of airborne infection during surgery. Lancet 1985; 1:386-388.
14. Lidwell OM. Clean air at operation and subsequent sepsis in the joint. Clin Orthop 1986;91-102.
15. Pittet D, Ducel G. Infectious risk factors related to operating rooms. Infect Control Hosp Epidemiol 1994; 15:456-462.
16. Whyte W, Hodgson R, Tinkler J. The importance of airborne bacterial contamination of wounds. J Hosp Infect 1982; 3:123-135.
17. Duguid JP, Wallace AT. Air infection with dust liberated from clothing. Lancet 2, 845-849. 1948.
18. Barrack RL, Sawhney J, Hsu J, Cofield RH. Cost analysis of revision total hip arthroplasty. A 5-year followup study. Clin Orthop 1999;175-178.
19. Bengtson S. Prosthetic osteomyelitis with special reference to the knee: risks, treatment and costs. Ann Med 1993; 25:523-529.
20. Sculco TP. The economic impact of infected joint arthroplasty. Orthopedics 1995; 18:871-873.
21. Peel AL, Taylor EW. Proposed definitions for the audit of postoperative infection: a discussion paper. Surgical Infection Study Group. Ann R Coll Surg Engl 1991; 73:385-388.
22. Ahl T, Dalen N, Jorbeck H, Hoborn J. Air contamination during hip and knee arthroplasties. Horizontal laminar flow randomized vs. conventional ventilation. Acta Orthop Scand 1995; 66:17-20.
23. Brown AR, Taylor GJ, Gregg PJ. Air contamination during skin preparation and draping in joint replacement surgery. J Bone Joint Surg Br 1996; 78:92-94.
24. Gosden PE, MacGowan AP, Bannister GC. Importance of air quality and related factors in the prevention of infection in orthopaedic implant surgery. J Hosp Infect 1998; 39:173-180.
25. Gruenberg MF, Campaner GL, Sola CA, Ortolan EG. Ultraclean air for prevention of postoperative infection after posterior spinal fusion with instrumentation: a comparison between surgeries performed with and without a vertical exponential filtered air-flow system. Spine 2004; 29:2330-2334.
26. Hubble MJ, Weale AE, Perez JV, Bowker KE, MacGowan AP, Bannister GC. Clothing in laminar-flow operating theatres. J Hosp Infect 1996; 32:1-7.
27. Hughes SP, Anderson FM. Infection in the operating room. J Bone Joint Surg Br 1999; 81:754-755.
28. Lidwell OM. Air, antibiotics and sepsis in replacement joints. J Hosp Infect 1988; 11 Suppl C:18-40.
29. Pittet D, Ducel G. Infectious risk factors related to operating rooms. Infect Control Hosp Epidemiol 1994; 15:456-462.
30. Humphreys H. Infection control team in the operating room: separating aspiration from reality! J Hosp Infect 1999; 42:265-267.
31. Charnley J. Postoperative infection after total hip replacement with special reference to air contamination in the operating room. Clin Orthop 1972; 87:167-187.

32. Clarke MT, Lee PT, Roberts CP, Gray J, Keene GS, Rushton N. Contamination of primary total hip replacements in standard and ultra-clean operating theaters detected by the polymerase chain reaction. Acta Orthop Scand 2004; 75:544-548.

4

TRANSFER OF BACTERIA BETWEEN BIOMATERIALS SURFACES IN THE OPERATING ROOM

an experimental study

Knobben BAS, Van der Mei HC, Van Horn JR, Busscher HJ

Journal of Biomedical Materials Research Part A 2005, accepted

Introduction

The number of biomaterials implants placed worldwide is huge and will only increase during the next few decades. Biomaterials implants are foreign bodies on which a biofilm can grow, provided bacteria are given the opportunity to adhere and multiply. Once a biofilm has formed, the bacteria within a biofilm are highly resistant to antibiotic treatment. In most cases, a prosthesis has to be removed temporarily until the infection has cleared fully from the surrounding tissue. This makes infection one of the worst complications, as most evident in orthopaedic implant surgery. Since many decades *Staphylococcus aureus* has been identified as a virulent micro-organism causing periprosthetic infection.[1;2] The coagulase-negative *Staphylococcus epidermidis* was long considered non- to low-virulent, but is now considered as the major source of intra-operative contamination and a cause of periprosthetic infection.[1-6] The obligate anaerobe *Propionibacterium acnes* was present in 62% of contaminated hip prostheses retrieved after removal due to chronic low-grade infection,[2] i.e. as frequently as *Staphylococcus* spp.

The most common cause of orthopaedic implant infection are bacteria entering the wound during surgery.[7;8] Intra-operative contamination is common in every operating room.[4;9-11] However, despite several technological and behavioural developments, bacteria can not be fully eliminated from an operating room.[12] Bacterial adhesion to and transfer between surfaces is a complicated process and with regard to the success of biomaterials implants, studies on bacterial adhesion and transfer should not be confined to biomaterials surfaces in the human body, but also encompass surfaces in the operating room, where the origin of many biomaterials related infections is found.

Hydrophobicity and roughness of the interacting surfaces are generally considered as important factors in bacterial adhesion, but also environmental conditions like moistness of the surface and the application of friction will affect bacterial transfer between surfaces. In clear contrast to what is currently being studied most in the literature (bacterial adhesion to surfaces) the problem in the clinical situation is much more to prevent transfer of bacteria from one surface to another. Contact lens induced keratitis is the result of bacterial transfer from the lens case to the lens and from the lens to the cornea. Similarly, intra-operative contamination is the result of a series of bacterial transfers from the skin of the patient or theatre personnel via instruments and other materials to the wound area.[13;14] Davis et al. identified materials that are frequently contaminated during elective orthopaedic surgery. In 14.5% of the procedures, the

light handles were contaminated, in 17% the theatre gowns and in 28.7% the gloves of the operating team.[4] The used sets of instruments were contaminated in 3.2% to 11.4% of the sampled cases. As a result, as much as 70% of all air-borne bacteria reach the wound via hands of the surgical personnel or by instruments used, while only 30% reach the wound directly via the air.[8]

The purpose of this study is to quantify the transfer of bacteria (the aerobes *S. epidermidis* and *S. aureus* and the anaerobe *P. acnes*) from one operating room material to another, while accounting for surface hydrophobicity and roughness, moistness and application of friction during transfer. As a possible clinical intervention method to prevent transfer, it was investigated whether dipping the gloves in a chlorhexidine splash-basin affected the viability of the transferred bacteria.

Materials and methods

Bacterial strains, culture conditions and harvesting

Three bacterial strains, *S. epidermidis* 8162, *S. aureus* 5434 and *P. acnes* 5198 isolated from patients with septic prosthetic loosening were employed. From these strains, a frozen stock was precultured at 37ºC on blood agar plates for 24 h aerobically (*S. aureus and S. epidermidis*) and for 48 h anaerobically (*P. acnes*). For the preparation of experimental cultures, colonies were inoculated into a 10 ml batch culture of Tryptone Soya Broth (TSB, Oxoid, United Kingdom) for 24 h at 37ºC under aerobic (*S. aureus* and *S. epidermidis*) and anaerobic (*P. acnes*) conditions. This preculture was used to inoculate a main culture of 200 ml TSB, which was allowed to grow for 16 h. Bacteria from this main culture were harvested in their stationary phase by centrifugation at 5000 g 5 min at 10ºC. The strains were washed twice with ultrapure water and resuspended in 10 ml ultrapure water. Finally, bacteria were suspended in 0.9% saline to a concentration of 1×10^8 cells ml^{-1}, as determined in a Bürker-Türk counting chamber. All bacteria were used immediately after harvesting.

Operating room materials

Bacterial transfer was studied between frequently contaminated materials, including latex operating gloves (Gammex, Ansell, Belgium), polyester theatre gowns (Gore-Prooftex, Rentex, Germany), polyvinylchloride (PVC) light handles and stainless steel broaches. Operating

gloves and theatre gowns were mounted onto sample stubs to obtain samples suitable for measurements. PVC light handles could also be mounted to allow easy measurements on a flat instrument piece. Gloves, theatre gowns and light handles were cleaned with 70% ethanol prior to measurements. Stainless steel samples were made from plate material, commercially purchased, ground down to grit number 1200, and subsequently polished with a diamond water-based suspension (Metadi 6 and 3 μm diamond suspension and Trident polishing cloth, Buehler, Lake Bluff) for 3 and 1.5 min, respectively. Both procedures were performed on a polishing machine with a 30 N load and with oppositely rotating axes (Phoenix Beta and vector grinder/polisher, Buehler, Lake Bluff). After polishing, the steel was cleaned by 5 min sonication in 2% alkaline cleaning agent followed by thorough rinsing with tap water, sonication in ethanol and rinsing in ultrapure water. After cleaning, the steel was passivated according to ASTM F86-91.

Measurement of surface hydrophobicity and roughness

Hydrophobicity of the materials was assessed through the measurement of water contact angles, employing the sessile drop technique and a homemade contour monitor. Water contact angles of 3 μl droplets were determined. For the measurements of bacterial cell surface hydrophobicity, bacteria were suspended in 10 ml ultrapure water. A cellulose acetate membrane filter with a pore diameter of 0.45 μm was put on a fritted glass support, and a bacterial deposit was obtained by filtration of the bacterial suspension under negative pressure. The filters, containing 10^8 bacteria per square millimetre, were placed on a metal sample disc with double-sided sticky tape and dried for 30-40 min in order to measure plateau water contact angles. Measurements for both materials and bacteria were performed in triplicate.

The roughness of the materials was measured with the aid of a profilometer (Proscan 2000, Scantron Industrial Products Ltd, Taunton, Somerset, UK). The samples were placed in a holder and mounted on the profilometer with the use of double-sided sticky tape. The slide was put below the laser to obtain height images in three dimensions of an area of one square centimetre. The height was measured in this area every 100 μm. The average roughness R_A was obtained from these images and indicates the average distance of the roughness profile to the centre plane of the profile. All measurements were done in triplicate.

Initial adhesion and bacterial transfer

Sterile donor materials (5 glove samples, 3 broach samples, 3 theatre gown samples and 2 light handle samples) with a diameter of 5 cm were exposed to different baths with the same bacterial suspension of 1 x 10^8 cells ml^{-1} for 15 min at room temperature (Figure 1A). After removal from the bacterial suspension, sterile filtration paper was used to remove excess suspension and the sample edges were cleaned with an alcohol soaked cotton swab.

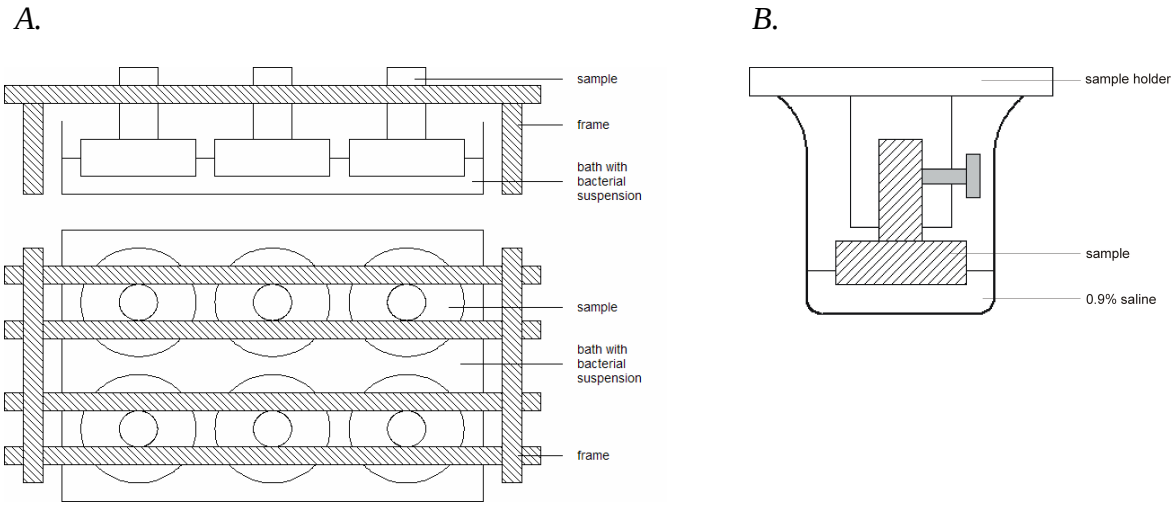


Figure 1. Inoculation and method of counting colony-forming units (CFUs) on the materials surface. Lateral and superior view of the samples hung in a bath with a bacterial suspension (A). The samples are fixed between two of the four layers of the frame. Lateral view of a sample in the sample holder (B). The holder is placed on a beaker with 20 mL of sterile 0.9% saline and then sonicated for 30 seconds.

For quantification of initial adhesion, one sample of each material was put in 20 ml of sterile 0.9% saline (Figure 1B) and sonicated for 30 s, after which serial dilutions were made (1, 10, 50 and 100 times) and plated on TSB agar. Plates were left to incubate at 37ºC for 24 h under aerobic conditions for the staphylococcal strains and for 48 h under anaerobic conditions for *P. acnes*. Finally, the number of CFUs was determined in order to yield the number of CFUs per unit area present on the donor material before transfer. The other samples were used to do the transfer experiments.

Table I shows the different bacterial transfers that were tested from one material surface to another. In all experiments the contact time was 10 s and the applied pressure 1.0 kg cm^{-2}. The experiments were performed both when the inoculum was still moist and after it was allowed to dry after inoculation. In case bacterial transfer from or to gloves was measured,

experiments were also performed with additional friction applied, consisting of 10 half-circle rotations during contact. Subsequently, the samples were handled as described above and in Figure 1.

Table I. Donor and recipient materials used to study the transfer of bacteria. All experiments were performed under a pressure of 1.0 kg cm^{-2} and a contact time of 10 s. Experiments with gloves were performed both with and without friction. Friction consisted of 10 half-circles of rotation during the 10 s contact time.[15]

Donor	Recipient
Glove moistened with inoculum / allowed to dry after inoculation	Glove Broach Theatre gown Light handle
Broach moistened with inoculum / allowed to dry after inoculation	Glove Theatre gown
Theatre gown moistened with inoculum / allowed to dry after inoculation	Glove Broach
Light handle moistened with inoculum / allowed to dry after inoculation	Glove

Intervention methods

In order to determine whether chlorhexidine is an effective antimicrobial agent to prevent transfer of *S. aureus*, *S. epidermidis* and *P. acnes*, gloves after bacterial inoculation were dipped in chlorhexidine-digluconate (4%, 0.4% and 0.04% in water) prior to transfer. The experiments were performed both with the inoculated gloves still moist and after air drying (1 min). After chlorhexidine dipping, gloves were either immediately handled or allowed to dry. Similar procedures were carried out with 0.9% saline as a control.

Statistical analysis

Statistical analyses were performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL). Differences between initial adhesion of *S. epidermidis*, *S. aureus* and *P. acnes* to the materials were determined with the two-sided Students t-test (accepting $p<0.05$ as the limit for statistical significance). Transfer was calculated as the percentage of CFUs cm^{-2} on the donating material that had transferred to the receiving material and was the mean of three experiments. Differences in transfer percentages for the three bacterial strains to and from the materials were again calculated using the two-sided Students t test ($p<0.05$). The same applies for the difference in transfer between moist and dry transfer and between transfer with and without the application of friction.

Finally, a univariate analysis was performed to test the independent variables "bacterial strain", "moistness", "friction", "donating" and "receiving" material for their correlation with bacterial transfer. The p values indicate the significance of the effect of an independent variable on the transfer ($p<0.05$). The percentage of the total variation in transfer that can be explained by an independent variable was expressed as the percentage of variance.

Results

Initial adhesion

Figure 2 compares the initial adhesion of the different bacterial strains to the various donor materials. Initial adhesion of *S. aureus* and *P. acnes* to the different donor materials is similar, but adhesion of *S. epidermidis* to gloves, theatre gowns and light handles is significantly ($p<0.05$) higher than for the two other strains. However, initial adhesion of *S. epidermidis* to the stainless steel broach is significantly ($p<0.05$) lower than of *S. aureus* and *P. acnes.*

The theatre gown attracts most bacteria, regardless of the strain involved, with almost similar numbers of *S. aureus* and *P. acnes* adhering to the broach. However, the broach attracted the lowest number of *S. epidermidis* of all materials involved. Adhesion of the strains to light handles was only slightly less than to theatre gowns.

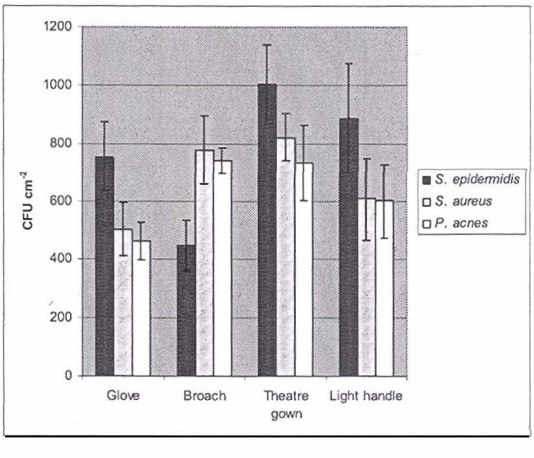


Figure 2. Initial adhesion of S. epidermidis, S. aureus and P. acnes to glove, broach, theatre gown and light handle. Mean values are shown in colony-forming units per square centimetre (CFU cm^{-2}). Error bars represent standard deviations over triplicate runs with separately cultured bacteria.

Transfer

Table II summarizes the bacterial transfer between different surfaces for transfer from a moist donor in the absence of friction. The mean transfer percentage of the tested transfers from moistened donors is 38% (SD=20.5) and ranges from 17 to 71%. The average transfer is generally lower from theatre gown and light handles than from gloves and broaches. Transfer from the broach was lowest for *S. aureus*, which is also the reason why the average transfer for *S. aureus* is lower than for the two other strains.

Transfer percentages for *S. epidermidis* are highest from glove to broach and from broach to theatre gown (both 67%) and lowest from light handle to glove (17%) and from theatre gown to glove (24%). Transfer percentages from the glove are significantly ($p<0.05$) higher to the broach than to the glove and to the light handle. In general, transfer percentages from the broach are significantly ($p<0.05$) higher than those from the theatre gown. When looking at the transfer percentages to the glove it can be seen that these are significantly ($p<0.05$) higher from the broach than from the theatre gown and from the light handle.

Table II. Mean transfer percentages for S. epidermidis, S. aureus and P. acnes in case of moist transfer without friction from one operating room material to another. Data are results of triplicate runs with separately cultured bacteria (± indicates standard deviation).

		S. epidermidis	***S. aureus***	***P. acnes***	**Average over strains**
Glove	Glove	33 ± 8	26 ± 5	36 ± 7	**32**
	Broach	67 ± 16	71 ± 14	33 ± 8	**57**
	Theatre gown	45 ± 6	40 ± 9	39 ± 11	**41**
	Light handle	29 ± 5	28 ± 10	61 ± 3	**39**
Average over materials		**44**	**41**	**42**	**42**
Broach	Glove	47 ± 8	24 ± 8	56 ± 9	**42**
	Theatre gown	67 ± 11	29 ± 8	57 ± 7	**51**
Average over materials		**57**	**27**	**57**	**47**
Theatre gown	Glove	24 ± 6	28 ± 5	29 ± 10	**27**
	Broach	32 ± 3	23 ± 6	19 ± 6	**25**
Average over materials		**28**	**26**	**24**	**26**
Light handle	Glove	17 ± 7	17 ± 4	48 ± 6	**27**
Average over all transfer		**40**	**32**	**42**	**38**

Transfer of *S. aureus* is comparable to the transfer of *S. epidermidis*, except for its transfer from the metallic broach. Transfer of *S. aureus* from broach to glove (24%) and theatre

gown (29%) is significantly ($p<0.05$) lower than observed for *S. epidermidis* (47% and 67%, respectively) and *P. acnes* (56% and 57%, respectively).

