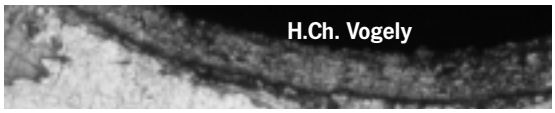
Infections in Orthopaedic Surgery

Clinical and Experimental Studies



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Infection in Orthopaedic Surgery

Clinical and Experimental Studies

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Clinical and Experimental Studies

Infecties in de Orthopaedie

Klinische en Experimentele Studies

(met een samenvatting in het Nederlands)

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- Vogely HCh, Pruijs JEH, Geelen SPM, Dhert WJA, Verbout AJ: The Outcome of Therapy of Osteomyelitis in Children. A Retrospective Study of 28 Patients. Eur J Orthop Surg Traumatol 1997; 7(3):181-185.
- Vogely HCh, Oner FC, Fleer A, Dhert WJA, Verbout AJ: Hematogenous infection of a total hip prosthesis due to *Clostridium perfringens*. Clin Infect Dis 1999; 28(1):157-158.
- Vogely HCh, Oosterbos CJM, Puts EWA, Nijhof MW, Nikkels PGJ, Fleer A, Tonino AJ, Dhert WJA, Verbout AJ: Effects of Hydroxyapatite Coating on Ti6Al4V Implant Site Infection in a Rabbit Tibial Model. J Orthop Res 2000: in press.
- Oosterbos CJM, Vogely HCh, Nijhof MW, Fleer A, Dhert WJA, Tonino AJ, Verbout AJ: Osseointegration of HA-coated and noncoated titanium implants in the presence of local infection. A comparative histological study in rabbits. Submitted for publication.
- Vogely HCh, Dhert WJA, Fleer A, Verbout AJ: The infected orthopaedic implant. An animal model to study the mechanisms of haematogenous infection of cementless implant materials. Eur J Orthop Surg Traumatol 1996; 6(2): 91-95.

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Contents

General Introduction

Some decades ago, the general expectations were that infectious illness would become less important in future than it had been until then. There was optimism about the possibility to exterminate notorious infectious diseases and trust in the action of antibiotics. Unfortunately, nowadays resistance is a serious problem and the micro-organisms that produce orthopaedic infections have become more difficult to kill. This resistance to standard antibiotic regimens makes proper antibiotic selection mandatory. Moreover it requires a long and sometimes intensive treatment, partially due to the difficulty to eradicate the causative organisms in an environment of poor vascularisation (such as in bone sequestra or adjacent to an endoprosthesis). Such a prolonged treatment of orthopaedic infections, in comparison with other infectious diseases, has a considerable physical and social impact on the patient and a relative high economic impact. Under the influence of managed care, the cost and effectiveness of various treatment alternatives have been questioned and efforts have been made to decrease hospitalisation and limit parental antibiotics.

Infections in orthopaedics, because of the nature of the substrate, are localised in bone and/or joints and are called osteomyelitis and arthritis. The infectious process of the bone and its marrow normally is caused by pyogenic micro-organisms, although other agents (e.g. those responsible for tuberculosis, viral or fungal infections) are possible as well. The commonest route is the haematogenous spread from a distant focus, but it can also be produced by extension of soft tissue infection adjacent to bone, or it can be initiated from an open wound or fracture. Septic arthritis can be produced by haematogenous spread to the synovium or by the aggravation of osteomyelitis involving the epiphysis or an intracapsular metaphysis. Direct contamination of the joint is another possibility.
The vascular architecture of the bone (i.e. the metaphysis) should predispose to the establishment of infection following bacteremia. The anatomical different patterns of vascularity between infant, child and adult explain the difference in clinical manifestations of the infection in children and adult. In children, the acute

haematogenous osteomyelitis occur more frequently than in adults, because of the vascular anatomy of growing bones, as well as the more frequent bacteremia and repeated traumata.

Infection reflects a complex interaction between the host, a micro-organism, and the mutual response of each to the other. The mere presence of bacteria in bone, whether from bacteremia or from direct inoculation, is not sufficient to produce an orthopaedic infection. Illness, malnutrition, trauma and inadequacy of the immune system may be factors that trigger bone and joint infections to become manifest. Infection occurs when an adequate number of a sufficiently virulent micro-organism overcomes the host's natural defences. Local factors (such as the vascularity) also play an important role in the development of infection. Besides, the relative absence of phagocytic cells in the metaphyses of bones may explain why acute haematogenous osteomyelitis is more common in this location.

In both the adult and paediatric population, *Staphylococcus aureus* is the most common causative agent for osteomyelitis and septic arthritis. However, depending on age, also other micro-organisms are frequently isolated. In neonates, group B streptococci and enterobacteriaceae are frequent causative agents; in older children haemolytic group A streptococci are isolated relatively frequently. This holds too for *Haemophilus influenzae* type B in children younger than 3 years of age, before the recent introduction of nation-wide vaccination.
Despite recent advances in diagnosis, antibiotic development and refined surgical techniques, thus a proper management of bone and joint infections remain a formidable challenge. It may require both antibiotic and surgical treatment. In acute osteomyelitis only antibiotic treatment may be sufficient, but then the micro-organism should be accurately identified and its antimicrobial susceptibility should be determined preferentially. When treatment is delayed, infection becomes chronic, resulting in pus and sequestra. In chronic osteomyelitis surgery is usually inevitable.

Orthopaedic surgery is evolving. The modern era of total hip arthroplasty is only about forty years old. During this relatively short period, the procedure has shown to be highly effective in improving physical function, social interaction and the overall health of patients. Development and improvement of these orthopaedic devices has led to new cemented implanting techniques, and cementless techniques and implants were introduced as well.

The introduction of implants in orthopaedic surgery has resulted in a new kind of infection, the prosthetic joint-related infections. Because of the presence of the foreign body there appears to be a higher susceptibility to infections, probably developing through direct contamination during implantation. However, also a haematogenous origin of infections has been documented, although it is probably less frequent.

In 1969 Charnley reported an infection rate for total hip arthroplasty of 9.5 per cent (Charnley 1969). In the following years, especially because of prophylactic antibiotics, the prevalence of an infected prosthesis was reduced considerably. As a result, in the more recent years, infection rates of 1 to 2 per cent have been reported for primary total hip arthroplasties (Charnley 1972; Fitzgerald 1973; Salvati 1982b; Lidwell 1986; Lidwell 1987; Maderazo 1988; Schutzer 1988; Garvin 1995).