The transfer of *P. acnes* proceeds along different lines than of the staphylococcal strains. Transfer of *P. acnes* from glove to light handle (61%) and from light handle to glove (48%) are significantly ($p<0.05$) higher than that of *S. epidermidis* (29% and 17%, respectively) and *S. aureus* (28% and 17%, respectively).

Influence of moistness and application of friction on bacterial transfer

Figure 3 shows that when the donor surface is allowed to dry prior to transfer, transfer percentages decrease significantly for all nine transfer pathways and all three bacterial strains when compared to moist surfaces without friction. On average over all nine pathways, the transfer of *S. epidermidis* decreased 2.7-fold, of *S. aureus* 1.5-fold and the transfer of *P. acnes* 1.7-fold.

The application of friction increases bacterial transfer from one material to another (see also Figure 3). The mean transfer percentage of *S. epidermidis* increased 1.6-fold, of *S. aureus* 1.8-fold and of *P. acnes* 1.5-fold compared to moist without friction.

Table III shows that all studied variables ("bacterial strain", "moistness", "application of friction" and "donating" and "receiving" material) have a significant influence on bacterial transfer, with the percentage of variance explained by moistness and application of friction being largest (41.0% and 36.5%, respectively).

Table III. Univariate analysis of variance of the transfer model used in this study. P-values show the significance of each factor. Percentages of variance indicate the strength of the influence of each factor on the transfer percentage.

Variable	Significance (p)	Percentage of variance
Bacterial strain	< 0.001	2.0
Moistness	< 0.001	41.0
Friction	< 0.001	36.5
Donating surface	< 0.001	3.7
Receiving surface	< 0.001	2.7

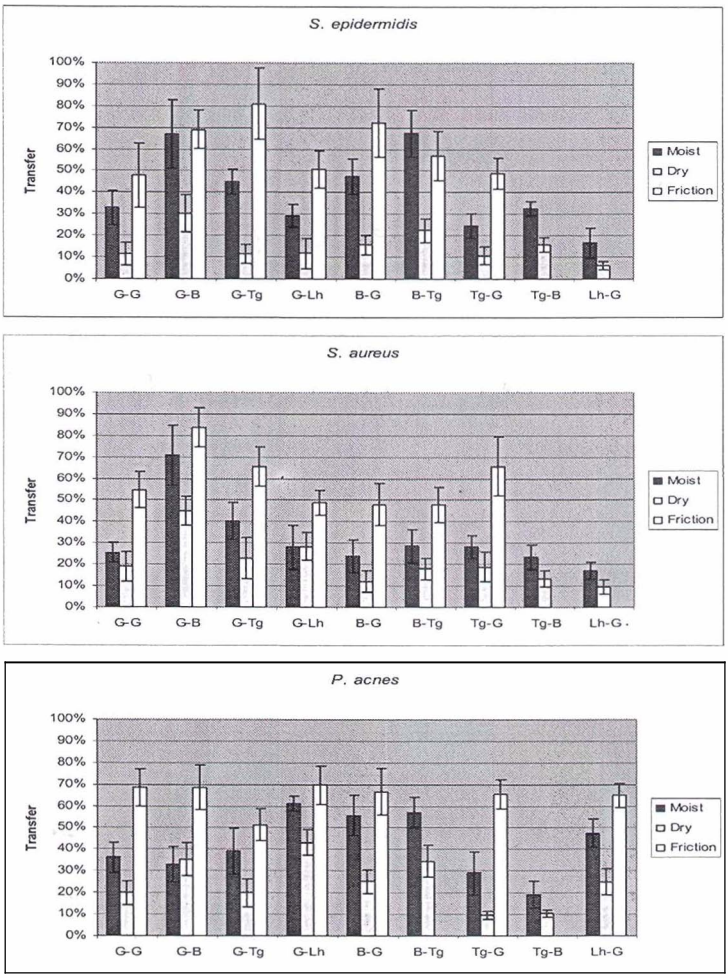


Figure 3. Transfer percentages for S. epidermidis, S. aureus and P. acnes from one operating room material to another. Mean transfer percentages are shown for moist transfer without friction (Moist), dry transfer without friction (Dry) and moist transfer with application of friction (Friction). Transfer from broach to theatre gown and vice versa were not performed. Error bars indicate standard deviations over triplicate runs with separately cultured bacteria. G = Glove; B = Broach; Tg = Theatre gown; Lh = Light handle.

Influence of hydrophobicity and roughness of bacterial strains and operating room materials on bacterial transfer

Table IV shows the mean water contact angles and the mean roughness of the surfaces of the operating room materials and the mean water contact angles of the bacterial strains. The stainless steel of the broach constituted the most hydrophilic surface and the polyester theatre gown was the most hydrophobic, likely also as a side-effect of its roughness. The

material is the roughest and the broach material the smoothest. The *S. aureus* and *P. acnes* strains employed are relatively hydrophilic, whereas *S. epidermidis* is a more hydrophobic strain.

Table IV. Hydrophobicity (determined by water contact angle measurements) and surface roughness (determined by AFM) of the operating room materials (glove, broach, theatre gown and light handle) and bacterial strains (S. epidermidis, S. aureus and P. acnes).

Material surface	Hydrophobicity (degrees)	Roughness (µm)
Glove	99	25
Broach	62	7.6
Theatre gown	136	35.4
Light handle	107	19.9
S. epidermidis	57	-
S. aureus	27	-
P. acnes	25	-

Figure 4 shows the average moist transfer percentages from the donating (A) and to the receiving operating room material surface (B) as a function of the hydrophobicity measured by water contact angles and roughness for *S. epidermidis*, *S. aureus* and *P. acnes* in a single parameter regression model. Transfer of *S. epidermidis* and *P. acnes* decreases with increasing hydrophobicity and roughness of the donating surface (Figure 4A-1 and 4A-2): the more hydrophobic and rough the material surface, the better the bacteria stick to it (i.e. the least transfer to the receiving surface). The only exception is the transfer of *S. epidermidis* from the light handle, which is surprisingly low (17%). *S. aureus* acts somewhat differently, mainly by sticking to the hydrophilic and smooth metallic broach surface on transfer.

When considering the hydrophobicity and roughness of the receiving material surface, transfer of the two staphylococcal strains is best to both the smooth and hydrophilic broach and to the rough and hydrophobic theatre gown, the latter especially for *S. epidermidis*. Transfer of *P. acnes* to a surface is worst when this surface is hydrophilic and smooth, transfer to the light handle is highest.

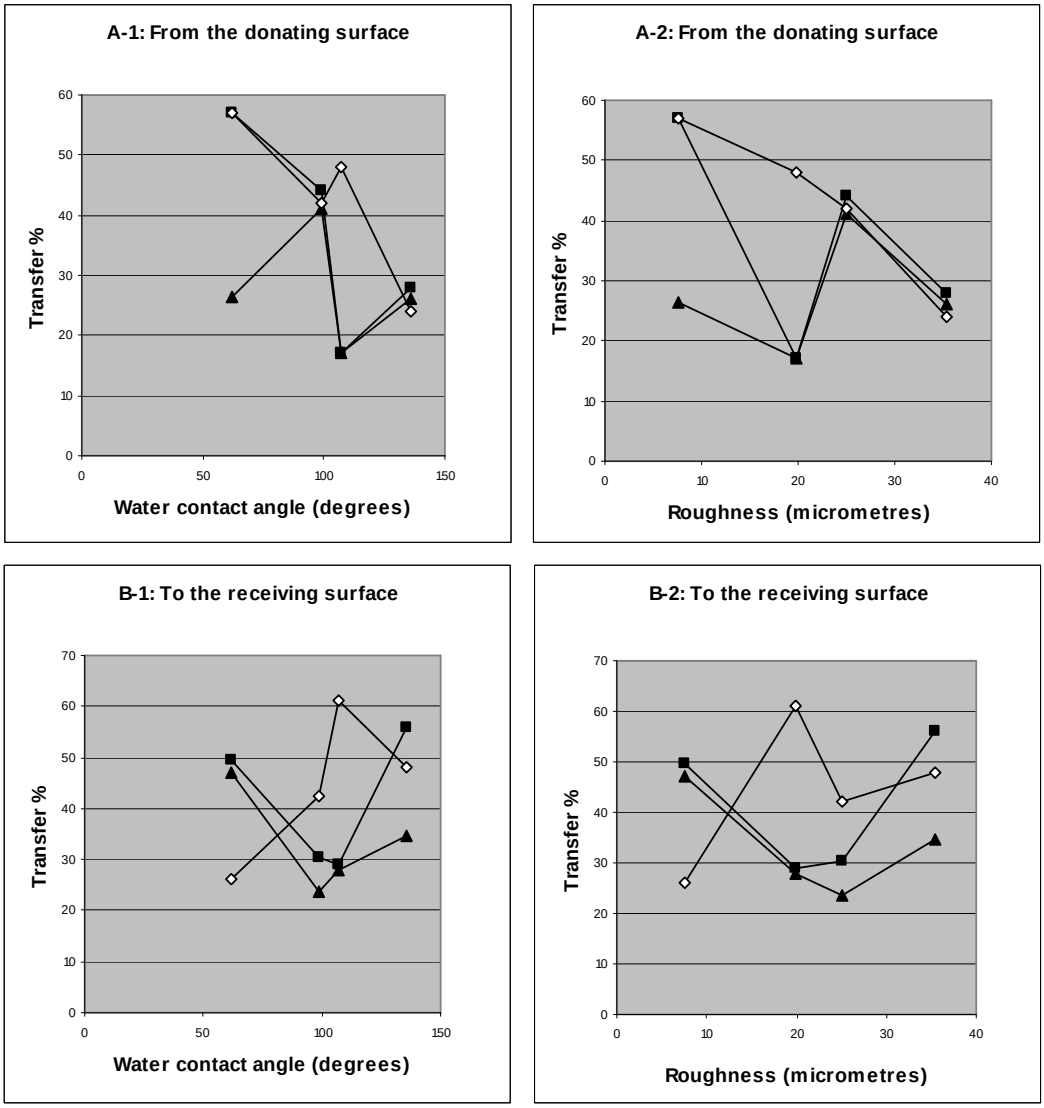


Figure 4. Average moist transfer percentages from the donating (A) and to the receiving operating room material surface (B) as a function of the hydrophobicity (A-1 and B-1) and roughness (A-2 and B-2) for S. epidermidis (■) *S. aureus* (▲) *and P. acnes* (◊).

Intervention

Table V shows that dipping the glove material in a 4% or 0.4% chlorhexidine solution kills all bacteria present, regardless of whether surfaces were dried prior to transfer or still moist. Dipping in 0.04% chlorhexidine was only effective under dried conditions, and under moist transfer conditions results were similar as for a 0.9% saline control.

Table V. Number of CFUs cm^{-2} still present on the sample after dipping in a saline (0.9%) or chlorhexidine solution (0.04, 0.4 and 4%). Experiments were performed when the inoculum was still moist and when the inoculum had been allowed to dry before dipping. After dipping half of the samples were allowed to dry before counting CFUs, the others were counted immediately.

	Inoculum still moist						Inoculum allowed to dry					
	Dipping fluid still moist			Dipping fluid allowed to dry			Dipping fluid still moist			Dipping fluid allowed to dry		
	SE	SA	PA	SE	SA	PA	SE	SA	PA	SE	SA	PA
0.9% saline	365	285	303	521	331	482	412	357	281	496	417	429
0.04% chlorhexidine	316	213	227	0	0	0	385	294	236	0	0	0
0.4% chlorhexidine	0	0	0	0	0	0	0	0	0	0	0	0
4% chlorhexidine	0	0	0	0	0	0	0	0	0	0	0	0

SE = *S. epidermidis*, SA = *S. aureus*, PA = *P. acnes*

Discussion

Transfer between glove, broach, theatre gown and light handle surfaces as well as initial adhesion onto these material surfaces was evaluated in this study for *S. epidermidis*, *S. aureus* and *P. acnes*. This is the first study to quantify this transfer of bacteria between different material surfaces used in the operating room. Most other studies focussing on bacterial adhesion or transfer are performed in the food sector or are contact lense related.[16-21] Several studies have focussed already on the initial adhesion of *S. epidermidis* and *S. aureus* to different material surfaces, but initial adhesion of *P. acnes* to comparable material surfaces was studied here for the first time.

Regarding initial adhesion, it is generally accepted that hydrophobic bacteria adhere to a greater extent than hydrophilic bacteria, especially to hydrophobic surfaces.[22] In this study, initial adhesion of the most hydrophobic bacterial strain used (*S. epidermidis*), is higher on all materials than the initial adhesion of *S. aureus* and *P. acnes*, except for the more hydrophilic metallic broach. This is in accordance with the generally accepted thought that bacteria with hydrophobic properties prefer to adhere to hydrophobic material surfaces; the ones with hydrophilic characteristics prefer hydrophilic surfaces.[17;23;24] Ramage et al., studying biofilm formation of *P. acnes*, *S. epidermidis* and *S. aureus* on PMMA (polymethylmethacrylate) bone cement and titanium alloys found that initial adhesion (within 30 min) to the titanium alloys was significantly higher for *S. epidermidis* than for *P. acnes* and *S. aureus*.[25] Faille et al. found that the more hydrophobic a material surface, the more likely bacteria will adhere to it.[17] With the exception of the hydrophilic *S. aureus* and *P. acnes* adhering best to the hydrophilic broach, similar conclusions can be drawn from our study.

Transfer was demonstrated to some extent with all bacterial strains and every tested material. The transfer that attracts the most attention is the transfer of the hydrophilic *S. aureus* from glove to broach and from broach to both glove and theatre gown. It appears that *S. aureus* transfers to the hydrophilic and smooth stainless steel very easily, and it sticks to it rather strongly, leading to low transfer percentages to other materials. This is again in accordance with the knowledge that hydrophilic strains adhere well to hydrophilic surfaces.[26]

All three bacteria adhere best to the theatre gown. Probably this has to do with the severe roughness of this material. A rough surface has a greater surface area and the depressions in the roughened surface provide more favourable sites for colonization.[27-29]

Transfer from the rough and hydrophobic theatre gown was low for all three bacterial strains. Because of the high roughness, a small contact area exists between the donating theatre gown surface and the other receiving surface, creating low transfer percentages. On the other hand, transfer to the theatre gown was quite high for all tested strains. Perhaps the hydrophobic nature of this material and some minor friction applied during the transfer experiments can account for this. In the discussion of the use of cotton or polyester theatre gowns this is quite interesting. A bacterial transfer study performed by Sattar et al. showed that a polyester-cotton blend releases bacteria much easier than cotton alone.[19] Comparison of fabrics indicate that disposable, polypropylene, spun bond laminate materials offer best protection.[30] In conclusion, it can be said that cotton gowns are more convenient to wear, but too permeable for bacteria (especially when wet); polyester-cotton drapes on the other hand are more inconvenient to wear, less permeable to bacteria, but apparently release attached bacteria more easily than cotton drapes.

P. acnes is increasingly being considered a potential pathogen to cause periprosthetic infection. Ramage et al. showed its possibility to grow a biofilm on orthopaedic implants and bone cement.[25] Our study shows that *P. acnes* transfers between all tested operating room material surfaces and that it transfers best away from the broach (56-57%) and between glove and light handle (61 and 48%). Combining these last findings with those of Davis et al., describing that 14.5% of the light handles are contaminated, it is obvious that the light handle issue still remains a problem.[4] Several studies have pointed out that light handles are often contaminated with bacteria, but few of them have given solutions. The proposed ‘compromise’ by Davis et al. is to manipulate the light handle with a sterile cloth, which is then discarded. Our proposed regime of dipping the gloves in a chlorhexidine splash-basin may further decrease bacterial adhesion and transfer into the wound.

Bacteria that are living in a biofilm are far more resistant to antibiotic treatment than planktonic bacteria, which makes the treatment of periprosthetic infection very difficult. During the transfer of bacteria in the operating room, the sessile bacteria are still in a monolayer and can easily be treated with chlorhexidine. Chlorhexidine has already been demonstrated to be effective against bacteria in such a state.[31-35] Intervention with this agent in the operating room by dipping the surgical gloves in a chlorhexidine splash-basin every ten minutes would be an easily applicable method to decrease bacterial transfer into the wound and hence lower the risk of postoperative infection.

This study examines the bacterial transfer between different material surfaces used in the operating room. Transfer (moist and without friction) was demonstrated to some extent with all three bacterial strains and with every tested material, ranging from 17 to 71%, and was influenced by the type of strain, moistness of the inoculum, the application of friction and the characteristics of both the donating and the receiving surface. Dipping the glove material in 4% or 0.4% chlorhexidine solutions killed all bacteria present, regardless of whether surfaces were dried or moist and thus prevented transfer.

References

1. Neut D, van Horn JR, van Kooten TG, van der Mei HC, Busscher HJ. Detection of biomaterial-associated infections in orthopaedic joint implants. Clin Orthop Relat Res 2003;261-8.
2. Tunney MM, Patrick S, Gorman SP et al. Improved detection of infection in hip replacements. A currently underestimated problem. J Bone Joint Surg Br 1998;80:568-72.
3. Al Maiyah M, Bajwa A, Mackenney P et al. Glove perforation and contamination in primary total hip arthroplasty. J Bone Joint Surg Br 2005;87:556-9.
4. Davis N, Curry A, Gambhir AK et al. Intraoperative bacterial contamination in operations for joint replacement. J Bone Joint Surg Br 1999;81:886-9.
5. Heal JS, Blom AW, Titcomb D, Taylor A, Bowker K, Hardy JR. Bacterial contamination of surgical gloves by water droplets spilt after scrubbing. J Hosp Infect 2003;53:136-9.
6. Rutala WA, Gergen MF, Jones JF, Weber DJ. Levels of microbial contamination on surgical instruments. Am J Infect Control 1998;26:143-5.
7. Lidwell OM. Air, antibiotics and sepsis in replacement joints. J Hosp Infect 988;11 Suppl C:18-40.
8. Whyte W, Hodgson R, Tinkler J. The importance of airborne bacterial contamination of wounds. J Hosp Infect 1982;3:123-35.
9. Hughes SP, Anderson FM. Infection in the operating room. J Bone Joint Surg Br 1999;81:754-5.
10. Knobben BAS, Engelsma Y, Neut D, van der Mei HC, Busscher HJ, van Horn JR. Intra-operative contamination influences wound discharge in orthopaedic implant surgery. Clin Orthop Relat Res 2006.
11. Maathuis PG, Neut D, Busscher HJ, van der Mei HC, van Horn JR. Perioperative contamination in primary total hip arthroplasty. Clin Orthop Relat Res 2005;136-9.
12. Knobben BAS, van Horn JR, van der Mei HC, Busscher HJ. Evalutation of measures to decrease intra-operative bacterial contamination in orthopaedic implant surgery. J Hosp Infect 2005;62(2):174-802.
13. Ha'eri GB, Wiley AM. Total hip replacement in a laminar flow environment with special reference to deep infections. Clin Orthop Relat Res 1980;163-8.
14. Howorth FH. Prevention of airborne infection during surgery. Lancet 1985;1:386-8.
15. Mbithi JN, Springthorpe VS, Boulet JR, Sattar SA. Survival of hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces. J Clin Microbiol 1992;30:757-63.
16. Bruinsma GM, van der Mei HC, Busscher HJ. Bacterial adhesion to surface hydrophilic and hydrophobic contact lenses. Biomaterials 2001;22:3217-24.
17. Faille C, Jullien C, Fontaine F, Bellon-Fontaine MN, Slomianny C, Benezech T. Adhesion of Bacillus spores and Escherichia coli cells to inert surfaces: role of surface hydrophobicity. Can J Microbiol 2002;48:728-38.
18. Midelet G, Carpentier B. Transfer of microorganisms, including Listeria monocytogenes, from various materials to beef. Appl Environ Microbiol 2002;68:4015-24.
19. Sattar SA, Springthorpe S, Mani S et al. Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using Staphylococcus aureus as a model. J Appl Microbiol 2001;90:962-70.
20. Vermeltfoort PB, van der Mei HC, Busscher HJ, Hooymans JM, Bruinsma GM. Physicochemical factors influencing bacterial transfer from contact lenses to surfaces with different roughness and wettability. J Biomed Mater Res B Appl Biomater 2004;71:336-42.
21. Whyte W, Bailey PV, Tinkler J, McCubbin I, Young L, Jess J. An evaluation of the routes of bacterial contamination occurring during aseptic pharmaceutical manufacturing. J Parenter Sci Technol 1982;36:102-7.
22. van Loosdrecht MC, Lyklema J, Norde W, Schraa G, Zehnder AJ. The role of bacterial cell wall hydrophobicity in adhesion. Appl Environ Microbiol 1987;53:1893-7.
23. Hogt AH, Dankert J, Feijen J. Adhesion of coagulase-negative staphylococci to methacrylate polymers and copolymers. J Biomed Mater Res 1986;20:533-45.
24. Satou N, Satou J, Shintani H, Okuda K. Adherence of streptococci to surface-modified glass. J Gen Microbiol 1988;134:1299-305.
25. Ramage G, Tunney MM, Patrick S, Gorman SP, Nixon JR. Formation of Propionibacterium acnes biofilms on orthopaedic biomaterials and their susceptibility to antimicrobials. Biomaterials 2003;24:3221-7.
26. An YH, Friedman RJ. Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. J Biomed Mater Res 1998;43:338-48.
27. Baker AS, Greenham LW. Release of gentamicin from acrylic bone cement. Elution and diffusion studies. J Bone Joint Surg Am 1988;70:1551-7.

28. McAllister EW, Carey LC, Brady PG, Heller R, Kovacs SG. The role of polymeric surface smoothness of biliary stents in bacterial adherence, biofilm deposition, and stent occlusion. Gastrointest Endosc 1993;39:422-5.
29. Quirynen M, van der Mei HC, Bollen CM et al. An in vivo study of the influence of the surface roughness of implants on the microbiology of supra- and subgingival plaque. J Dent Res 1993;72:1304-9.
30. Granzow JW, Smith JW, Nichols RL, Waterman RS, Muzik AC. Evaluation of the protective value of hospital gowns against blood strike-through and methicillin-resistant Staphylococcus aureus penetration. Am J Infect Control 1998;26:85-93.
31. Douw CM, Bulstra SK, Vandenbroucke J, Geesink RG, Vermeulen A. Clinical and pathological changes in the knee after accidental chlorhexidine irrigation during arthroscopy. Case reports and review of the literature. J Bone Joint Surg Br 1998;80:437-40.
32. Reading AD, Rooney P, Taylor GJ. Quantitative assessment of the effect of 0.05% chlorhexidine on rat articular cartilage metabolism in vitro and in vivo. J Orthop Res 2000;18:762-7.
33. Rombouts JJ, Wery PE, Delloye C, Noel H, Vincent A. [Proper use of irrigation solutions in orthopedic surgery. Apropos of a case of chondrolysis due to chlorhexidine]. Acta Orthop Belg 1986;52:685-702.
34. Saatman RA, Carlton WW, Hubben K, Streett CS, Tuckosh JR, DeBaecke PJ. A wound healing study of chlorhexidine digluconate in guinea pigs. Fundam Appl Toxicol 1986;6:1-6.
35. Severyns AM, Lejeune A, Rocoux G, Lejeune G. Non-toxic antiseptic irrigation with chlorhexidine in experimental revascularization in the rat. J Hosp Infect 1991;17:197-206.

5

EVALUATION OF INTRA-OPERATIVE CULTURES IN ORTHOPAEDIC IMPLANT LOOSENING

Knobben BAS, Van der Mei HC, Busscher HJ, Van Horn JR

Journal of Bone and Joint Surgery (Br) 2005, submitted

Introduction

Prosthetic loosening is a common complication in orthopaedic implant surgery. Loosening is based either on mechanical failure (aseptic loosening) or on an infectious process surrounding the prosthesis (septic loosening). The percentage of septic loosenings in primary arthroplasty is approximately 1.5% for hip and 2.5% for knee implants and is much lower than the percentage of aseptic loosening.[1] The percentage however, of prosthetic joint infection after revision arthroplasty is 3.2% for hips and 5.6% for knees,[1] and can be as high as 40% for failed hip arthroplasties with a positive intra-operative culture.[2]

The exclusion of the diagnosis septic loosening is imperative in order to determine the proper management of patients in need of revision surgery, because both surgical management and outcome may differ depending on whether the arthroplasty loosening is infectious or mechanical in origin.[3-5] A wrong diagnosis will lead to treatment failure, increased morbidity and added costs to the healthcare system. The estimated cost of treating an infected arthroplasty is over $50,000 per episode, whereas a simple replacement due to aseptic loosening costs about $20,000.[6]

The incidence of prosthetic joint infection is grossly underestimated by current culture detection methods.[5;7;8] No single test is able to show the presence of periprosthetic infection in every case.[9] Low-grade infections in particular are difficult to distinguish from aseptic failure, often presenting only early loosening and persisting pain, or no clinical signs of infection at all.[10] The detection of bacteria in the tissue or biomaterial scrapings can be limited due to a low inoculum, or the formation of small-colony variants of *Staphylococcus aureus* and *Staphylococcus epidermidis*. In addition, concurrent treatment with antimicrobial agents before microbiological sampling can prevent bacterial growth in the laboratory and hence also limit detection.[11] It is therefore imperative, that current clinical practice with regard to the detection and subsequent treatment of prosthetic joint infection be reassessed. Moreover, similar as with intra-operative contamination during primary arthroplasties, false-positive (contamination during sampling or culturing process) or false-negative test results may occur with an impact on the treatment modality chosen.[12-14]

A recent study performed in our hospital on intra-operative culturing during revision surgery, revealed that the new routine hospital culturing method (used at that time) showed microbial growth in only 41% of the cases diagnosed as suspected septic loosening by the orthopaedic surgeon. A newly developed method of research laboratory culturing in which

both periprosthetic tissue and scrapings from the prosthesis surface itself are cultured both aerobically and anaerobically for a prolonged period of time, showed microbial growth in 64% (tissue culturing) to 86% (tissue and removed prosthesis) of the cultures.[15] Other studies showed that the detection of prosthetic joint infection can be improved by ultrasonication of the prosthesis[16] or PCR, detecting bacterial DNA in aseptically loosened total hip arthroplasties.[17]

The aim of this study is to re-evaluate our detection method of extensive culturing of both excised tissue and scrapings from the removed prosthesis during revision surgery of hip and knee, initially clinically diagnosed either as septic or aseptic loosening. Subsequently, it is investigated what the positive and negative predictive values of different intra-operative culturing types are for developing deep periprosthetic infection after the revision of the prosthesis. In this re-evaluation, we attempt to account for the fact that intra-operative contamination may occur during revision of a truly aseptically loosened prosthesis by comparing patients revised in an operating room with conventional airflow and with laminar airflow.

Materials and methods

Patients

In this study patients diagnosed with loosening of hip or knee implants undergoing revision surgery in our hospital were included in the period ranging from January 2003 to January 2004. All patients underwent standardized preoperative hygiene procedures. Routine antibiotic prophylaxis consisted of cefazoline, 1000 mg intravenously, twenty minutes before the operation for patients with aseptic loosening. Patients with suspected septic loosening often were already treated with antibiotics. All patients received postoperative anticoagulation (nadroparine, 0.3 mL subcutaneously combined with acenocoumarol orally). In total, 29 men and 30 women with a mean age of 68 years were included (see also Table I).

The prosthetic parts being revised were 11 total hip prostheses, 17 cups, 15 stems, 15 total knee prostheses and 1 femoral part of a total knee prosthesis. Before insertion of the primary prosthesis the original pathology was osteoarthritis in 44 cases, rheumatoid arthritis in 12 cases and avascular necrosis in 3 cases. During this primary insertion, cement was used in 34 of the 59 cases. The mean time the implant had been present in the body was 9.0 years. The

indication for revision was suspected septic loosening in 14 cases and suspected aseptic loosening in 45 cases.

Table I. Baseline characteristics of the three groups.

	Septic loosening (N=14)	Aseptic loosening (N=45)	Total group (N=59)
Mean age (± SD) (in years)	66.7 (52.8-80.6)	67.9 (61.8-74.0)	67.6 (55.3-79.9)
Male/female	8/6	21/24	29/30
Cement/no cement	9/5	25/20	34/25
Time prosthesis in situ (± SD) (in years)	0.7 (0.2-1.2)	11.4 (5.6-17.2)	9.0 (2.2-15.8)
Prosthetic component			
- THA	6	5	11
- Cup		17	17
- Stem		15	15
- TKA	8	7	15
- Femoral part TKA		1	1
Osteoarthritis	10	34	44
Rheumatoid arthritis	3	9	12
Avascular necrosis	1	2	3

THA: total hip arthroplasty
TKA: total knee arthroplasty

Intra-operative culturing

New routine hospital culturing

During revision surgery at least three tissue samples were obtained of suspected infected areas (including capsular tissue and membrane tissue) and an aspirate of joint fluid was taken upon entering the capsule. Within 2 hours after sampling, tissue samples and aspirate were transported to the hospital laboratory and handled within 1 to 4 hours. Samples were incubated for three weeks on blood and chocolate agar at 35°C under aerobic conditions; plate inspection occurred during the first four days and at days 7, 14 and 21. Samples were also incubated on brucella blood agar for 10 days at 35°C under anaerobic conditions; these plates were inspected at days 2, 4, 6, 8 and 10. Subsequently, Gram staining was done and strains were identified by growing on selective agar or performing specific tests.

Research laboratory tissue culturing

The excised tissue samples and joint fluid aspirate were transported to our biomaterial research laboratory within 2 to 4 hours to be handled immediately. The tissue samples were streaked on blood agar plates and the joint fluid aspirate was put on these plates as well. The agar plates were incubated for 7 days at 37°C both aerobically and anaerobically. Plates were inspected during the first 4 days and at day 7. Positive samples were taken for Gram-staining.

Subsequently, a catalase test and DNase test were performed to identify Coagulase negative staphylococci (CNS) and *S. aureus*.

Research laboratory biomaterial culturing

The explanted prosthetic parts were put in a sterile organ bag and transported in cooled (4°C) reduced transport fluid (NaCl 0.9g/L, $(NH_4)_2SO_4$ 0.9g/L, KH_2PO_4 0.45g/L, $MgSO_4$ 0.19 g/L, K_2HPO_4 0.45 g/L, ethylenediaminetetraacetic acid 0.37 g/L, L-Cysteine HCl 0.2 g/L: pH 6.8) and also transported to our biomaterial research laboratory within 2 to 4 hours to be handled immediately. As many parts as possible of every prosthesis were scraped with surgical knives after which the knife was streaked on blood agar plates. The plates were handled as described above.

Postoperative infectious complications

In order to investigate whether infectious complications occurred related to the revision surgery, all patients were followed for at least 18 months. First, patients were monitored during their stay at the orthopaedic ward. After their discharge from the hospital during the postoperative controls at standard times after surgery, C-reactive protein level (CRP), erythrocyte sedimentation rate (ESR) and a white blood cell count were performed. In the absence of other foci for infection, a prosthesis was considered infected in case of an increase of infection parameters.

Conventional versus laminar airflow

Patients were included during use of a conventional air flow system, known to yield 34% intra-operative contamination, and after installation of a new, laminar flow system (see Table II for specifications of both air flow systems), reducing the number of intra-operative contamination to 9%.[18] By comparing the occurrence of intra-operative contamination under both conditions for aseptic and septic loosening, its role during revision surgery and the development of deep periprosthetic infection will be assessed.

Statistical analysis

The Pearson's Chi-square test for categorical data was used to test differences between the groups, when all cells of the contingency table contained at least 5 persons. Otherwise the Fisher's exact test was used. Statistical calculations were performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL). For the three types of intra-operative culturing

predicting the occurrence of deep periprosthetic infection both the positive and the negative predicative value were calculated.

Table II. Characteristics of the old conventional and the new laminar airflow system as used in this study.

	Old situation: Conventional airflow system	**New situation: Laminar airflow system**
Total air	2700 m^3 /h	8100 m^3 /h
Fresh air	2700 m^3 /h	2700 m^3 /h
Recirculating air	None	5400 m^3 /h
Plenum size	240 x 300 cm (7.2 m^2)	320 x 320 cm (10.2 m^2)
Type HEPA* filter	Cassette filter	Plate filter
Bottom layer ceiling	Perforated steel	Polyester distribution cloth
Air conduction	None	Glass panels extending from ceiling
Air inflow speed	10 cm/sec	20 cm/sec
Airflow principle	Mixing	Diluting
Ventilation of fresh air	22/h	22/h
Total Ventilation of air	22/h	60/h
Dilution at operating table	22/h	240/h

* High Efficiency Particulate Air filter

Results

Intra-operative culturing

In the total group of 59 patients, new routine hospital culturing showed microbial growth in 11 of the 59 (18.6%) cases in at least one of the cultures. The research laboratory tissue culturing performed in our laboratory revealed bacteria in 22/59 (37.3%) cases and the culturing of the biomaterial showed growth in 30/59 (50.8%) of the cases. Table III lists the type of organism cultured and their frequency, found with the three techniques. It can be seen that CNS was identified with biomaterial culturing in 22 of the 38 (57.9%) positive samples, *S. aureus* was seen in 6/38 (15.8%) samples and *P. acnes* in 3/38 (7.9%) samples. From biomaterial culturing, more than one bacterial strain was discovered in 8 cases.

Table III. Organisms found with the three culturing methods.

Organism	New routine hospital culture (Number)	Research laboratory tissue culture (Number)	Biomaterial culture (Number)
Aerobes:			
- CNS	7	15	22
- *S. aureus*	3	5	6
- Gram-positive cocci	1	3	3
- Gram-positive rods		1	1
- Gram-negative rods	1	1	1
Anaerobes:		4	
- *P. acnes*	1	1	3
- Gram-positive cocci	1	1	1
- Gram-negative rods	1		1
	15	31	38

Postoperative infectious complications

In the group of 14 patients undergoing surgery because of septic loosening, new routine hospital culturing showed microbial growth in 8 of the 14 (57.1%) cases, research laboratory tissue culturing performed in our laboratory revealed bacteria in 9/14 (64.3%) cases and the biomaterial culturing showed growth in all 14 cases. All 14 patients were treated with a two-stage revision combined with intravenous antibiotic therapy, after which 10/14 patients still showed infectious complications: 2/14 patients needed additional antibiotic treatment after reimplantation, 5/14 patients needed lavage on one or more occasions before reimplantation of the prosthesis, and 3/14 patients eventually had their prosthesis removed (1 girdlestone and 2 knee-arthrodeses). After 18 months of follow-up 1/14 patient had died because of sepsis and 3/14 still had elevated CRP and ESR-levels in their blood.

As demonstrated in table IV, the group of 45 patients with suspected aseptic loosening showed microbial growth in one or more cultures during new routine hospital culturing in only 4/45 (8.9%) cases, while the research laboratory tissue culturing performed in our laboratory showed growth in 13/45 (28.9%) cases, and the biomaterial culturing in 16/45 (35.6%) cases.

After a follow-up of at least 18 months 12/45 (26.7%) patients had developed a deep periprosthetic infection. It appeared that all 4 cases with a positive new routine hospital culture had developed a deep periprosthetic infection, but that also 8/27 cases with negative new routine hospital cultures had developed one. Of the 13 patients with positive research laboratory tissue cultures 8 developed an infection, meaning that 4/32 with negative research laboratory tissue cultures also developed an infection. Of the 16 patients with positive

biomaterial cultures 12 developed a deep periprosthetic infection. The remaining 29 patients with no growth in biomaterial cultures did not develop a deep periprosthetic infection during the follow-up of at least 18 months.

Table IV also shows that the negative predictive value of biomaterial culturing is 100%, suggesting that no septic loosening was missed with this method, whereas the negative predictive value of new routine hospital culturing was only 80%. In this case that means that 8 septic loosenings were wrongfully treated as aseptic ones, resulting in deep periprosthetic infection.

Table IV. Number of patients with suspected aseptic loosening developing deep periprosthetic infection (DPI) within the first 18 months of follow-up for each culturing type and the combination of research laboratory tissue culturing and biomaterial culturing. The positive predictive value and the negative predictive value are also shown.

	Bacterial growth		**No bacterial growth**		**PPV**	**NPV**
	DPI	No DPI	DPI	No DPI		
NRHC	4	0	8	33	100%	80%
RLTC	8	5	4	28	62%	88%
BC	12	4	0	29	75%	100%
RLTC and BC	12	6	0	27	67%	100%

NRHC: new routine hospital culturing; RLTC: research laboratory tissue culturing; BC: biomaterial culturing; DPI: deep periprosthetic infection; PPV: positive predictive value; NPV: negative predictive value.

Conventional versus laminar airflow

In order to compare the old and new operating room situation, the research laboratory culture method is used (the combination of research laboratory tissue culturing and biomaterial culturing). As can be seen from Figure 1A, 7 suspected septic loosenings were operated upon in the operating theatre with the conventional airflow system, and also 7 suspected septic loosenings were treated in the new situation with laminar airflow. It can be seen that both the 7 patients with suspected septic loosening in the old operating theatre and the 7 patients with suspected loosening in the new operating theatre showed microbial growth on research laboratory culturing.

Figure 1B shows that in the old situation 15/23 (65.2%) aseptic loosenings showed microbial growth in the research laboratory tissue and biomaterial cultures. In the operating theatre with the new laminar flow system the number of times bacterial growth was seen in the group with aseptic loosening was significantly smaller (3/22: 13.6%) than in the old operating theatre ($p=0.001$). Of the positive cultures in the old operating theatre 9/15 patients (60%)

developed a deep periprosthetic infection, compared to 3/3 patients (100%) with positive cultures in the new operating theatre.