Although infection rates have decreased substantially over the past few decades, infection still is a source of considerable morbidity because the incidence of total joint replacement is increasing (Okhuijsen 1998). Therefore in absolute numbers, infection of a prosthesis still constitutes a major and increasing burden to the health care system (Sculco 1993).

As mentioned above, within the category of prosthetic joint-related infections both the haematogenous type as well as those caused by direct contamination have to be distinguished. One states that haematogenous infection is more likely to occur in patients who are immunosuppressed, such as those who have been treated with immunosuppressive medications for inflammatory arthritis. In fact it is not clear if this is caused by the treatment of the disease or

by the underlying disease itself. Recurrent bacteremia, such as occur in patients who are repeatedly and recurrently catheterised, dental manipulation, respiratory infection, remote periprosthetic infection, open skin, endoscopy and contamination of the operative site are other causes of haematogenous infections. The skin has shown to be the most frequent source of a haematogenous infection.

Operative debridement and antibiotic therapy are the mainstays of treatment of infection of a hip arthroplasty. The debridement includes the excision of all infected and necrotic tissue and eventually removal of the prosthesis and cement. A still unresolved question is, whether after removal of the prosthesis, a new prosthesis should be replaced immediately or in a later phase.

An increasing knowledge of mechanisms, diagnosis and treatment of orthopaedic infections has led to a more successful eradication of the infection for more patients than several decades ago, however many questions still remain unanswered. Until now, there has been a rapid increase in the use of biomaterials in orthopaedic surgery, both with respect to the actual number of implants as with respect to an increasing variety of implant materials, all with different material (biocompatibility) characteristics.

The author's interest in orthopaedic infections was initiated by the impact of these infections on patients. It stimulated to a study, as presented in Chapter 3, of a population of children with osteomyelitis, in which the haematogenous route is by far the most important pathway for bone infection. In Chapters 4 and 5 clinical aspects of prosthetic joint-related infections are described. At first, an attempt is made to define the outcome of this difficult and heterogeneous population. Then, emphasising the significance of the haematogenous route for prosthetic infections, an unusual complication of an abdominal infection, i.e. prosthetic hip infection, is presented. Before these clinical data, the literature is presented in Chapter 2. In Chapter 6 a summary of the foregoing chapters 2 to 5 is given and aims for experimental studies are formulated. These

experimental studies are presented in Chapters 7 and 8. In Chapter 7 the question of infection susceptibility in relation with implant surface characteristics and biocompatibility is addressed. New implant materials have been developed during the last decade, with the goal of increased biocompatibility, while "bacterial compatibility" did not receive much attention. Are bioactive implant surfaces less susceptible to bacterial colonisation, as Gristina's "race for the surface" would suggest, or are there other aspects, which in turn determine the clinical success of an infected implant? And what is the influence of infection on the ingrowth of noncemented prostheses? Chapter 8 focuses on a further understanding of the haematogenous route as a cause for bone or prosthesis infection by the development of animal model in the rabbit. Finally, Chapter 9 contains a summary and discussion of this thesis.

Review of the Literature

2.1. Introduction

The replacement of the hip joint by a total joint arthroplasty has been performed for almost 40 years. The procedure has proven to be highly effective in improving physical function, social interaction and over-all health of millions of patients (Laupacis 1993).
In the beginning, this total joint replacement procedure was complicated by a rate of infection of almost 10 percent (Charnley 1969). Although infection rates have decreased substantially over the past few decades, infection still is a source of considerable morbidity. This reduction in infection rate has been accompanied by an increase in the incidence of total joint replacement (Okhuijsen 1998). Therefore in absolute numbers, infection of a prosthesis still constitutes a major burden to the health care system and is a complication that remains a considerable concern (Sculco 1993). In the current chapter, various general aspects of an infected total joint arthroplasty such as epidemiology, risk factors, classification, diagnostic tools, and treatment options will be described.

2.2. Epidemiology and risk factors of infection of joint replacement

In 1969 Charnley reported an infection rate for total hip arthroplasty of 9.5 per cent (Charnley 1969). In the following years, various precautions such as prophylactic antibiotics, ultraclean-air operation rooms, better operative techniques, different modes of fixation of the implant and careful selection of patients, considerably reduced the prevalence of an infected prosthesis. As a result, in the more recent years, infection rates of 1 to 2 per cent have been reported for primary total hip arthroplasties (Charnley 1972; Fitzgerald 1973; Salvati 1982b; Lidwell 1986; Lidwell 1987; Maderazo 1988; Schutzer 1988; Garvin 1995). In the Mayo Clinic, between 1969 and 1996, the prevalence of infection of a primary total hip prosthesis and a primary total knee prosthesis, was 1.3 per cent and 2.0 per cent respectively (Hanssen 1999). The rate of deep infection was influenced by previous surgery of the affected joint

(Fitzgerald 1977; Poss 1984; Wilson 1990). For revision operations, the rate of infection was 3.2 per cent and 5.6 per cent for hip and knee arthroplasty respectively (Hanssen 1999).

The infection rate of a total joint arthroplasty is higher in patients with rheumatoid arthritis than with osteoarthritis (Charnley 1972; Ahlberg 1978; Stinchfield 1980; Glynn 1983; Ainscow 1984; Poss 1984; Bengtson 1987; Maderazo 1988; Wilson 1990). Also patients suffering from diabetes mellitus are at an increased risk for a deep total joint infection (England 1990; Papagelopoulos 1996). However, Moeckel et al. did not find a higher rate of infection of total hip arthroplasties in patients with diabetes mellitus (Moeckel 1993).

Another factor influencing infection is poor nutrition. Wound complications occurred more frequently in patients with malnutrition as indicated by a serum albumin level of less than 35 grams per litre (Jensen 1982; Greene 1991; Smith 1991). Other risk factors for wound complications and deep infection are for instance obesity, recurrent urinary-tract infection, oral use of steroids (Wilson 1990) and psoriasis (Stern 1989).

Prolonged preoperative hospital stay is another potential factor that increases the rate of infection of an arthroplasty. According to Cruse and Foord, the rate of infection was 1.1 per cent for patients who had been admitted to the hospital on the day of operation compared with 4.3 per cent for those who had been hospitalised for two weeks before the operation (Cruse 1973).

Other factors that are associated with an increased infection rate are the presence of structural bone graft (Schutzer 1988), the use of metal-metal prosthesis (Poss 1984), and the use of constrained prostheses (Poss 1984; Bengtson 1987).

A specific group are the haematogenous infections. This type of infections is likely to occur in patients who are immunosuppressed, such as those who have had a renal transplant or who have been treated with immunosuppressive medications for inflammatory arthritis, and those who have recurrent bacteremia, such as drug abusers and patients who are repeatedly and recurrently catheterised (Irvine 1974; Hughes 1979). Also responsible for a haematogenous infection may be dental manipulation (Rubin 1976; Hunter 1977;

Hughes 1979; Stinchfield 1980), respiratory infection (Hunter 1977), remote periprosthetic infection (Hunter 1977), open skin (Ainscow 1984; Maderazo 1988; Deacon 1996), endoscopy (Vanderhooft 1994) and contamination of the operative site (Hughes 1979). It is usually not possible to trace the primary focus of infection, but in a literature review Deacon reported on 180 cases of suspected haematogenous infections of a prosthetic joint (Deacon 1996). The skin was the most frequent source, and other frequently found foci were the genito-urinary tract, the mouth and the respiratory tract.

The cost of treatment of a total hip prosthesis infection was at least 50,000 dollars per patient in 1986 in the United States (Maderazo 1988), and currently these costs may very well be higher. The costs per year to treat the total of 3,500 to 4,000 total hip infections was estimated between 150 and 200 million dollars (Sculco 1993). These costs are considerably higher after revision procedures.

2.3. Classification of prosthesis-related infections of the hip

A classification system of infection of orthopaedic implants was proposed by Coventry (Coventry 1975) and later amended by Fitzgerald et al. (Fitzgerald 1977) (Table 2.1). This three-stage system is based on the mode or timing of the presentation of infection. Stage-I infections occur in the immediate postoperative period as an acute fulminating infection. The infection is caused by contamination at the time of the operation or by infected haematomas or superficial wound infections. This type of infection present within the first 3 months, usually becomes manifest within 4 weeks after the operation. The diagnostic challenge is to determine whether or not a superficial infection has penetrated deep into the fascia. Stage-II infections are believed to originate also at the time of the operation. Because of a small inoculum or the low virulence of the organism, the onset of the symptoms is delayed. Mostly the infection presents itself in the form of an indolent, chronic low-grade infection. Pain is often present from the beginning of the original

procedure. The patient is usually seen between 6 weeks and 24 months after the operative procedure. This type of infection represents a diagnostic challenge, because the findings on examination of the hip in a patient who has a stage-II infection usually are not specific and are similar to those associated with aseptic loosening. Stage-III infections are thought to be caused by haematogenous spread from a distant focus to a previously asymptomatic hip. This type of infection appears usually 2 years or more after the operative procedure. Early diagnosis may allow salvage of the joint by means of complete debridement. A delay in the diagnosis may necessitate a one- or two-stage exchange procedure.

To facilitate the management of the patients with an infected hip arthroplasty, Estrada described a new classification that comprises four categories (Table 2.1), and he also makes general suggestions for treatment (Estrada 1993). In the first category, peroperative unexpected "positive intraoperative cultures" are present. Infection is diagnosed after two or more positive specimens, obtained intraoperatively from different sites of the hip. The cultures must be positive for the same organism. He suggests that the infection should be treated by intravenous antibiotics without operative intervention. The second category includes patients with an "early postoperative infection", apparent within one month after operation, and debridement with intravenous administration of antibiotics are the treatment suggestions. More than one month after operation, the infection is classified as "late chronic" and is characterised by an insidious clinical onset. As treatment suggestions for this stage, removal of the components and intravenous antibiotics are mentioned, followed by various other options. The last category is the "acute haematogenous infection", characterised by an acute onset of clinical symptoms in a previously well functioning hip. If the prosthesis is well fixed, treatment is suggested according to the "early postoperative infection", and if the prosthesis is found to be loose, the treatment should be similar to that for a "late chronic infection".

Table 2.1. Classification of prosthetic-related infections according to Fitzgerald and Estrada.

Classification	
Category	**Definition**
Fitzgerald et al. (Fitzgerald 1977):	
Stage-I (acute fulminating infection)	Developed within 3 months after surgery, usually within the first month
Stage-II (delayed sepsis)	Developed as a creeping, indolent infection within the first 24 months
Stage-III (late haematogenous infection)	Caused by a haematogenous spread to a previously asymptomatic hip, usually 2 years or more after the arthroplasty
Estrada et al. (Estrada 1993):	
Positive intraoperative culture	Two or more intraoperative specimens positive for the same organisms
Early postoperative infection	Apparent within 1 month of surgery
Late chronic infection	Presenting >1 month after surgery, with an insidious clinical onset
Acute haematogenous infection	Acute onset of clinical symptoms in a previously well-functioning joint

2.4. Causative organisms

From the majority of the patients with an infected hip arthroplasty, Gram-positive micro-organisms are isolated (Table 2.2). More than 50 percent of the Gram-positive isolates are staphylococci. Coagulase-negative staphylococci (CNS) and *Staphylococcus aureus* are isolated in almost equal numbers. During surgical debridement of an infected arthroplasty, Gram-negative bacilli are isolated from only 18 per cent of the cultured micro-organisms. Gram-negative bacilli have rarely been cultured from wounds that were closed and did not have sinus tract drainage at some time during the postoperative course. Because Gram-negative bacilli are usually isolated from mixed infections, it seems that the Gram-neg-

ative bacilli are usually secondary invaders of open, draining wounds in patients with deep sepsis of an arthroplasty of the hip (Lotke 1992). Anaerobic micro-organisms are isolated in 10 per cent of the positive cultures.

Table 2.2.
Overview of causative micro-organisms of prosthetic infections as reported to the literature between 1973 and 1998.