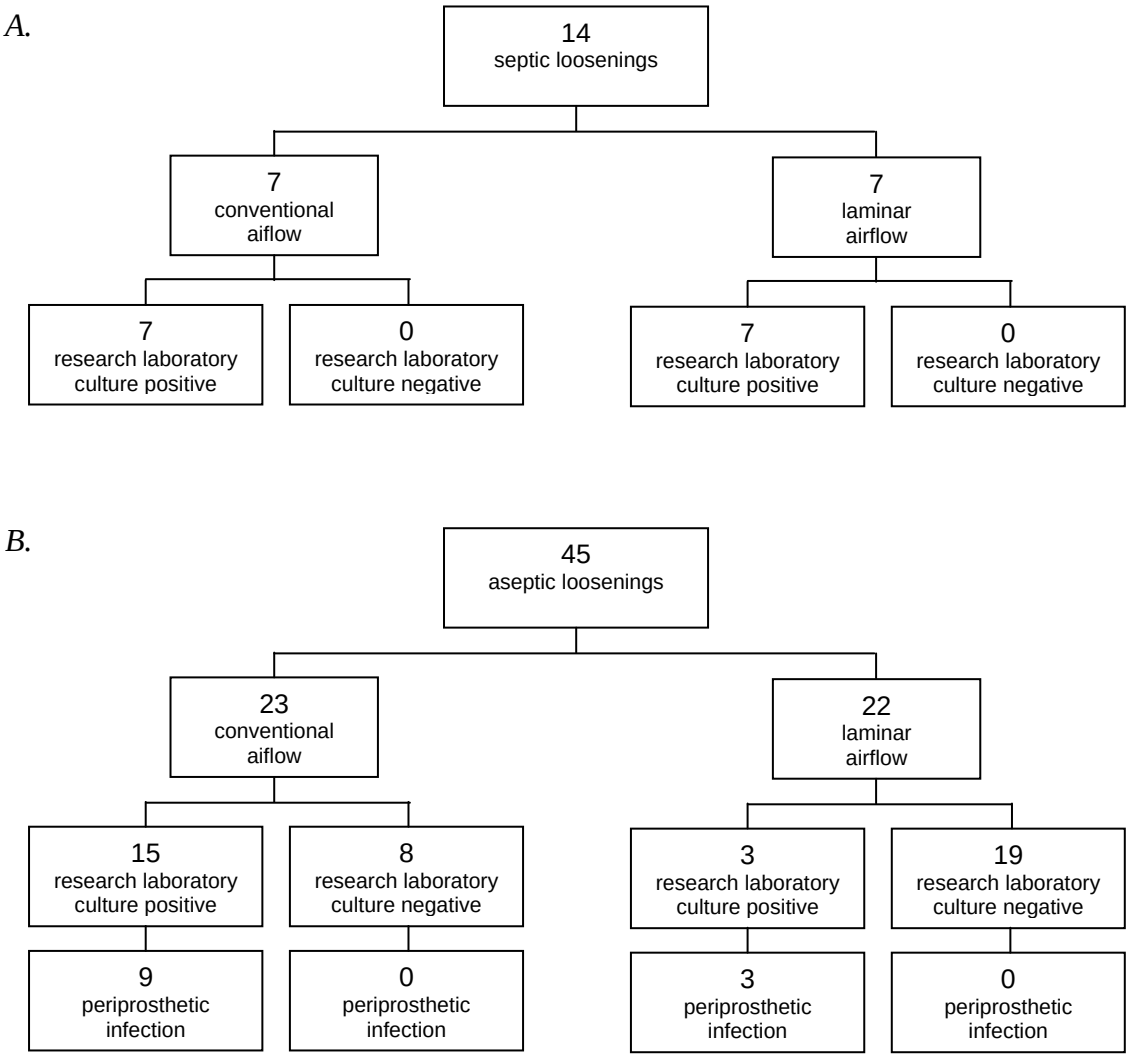


Figure 1. Septic and aseptic loosening in the operating theatre with conventional and with laminar airflow. The results of research laboratory tissue and biomaterial culturing for the suspected septic loosening is shown (A) as well as the outcome of the research laboratory tissue and biomaterial culturing in terms of periprosthetic infection for the suspected aseptic loosenings (B).

Discussion

This study shows the importance of biomaterial culturing in orthopaedic implant revision surgery in differentiating between septic and aseptic loosening. To our knowledge this is the first study to investigate the positive and negative predictive value of different intra-operative culturing types for developing deep periprosthetic infection after the revision of the prosthesis. This study also points out that in some cases in which the intra-operative culturing was positive for bacterial growth, this was probably due to intra-operative contamination and not due to an aseptic loosening that was wrongfully considered septic.

Biomaterial culturing showed microbial growth in our study in 100% of suspected septic loosenings and in 36% of the suspected aseptic ones, whereas new routine hospital culturing only showed growth in 57% of the septic cases and in only 9% of the suspected aseptic loosenings. These results are in accordance with the study by Neut et al. performed in our hospital.[15] From table III it can be seen that the bacteria that were found the most are CNS (N=20), *S. aureus* (N=6) and anaerobes (N=6), of which half were determined as *P. acnes*. This is in accordance with other studies regarding this topic.[8;10;16;19-20] All three bacteria have also been proven to be able to grow biofilms on prostheses.[21]

In most cases of suspected septic loosening patients were operated upon while being treated already with antibiotics. It is recommended however to discontinue antimicrobial therapy at least two weeks before tissue sampling.[22] This might explain why in only 57-64% of the suspected septic loosenings bacterial growth was shown in the tissue culturing. On the other hand, it did not seem to affect the sensitivity of culturing biomaterial scrapings (100%), evidently because micro-organisms growing in a biofilm are up to 1,000 times more resistant to growth-dependent antimicrobial agents than their planktonic (free-living) counterparts.[23;24] A limitation to this study regarding the follow-up is the fact that patients with positive new routine hospital cultures received antibiotics against the bacteria identified. Nevertheless, all four cases developed deep periprosthetic infection.

Regarding the discussion whether aseptic loosening exists or if its part of the prosthetic loosenings is overrated, our results suggest that with biomaterial culturing 36% (16/45 cases) were in fact septic loosenings. New routine hospital culturing only showed growth in 9% (4/45) of the cases, meaning that 12 cases (27%) were wrongfully treated as aseptic loosenings.

Diagnosing prosthetic loosening is extremely difficult. Many hospitals use preoperative aspiration as some kind of golden standard. It is believed by some that a combination of

preoperative and intra-operative tests is needed for an accurate diagnosis of infection of prosthetic joint infections.[10] Others state that there is no generally established definition of a deep infection, most diagnostic tools are hampered by varying accuracy, and the current low prevalence of deep infection make new diagnostic tools (such as PCR) difficult to evaluate.[8;9;17;25;]

Intra-operative culturing is considered to provide the most accurate specimens for microbiological cultures and is frequently used as the reference standard for diagnosing orthopaedic implant infection.[8;16;26] Several investigators have suggested ways to perform intra-operative tissue sampling and culturing and suggest that at least three intra-operative tissue specimens should be sampled for culture.[20;27] Atkins et al. recommend that five or six specimens be sent, and that the cut-off for a definite diagnosis of infection be three or more operative specimens that yield an indistinguishable organism.[19] Others state that intra-operative culturing during revision total hip surgery is an unreliable predictor of sepsis, but they only cultured three joint fluid samples, not tissue samples.[28] Alternatives are biomaterial culturing or histology. The latter has been proven to be better than intra-operative tissue sampling.[16;20]

Regarding biomaterial culturing, Spanghel et al. found that there was no substantial difference between the results on culture of tissue compared with those on culture of material obtained by swabbing of the prosthesis.[27] Our results show otherwise. This is because bacteria on a biomaterial grow in a biofilm mode of growth, which firmly anchors and protects the bacteria from being swabbed off. Alternatives for scrapings are multifocal laser scanning of the biomaterial or ultrasonication of the prosthesis.[15;16] A disadvantage of biomaterial culturing is the risk of contamination during the prosthesis culturing process may be high, hence leading to false-positive results.[22] The somewhat low positive predictive value of biomaterial culturing for developing periprosthetic infection might be explained by that.

A factor that might be overlooked in most studies regarding septic and/or aseptic loosenings is the role of intra-operative contamination. Therefore we compared the suspected aseptic loosenings that were operated upon in an operating theatre with conventional airflow with the ones that were treated in an operating theatre with laminar airflow. We found that the number of times bacterial growth was seen in the operating theatre with the new laminar flow system was significantly smaller than in the old operating theatre (P=0.001), suggesting that in many cases intra-operative contamination might have played a key role and this may have caused the deep periprosthetic infection in some of the 9 patients. Another study performed in our hospital confirms this, as with primary arthroplasty the intra-operative bacterial

contamination level dropped from 34% to 9% when the airflow system was replaced, among other things.[18]

In conclusion, culturing of biomaterial scrapings is imperative in differentiating between septic and aseptic prosthetic loosening, with a positive predictive value of 75% and a negative predictive value of 100% for the occurrence of periprosthetic infection after revision arthroplasty. The increased incidence of infection after revision surgery as compared to primary arthroplasties may to our mind be partly due to the fact that the revision took place in a septic environment that hitherto had not been recognized as such and had been treated as an aseptic and biomechanical loosening.

References

1. Hanssen AD, Rand JA. Evaluation and treatment of infection at the site of a total hip or knee arthroplasty. Instr Course Lect 1999;48:111-122.
2. Dupont JA. Significance of operative cultures in total hip arthroplasty. Clin Orthop Relat Res 1986;122-127.
3. Hamblen DL. Diagnosis of infection and the role of permanent excision arthroplasty. Orthop Clin North Am 1993;24:743-749.
4. Masterson EL, Masri BA, Duncan CP. Treatment of infection at the site of total hip replacement. Instr Course Lect 1998;47:297-306.
5. Morscher E, Babst R, Jenny H. Treatment of infected joint arthroplasty. Int Orthop 1990;14:161-165.
6. Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS, Osmon DR. Risk factors for prosthetic joint infection: case-control study. Clin Infect Dis 1998;27:1247-1254.
7. Hamblen DL. Diagnosis of infection and the role of permanent excision arthroplasty. Orthop Clin North Am 1993;24:743-749.
8. Tunney MM, Patrick S, Curran MD, Ramage G, Hanna D, Nixon JR, Gorman SP, Davis RI, Anderson N. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J Clin Microbiol 1999;37:3281-3290.
9. Virolainen P, Lahteenmaki H, Hiltunen A, Sipola E, Meurman O, Nelimarkka O. The reliability of diagnosis of infection during revision arthroplasties. Scand J Surg 2002;91:178-181.
10. Trampuz A, Zimmerli W. Prosthetic joint infections: update in diagnosis and treatment. Swiss Med Wkly 2005;135:243-251.
11. Widmer AF. New developments in diagnosis and treatment of infection in orthopedic implants. Clin Infect Dis 2001;33 Suppl 2:S94-106.
12. Davis N, Curry A, Gambhir AK, Panigrahi H, Walker CR, Wilkins EG, Worsley MA, Kay PR. Intraoperative bacterial contamination in operations for joint replacement. J Bone Joint Surg Br 1999;81:886-889.
13. Knobben BAS, Engelsma Y, Neut D, Van der Mei HC, Busscher HJ, Van Horn JR. Intra-operative contamination influences wound discharge in orthopaedic implant surgery. Clin Orthop Relat Res 2006.
14. Maathuis PG, Neut D, Busscher HJ, van der Mei HC, van Horn JR. Perioperative contamination in primary total hip arthroplasty. Clin Orthop Relat Res 2005;136-139.
15. Neut D, van Horn JR, van Kooten TG, van der Mei HC, Busscher HJ. Detection of biomaterial-associated infections in orthopaedic joint implants. Clin Orthop Relat Res 2003;261-268.
16. Tunney MM, Patrick S, Gorman SP, Nixon JR, Anderson N, Davis RI, Hanna D, Ramage G. Improved detection of infection in hip replacements. A currently underestimated problem. J Bone Joint Surg Br 1998;80:568-572.
17. Clarke MT, Roberts CP, Lee PT, Gray J, Keene GS, Rushton N. Polymerase chain reaction can detect bacterial DNA in aseptically loose total hip arthroplasties. Clin Orthop Relat Res 2004;132-137.
18. Knobben BAS, van Horn JR, van der Mei HC, Busscher HJ. Evalutation of measures to decrease intra-operative bacterial contamination in orthopaedic implant surgery. J Hosp Infect 2005;62(2):174-802.
19. Atkins BL, Athanasou N, Deeks JJ, Crook DW, Simpson H, Peto TE, McLardy-Smith P, Berendt AR. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. The OSIRIS Collaborative Study Group. J Clin Microbiol 1998;36:2932-2939.
20. Pandey R, Berendt AR, Athanasou NA. Histological and microbiological findings in non-infected and infected revision arthroplasty tissues. The OSIRIS Collaborative Study Group. Oxford Skeletal Infection Research and Intervention Service. Arch Orthop Trauma Surg 2000;120:570-574.
21. Ramage G, Tunney MM, Patrick S, Gorman SP, Nixon JR. Formation of Propionibacterium acnes biofilms on orthopaedic biomaterials and their susceptibility to antimicrobials. Biomaterials 2003;24:3221-3227.
22. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med 2004;351:1645-1654.
23. Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis 2002;8:881-890.
24. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet 2001;358:135-138.
25. Ince A, Rupp J, Frommelt L, Katzer A, Gille J, Lohr JF. Is "aseptic" loosening of the prosthetic cup after total hip replacement due to nonculturable bacterial pathogens in patients with low-grade infection? Clin Infect Dis 2004;39:1599-1603.
26. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. JAMA 1998;279:1537-1541.

27. Spangehl MJ, Masri BA, O'Connell JX, Duncan CP. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. J Bone Joint Surg Am 1999;81:672-683.
28. Padgett DE, Silverman A, Sachjowicz F, Simpson RB, Rosenberg AG, Galante JO. Efficacy of intraoperative cultures obtained during revision total hip arthroplasty. J Arthroplasty 1995;10:420-426.

6

INTRA-OPERATIVE CULTURING MAY LEAD TO COST REDUCTION IN ORTHOPAEDIC IMPLANT SURGERY

Knobben BAS, Stant AD, Busscher HJ, Van Horn JR, Van der Mei HC

Journal of Bone and Joint Surgery (Br) 2005, submitted

Introduction

Osteoarthritis is a slowly progressive degenerative disease that afflicts more than two-thirds (68%) of persons older than 55 years of age,[1] and becomes more prevalent with advancing age.[2;3] Presently, 43 million individuals have arthritis and by the year 2020, it is estimated that 59.4 million persons will be affected by this disease world wide.[1] Therewith arthritis is the most frequently reported chronic condition in the elderly. The Centres of Disease Control and Prevention in 1994 reported that by the year 2020, arthritis will have the largest increase in numbers of new patients of any disease in the United States.[4] By the year 2030, it is estimated that there will be an 85% increase in knee replacements and an 80% increase in hip replacements.

Osteoarthritis has a significant impact on psychosocial and physical function and is the leading cause of disability in later life.[5] Osteoarthritis however, is not only a disease of old age. Age of onset varies depending on the involved joint[2;3] and involves more than three out of every hundred persons below age 45 and more than 25 out of every hundred persons between the ages of 45 and 64 suffer from this disabling disease.[6;7] There are significant out-of-pocket costs and loss of earnings due to changes in occupation and in domestic duties.[5] Charges in 1993 in a managed care organization attributable to osteoarthritis per person-year were twice the rate as in patients without arthritis.[8] The high prevalence of osteoarthritis in the population is reflected in the high costs to treat patients suffering from this affliction. The cost of arthritis in the year 2000 was estimated at 95 billion dollars.[1]

Like all biomedical devices, total hip replacements can wear out and have a life expectancy of 10 to 30 years of service.[9] Approximately 10% of all hip replacements will fail and require revision surgery.[9] Revision surgery is also considered to be cost-effective,[10;11] but is more costly, may require a significantly longer hospital stay, incurs higher complication rates, and has a poorer prognosis than the original joint replacement procedure.[10-17]

Refinements in sterilization and improvement in the quality of bearing surfaces are expected to improve longevity and reduce the need for revision surgery. In the United States alone, $200-250 million is spent annually on treating infected joints.[18] Bacteria are most likely present in every operating room, and whether a prosthesis can actually be implanted without intra-operative bacterial contamination remains an open question. Previous studies have shown that intra-operative bacterial contamination occurred in 36% of all cases during insertion of a

primary prosthesis, with significant postoperative consequences, including deep periprosthetic infection and prolonged wound discharge.[19]

This study encompasses an economic evaluation of prosthetic joint infections. Economic evaluations in medicine are aimed at the quality-cost-ratio of care. In the study presented now the economic aspects of illness of failing prostheses properly dealing with bacterial contamination during prosthetic replacements in orthopaedic surgery and the ensuing biomaterials related infection together with the complete eradication of the infection leading to complaint free cure for the patient were determined by means of a 'cost-of-illness' approach. Firstly, the scope of the social costs generated by patients who undergo a primary or revision-operation for a hip or knee implant are evaluated, as well as the cost increase upon development of a deep periprosthetic infection.

Furthermore, it was investigated whether there are differences in these costs between patients with positive and negative intra-operative cultures in order to demonstrate that intra-operative culturing could reduce societal costs associated with periprosthetic infection due to intra-operative contamination.

Materials and methods

Patients

We prospectively analyzed primary and revision hip and knee arthroplasties in the Orthopaedic Department of the University of Groningen Medical Centre, Groningen, The Netherlands. The study and its protocol were approved by the hospital Ethical Committee. In order to obtain a representative sample over the predefined inclusion period of one year (thus minimizing periodic effects), we used a list of random numbers, generated by computer, which determined whether intra-operative culture methods would be applied for that patient. All patients were followed for registration of total costs and complications, especially deep periprosthetic infection. We restricted the number of patients that were included so as to minimize the burden for the personnel involved, since the protocol was not yet part of standard practice at the time the study was conducted. We included 50 patients undergoing a primary knee or hip arthroplasty and 40 patients undergoing a revision of their arthroplasty. Patients with a positive intra-operative culture formed the study group, and patients without intra-operative contamination were the control group.

Patients in whom a second prosthesis on another joint was inserted during the eighteen months of the follow-up were excluded from the study. Surgery took place in an operating theatre where conventional air flow was used, and the operating team wore disposable impervious drapes. At the end of surgery, drains were placed at the operation site in all patients. All 50 patients in the primary group received antimicrobial prophylaxis (cefazoline, 1000 mg intravenously) twenty minutes before the operation. The patients in the revision group received different kinds of antimicrobial prophylaxis, depending on the indication of surgery. All included patients received postoperative anticoagulation (nadroparine, 0.3 mL subcutaneously combined with acenocoumarol orally).

Measurement of intra-operative contamination

During primary arthroplasties, samples were taken intra-operatively at different stages of the procedure, consisting of four instrument swabs and two portions of removed bone, as described in a previous study.[20] During revision surgery, three tissue samples were obtained of suspected infected areas (including capsular tissue and membrane tissue), an aspirate of joint fluid was taken upon entering the capsule, and the explanted biomaterial was cultured, using the method described by Neut et al.[21] During some procedures, a clean swab was taken out of the charcoal medium in the operating room and left in the open for a short while after which it was put back in the medium in order to check whether contamination occurred during transport and culturing of the samples. Cultured material was considered contaminated, when bacterial growth was observed, regardless of the amount of growth. The study was performed blind, without informing the orthopaedic surgeon on the test result in order to ensure that all patients were treated according to protocol regardless of the results of the evaluation.