Reference	CNS	*S. aureus*	Strepto-cocci	other Gram pos.	Gram neg.	anaerobes	other	no. of isolates
	%	%	%	%	%	%	%	
Murray 1973	27	33	7	-	33	-	-	15
Fitzgerald 1977	16	20	18	-	24	20	-	49
Carlsson 1978	29	28	12	2	6	23	-	102
Hughes 1979	70	10	3	3	3	7	3	30
Buchholz 1981	5	40	8	11	23	12	2	695
Inman 1984	38	18	23	3	11	8	-	66
Fitzgerald 1985	32	27	12	6	17	6	-	142
Antti-Poika 1989	8	35	28	-	30	-	-	40
McDonald 1989	34	17	17	8	16	7	-	110
Schmalzried 1992	12	33	10	4	37	4	-	51
Garvin 1993	38	19	13	6	11	13	1	96
Garvin 1994a	30	19	16	9	19	7	-	43
Raut 1994	33	21	15	17	10	2	2	52
Nestor 1994	42	18	13	-	16	11	-	45
Lieberman 1995	29	16	21	-	29	4	-	68
Raut 1995	52	9	10	22	6	1	-	162
Lai 1996	26	42	2	5	21	-	5	43
Lachiewicz 1996	38	29	10	-	14	10	-	21
Tsukayama 1996	38	22	14	-	14	8	3	147
Ure 1998	41	23	23	5	-	9	-	22
Ostendorf 1998	38	18	16	2	16	10	-	209
Total	26	27	12	7	18	9	1	2208

There is a shift in the types of bacteria found in peroperative cultures during the last two decades. More infections with *Staphylococcus epidermidis* are seen nowadays (Ostendorf 1998), while for instance less anaerobic micro-organisms were isolated from patients with infected arthroplasties (Fitzgerald 1973; Ostendorf 1998). *Peptococcus species* are the most frequent anaerobic isolates recovered.
One of the main items in the successful management of the infected arthroplasty is the virulence of the causal organism. McDonald et al. (McDonald 1989) demonstrated that patients from whom a causal organism of low virulence was isolated, had a lower incidence of recurrent sepsis than those patients from which a more virulent organism was isolated. The less virulent organisms included CNS, anaerobic cocci, and streptococci other than group D streptococci. The virulent causal organisms included methicillin-resistant staphylococci, gram-negative bacilli, group D streptococci and enterococci. Especially, organisms that secrete glycocalyx should be considered to be more virulent causal organisms (McDonald 1989).

2.5. Diagnosis of infection

An outline of the various data and investigations on which the diagnosis orthopaedic implant infection may be based, is presented in Table 2.3. This includes clinical data, laboratory investigations and imaging studies. Each of these will be discussed briefly.

2.5.1. Clinical Presentation

Pain is the major presenting symptom of most patients with a deep infection. Systemic signs of infection, such as fever, chills and sweating may also be present. The region of the hip may be erythematous, swollen, fluctuant and tender. Sometimes wound drainage is present, usually purulent. However, these symptoms are not always present, so it is not always possible to establish a clear conclusive diagnosis. A history of persisting pain or prolonged drainage after the operation and a careful physical examination can help to guide the appropriate investigations.

Table 2.3.
Overview of various investigations that may lead to the diagnosis of infection.

Diagnosis of infection		
Preoperative investigations		
History and examination		Fever, local pain and erythema
Haematological investigations	ESR	>30 mm/hr
	CRP	>10 mg/l
	WBC	>11.0x10^9/l
Radiography	Radiographs	Osteolysis, periosteal reaction
	Arthrographs	Loosening
Radionuclide imaging	Technetium-99m bone	Increased uptake
	Gallium-67 citrate	Increased uptake
	Indium-111-labeled leukocytes	Increased uptake
	Indium-111-labeled Ig-G	Increased uptake
Preoperative aspiration	Gram-staining	Presence of bacteria
	Culture	Positive culture
Peroperative investigations		
Surgical judgement		Pus
Peroperative sampling	Gram-staining	Presence of bacteria
	Culture	More than one-third of cultures are positive
	Polymerase chain reaction	Presence of bacterial DNA
Frozen section		More than 5 polymorphonuclear leukocytes per high-power field

2.5.2. Laboratory examinations

White blood-cell count (WBC)

White blood-cell count is rarely elevated in patients who have an infection following a total hip arthroplasty and is therefore not helpful for diagnosing an infection of an arthroplasty (Eftekhar 1984; Spangehl 1999). Considering white blood-cell count to be elevated when it is more than 11.0x10^9 per litre, sensitivity is only 0.20 and specificity 0.96 (Spangehl 1999). When the white blood-cell count is increased, a systemic manifestation of infection is usually clinically obvious.

Erythrocyte sedimentation rate (ESR)

The erythrocyte sedimentation rate is a non-specific haematological test. Responsible for an elevation of the erythrocyte sedimentation rate are acute-phase reactants. These positively charged macromolecules are produced in the liver as a response to a variety of conditions such as inflammatory diseases, infection and neoplastic processes (Covey 1987). The discriminating value chosen to differentiate between septic and aseptic conditions has been described to be above 30 to 35 millimetres per hour (Sanzen 1989; Thoren 1991; Roberts 1992). A specificity of 0.85 for an elevated erythrocyte sedimentation rate was reported, and a sensitivity of 0.82 (Spangehl 1999). The erythrocyte sedimentation rate may remain elevated for months after an uncomplicated total hip replacement (Shih 1987).

C-reactive protein level (CRP)

Another acute-phase reactant, C-reactive protein, is synthesised in the liver. Under normal conditions, it is found in trace amounts (Sanzen 1989). Also C-reactive protein increases in a non-specific manner as a result of infection, inflammatory disease neoplastic disorder and surgery. The C-reactive protein level elevates to maximum values within 48 hours after an operation and returns to normal trace amounts in approximately 2 to 3 weeks (Aalto 1984; Shih 1987; Niskanen 1996). Because of this ability to return to a normal value much faster then the erythrocyte sedimentation rate, it is a more sensitive indicator of infection, especially in the early post-operative period (White 1998; Spangehl 1999). A C-reactive protein value higher than 10 milligrams per litre should be considered to be suggestive of infection. For this parameter, a sensitivity of 0.96 and a specificity of 0.92 were reported (Spangehl 1999).

2.5.3. Imaging techniques

Radiography

Plain radiographs are of limited value for the diagnosis of infection of an orthopaedic implant, particularly in differentiating between aseptic loosening and infection. Many radiographic find-

ings, such as loosening, osteolysis (Huddleston 1988; Barrack 1993) and endosteal scalloping (Lyons 1985) are common in both septic and aseptic failure (Figure 2.1).
Periostal new-bone formation has been considered by some to be pathognomonic of deep infection (Fitzgerald 1992).