Follow-up

In order to investigate whether infectious complications occurred post-operatively all patients were followed up for 18 months. During the standard regular checkups after surgery C-reactive protein assay, erythrocyte sedimentation rate and a white blood cell count were done. A prosthesis was considered infected in case of elevated infection parameters when other foci of infection were carefully excluded, as judged by the orthopaedic surgeon.

Economic evaluation

The economic evaluation was conducted from a societal persepective; both medical costs and costs outside the healthcare sector were assessed. The time-horizon of the evaluation covered a period of twelve months. The robustness of the results of the economic evaluation was examined by means of various sensitivity analyses. Costs were not discounted due to the relatively short time-horizon of the study. The types of costs that were included in the analyses are noted in Table I.

Table I. Cost categories and types of costs.

Direct medical costs	Direct non-medical costs	Indirect non-medical costs
Inpatient and semi-inpatient care	Informal care	Productivity losses (un)paid work
Medical intervention, surgery	Travel costs	Productivity losses without absence from work
General health care	Out-of-pocket costs	
Medication		

Costs of informal care were registered in detail in the present study. Besides informal care consisting of household work, various other forms of support that family members or acquaintances can provide were also assessed, like accompanying patients to healthcare professionals. Out-of-pocket costs are various additional costs directly related to the illness, like costs of adjustments in the house related to experienced physical problems. Costs of productivity losses due to illness-related absence from work were estimated by means of the friction cost method.[22] In addition, costs of decreased productivity without absence from work were also assessed. Costs related to the inability to perform voluntary work were estimated by hourly wages for professional household workers.

Cost data were registered prospectively for all patients included in the study. Most of the information was collected by means of a detailed questionnaire on costs as incurred by the patient and his or her family. This questionnaire was sent to the patient three (T1), six (T2), and 12 months after inclusion (T3). The questionnaire assessed, among others, admissions to hospitals, contacts with healthcare professionals, and absence from work. Additional information was collected from healthcare professionals involved who were interviewed for

instance on the prescribed medication.

In order to facilitate comparisons with other economic evaluations, unit prices, i.e. the price of one unit of each included cost type, were mainly based on Dutch standard prices.[23] True costs of used resources were estimated when standard prices were not available. Costs of surgery were estimated by means of the College of Dutch Healthcare Rates. All unit prices were based on the price level of the Euro in the year 2004. Reference prices established for previous years were adjusted to prices of 2004 by applying the consumer price index.

Statistical analysis

Total costs per cost category will be described for patients in the primary and revision groups, presented results will differentiate between positive and negative culture outcomes within these groups. Total costs during the study were log transformed, due to the skewly distributed costs, and subsequently analysed using mixed model methodology (SPSS 12). Mixed models are strongly preferred for longitudinal analyses since all available data can be used, including data of patients for whom not all the measurements are available. In the analyses, a level of significance of $P<0.05$ was assumed.

Results

Patient groups and culturing results

Results of the analyses are based on the data of 50 patients in the primary and 35 in the revision group. One patient in the revision group was operated on different joints on several occasions, and was accidentally included twice in the study. This patient was excluded from all analyses, leaving a group of 38 revision patients. During the one-and-a-half-year year follow-up three patients in the revision group died, all because of reasons not related to the prosthesis. Therefore, data of these patients were excluded from most analyses, except from the mixed model and sensitivity analyses.

The primary group of 50 patients consisted of 34 women and 16 men, receiving 36 hip and 14 knee prostheses. The mean age was 65.8 years (40-84) and the indication for surgery was osteoarthritis in 40 patients and rheumatoid arthritis in 10 patients. The revision group of 35 patients consisted of 24 women and 11 men, having a revision of 8 total knee prostheses, 14 total hip prostheses, 1 stem prosthesis and 12 cups. The indication for surgery was aseptic

loosening in 25 cases and septic loosening in 10 cases. The mean age was 65.9 years (37-91) and 9 patients suffered from rheumatoid arthritis.

In the primary group intra-operative culturing gave bacteria in 21 of the 50 cases (42%) and in the revision group in 23 of the 35 cases (65.7%). Microbial growth was found during 13 of the 25 revisions (52%) because of aseptic loosening and in all 10 revisions because of septic loosening. The control swab was negative, i.e. it yielded no growth at all times. In both the primary and the revision group base characteristics like “rheumatoid arthritis”, “gender”, and “hip or knee prosthesis” were not significantly different in the groups with and without intra-operative contamination.

Total costs of primary or revision surgery

Within the follow up of 18 months 2 patients with a hip prosthesis developed a deep periprosthetic infection, with a cost of €45,034 and €59,180. The mean total cost of patients without a deep periprosthetic infection (N=48) was €15,376, ranging from €5890 to €53,247.

The mean total costs of a primary hip because of osteoarthritis without periprosthetic infection and without rheumatoid arthritis (N=27) were €12,982 (€5890 - €53,247), and the total costs of a primary knee because of osteoarthritis without periprosthetic infection (N=12) were €12,366 (€6371 - €23,094). The costs for patients without periprosthetic infection suffering from rheumatoid arthritis (€26,573) were twice as high as for patients with osteoarthritis (€12,793).

The mean total costs of a revision of a prosthesis accounted €41,356, with a minimum of €10,360 and a maximum of €123,829. The mean costs were €60,290 for revision of a total knee prosthesis, €40,387 for revision of a total hip prosthesis, €30,746 for revision of a cup, and revision of the stem prosthesis was €30,768. The mean total costs of revision of a prosthesis with indication of aseptic loosening (N=25) were €36,798, and those of a prosthesis with indication of septic loosening (N=10) were €52,750. Again the costs for patients with rheumatoid arthritis were almost twice as high (€63,916) as for patients without osteoarthritis (€33,547).

Overview cost categories with and without intra-operative contamination

Table II shows the direct medical costs generated by both groups during the 12 months of the study. The results displayed differentiate between patients with positive and negative intra-operative culture outcomes. Costs of hospital and supplemental admissions were

substantial in all groups; costs of supplemental admissions were higher than those of admissions related to the initial surgery when the implant was placed. Considerable costs were related to contacts with physiotherapists and homecare. Overall, costs in the revision group were much higher than costs in the primary group.

Table II. Direct medical costs (€) incurred during T0 through T3.

Types of costs	PI group (N=50)		RO group (N=35)	
	Positive culture Mean costs (SD)	**Negative culture Mean costs (SD)**	**Positive culture Mean costs (SD)**	**Negative culture Mean costs (SD)**
Inpatient and semi-inpatient care				
Initial hospital admission	6189 (6833)	2596 (1014)	13738 (10794)	5948 (3113)
Supplemental admissions	7639 (9576)	3617 (5519)	18509 (18619)	6423 (6741)
Day care	0 (-)	8 (43)	19 (64)	36 (129)
Medical interventions				
Primary surgery	1445 (32)	1446 (32)	-	-
Revision surgery	2449 (755)	0 (-)	2515 (632)	2084 (205)
Implants	2511 (642)	2582 (633)	3103 (2160)	2693 (1181)
General health care				
General practitioner	16 (33)	7 (18)	21 (17)	25 (22)
Physiotherapist	852 (619)	461 (236)	1285 (740)	915 (501)
Ergotherapist	0 (-)	0 (-)	6 (28)	0 (-)
Alternative health care	0 (-)	4 (22)	0 (-)	0 (-)
Home care	215 (539)	121 (457)	775 (995)	771 (1132)
Emergency care	7 (31)	10 (36)	6 (28)	0 (-)
Other general health care	0 (-)	1 (5)	9 (35)	2 (7)
Medication				
Antibiotics	39 (95)	1 (4)	404 (577)	0 (-)

Table III shows the costs generated outside the health care sector. Costs of the various types of informal care were substantial in all groups. None of the patients in the primary group was working during the study, therefore there were no productivity losses for paid work in this group. In the revision group costs of productivity losses of paid work was considerable.

Table III. Direct and indirect non-medical costs (€) incurred during T0 through T3.

Types of costs	PI group (N=50)		RO group (N=35)	
	Positive culture Mean costs (SD)	Negative culture Mean costs (SD)	Positive culture Mean costs (SD)	Negative culture Mean costs (SD)
Direct non-medical costs				
Travel costs	28 (53)	11 (29)	119 (445)	22 (32)
Informal care (household work)	1685 (2086)	1205 (1977)	2114 (3470)	1050 (1882)
Other informal care	733 (1340)	828 (2303)	1798 (1628)	971 (878)
Non-prescribed medication	13 (61)	10 (56)	0 (1)	1 (3)
Out-of-pocket costs	253 (782)	140 (428)	764 (2532)	65 (93)
Indirect non-medical costs				
Productivity losses paid work	0 (-)	0 (-)	2634 (5371)	1246 (3091)
Productivity losses voluntary work	151 (400)	60 (254)	210 (875)	176 (372)
Productivity losses paid work without absence	0 (-)	0 (-)	11 (55)	0 (-)

Total costs during the study with and without intra-operative contamination

An overview of the total costs during the study is provided in Figure 1A and B. Most of the costs were generated during T0-T1. Total costs of patients with positive culture outcomes were considerably higher at each measurement than costs of patients with negative culture outcomes in both the primary and revision group.

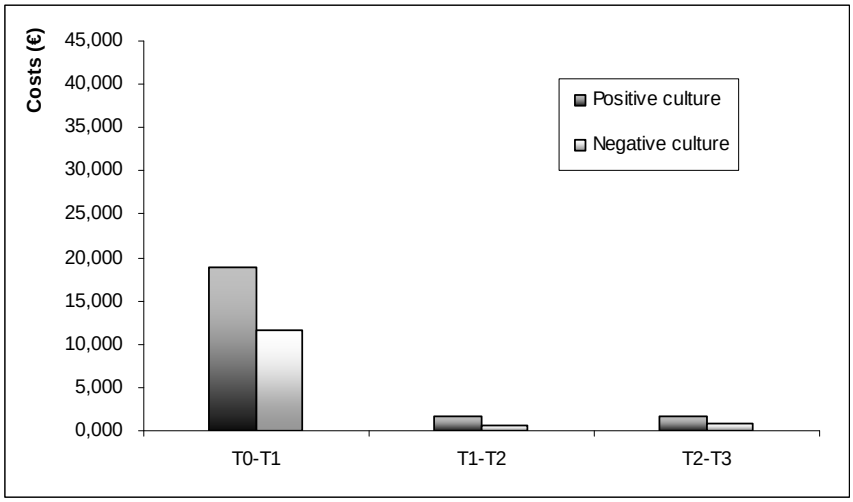
Longitudinal analyses of costs were conducted by means of mixed model methodology, results are presented in Table IV. In the primary group, there was no significant effect of culture results on total costs. However, the effect of time was significant, costs during the initial measurement period (T0-T1) were substantially higher than costs during later measurements (T1-T2 and T2-T3). In the revision group a significant effect of culture results was found, patients with positive culture outcomes generated significantly higher costs than patients with negative culture outcomes. Furthermore, the effect of time was significant as well. The interaction between culture and time approached statistical significance in the revision group; there are indications that total costs of patients with positive culture outcomes demonstrate a different pattern over time than costs of patients with negative culture outcomes.

Table IV. Cost analysis; ANOVA (ANalysis Of VAriance between groups) table for the mixed effect analyses.

Outcome measure with modeleffects	Siginifance (p)
Total costs primary group	
Culture	0.45
Time	<0.001
Culture * Time	0.77
Total costs revision group	
Culture	<0.01
Time	<0.001
Culture * Time	0.05

Mixed effect analyses included a random effect of subject. Analyses were conducted after log transformation of the cost data.

A. Primary group



B. Revision group

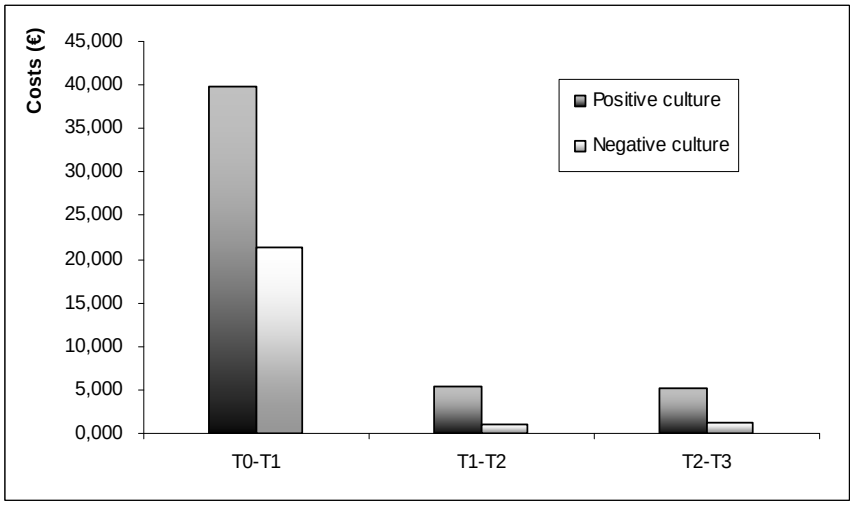


Figure 1. Mean total costs during study (differentiated between positive en negative culture outcomes).

Sensitivity analyses

The conducted sensitivity analyses consisted of varying the most influential types of costs, i.e. costs that amounted to at least 5% of total costs. The identified types of costs were costs related to surgery, implants and hospital and nursing home admissions. For the primary group, (household) informal care was also included in the sensitivity analyses, whereas productivity losses were included for the revision group. The variation consisted of increasing the identified cost types in one group with 20%, while at the same time decreasing costs with 20% in the other group. The consequences for (differences in) total costs are presented in Table V. Mean costs of patients with positive culture outcomes were considerably higher for all the conducted sensitivity analyses, which was most evident for patients in the revision group.

Table V. Sensitivity analyses: variation of influential cost types.

Sensitivity analyses	PI group (N=50)			RO group (N=35)		
	Positive culture Mean costs	Negative culture Mean costs	Difference in costs*	Positive culture Mean costs	Negative culture Mean costs	Difference in costs*
Standard analyses	22009	13107	8902	50578	23681	26897
+20% positive culture, -20% negative culture	25949	10818	15131	59082	19817	39265
-20% positive culture, +20% negative culture	18068	15396	2673	42073	27545	14528

* Difference in mean total costs between patients with positive and negative culture outcomes

Discussion

This study focussed on the total costs of primary and revision arthroplasty in a tertiary care unit in The Netherlands. As far as we know, this is the first study that, besides the direct medical costs, also included the direct and indirect non-medical costs in the first 12 months after surgery in the calculations. Our results show that the mean total costs generated by patients undergoing surgery for primary hip or knee arthroplasty are €16,846, and the mean total costs of patients undergoing revision surgery are €41,356.

The total amount of costs generated during the study was largely influenced by costs related to surgery, implants, hospital admission, and subsequent nursing home admission.

These results are in accordance with other studies, taking into account that they only included the direct medical costs.[12;14-16] By far the most costs were generated during the first three months of the study (T0-T1). The costs for patients suffering from rheumatoid arthritis were twice as high as for patients without rheumatoid arthritis, in both the primary and the revision group.

Intra-operative bacterial contamination is likely to be present in every operating theatre. However, scepticism about the importance of intra-operative contamination still remains. Although it is generally believed that every operating room is contaminated to some extent, it is not always clear whether this contamination is a risk for periprosthetic infection. We believe that every bacterium colonising a primary prosthesis, but not identified and eradicated, will likely infect the new prosthesis and put it at risk of failure. Previous studies of our group have pointed out that our methods of intra-operative culturing are significantly associated with postoperative infectious complications, including deep periprosthetic infection after primary arthroplasty and re-infection after revision surgery.[19;24]

Besides the devastating effect a deep periprosthetic infection has on the patient, it is also associated with very high costs to the healthcare system. Although only two patients from the primary group developed a deep periprosthetic infection, our results show that when a primary prosthesis gets infected the costs increase more than three times. The mean costs for a septic revision were €52,750 (N=10), which also resembles the results of previously performed studies on this topic.[15;18] Prevention of periprosthetic infection is therefore imperative, also from an economic point of view. In a previous study we proved that installing a new laminar air flow system and taking behavioural measures in the operating room, drastically decreased the percentage of intra-operative contamination from 33% to 5%.[20] In another study we found that the percentage of periprosthetic infection after revision of an aseptically loosened prosthesis decreased from 39% in the operating theatre with conventional airflow to 14% in the new theatre with laminar airflow.[24] Although installing the new airflow system in our two operating rooms involved high costs (€540,000), from the results of our studies it seems likely to be cost-effective as the incidence of postoperative infectious complications (including periprosthetic infection) are prevented from happening.

In this study, differences in costs between patients with positive and negative culture outcomes were analysed. Total costs of patients with positive culture outcomes were considerably higher at each time of measurement in both the primary and revision group. Although power analyses were not based on economic outcomes we found that in the revision

group costs were significantly higher for patients with positive culture outcomes compared to those with negative outcomes. Treatment specifically aimed at these patients could subsequently lead to a decrease in periprosthetic infections and hence considerable cost savings. As described earlier, intra-operative culturing during primary arthroplasty can lead to early diagnosis compared with the current clinical practice, since current treatment modalities usually include culturing of wound discharge about five days after surgery prior to administration of antibiotics.[19] Therewith, intra-operative culturing during revision arthroplasty can give early indications of infection, that may warrant antibiotic treatment before bacteria have settled on the implant surfaces in their mature biofilm state of growth. This will lead to a decrease in postoperative infectious complications and an associated decrease in medical and non-medical costs. As both culture methods do not lengthen operating time and are not expensive, the authors recommend that intra-operative cultures be routinely conducted during both primary and revision arthroplasty, both from an economic as well as a medical perspective.

References

1. Elders MJ. The increasing impact of arthritis on public health. J Rheumatology Suppl 2000;60:6-8.
2. Acheson RM, Collart AB. New Haven Survey of Joint Diseases: XVII. Relationship between some systemic characteristics and osteoarthrosis in a general population. Ann Rheum Dis 1975;34:379-387.
3. Peyron JG. Osteoarthritis: The epidemiologic viewpoint. Clin Orthop Relat Res 1986;13:13-19.
4. Simon LS.Osteoarthritis: a review. Clin Cornerstone 1999;2:26-37.
5. March LM, Bachmeier CJ. Economics of osteoarthritis: a global perspective. Baillieres Clin Rheumatol 1997;11:817-34
6. National Center for Health Statistics. Current estimates from the National Health Interview Survey, 1988. Vital Health Stat 10 1989;1-250.
7. Kelsey JL, Hochberg MC. Epidemiology of chronic musculoskeletal disorders. Am Rev Public Health 1988;91:379-401.
8. Felson DT. Epidemiology of Osteoarthritis: Prevalence and Risk Factors. Osteoarthritis Disorders, American Academy of Orthopaedic Surgeons, 1995. pagina's
9. MacLean CH, Knight K, Paulus H, Brook RH, Shekelle PG. Costs attributable to osteoarthritis. J Rheumatol 1998;25:2213-8.
10. Iorio R, Healy WL, Richards JA. Comparison of the hospital cost of primary and revision total knee arthroplasty after cost containment. Orthopedics 1999;22:195-9.
11. Saleh KJ, Wood KC, Gafni A, Gross AE. Immediate surgery versus waiting list policy in revision total hip arthroplasty: an economic evaluation. J Arthroplasty 1997;12:1-10.
12. Barrack RL. Economics of revision total hip arthroplasty. Clin Orthop Relat Res 1995;209-14.
13. Barrack RL, Sawhney J, Hsu J, Cofield RH. Cost analysis of revision total hip arthroplasty. A 5-year follow-up study. Clin Orthop Relat Res 1999;175-8.
14. Best JT. Revision total hip and total knee arthroplasty. Orthop Nurs 2005;24:174-9.
15. Hebert CK, Williams RE, Levy RS, Barrack RL. Cost of treating an infected total knee replacement. Clin Orthop Relat Res 1996;140-5.
16. Lavernia CJ, Drakeford NK, Tsao AK, Gittelsohn A, Krackow KA, Hungerford DS. Revision of primary hip and knee arthroplasty. A cost analysis. Clin Orthop Relat Res 1995;209-14.
17. Okhuijssen SY, Dhert WJ, Faro LM, Keet JG, Schrijvers AJ, Verbout AJ. An estimate of the intramural costs of the placement of a total hip prosthesis. Ned Tijdschr Geneeskd 1998;142:1450-5.
18. Sculco TP. The economic impact of infected joint arthroplasty. Orthopedics 1995;18:871-3.
19. Knobben BAS, Engelsma Y, Neut D, van der Mei HC, Busscher HJ, van Horn JR. Intra-operative contamination influences wound discharge in orthopaedic implant surgery. Clin Orthop Relat Res 2006.
20. Knobben BAS, van Horn JR, van der Mei HC, Busscher HJ. Evalutation of measures to decrease intra-operative bacterial contamination in orthopaedic implant surgery. J Hosp Infect 2005;62(2):174-802.
21. Neut D, van Horn JR, van Kooten TG, van der Mei HC, Busscher HJ. Detection of biomaterial-associated infections in orthopaedic joint implants. Clin Orthop Relat Res 2003;261-8.
22. Koopmanschap MA, Rutten FFH, Van Ineveld BM, Van Roijen L. The friction cost method for measuring indirect costs of disease. J Health Econ 1995;14:171-89.
23. Oostenbrink JB, Bouwmans CAM, Koopmanschap MA, Rutten FFH. Manual for cost studies, methods and standard prices for economic evaluation in health care. Amstelveen: Health Care Insurance Council, 2004.
24. Knobben BAS, van der Mei HC, Busscher HJ, van Horn JR. Evaluation of intra-operative cultures in orthopedic implant loosening. J Bone Joint Surg (Br) 2006 (submitted).

7

GENERAL DISCUSSION

Introduction

The prevalence of osteoarthritis in the population is high and as most adequate treatment modalities for it are expensive, the total expenditure of treating this affliction is very high. The cost of arthritis in the year 2000 in the United States alone was estimated at 95 billion dollars.[1] In 1996 over 607,000 hip and knee replacements were performed in the United States.[2] By the year 2030, it is estimated that there will be an 85% increase in knee replacements and an 80% increase in hip replacements.[3] Total hip replacements can wear out and have a life expectancy of 10 to 30 years of service.[4] Approximately 10% of all hip replacements will fail and require revision surgery.[4] Revision surgery is more costly, requires a significantly longer hospital stay, incurs higher complication rates, and has a poorer prognosis than the original joint replacement procedure.[5;6] Probably the worst complication is periprosthetic infection. It constitutes a disaster for both patient and doctor. Conservative estimates of infection rates average 1-2% for hip implants and 2-4% for knee implants. The number of joint replacements is expected to increase drastically in the next twenty years and if the infection rate is not reduced, also the incidence of infection will increase, yielding increased morbidity, hospital stay and costs to the healthcare system.

The ultimate goal of this study was to assess the predictive value of microbiological analysis of the used set of instruments and removed bone chips during primary arthroplasty and of the removed prosthesis during revision surgery. Eventually, this will lead to the identification of patients with a higher risk of deep periprosthetic infection, in order to handle this group of patients accordingly with early and appropriate treatment.

On intra-operative culturing during primary arthroplasty

Charnley already recognised in 1972 that intra-operative contamination was a major threat to the success of total joint replacements, but others stated that its role as a cause of deep infection was highly overrated.[7;8] Several studies on intra-operative culturing of equipment and bacterial analysis of air samples have been performed, yielding conflicting conclusions on relationships with postoperative infections.[9-13] Scepticism about the importance of intra-operative contamination therefore still remains. Although it is generally believed that every

operating room is contaminated to some extent, it is not always clear whether this contamination is a risk for periprosthetic infection. We believe that any bacteria colonising the primary prosthesis, but not identified and eradicated, may infect the new prosthesis and put it at risk of (renewed) failure.

During the period of intra-operative culturing in this thesis contamination was demonstrated during 33-36% of the primary arthroplasty operations (in the operating room with conventional airflow). The association between intra-operative contamination and the occurrence of a periprosthetic infection appeared to be highly significant. The cultures of removed bone chips yielded results of which the negative predictive value, the sensitivity, and the specificity for the occurrence of periprosthetic infection are excellent (see Table I). Subsequently, the mean costs per patient with a positive intra-operative culture was drastically higher than for the patients with a negative intra-operative culture (see Figure 1). Moreover, it was shown that the mean costs per patient with a deep periprosthetic infection (> €45,000) were three times higher than those for patients that didn't develop a deep periprosthetic infection (€15,000). Therefore, we consider our culture method (mainly the culturing of bone chips) to be an effective instrument in the battle against periprosthetic infection.

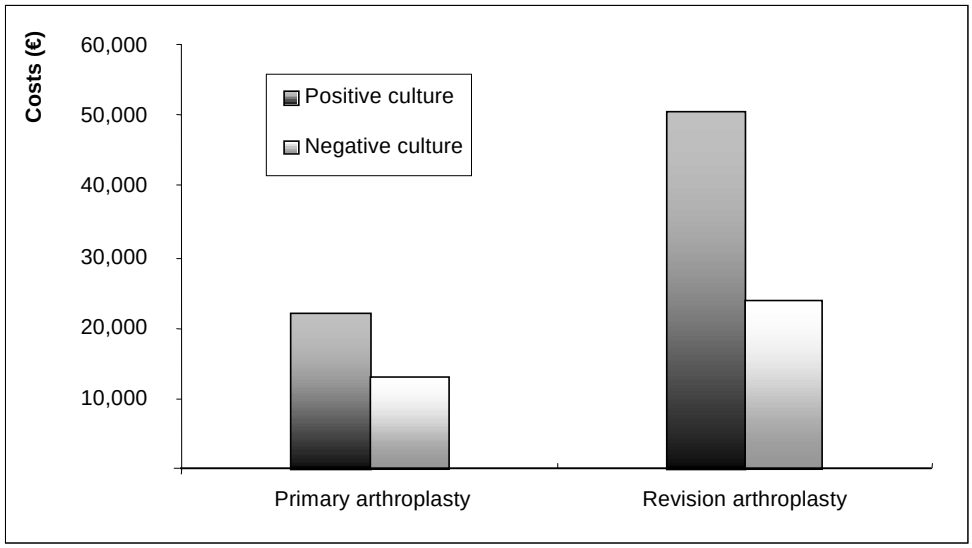


Figure 2. Mean total costs per patient, differentiated between positive en negative intra-operative culture outcomes.

On intra-operative culturing during revision arthroplasty

The percentage of septic loosenings in primary arthroplasty is approximately 1.5% for hip and 2.5% for knee implants and is much lower than the percentage of aseptic loosening.[14] The percentage however, of prosthetic joint infection after revision arthroplasty is 3.2% for hips and 5.6% for knees,[14] and can be as high as 40% for failed hip arthroplasties with a positive intra-operative culture.[15] It is imperative to exclude septic loosening in order to determine the proper management of patients in need of revision surgery, because both surgical management and outcome may differ depending on whether the arthroplasty loosening is infectious or mechanical in origin.[16-18] A wrong diagnosis will lead to treatment failure, increased morbidity and added costs to the healthcare system.

The incidence of prosthetic joint infection is grossly underestimated by current culture detection methods.[18-20] No single test is able to show the presence of periprosthetic infection in every case.[21] Loosening due to a low-grade infection in particular is difficult to distinguish from aseptic failure, as it often presents as (persisting or recurring) pain sometimes in combination with discrete signs of radiological loosening with limited or no clinical signs of infection at all.[22] A recent study of our group showed that an extensive culture technique of both excised tissue and of scrapings of the removed prosthesis is more sensitive for detecting bacteria than routine hospital culturing.[23] However, similar as with intra-operative contamination during primary arthroplasties, false-positive (contamination during sampling or culturing process) or false-negative test results may occur with an impact on the treatment modality chosen.[24;25]

Table I. The positive and negative predictive values (PPV and NPV, respectively), and the sensitivity (Sens) and specificity (Spec) of intra-operative culturing for the occurrence of periprosthetic infection. Cultures were taken during primary (instrument swabs and bone chips) and during revision surgery (extensive tissue and biomaterial culturing), both with conventional and with laminar airflow in the operating theatre.

		CONVENTIONAL AIRFLOW				LAMINAR AIRFLOW			
		PPV (%)	NPV (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Sens (%)	Spec (%)
PRIMARY	Instruments	4.8	92.4	14.2	92.4	0	98.5	0	93
	Bone chips	35.3	98.8	85.7	88.2	25	100	100	95.7
REVISION	Tissue	54.5	75	66.7	64.3	100	95	33.3	100
	Biomaterial	69.2	100	100	71.4	100	100	100	100

This thesis shows that the extensive culture technique is more sensitive than hospital culturing during both septic and aseptic loosening, and also has high predictive values, sensitivity and specificity for the occurrence of (re-)infection after revision of suspected aseptic loosenings. In particular the culturing of the scrapings of the biomaterial is very predictive, sensitive and specific (see table I). Moreover, the mean costs per patient with a positive intra-operative culture were more than twice as high as the mean costs per patient with a negative culture (see Figure 1).

On reducing intra-operative contamination

Intra-operative contamination is common in every operating room.[26-28] However, there are ways to decrease this phenomenon to a minimum by implementing a policy which is based on a behavioural and systemic approach. In the behavioural approach, preventive measures focus on reducing the number of air-borne particles in the operating room through disciplinary measures. Simple and cheap measures include limiting the number of personnel in the operating room, while also movements of personnel in the operating room should be restricted to a minimum, as it has been shown that increased activity enhances the dispersion of bacteria.[29] A systemic approach consists of improving the airflow system. The introduction of laminar airflow systems has greatly reduced infection in orthopaedic implant surgery. Laminar flow, as opposed to turbulent flow, allows air-borne particles to pass the operating area and prevent them from landing in the wound area. In a downflow laminar system for example, the unidirectional air enters the operating room in the ceiling above the operating area through filters. This study shows a significant decrease of intra-operative contamination after implementing a behavioural and a systemic alteration, both during primary arthroplasty and revision arthroplasty of aseptic loosened prostheses (see Figure 2). The majority of the individual parameters combined in our interventions, have already been proven to reduce contamination in the operating room,[14;27;30-36] but their combined effects were not yet determined. However, the combination of all these parameters evidently creates the most effective weapon against infection. As the total costs for treating a septic loosened prosthesis were estimated to be €52,750, the costs for building a laminar airflow system (in our hospital €540,000 for two operating theatres) will be recovered when only eleven periprosthetic infections have been prevented.

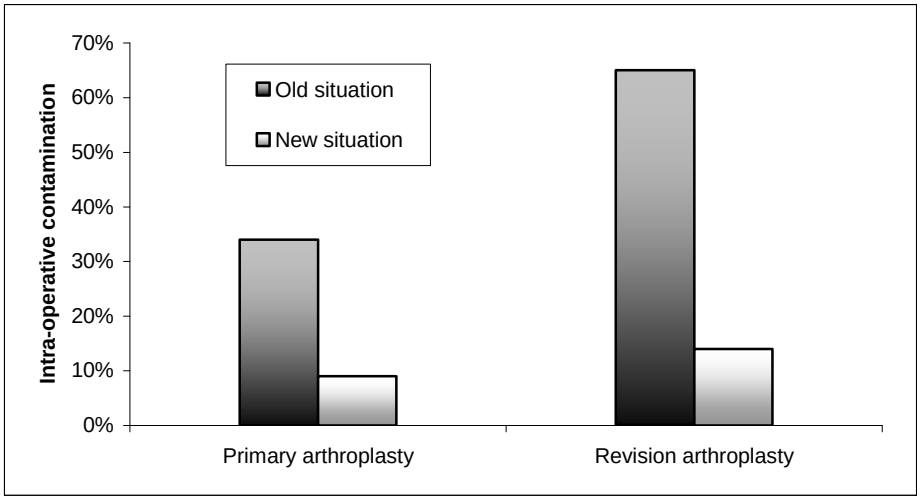


Figure 2. Percentage of primary and revision arthroplasties that were contaminated intra-operatively, measured before (old situation) and after systemic and behavioural interventions took place (new situation).

Intra-operative contamination varies during surgery and during the day. One can expect that the longer an operation lasts, implicating an increased exposure time, the more bacteria are present in the operating area and thus gain access to the wound. Our results furthermore show significantly more contamination during the early phase of a procedure than during the late phase. Just prior to an operating procedure extensive movement is occurring in the operating area for the final preparation, positioning and draping of the patient. After this high peak of initial movement it is from then on as limited as possible during the rest of the procedure. Consequently, it is not surprising that the samples taken in the initial phase of the operation showed a higher contamination rate than those taken during the late phase. These results coincide with the results of measurements we did of air particles about 50 centimeters from the operating wound. Samples taken just before surgery showed the highest number of particles, followed by a decrease at 30 minutes after incision. At the end of surgery, counts increased somewhat, but not to the initial values. Noteworthy is that during the fourth and last operation of the day the counts increased markedly, probably caused by people who are already cleaning up things and hence are moving about a lot.

As bacteria can never be fully eliminated from an operating room, we also studied transfer of bacteria between different operating room materials. Bacterial adhesion to and transfer between surfaces is a complicated process and with regard to the success of biomaterials implants, studies on bacterial adhesion and transfer should not be confined to biomaterials surfaces on the surface of and inside the human body, but should also include

surfaces in the operating room, where the origin of many biomaterials related infections is found. Transfer was demonstrated to some extent with all bacterial strains and with every tested material, ranging from 17 to 71%, and was influenced by the bacterial strain, moistness of the inoculum, the application of friction and the roughness and hydrophobicity of both the donating and the receiving surface. Reducing this transfer, for example by changing surface properties, can eventually reduce the number of bacteria that enter the operating wound.

On the clinical significance of this thesis

Factors leading to periprosthetic infection must be considered with respect to the patient, the wound, the operating-room environment, and microbiological characteristics of the infecting organism.

In current clinical practice patients who have to undergo an arthroplasty are screened pre-operatively mainly to see whether a patient is healthy enough to withstand surgery. We suggest that patients should also be screened on risk factors for postoperative infection, not to exclude patients from surgery, but to know whether they are at higher risk or not. Known risk factors are rheumatoid arthritis and other immunocompromising diseases, diabetes, poor nutrition, obesity, urinary tract infection, oral use of steroids, previous operations on the affected joint, and a history of joint infection.[14] These risk factors should be eliminated as much as possible before surgery takes place (i.e. poor nutrition, obesity, urinary tract infection, oral use of steroids).

Intra-operatively, cultures should be taken during both primary and revision arthroplasty. As shown earlier in this thesis, culturing of removed bone chips during primary arthroplasty and culturing of (scrapings of) the removed biomaterial during revision surgery is a very sensitive and specific diagnostic instruments for predicting periprosthetic infection. Both procedures are not expensive and do not lengthen the operative procedure.

Radical alterations in behaviour and airflow system in an operating room can decrease intra-operative contamination. To maintain these low bacterial counts, both the airflow system and behaviour have to be monitored constantly and consistently. Both the manufacturer of the airflow system and the hospitals infection control officer (for example a consultant microbiologist) should advice on the microbiological performance of the airflow system and therefore have responsibility for the monitoring thereof. An infection committee should

monitor the behavioural changes and report frequently to the people working in the operating room. Both positive and negative feedback help maintain the reduction in dispersion of bacteria. Finally, it is important to emphasise that all personnel working in the operating room, including surgeons, operating room assistants, anaesthesiologists and cleaning personnel adhere to the hygiene protocol very strictly.

Bacteria that are living in a biofilm are far more resistant to antibiotic treatment than planktonic bacteria, which make the treatment of periprosthetic infection very difficult. During the transfer of bacteria in the operating room, the sessile bacteria are still in a monolayer and can easily be treated with chlorhexidine which has already been demonstrated to be effective against bacteria in such a state.[37-41] Intervention with this agent in the operating room by dipping the surgical gloves in a chlorhexidine splash-basin every ten minutes would be an easily applicable method to decrease bacterial transfer into the wound and hence lower the risk of postoperative infection.

Post-operatively, the wound should be carefully monitored. If there is an obvious infection of the wound, then intervention should take please immediately. This is also the case if large haematoma or other sites of infection are present in the patient.[42] These are the clear cases. In most cases, however, it is not clear whether there is an infection or not. This thesis shows that a wound which is not dry within four days constitutes a risk factor for periprosthetic infection. It also shows that intra-operative contamination is significantly associated with this prolonged wound discharge and with periprosthetic infection. Therefore, we recommend that every wound that keeps discharging for 5 days or more receives extra attention (see Figure 3).

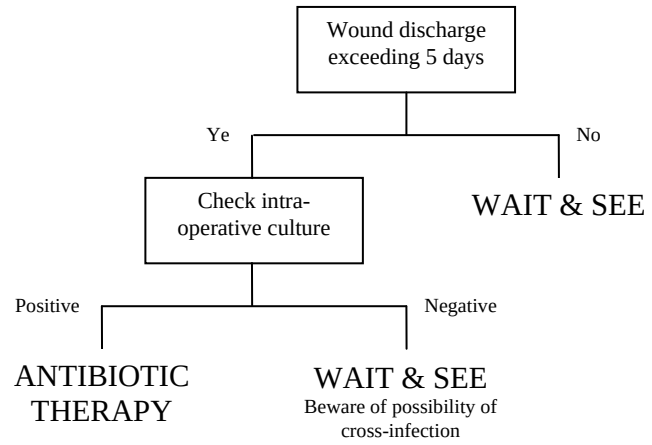


Figure 3. Flowchart for the treatment of postoperative prolonged wound discharge after insertion of a hip or knee prosthesis.

First of all the results of the intra-operative culture should be checked. In the case of contamination the patient should receive antibiotic treatment aimed at the micro-organism(s) found. In the case of prolonged wound discharge but negative intra-operative cultures the following should be considered: although the wound was not contaminated intra-operatively, there is still a risk the wound gets cross-infected on the ward. The wound should therefore be handled and monitored very carefully until it closes, preferably with determination of infection parameters in the blood (i.e. C-reactive protein). More research on this is strongly recommended.