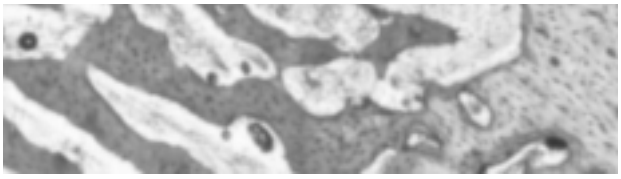


Figure 2.1
X-ray of a patient with an infected total hip prosthesis. There is obvious osteolysis around the femoral component.

Most patients who have an early postoperative or an acute haematogenous infection show no signs of loosening. In cemented prosthesis, loosening is categorised as possible, probable and definite loosening (Harris 1982). Possible loosening is defined as a radiolucent line, occupying 50 to 100 per cent of the bone-cement interface which was not visible on the radiographs taken immediately postoperatively. Probable loosening is defined as a continuous radiolucent line surrounding the entire cement mantle. Definite loosening is defined as a radiolucent line at the stem-cement interface,

fracture of the cement mantle or migration of the prosthesis. However, the clinical significance of a radiolucent line on radiographs has been questioned (Jasty 1991; Kwong 1992). On the acetabular side definitive loosening is defined by migration of the socket or cement mantle, protrusio acetabuli or acetabular fracture (Lyons 1985). Loosening is often subtle and easier to distinguish if old radiographs are reviewed. Arthrography can improve the accuracy of radiographs in the diagnosis of loosening (O'Neill 1984; Harris 1986). Arthrography does not have notable advantages compared with plain radiography when used for the diagnosis of loosening of the femoral component. It is of more benefit when used for the assessment of loosening of a cemented socket (O'Neill 1984).

Radionuclide imaging

Technetium-99m bone scintigraphy is highly sensitive, but not specific for the diagnosis of infection after a hip arthroplasty (Nijhof 1997b). Multiple conditions, such as fractures, tumours, heterotopic ossification and inflammatory disorders can also result in an increased uptake.

Focal uptake can be present for as long as one year after an uncomplicated hip replacement and for more than two years after insertion of a cementless implant (Oswald 1989).

Gallium-67 citrate is an isotope that accumulates in areas of inflammation. Because any inflammation may cause an increased uptake, it is not specific (Wegener 1991). Although more accurate than single isotope scans, sequential technetium-gallium scintigraphy still lacks sufficient accuracy to be clinically useful for diagnosing a prosthetic infection. Merkel et al. and Kraemer et al. reported a sensitivity of 0.50 and 0.38 and a specificity of 0.78 and 1.00 (Merkel 1985; Kraemer 1993).

Indium-111-labeled leukocytes are useful for the diagnosis of increased vascularity and leukocyte accumulation. The sensitivity for diagnosing an infection after hip replacement is high, but the specificity is poor (1.00 and 0.41-0.50, respectively) (Wukich 1987; Johnson 1988). In an attempt to increase its ability to aid in the diagnosis of infection, indium-111-labeled leukocyte scanning was, in a sequential protocol, combined with technetium scanning. The

combined scans generally had higher sensitivities and specificities (0.89-1.00 and 0.95-0.98, respectively) (Johnson 1988; Palestro 1990).

In Indium-111-labeled immunoglobulin-G scintigraphy, the radiopharmaceutical agent is labelled to a carrier that targets areas of acute inflammation. The advantage of this method compared with indium-111-labeled leukocyte scintigraphy is that the lengthy laboratory preparation and handling of possible infected blood can be avoided. The first studies prospectively compared results of indium-111-labeled immunoglobulin-G scintigraphy with standard three-phase technetium-99m scintigraphy, with promising results (sensitivity 0.92, specificity 0.88) (Oyen 1992). Additional studies showed that Indium-111-labeled immunoglobulin-G scintigraphy is a very sensitive tool for detection of infectious bone and joint disease and is a highly sensitive (1.00) and fairly specific tool for detecting late infection of total hip and total knee arthroplasties (0.82 and 0.50, respectively) (Nijhof 1997b; Nijhof 1997c).

2.5.4. Microbiological examinations

The role of aspiration to obtain material for culturing has generated considerable debate. By some authors aspiration was advocated for all patients who had a failed hip replacement (Fitzgerald 1977; Roberts 1992). More recently however, other authors favour a more limited role, with aspiration being used to confirm a clinical suspicion of infection or to support (or to reject) the findings of other preoperative investigations (Barrack 1993; Tigges 1993; Fehring 1996; Lachiewicz 1996; Mulcahy 1996). A great benefit of aspiration at suspected infection is the ability to identify the organism and its antibiotic sensitivity profile, which may influence the preoperative planning as well as the type of antibiotics chosen for therapy. Investigations reported a wide range of sensitivity (0.50-0.93) and specificity (0.82-0.97) for aspiration (Roberts 1992; Barrack 1993; Kraemer 1993; Tigges 1993; Fehring 1996; Lachiewicz 1996; Mulcahy 1996; Spangehl 1999). The wide variation in positive results may be due in part to the technique of aspiration (Spangehl 1999). To reduce the number of false-positive results, and thereby improve accuracy, a standard protocol should

be established. To reduce the number of false-negative results, all antibiotics must be discontinued for 2 to 3 weeks before aspiration. Local anaesthetics should be used only in the skin and not in the joint as they may be bacteriostatic. If not enough fluid is obtained, the joint can be irrigated with saline solution and reaspirated. A needle biopsy of synovial tissue also should be performed at the time of the aspiration (Spangehl 1998).

A Gram stain is a simple investigation to establish the presence or absence of bacteria. The Gram stain may be specific, but it lacks any acceptable level of sensitivity (0-0.23) (Kraemer 1993; Feldman 1995; Chimento 1996; Della Valle 1999b; Spangehl 1999). Therefore the Gram stain is not reliable for determining the presence of an infection in patients who undergo a reoperation after a hip arthroplasty.

Peroperative cultures are often used as the standard for the diagnosis of infection after an arthroplasty. However, the results are not 100 per cent accurate. A recent study reported a rate of peroperative false-positive cultures of 3 per cent for patients who had a revision hip replacement (Spangehl 1999). Several other investigators have reported rates of false-positive cultures as high as 29 percent in association with total hip arthroplasty (Murray 1973; Tietjen 1977). To avoid false results, a careful sample acquisition protocol is important. Preoperative antibiotics should be withheld until samples for cultures have been taken. Clean instruments should be used. The samples should be taken from an area that has not been cauterised and prior to irrigation of the woundbed. To decrease the chance of colonisation and to allow for subsequent administration of antibiotics the samples are taken immediately after the pseudocapsule has been opened. A minimum of 3 samples should be obtained from close to the surface of the prosthesis and from inflamed tissue and sent immediately to the microbiology laboratory for processing (Spangehl 1998). Cultures should not be considered negative until the final results of the broth subcultures are available. These final results should be interpreted in the context of all preoperative and perioperative findings (Spangehl 1998).