References

1. Elders MJ. The increasing impact of arthritis on public health. J Rheumatol Suppl 2000;60:6-8.
2. AHCPR, Center for Organisation and Delivery Studies Healthcare Costs and Utilisation Project. Table 2: Statistics for 1996 HCUP Nationwide Inpatient Sample, by multi-level CCS procedure.
3. National Center for Health Stastics. American Academy and American Association Orthopaedic Surgeons Bulletin 1999;47:14.
4. Schulte KR, Callaghan JJ, Kelley SS, Johnston RC. The outcome of Charnley total hip arthroplasty with cement after a minimum twenty-year follow-up. The results of one surgeon. J Bone Joint Surg Am 1993;75:961-975.
5. Iorio R, Healy WL, Richards JA. Comparison of the hospital cost of primary and revision total hip arthroplasty after cost containment. Orthopedics 1999;22:185-189.
6. Saleh KJ, Wood KC, Gafni A, Gross AE. Immediate surgery versus waiting list policy in revision total hip arthroplasty. An economic evaluation. J Arthroplasty 1997;12:1-10.
7. Charnley J. Postoperative infection after total hip replacement with special reference to air contamination in the operating room. Clin Orthop Relat Res 1972;87:167-187.
8. Humphreys H. Infection control team in the operating room: separating aspiration from reality! J Hosp Infect 1999;42:265-267.
9. Davis N, Curry A, Gambhir AK, Panigrahi H, Walker CR, Wilkins EG, Worsley MA, Kay PR. Intraoperative bacterial contamination in operations for joint replacement. J Bone Joint Surg Br 1999;81:886-889.
10. Lawal OO, Adejuyigbe O, Oluwole SF. The predictive value of bacterial contamination at operation in post-operative wound sepsis. Afr J Med Med Sci 1990;19:173-179.
11. Lidwell OM, Lowbury EJ, Whyte W, Blowers R, Stanley SJ, Lowe D. Airborne contamination of wounds in joint replacement operations: the relationship to sepsis rates. J Hosp Infect 1983;4:111-131.
12. Robinson AH, Drew S, Anderson J, Bentley G, Ridgway GL. Suction tip contamination in the ultraclean-air operating theatre. Ann R Coll Surg Engl 1993;75:254-256.
13. Taylor GJ, Leeming JP, Bannister GC. Assessment of airborne bacterial contamination of clean wounds: results in a tissue model. J Hosp Infect 1992;22:241-249.
14. Hanssen AD, Rand JA. Evaluation and treatment of infection at the site of a total hip or knee arthroplasty. Instr Course Lect 1999;48:111-122.
15. Dupont JA. Significance of operative cultures in total hip arthroplasty. Clin Orthop Relat Res 1986;122-127.
16. Hamblen DL. Diagnosis of infection and the role of permanent excision arthroplasty. Orthop Clin North Am 1993;24:743-749.
17. Masterson EL, Masri BA, Duncan CP. Treatment of infection at the site of total hip replacement. Instr Course Lect 1998;47:297-306.
18. Morscher E, Babst R, Jenny H. Treatment of infected joint arthroplasty. Int Orthop 1990;14:161-165.
19. Hamblen DL. Diagnosis of infection and the role of permanent excision arthroplasty. Orthop Clin North Am 1993;24:743-749.
20. Tunney MM, Patrick S, Curran MD, Ramage G, Hanna D, Nixon JR, Gorman SP, Davis RI, Anderson N. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J Clin Microbiol 1999;37:3281-3290.
21. Virolainen P, Lahteenmaki H, Hiltunen A, Sipola E, Meurman O, Nelimarkka O. The reliability of diagnosis of infection during revision arthroplasties. Scand J Surg 2002;91:178-181.
22. Trampuz A, Zimmerli W. Prosthetic joint infections: update in diagnosis and treatment. Swiss Med Wkly 2005;135:243-251.
23. Davis N, Curry A, Gambhir AK, Panigrahi H, Walker CR, Wilkins EG, Worsley MA, Kay PR. Intraoperative bacterial contamination in operations for joint replacement. J Bone Joint Surg Br 1999;81:886-889.
24. Clarke MT, Roberts CP, Lee PT, Gray J, Keene GS, Rushton N. Polymerase chain reaction can detect bacterial DNA in aseptically loose total hip arthroplasties. Clin Orthop Relat Res 2004;132-137.
25. Neut D, van Horn JR, van Kooten TG, van der Mei HC, Busscher HJ. Detection of biomaterial-associated infections in orthopaedic joint implants. Clin Orthop Relat Res 2003;261-268.
26. Hughes SP, Anderson FM. Infection in the operating room. J Bone Joint Surg Br 1999;81:754-755.
27. Maathuis PG, Neut D, Busscher HJ, van der Mei HC, van Horn JR. Perioperative contamination in primary total hip arthroplasty. Clin Orthop Relat Res 2005;136-139.
28. Duguid JP, Wallace AT. Air infection with dust liberated from clothing. Lancet 2 1948;845-849.

29. Ahl T, Dalen N, Jorbeck H, Hoborn J. Air contamination during hip and knee arthroplasties. Horizontal laminar flow randomized vs. conventional ventilation. Acta Orthop Scand 1995;66:17-20.
30. Brown AR, Taylor GJ, Gregg PJ. Air contamination during skin preparation and draping in joint replacement surgery. J Bone Joint Surg Br 1996;78:92-94.
31. Gosden PE, MacGowan AP, Bannister GC. Importance of air quality and related factors in the prevention of infection in orthopaedic implant surgery. J Hosp Infect 1998;39:173-180.
32. Gruenberg MF, Campaner GL, Sola CA, Ortolan EG. Ultraclean air for prevention of postoperative infection after posterior spinal fusion with instrumentation: a comparison between surgeries performed with and without a vertical exponential filtered air-flow system. Spine 2004;29:2330-2334.
33. Hubble MJ, Weale AE, Perez JV, Bowker KE, MacGowan AP, Bannister GC. Clothing in laminar-flow operating theatres. J Hosp Infect 1996;32:1-7.
34. Lidwell OM. Air, antibiotics and sepsis in replacement joints. J Hosp Infect 1988;11 Suppl C:18-40.
35. Hughes SP, Anderson FM. Infection in the operating room. J Bone Joint Surg Br 1999;81:754-755.
36. Pittet D, Ducel G. Infectious risk factors related to operating rooms. Infect Control Hosp Epidemiol 1994;15:456-462.
37. Douw CM, Bulstra SK, Vandenbroucke J, Geesink RG, Vermeulen A. Clinical and pathological changes in the knee after accidental chlorhexidine irrigation during arthroscopy. Case reports and review of the literature. J Bone Joint Surg Br 1998;80:437-440.
38. Reading AD, Rooney P, Taylor GJ. Quantitative assessment of the effect of 0.05% chlorhexidine on rat articular cartilage metabolism in vitro and in vivo. J Orthop Res 2000;18:762-767.
39. Rombouts JJ, Wery PE, Delloye C, Noel H, Vincent A. [Proper use of irrigation solutions in orthopedic surgery. Apropos of a case of chondrolysis due to chlorhexidine]. Acta Orthop Belg 1986;52:685-702.
40. Saatman RA, Carlton WW, Hubben K, Streett CS, Tuckosh JR, DeBaecke PJ. A wound healing study of chlorhexidine digluconate in guinea pigs. Fundam Appl Toxicol 1986;6:1-6.
41. Severyns AM, Lejeune A, Rocoux G, Lejeune G. Non-toxic antiseptic irrigation with chlorhexidine in experimental revascularization in the rat. J Hosp Infect 1991;17:197-206.
42. Saleh K, Olson M, Resig S, Bershadsky B, Kuskowski M, Gioe T, Robinson H, Schmidt R, McElfresh E. Predictors of wound infection in hip and knee joint replacement: results from a 20 year surveillance program. J Orthop Res 2002;20:506-515.

8

SUMMARY

As described in **Chapter 1** infection is one of the most common complications in surgery. In particular deep periprosthetic infections in orthopaedic surgery constitute a disaster for both patient and doctor. Conservative estimates of infection rates average 1-2% for hip implants and 2-4% for knee implants. The number of joint replacements is expected to double in the next twenty years and if the infection rate is not reduced, also the incidence of infection will double, yielding increased morbidity, hospital stay and costs to the healthcare system. Deep prosthetic infections can be subdivided in (i) early (within three months after surgery), (ii) delayed (within one-and-a-half to two years after surgery) or (iii) late infections. Both early and delayed infections can be caused during surgery by direct contact with the wound, airborne colonisation or by cross-infection on the ward. Late infection is considered mostly to be caused by blood-borne contamination, for example during insertion of a urinary catheter, infection of an intravenous canula, skin or dental sepsis. This thesis focuses on the early and delayed infections caused by intra-operative contamination.

Intra-operative bacterial contamination may be present in every operating room, and constitutes a possible risk for postoperative wound healing problems and periprosthetic infection, but to what extent remains unclear. In **Chapter 2** the results of a study is presented in which we investigated whether bacterial contamination of the instruments and bone during primary prosthesis insertion was associated with prolonged wound discharge, and subsequent periprosthetic infection. During 100 total hip arthroplasties, four intra-operative cultures were taken from the instruments and two portions of removed bone. Postoperatively, the duration of wound discharge was monitored, taking day 5 as the cut-off point. All patients were followed for two years to find out whether periprosthetic infection occurred. Bacterial contamination was present during 36 operative procedures (36%). A significant association was found between intra-operative contamination and prolonged wound discharge, with a relative risk (RR) of 2.5. The culturing of removed bone had a positive predictive value of 81-90% for prolonged wound discharge. Other factors associated with prolonged wound discharge were rheumatoid arthritis (RR 6.4), use of cement (RR 1.6) and increased blood loss (RR 1.5). We conclude that there is a significant association between intra-operative contamination, prolonged wound discharge and periprosthetic infection.

In **Chapter 3** the aim was to evaluate whether behavioural and systemic measures in the operating theatre will decrease intra-operative contamination during total hip or knee

replacements. The influence of these measures on subsequent prolonged wound discharge, superficial surgical site infection and deep periprosthetic infection during an 18 month follow-up is also investigated. During 207 procedures, four swabs were taken from instruments at the beginning and at the end of the procedure. Removed material from the bone (acetabulum and femur in case of the hip joint; femur and tibia in case of the knee joint) was tested for contamination as well. At first, 70 operations in an old situation were included (control group), after which the first behavioural measure was introduced: better use of the area directly beneath the plenum. During 67 operations in this new situation cultures were taken (group 1), followed by the introduction of a strict protocol based on the adherence to operating room-rules and the installation of a new laminar flow system. 70 operations (group 2) were monitored after this second intervention. The control group showed intra-operative contamination in 23/70 (32.9%) of the cases, group 1 showed contamination in 34.3% of the cases (23/67) and group 2 showed contamination in 6/70 cases, corresponding to 8.6%. The parameters prolonged wound discharge and superficial surgical site infection also decreased drastically in group 2 as did the incidence of deep periprosthetic infection, but this did not reach statistical significance. This study shows that the combination of systemic and behavioural changes in an operating room significantly decreases the incidence of intra-operative bacterial contamination, subsequent prolonged wound discharge and superficial surgical site infection. After 18 months of follow up there was also a decrease in deep periprosthetic infection.

Bacterial adhesion to and transfer between surfaces is a complicated process. With regard to the success of biomaterials implants, studies on bacterial adhesion and transfer should not be confined to biomaterials surfaces in the human body, but should also encompass surfaces in the operating room, where the origin of many biomaterials related infections is found. The purpose of **Chapter 4** was to quantify the transfer of *Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes* from one operating room material to another, while accounting for surface hydrophobicity and roughness, moistness and application of friction during transfer. The tested operating room materials were glove, broach, theatre gown and light handle. As a possible clinical intervention method to prevent transfer, it was investigated whether dipping the gloves in a chlorhexidine splash-basin affected the viability of the transferred bacteria. Transfer (moist and without friction) was demonstrated to some extent with all bacterial strains and with every tested material, ranging from 17 to 71%, and was

influenced by the bacterial strain, moistness of the inoculum, the application of friction and the characteristics of both the donating and the receiving surface. Dipping the glove material in 4% or 0.4% chlorhexidine solutions killed all bacteria present, regardless of whether surfaces were dried or moist and thus prevented transfer.

The aim of the study as described in **Chapter 5** was to evaluate our research laboratory tissue and biomaterial culturing (RLTC and BC, respectively) during revision surgery of hip and knee, initially clinically diagnosed either as septic or aseptic loosening. The results are compared with the new routine hospital culturing (NRHC) method. In total, intra-operative culturing was performed in 59 consecutive patients who underwent revision of their prosthesis. The indication for revision was suspected septic loosening in 14 cases (7 with conventional and 7 with laminar airflow) and suspected aseptic loosening in 45 cases (23 with conventional and 22 with laminar airflow). In order to investigate whether infectious complications occurred related to the revision surgery, all patients were followed for at least 18 months. In the group of 14 patients with septic loosening, NRHC showed microbial growth in 8 of the 14 (57.1%) cases, RLTC revealed bacteria in 9/14 (64.3%) cases and BC showed growth in all 14 cases. Alternatively, the group of 45 patients with suspected aseptic loosening showed microbial growth during NRHC in only 4/45 (8.9%) cases, while RLTC showed growth in 13/45 (28.9%) cases, and BC in 16/45 (35.6%) cases. After follow-up it seemed that BC had a positive predictive value of 75% and a negative predictive value of 100% for the (re-)occurrence of periprosthetic infection after revision arthroplasty for suspected aseptic loosening. In the operating theatre with conventional airflow RLTC and BC showed microbial growth in 15/23 cases (65%), compared to 3/22 (14%) with laminar airflow, suggesting that in many cases intra-operative contamination might have played a key role.

Chapter 6 encompasses an economic evaluation of prosthetic joint infections. Firstly, the scope of the social costs generated by patients who undergo a primary or revision-operation for a hip or knee implant was evaluated, as well as the cost increase upon development of a deep peri-prosthetic infection. Subsequently, it was investigated whether there are differences in these costs between patients with positive and negative intra-operative cultures in order to demonstrate that intra-operative culturing is a cost-effective means in clinical practice to prevent a possible peri-prosthetic infection due to intra-operative contamination. The mean total costs of placing a primary prosthesis was €16.846 (€5890 - €59.180). Within the follow

up of 18 months 2 patients with a hip prosthesis developed a deep periprosthetic infection, with a cost of €45.034 and €59.180. The mean total cost of patients without a deep periprosthetic infection (N=48) was €15.376 (€5890 - €53.247). Revision of an aseptic loosened prosthesis (N=25) had a mean total costs of €36.798, and revision of a septic loosened prosthesis €52.750 (N=10). Total costs of patients with positive culture outcomes were considerably higher than costs of patients with negative culture outcomes in both the primary and revision group. These patients could be identified early using the culture techniques applied in the current study. As used culture methods do not lengthen operating time and are not expensive the authors recommend that intra-operative cultures be routinely conducted during both primary and revision arthroplasty, both from an economic as well as a medical perspective.

As indicated in the **General Discussion** (Chapter 7), this thesis shows that to prevent and treat periprosthetic infection appropriately, it is necessary to take measures pre-, intra-, and post-operatively. Pre-operatively by screening the patients, intra-operatively by taking cultures and altering operating room discipline and airflow system, and potentially by decreasing bacterial transfer, and post-operatively by monitoring wound discharge and measuring also other infection parameters. As treating an infected prosthesis is proven to be very expensive, it seems cost-effective to take all these measures.


NL
NEDERLANDSE
SAMENVATTING

Infectie is een van de meest voorkomende chirurgische complicaties **(Hoofdstuk 1)**. In het bijzonder de diepe periprothetische infectie in de orthopedische chirurgie is rampzalig voor zowel patiënt als arts. Het infectiepercentage wordt geschat op 1-2% na heuparthroplastiek en 2-4% na kniearthroplastiek. Het aantal gewrichtsvervangende operaties zal naar verwachting in de komende twintig jaar verdubbelen. Indien het infectiepercentage niet afneemt zal ook het aantal periprothetische infecties verdubbelen, met als gevolg toename van morbiditeit, langdurige ziekenhuisopnames en toename van de kosten voor de gezondheidszorg. Diepe periprothetische infecties kunnen worden onderverdeeld in (i) vroeg (binnen drie maanden na plaatsing), (ii) vertraagd (binnen anderhalf tot twee jaar na plaatsing) en (iii) laat. Zowel de vroege als de vertraagde vorm kunnen worden veroorzaakt door bacteriële contaminatie van de wond door direct contact, door contaminatie die door de lucht wordt aangevoerd of door contaminatie van de wond na de operatie op de verpleegafdeling. De late infecties worden over het algemeen veroorzaakt door hematogene contaminatie, bijvoorbeeld na het plaatsen van een urinewegcatheter, een infectie van een intraveneuze lijn of een huid- of tandinfectie. Dit proefschrift is gericht op de vroege en vertraagde infecties, die veroorzaakt worden door intra-operatieve bacteriële contaminatie.

Intra-operatieve bacteriële contaminatie is mogelijk aanwezig in elke operatiekamer en vormt een mogelijk risico voor postoperatieve wondgenezingsproblemen en periprothetische infectie, maar in welke omvang blijft vooralsnog onduidelijk. In **Hoofdstuk 2** worden de resultaten gepresenteerd van een studie waarin werd onderzocht of bacteriële contaminatie van het instrumentarium en van verwijderde botsnippers tijdens het inbrengen van een primaire prothese geassocieerd was met verlengde wondlekkage en periprothetische infectie. Tijdens het plaatsen van 100 primaire heupprothesen werden vier kweken genomen van het instrumentarium en twee porties met botsnippers werden op kweek gezet. Postoperatief werd gekeken hoelang het duurde voordat de wond droog en dicht was, waarbij de 5^{e} dag na de operatie als afkappunt werd gebruikt. Alle patiënten werden gedurende twee jaar vervolgd om te kijken of er zich een periprothetische infectie voordeed. Bacteriële contaminatie werd gemeten tijdens 36 van de 100 operaties (36%). Er werd een significante associatie gevonden tussen intra-operatieve bacteriële contaminatie en optreden van verlengde wondlekkage, met een relatieve risicofactor (RR) van 2,5. Het kweken van de botsnippers had een positief voorspellende waarde van 81-90% voor het optreden van verlengde wondlekkage. Andere

factoren die geassocieerd waren met verlengde wondlekkage waren rheumatoïde artritis (RR 6,4), het gebruik van cement (RR 1,6) en verhoogt bloedverlies tijdens de operatie (RR 1,5). Concluderend werd er een significante associatie gevonden tussen intra-operatieve contaminatie, verlengde wondlekkage en periprothetische infectie.

In **Hoofdstuk 3** was het doel om te evalueren of gedrags- en systemische maatregelen in de operatiekamer een afname van intra-operatieve contaminatie tot gevolg zouden hebben tijdens primaire knie- en heuparthroplastieken. De invloed van deze maatregelen op verlengde wondlekkage, postoperatieve wondinfectie en periprothetische infectie werd ook onderzocht. Tijdens 207 operaties werden vier instrumentariumkweken genomen, twee tijdens de vroege fase van de operatie en twee tijdens de late fase. Verwijderde botsnippers (van acetabulum en femur bij heuparthroplastieken en van femur en tibia bij kniearthroplastieken) werden ook op kweek gezet. Allereerst werden 70 operaties in de oude operatiekamer met conventionele airflow geïncludeerd (controlegroep), waarna de eerste gedragsmaatregelen werden doorgevoerd: beter gebruik van het gebied precies onder het plenum. Tijdens 67 operaties in deze nieuwe situatie werden weer kweken genomen (groep 1), waarna een strikt protocol met vele gedragsmaatregelen werd ingevoerd en een laminair airflow systeem in gebruik werd genomen. In deze nieuwe situatie werden weer tijdens 70 operaties kweken afgenomen (groep 2). In de controlegroep waren 23 van de 70 operaties gecontamineerd (32,9%), in groep 1 bleek 23 van de 67 ingrepen gecontamineerd (34,4%) en in groep 2 werd tijdens 6 van de 70 ingrepen contaminatie gevonden (8,6%). Het optreden van verlengde wondlekkage en postoperatieve wondinfectie bleek ook drastisch afgenomen in groep 2, evenals periprothetische infectie, zij het dat dit laatste niet statistisch significant was. Deze studie toonde aan dat de combinatie van gedrags- en systemische maatregelen in een operatiekamer het optreden van intra-operatieve contaminatie, postoperatieve verlengde wondlekkage en wondinfectie significant doet afnemen. Na anderhalf jaar follow-up bleek ook de incidentie van periprothetische infectie afgenomen te zijn.