New molecular biological technology can be used to determine the presence of bacterial DNA and RNA. Polymerase chain reaction

enables the production of large amounts of specific sequences of target DNA from small quantities of starting material. Next, the DNA can be identified by several techniques (Clarke 1992; Rapley 1992; Garvin 1995; Mariani 1998). The disadvantage of the technique of polymerase chain reaction is susceptibility to contamination. Whether this technique is too sensitive, leading to a high rate of false-positive results, remains to be determined (Nijhof 1998).

2.5.5. Peroperative judgement of infection

Even though preoperative investigations have been normal, some arthroplasties may appear to be infected to the surgeon during the operative procedure. Formation of an abscess and pus are the most obvious signs of infection. Signs such as a diffuse synovitis, turbid joint fluid and formation of slime are all suspicious. Keeping in mind that preoperative investigations are not 100 per cent accurate, the appearance of the joint is occasionally so clearly suspicious for infection that the surgeon will not proceed with a normal single-stage exchange procedure. However false impressions do occur. For example, severe accumulations of wear debris can induce a reaction that is similar in macroscopical appearance to that of an infection.

There are various options for patients in whom the macroscopic appearance looks like an infection, despite negative findings of preoperative investigations. The first option is to remove the prosthesis, obtaining multiple cultures and performing a radical debridement. The postoperative clinical situation of the patient and the results of the cultures should then either lead to the decision to create a definitive Girdlestone situation or to do a two-stage exchange. A second option is to leave the prosthesis in place, obtain multiple specimens for culture and reoperate at a later date after the results of the cultures are available. The last option is to perform a one-stage exchange procedure after a radical debridement. The results of the peroperative cultures should direct decisions as to the necessity of further antibiotic treatment (Spangehl 1998).

2.5.6. Histological examination

For diagnosing infection, peroperative frozen sections of periprosthetic tissue have become a valuable tool. Mirra et al. stated that five polymorphonucleair leukocytes per high-power field is conclusive for the diagnosis of infection and that lack of polymorphonucleair leukocytes is conclusive for absence of infection (Mirra 1976). Various investigators reported a sensitivity of more than 0.80 and a specificity of more than 0.90 (Athanasou 1995; Feldman 1995; Lonner 1996; Spangehl 1999). However, Fehring reported a much lower sensitivity for the use of frozen sections in the diagnosis of infection (Fehring 1994; Fehring 1996). It is important that tissue samples should be taken from the areas that appear to be actually infected. In addition, as a result of substantial interobserver variations, the pathologist should be experienced in preparation and interpretation of the specimens (Spangehl 1998). The sensitivity of frozen sections at the time of reoperation, for the detection of persistent infection after resection arthroplasty and treatment with antibiotics, is however poor (Della Valle 1999a).

Table 2.4. Overview of the various options for treatment of an infected arthroplasty. Antibiotics are always indicated.

Treatment options	
Antibiotics	No surgical treatment
Debridement	Prosthesis remains in situ
One-stage revision	Surgical debridement plus exchange arthroplasty
Two-stage revision	Removal of the prosthesis, surgical debridement and exchange in a second stage
Resection arthroplasty	Removal of the prosthesis and surgical debridement
Disarticulation	Separation of the leg
Arthrodesis	Fusion of the hip

2.6. Treatment of the infected prosthesis (Table 2.4)

2.6.1. Antibiotics without surgical treatment

Antibiotic treatment without operative intervention is most commonly used in an attempt to achieve chronic suppression of a

prosthetic infection. This treatment is used in patients who are medically unfit to undergo a major operation or when they refuse an operative treatment (Goulet 1988; Segreti 1998). The infection is suppressed so that the symptoms are minimised, but it is not eradicated. The infecting organism must be known and must be sensitive for the used antibiotics. The selected antibiotics should be effective orally and should be well tolerated by the patient. Side effects such as diarrhoea and the emergence of resistant strains are the most common causes of failure of this treatment.

2.6.2. Debridement without removal of the prosthesis

Direct postoperative and acute late haematogenous infections can be treated with adequate debridement and appropriate systemic antibiotics. Tsukayama et al. (Tsukayama 1996) emphasised the importance of limiting this method of treatment to early postoperative infections that had developed less then one month postoperatively. Seventy-one per cent of the infections that developed less than one month after the operation were eradicated by debridement and intravenous administration of antibiotics.
The primary difficulty appears to be the lack of accuracy with which acute infections can be distinguished from the chronic ones. Patients in whom the distinction between acute and chronic infection cannot be made with confidence should be managed as if they have a chronic infection (Masterson 1998).

2.6.3. One-stage exchange arthroplasty

The one-stage exchange procedure was introduced by Buchholz and Gartman in 1972. This treatments consist of excision of the infected components, surgical debridement, and an immediate reconstruction with a cemented arthroplasty (Buchholz 1972).
A one-stage procedure could be desirable because of the avoidance of additional operative procedures. This is particularly important for patients who have several major medical problems, for whom the risks of additional procedures are high. However, the potential advantages must be weighted against the slightly lower rates of eradication of infection that are observed after one-stage compared with two-stage procedures. The difficulty of removing a solidly

fixed cemented prosthesis without destroying the remaining bone stock can be a problem. Especially when bone stock is deficient, the insertion of an implant with cement is not appropriate in many revision procedures (Masterson 1998).
Garvin and Hansen summarised results of one-stage exchange arthroplasties (Garvin 1995). They found that the cumulative success rate from sixteen reports of these procedures performed with antibiotic-impregnated cement was 82 per cent. The cumulative success rate from four reports of one-stage exchange arthroplasty performed without local delivery of antibiotics was 58 per cent.

2.6.4. Two-stage exchange arthroplasty

The main aspects of a two-stage exchange arthroplasty include removal of the implant including all cement, and dead or necrotic tissue. Usually, antibiotic loaded bone cement (beads or a cement spacer) is left behind. Intravenous antibiotics are administered postoperatively. In a second stage a new prosthesis is implanted.
The success of eradication of infection after a two-stage procedure is higher as compared with a one-stage exchange procedure. Garvin and Hanssen (Garvin 1995) reported the cumulative rate of eradication of infection to be 91 per cent in a review of twelve reports of two-stage exchange procedures in which antibiotic-impregnated cement was used. As mentioned in the previous paragraph, the success rate from sixteen studies of one-stage procedures with antibiotic-impregnated cement was 82 per cent.
There is no consensus on duration and the route of administration of antibiotic therapy. Some evidence exists that the use of intravenous therapy for less than four weeks is associated with a higher rate of recurrence when the infection is caused by highly virulent organisms, such as gram-negative bacilli and group-D streptococci (McDonald 1989). Most protocols have included six weeks of intravenous administration of antibiotics (Tsukayama 1996), which is considered to be more reliable than oral administration.
There is also no consensus on the interval between the first and the second stage. This is, of course, also dependent upon various clinical and bacteriological parameters. This interval is reported to vary

widely, from 6 days to more than 6 years postoperatively (McDonald 1989).
The use of bone allograft for reconstruction after an infection is associated with a higher rate of recurrent infection because the allograft might act as a sequestrum. Therefore major bone loss has been regarded by some authors as a contraindication for reimplantation after an infection (Salvati 1982a).
Most centres described results of reimplantation using components inserted with cement. Nestor et al. (Nestor 1994) and Lai et al. (Lai 1996) described results of two-stage exchange procedures that were performed with a cementless prosthesis. In these studies, infection recurred in 18 per cent and 13 per cent, respectively.

2.6.5. Resection arthroplasty

Resection arthroplasty or Girdlestone procedure may be the most appropriate definitive treatment for patients who are psychologically or medically unfit to have an additional reconstructive procedure and those who are mentally unable to cooperate with the postoperative restrictions and rehabilitation protocols after a complex reconstruction. Also a severe deficiency of bone stock and poor quality of local soft tissue are indications for an excision arthroplasty (de Laat 1991; Lieberman 1994).
This procedure is highly effective in controlling the infection and reducing pain (Bourne 1984; McElwaine 1984; Grauer 1989; Forgon 1990; de Laat 1991; Castellanos 1998). However, it is also associated with a considerable loss of function. After an excision arthroplasty patients walk poorly, with a frequent need for walking aids (Ahlgren 1980; Bittar 1982; Bourne 1984; McElwaine 1984; Grauer 1989). Patients have a prominent lurching gait, velocity and cadence are reduced, and their energy expenditure is increased (Waters 1987). In addition, patients need external shoe-lifts because of limb-shortening, varying from 3 to 11 centimetres (Ahlgren 1980; Bourne 1984; Grauer 1989; Castellanos 1998). In summary, the Girdlestone procedure is a reasonable salvage operation for some complications following hip surgery, but as a result of the considerable impairment of function, reimplantation of a total hip prosthesis is recommended whenever possible (Schroder 1998).

2.6.6. Other options

Arthrodesis of the hip after a periprosthetic infection has been reported by Kostuik and Alexander (Kostuik 1984). Indications for the procedure were a relative absence of bone and poor quality of the local soft tissues. This treatment was chosen in seven, relatively young male persons with strenuous functional demands. All hips fused. Although they had a mean leg-length discrepancy of 4.6 centimetres, all patients were able to walk.

Disarticulation at the hip or amputation also has been reported, but fortunately it is rarely performed. Indications are life-threatening infection, severe loss of soft tissue and bone stock and vascular injury (Fenelon 1980).

2.6.7. Future treatments

Improvement of antimicrobal therapy by new systems for the local delivery of antibiotics is one of the areas of development. The goal is to achieve local therapeutic bactericidal concentrations of the drugs (Garvin 1994b). The advantages are that clinical parental therapy is not necessary and patients can be discharged from hospital sooner. One-stage therapy may be facilitated with the use of this technique. Also the local therapy would minimise the risk of systemic toxicity which is associated with long-term intravenous therapy (Callaghan 1985; Garvin 1994b).

Another area of improvement includes the possibility to amplify cell-mediated immune responses against infection (Myrvik 1993). Patients may be managed prophylactically by activation of a natural initiating mechanism for amplification of the cell-mediated immune response. Research will determine the effectiveness of priming of macrophages as an immune augmentation against pathogens that are most likely to be encountered at the time of hip arthroplasty (Myrvik 1993).

2.7. Microbiological aspects of implant related infections

In the pathogenesis of infection, adhesion of bacteria to the surface of human tissue or an implanted biomaterial surface is an

important step (Gristina 1983; Fitzgerald 1989; Sugarman 1989; An 1996a). However, the exact mechanisms by which such infections occur still remain unclear. It is well known that after adherence of certain strains of bacteria to an implant surface, a layer of slime can be secreted, which makes bacteria less accessible to the host defence system (Gray 1984; An 1996a) and decreases antibiotic susceptibility significantly (Sheth 1985; Gristina 1987b; Gristina 1989; Naylor 1990). These bacteria can remain "quietly" on the surface of a material for a long period of time. However, when environmental influences, such as a decreased host immune function or poor tissue ingrowth around a prosthesis, change, these bacteria can result in a manifest clinical infection. The adhesion of bacteria is a very complicated process. Besides the characteristics of the bacteria itself, surface factors of the target material and environmental factors affect this process. In the following paragraphs, various aspects related to bacterial adhesion and colonisation in relationship with (orthopaedic) implants will be described.

2.7.1. Bacterial characteristics

Bacterial capsules

Bacterial capsules are firmly adherent as a discrete covering layer with a distinct margin on the bacterial cells at the outside of the cell wall. They can be seen as a clear zone surrounding the bacterial cell and are separated from extracellular slime. Most capsules are composed of polysaccharides and proteins (Sutherland 1983). According to a number of studies, cell surface polysaccharides and proteins act as bacterial adhesins (Hogt 1986; Kroncke 1990). Surface hydrophobicity of different encapsulated coagulase-negative staphylococci (CNS) differs. The presence of a more hydrophilic capsule reduces adhesion (Hogt 1983). Several studies have suggested that the capsules of *Staphylococcus aureus* and *Staphylococcus epidermidis* are both important factors in the pathogenesis of infection with these bacteria (Smith 1977; Ichiman 1981).

Bacterial surface structures

Surface structures of bacteria are distinguished in flagella, sex

pili, fimbriae and fibrillae.