De adhesie van bacteriën aan en de overdracht tussen verschillende oppervlakken is een gecompliceerd proces. Ten aanzien van het slagen van biomedische implantaten, zou niet alleen onderzoek moeten worden gedaan naar de oppervlakken van de implantaten zelf, maar ook naar verschillende oppervlakken in de operatiekamer, alwaar de meeste biomateriaal gerelateerde infecties hun oorzaak vinden. Het doel van **Hoofdstuk 4** was het kwantificeren

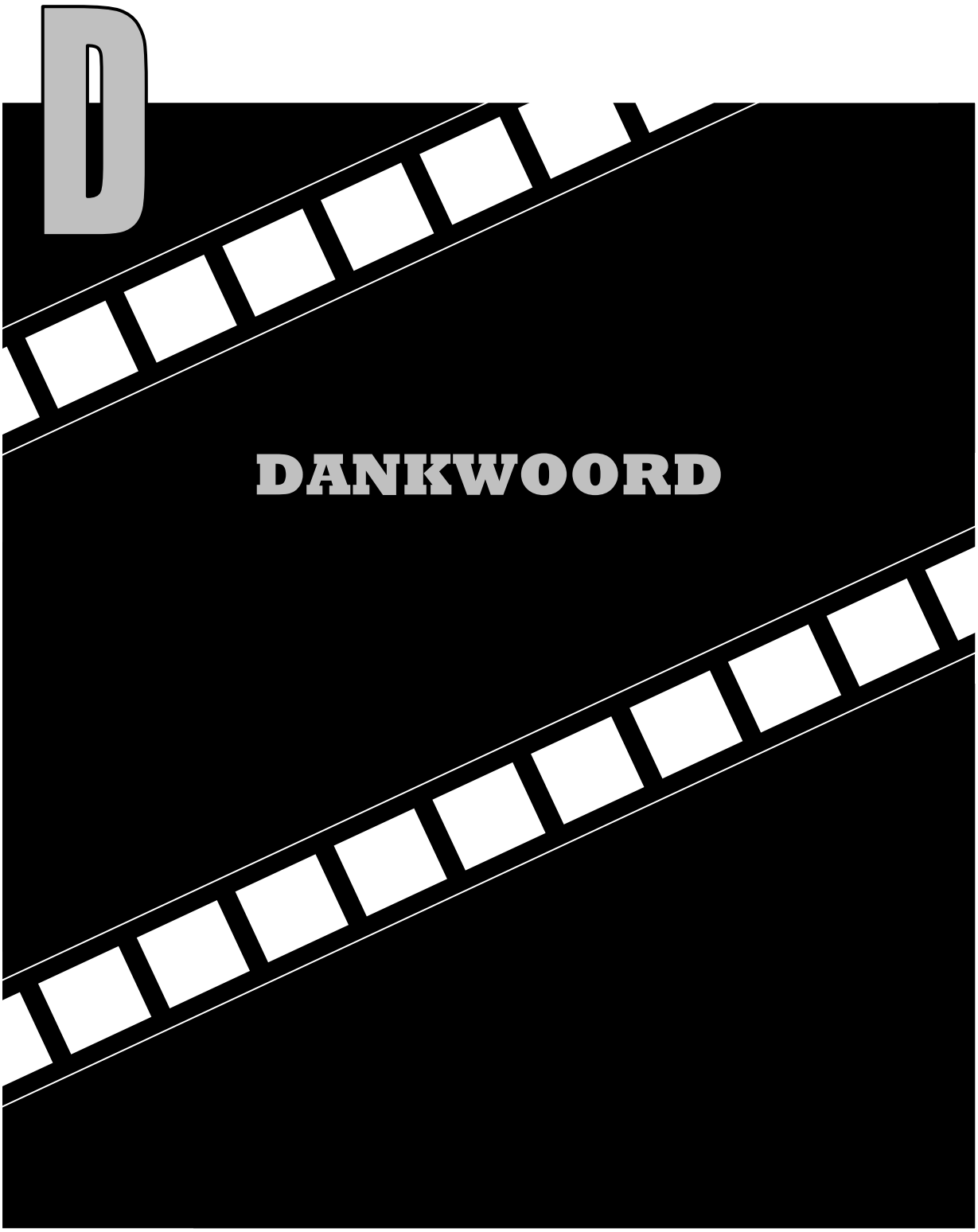
van de overdracht van *Stafylococcus aureus*, *Stafylococcus epidermidis* en *Propionibacterium acnes* van het ene operatiekameroppervlak naar het andere, rekening houdend met factoren als hydrofobiciteit en ruwheid van deze oppervlakken, vochtigheid en het toepassen van frictie tijdens de overdracht. De onderzochte materialen waren een operatiehandschoen, een botfrees, operatiekleding en een handvat van de operatielamp. Als mogelijke klinische interventie om overdracht tegen te gaan, werd getest of het dippen van operatiehandschoenen in een chloorhexidine-badje de levensvatbaarheid van overgedragen bacteriën zou beïnvloeden. Overdracht (vochtig en met frictie) werd aangetoond met alle drie de bacteriestammen en tussen alle vier de materialen, variërend van 17 tot 71%. De overdracht werd beïnvloed door de soort bacteriestam, vochtigheid van het overdrachtsoppervlak, het toepassen van frictie en de eigenschappen van zowel het “schenkende” als het “ontvangende oppervlak”. Het dippen van de operatiehandschoen in 4% of 0,4% chloorhexidine-oplossingen doodde alle aanwezige bacteriën, ongeacht of het inoculum nog nat was of al was opgedroogd.

Het doel van de studie in **Hoofdstuk 5** was het evalueren van de door ons gebruikte weefsel- en prothesekweektechnieken tijdens het reviseren van heup- en knieprothesen, zowel als het om septische als om aseptische loslating (klinische diagnose) ging. De resultaten werden vergeleken met de normale weefselkweek uitgevoerd door de afdeling medische microbiologie van het ziekenhuis. Intra-operatieve kweken werden afgenomen tijdens 59 revisie-ingrepen. In 14 gevallen was de indicatie septische loslating (7 met conventionele en 7 met laminaire airflow) en in 45 gevallen aseptische loslating (23 met conventionele en 22 met laminaire airflow). Alle patiënten werden gedurende 18 maanden vervolgd om te zien of infectieuze complicaties optraden, gerelateerd aan de revisie-ingreep. In de groep van 14 patiënten met verdenking van een septische loslating, werd met de ziekenhuisweefselkweek in 8 gevallen (57,1%) een bacterie aangetoond, met onze weefselkweektechniek in 9 gevallen (64,3%) en met onze prothesekweek in 100% van de gevallen. In de groep van 45 patiënten met verdenking van een aseptische loslating werd met de ziekenhuisweefselkweek bij 4 patiënten een bacterie gevonden (8,9%), met onze weefselkweektechniek bij 13 patiënten (28,9%) en met onze prothesekweek bij 16 patiënten (35,6%). Na follow-up bleek dat de prothesekweek een positief voorspellende waarde van 75% en een negatief voorspellende waarde van 100% had voor het ontstaan van een (re)infectie na de revisie-ingreep bij verdenking van een aseptische loslating. In de operatiekamer met conventionele airflow waren onze weefsel- en/of prothesekweken positief in 15 van de 23 gevallen (65,2%) met verdenking

aseptische loslating en in de operatiekamer met de laminaire airflow in 3 van de 22 gevallen (13,6%). Dit kan mogelijk worden verklaard door intra-operatieve contaminatie, die is opgetreden in de operatiekamer met laminaire airflow.

Hoofdstuk 6 beschrijft een economische evaluatie van periprothetische infecties. Allereerst werd bekeken welke totale kosten werden gegenereerd door patiënten die een primaire of revisie-ingreep van hun heup of knie ondergingen, evenals de toename van kosten als zich een periprothetische infectie voordoet. Vervolgens werd onderzocht of er verschillen in kosten waren tussen patiënten met positieve en met negatieve intra-operatieve kweken, om aan te tonen dat het afnemen van intra-operatieve kweken een kostenbesparend middel kan zijn dat intra-operatieve contaminatie aantoont, waardoor een periprothetische infectie wellicht kan worden voorkomen. De gemiddelde totale kosten per patiënt met een primaire prothese bedroegen €16.846 (€5.890 - €59.180). Binnen de follow-up van twee jaar ontwikkelden 2 van de 50 primaire patiënten een periprothetische infectie. Deze twee patiënten kostten €45.034 en €59.180. De gemiddelde totale kosten per patiënt zonder een periprothetische infectie (N=48) waren €15.376 (€5.890 - €53.247). Patiënten die een revisie vanwege aseptische loslating hadden ondergaan (N=25), kostten gemiddeld €36.798 en patiënten met een revisie vanwege septische loslating €52.750 (N=10). De totale kosten van patiënten met een positieve intra-operatieve kweek waren aanzienlijk hoger dan de kosten van patiënten met een negatieve kweek, zowel in de primaire als in de revisiegroep. Deze patiënten kunnen met onze kweekmethode vroeg geïdentificeerd worden. Daar de kweekmethoden niet duur zijn en de operatie niet wezenlijk verlengen, wordt het intra-operatief kweken tijdens primaire en revisie-ingrepen zeer aanbevolen, zowel vanuit medisch als vanuit economisch perspectief.

Zoals besproken in de **Generale Discussie** (Hoofdstuk 7) toont dit proefschrift aan dat het nodig is om zowel pre-operatieve, intra-operatieve als postoperatieve maatregelen te nemen, om in de toekomst de periprothetische infectie te kunnen voorkomen, dan wel adequaat te kunnen behandelen. Pre-operatief dienen de patiënten goed te worden gescreend, intra-operatief dienen kweken genomen te worden en, indien nodig, gedrags- en systemische veranderingen worden doorgevoerd en postoperatief dient de wondheling goed te worden gecontroleerd. Aangezien het behandelen van een prothetische infectie bewezen erg duur is, lijkt het nemen van al deze maatregelen kosteneffectief te zijn.


D
DANKWOORD

Terwijl ik dit schrijf besef ik dat dit wellicht het moeilijkste hoofdstuk van dit proefschrift is. Ik heb het gevoel dat ik zoveel mensen moet bedanken. Niet per se voor de hulp bij het vervaardigen van dit proefschrift, maar meer voor het me op de been houden toen mijn eigen benen dat niet meer zo goed konden.

Henk en Henny, bedankt voor de goede samenwerking. Jullie beider kwaliteiten zijn gecombineerd een sterk wapen in het land van onderzoek en promoveren. Henk, tevens bedankt voor de goede gesprekken. Daar heb ik veel aan gehad.

Professor Van Horn, bedankt voor de vele mogelijkheden die u me heeft geboden om te zijn waar ik nu ben. Ik heb veel van u geleerd en hoop er nog ver mee te komen. Geniet van uw leven in het prachtige Suriname. Wellicht dat ik het ooit nog met eigen ogen zal zien.

Professoren Bulstra, Verhaar en Degener. Bedankt voor het accepteren van de uitnodiging om in de leescommissie van dit proefschrift zitting te nemen (en er vervolgens veel tijd aan te hebben besteed). We komen elkaar vast nog tegen de komende jaren.

Bertram, tja, Bertram. Waar zal ik beginnen. Collega onderzoeker, collega-in-spe orthopedisch chirurg, vriend, kamergenoot, statistisch ondersteuner, kaftmedeontwerper… Kortom te veel om op te noemen. Oja, nog eentje: paranimf!

Lars, tja, Lars. Van het zelfde laken een pak. Oud-huisgenoot, dispuutsgenoot, mede-PPCC-er, vriend, collega-in-spe orthopedisch chirurg, mijn trouwe steun en toeverlaat… En inderdaad, hoe kan het ook anders: paranimf!

Inges, Alette, Natalie, Paul, Barbara en Oscar (kortom het Braincenter). Bedankt voor de gezelligheid! Het was echt super om met jullie een kamer te delen.

Martin, Geert, Patrick, Daniëlle, Jan R, Dennis Stant, Martin Been, Marja de Groot-Heupner, Andre Bus en de leden van de Orthopedie-infectiecommissie. Bedankt voor de hulp bij het onderzoek (en de gezelligheid tijdens de congressen)!

Ellen van Drooge en Ina Heidema, fantastisch hoe jullie altijd blijven meedenken.

Florence Postollec, dank voor je gezelligheid en dat ik je paranimf mocht zijn.

Rest van Orthopedie en BME (kortom iedereen die ik vergeet), bedankt!

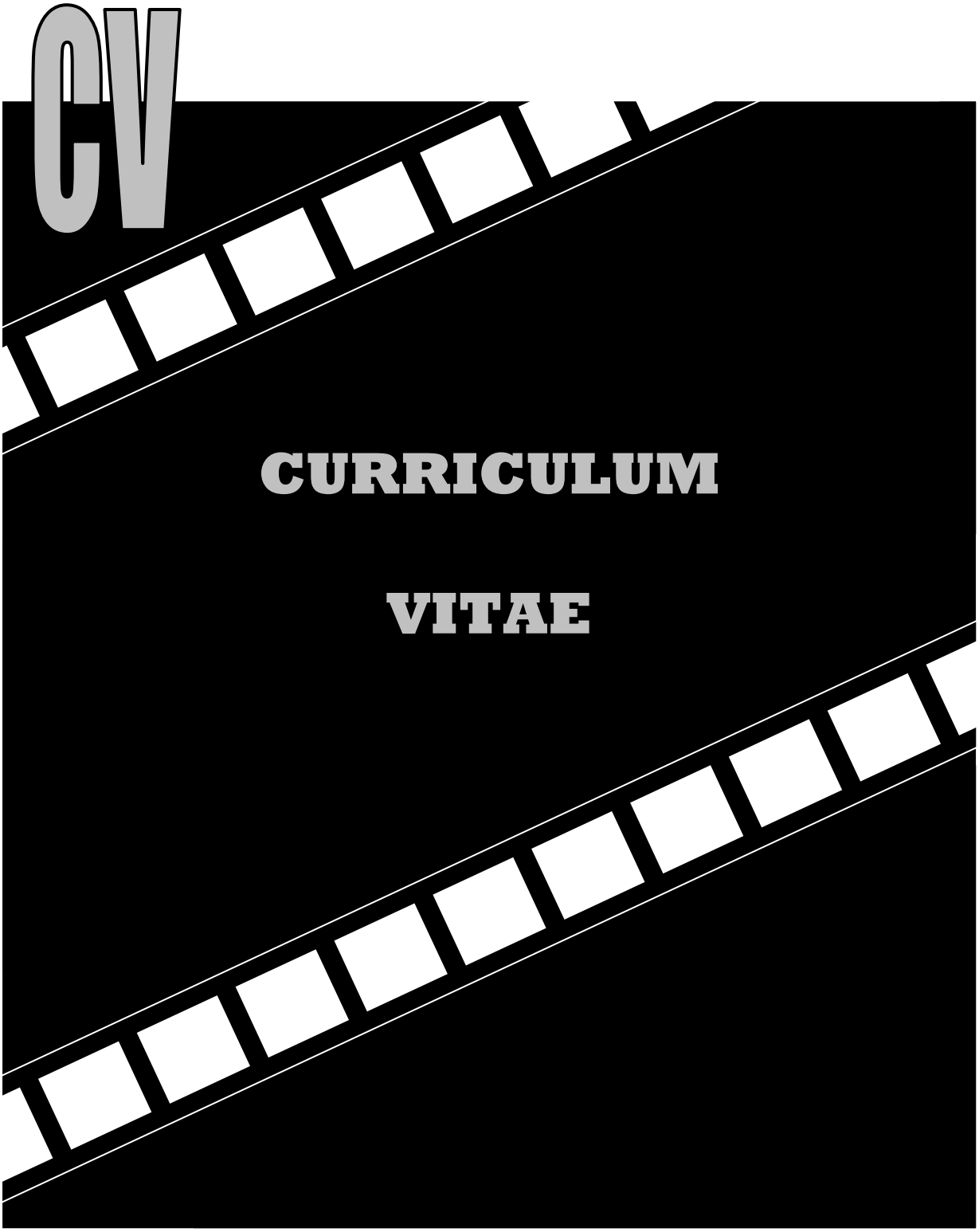
In willekeurige volgorde: Bunny, Do!, Renata (thanks for bringing the sun), Sioe Hoey, Ard, Marc, Crudo, Ellen, Gijs, Gedert T, Jimmy, Yde, Schuur, Bas H., Verdonk, Ed, Hans, Temmink, Willem, Louie, Daf, Struikie, Bossie (en andere clubgenootjes), Hilke, Lucas, Ingrid, DJ, Esther, de rest van Cambrinus en Usus (en nu vergeet ik er toch nog een hoop) bedankt voor alle gezelligheid!!

Klotedief, dank voor het jatten van mijn proefschrift. Hierdoor is het uiteindelijk beter geworden.

TOPNAAD, bedankt voor alles! En dat is heel veel!

Mam, Jan, Janneke (en Bas natuurlijk) en Marijn. Bedankt voor de altijd aanwezige steun en interesse! Ben blij dat jullie mijn familie zijn.

Pap, deze is voor jou, dat weet je!


CV
CURRICULUM
VITAE

The author of this thesis was born in Nieuw Heeten, The Netherlands, on January 31st in 1976. After graduating from the Scholengemeenschap Holten he commenced Technical Engineering at the Technical University in Twente. After completing his propedeutics he started with medical school at the university in Groningen. In 2002 he graduated and started working as medical doctor at the Department of Orthopaedic Surgery (headed by Prof. Dr. J.R. van Horn) of the University Medical Center in Groningen. During that time he started working on a Dutch problem-based textbook in orthopaedics, called "Probleemgeoriënteerd denken in de orthopedie: een praktijkboek voor de opleiding en de kliniek", which was published in March 2004. In the meantime he started working on this thesis at the Department of Biomedical Engineering in Groningen (headed by Prof. Dr. Ir. H.J. Busscher). Currently he is working at the Department of Surgery of the Martini Hospital Groningen (headed by dr. P.C. Baas and dr. G.I.J.M. Beerthuizen) prior to further specialization to orthopaedic surgeon.

Publications:

- Knobben BAS, Van Horn JR, Van der Mei HC, Busscher HJ. Evaluation of measures to decrease intra-operative bacterial contamination in orthopaedic implant surgery. Journal of Hospital Infections 2006. Feb;62(2):174-80.
- Knobben BAS, Engelsma Y, Neut D, Van der Mei HC, Busscher HJ, Van Horn JR. Intra-operative contamination influences wound discharge and periprosthetic infection. Clinical Orthopaedics and Related Research. Accepted 2006.

Book:

- Ensing GT, Knobben BAS, Houweling ST, Verhaar JAN, Van Horn JR. Probleemgeoriënteerd denken in de orthopedie: een praktijkboek voor de opleiding en de kliniek. Leusden, de Tijdstroom 2004. ISBN 905898060X.