Flagella are long helical filaments extending from the cell surface, which generate propulsive forces and enable bacteria to move in their environment. Their diameter is 15-20 nm and they may be as long as many times the length of the bacterial cell. Flagella are composed of many polypeptides that form a filamentous hook and a complex basal body that interacts with both outer and inner membranes (Beveridge 1981).

Fimbriae (or pili) are a group of rigid, straight, filamentous appendages on a bacterial surface. (Figure 2.2) They are 3-8 nm in diameter and may be as long as 15-20 μm. There are generally several hundred fimbriae over the entire surface of the bacterial cell. Fimbriae arise from proteins in the outer surface of the outer membrane and may stretch out for large distances beyond the outer membrane (Brinton 1965). Bacterial fimbriae are polymers consisting of identical protein subunits (Jones 1983). They are best characterised in Gram-negative bacteria and are accepted as a major adhesive structure on the bacterial surface. Those who are responsible for adhesion to other bacteria are called 'sex pili'. These fimbriae contain structures, which are responsible for adhesive activities (adhesins). These adhesions or adhesive submits may mediate bacte-

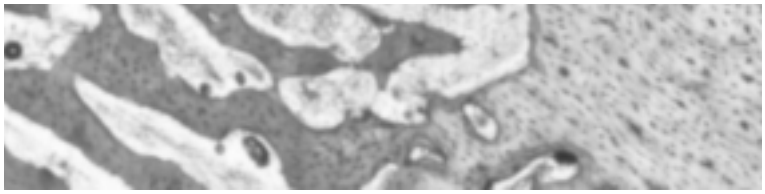


Figure 2.2
Transmission electron micrograph, showing the surface structure of a Staphylococcus epidermidis cell, clearly demonstrating filamentous "fimbriae-like" surface appendices. Black dots depict immuno-gold labelling of an adhesin for polymer(polysterene) surface. Bar = 0.1 μm.

rial adhesion (de Graaf 1988; Hoschutzky 1989). Fimbriated bacteria are also often more hydrophobic than non-fimbriated ones. Hydrophobicity is also dependent on the type of fimbriae (Lindahl 1981), and adhesion is mediated by fimbriae-dependent surface hydrophobicity. In summary, it is believed that bacterial fimbriae are related to virulence, and bacteria that possess fimbriae are more infectious than their non-fimbriated variants (Klemm 1985).
Fibrillae are more amorphous surface adhesive structures. They have not the regular filamentous forms of fimbriae. Fibrillae are seen at the surface of various streptococci and they are thought to provide a better adhesion onto hydroxyapatite substrata (Phillips 1981).

Slime and glycocalyx

The extracellular substance produced by the bacteria is defined as "slime" (Figure 2.3). Capsules and slime are both subclasses of extracellular polymeric substances and are usually polysaccharides. A glycocalyx is defined as the accumulated biomass of bacteria and their extracellular substances (slime) on a solid surface (An 1998). In addition to adhesion, slime production of bacteria appears to play a primary role in the pathogenesis of most prosthesis

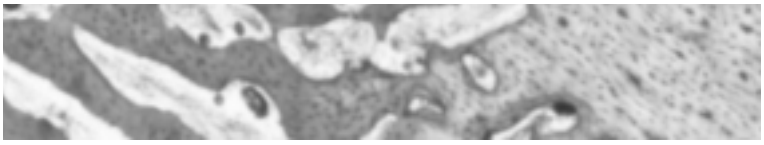


Figure 2.3
Scanning electron micrograph, showing slime layer produced by coagulase-negative staphylococci on the surface of a polyethylene catheter.
Bar = 4 µm (From Peters 1982, reprinted with permission)

infections (Dougherty 1989; Tsai 1992). Bacterial strains that do not produce slime are less adherent and less pathogenic (Christensen 1982; Christensen 1983; Davenport 1986). Slime is thought to be important for intercellular connection during surface colonisation. The current concept is that the production of slime will be especially important for events after the initial phase of adhesion, which include colonisation of various surfaces, protection against phagocytosis, interference with the cellular immune response (Buret 1991), and protection against antibiotics (An 1998).

Physicochemical characteristics of bacteria

The adherence characteristics of a specific bacterial strain are dependent upon the specific physicochemical characteristics of the substrate (material surface) and the bacteria. These physicochemical characteristics of bacteria, such as hydrophobicity and surface charge, differ for various species and strains.

Hydrophobicity of bacterial surface

Hydrophobicity of the bacterial surface is an important physical factor for adhesion. This is particularly the case when surfaces of the materials are either hydrophilic or hydrophobic. Growth medium, bacterial growth phase, and bacterial surface structure influence the hydrophobicity. In general, it can be said that bacteria with hydrophobic surface properties adhere preferentially on hydrophobic material surfaces; the ones with hydrophilic characteristics adhere preferentially on hydrophilic surfaces (Hogt 1983; Satou 1988a; Satou 1988b). Usually, hydrophobic bacteria adhere to a greater extent than hydrophilic bacteria (van Loosdrecht 1987b).

Surface charge of bacteria

Another physical factor for bacterial adhesion is the bacterial surface charge (Hogt 1985). Most particles acquire an electric charge in aqueous environment due to the ionisation of their surface molecules/groups. The surface charge attracts ions of opposite charge in the medium and results in the formation of an electric double layer. A high surface charge is accompanied by a hydrophilic characteristic of the bacteria, but a hydrophobic bacterium may still have a

rather high surface charge (Hogt 1985). The surface charge of bacteria is influenced by growth medium, bacterial growth phase and bacterial surface structure and varies between bacterial species.
Long-range electrostatic forces may influence the initial attachment of bacteria onto solid surfaces. However, several studies of bacterial adhesion have shown that adhesion of many bacteria to different surfaces was not significantly affected by the relative surface charge of the bacteria (Abbott 1983; Hogt 1985; Harkes 1991). This in contradiction to several other studies, which showed that bacterial surface charge does affect adhesion of bacteria to different surfaces (van Loosdrecht 1987a; Gilbert 1991).

2.7.2. Material characteristics

In addition to the characteristics of the bacterial surface, the surface of the substrate or material is a determining factor in bacterial adhesion and colonisation. Such material surface parameters include chemical composition of the material, surface roughness or physical configuration, surface charge, and hydrophobicity. Also, surface energy, empty binding sites and hydrophobic and hydrophilic characteristics can be quickly altered by either the absorption or binding of serum proteins and formation of biofilms (Gristina 1987a).

Chemical composition of the material

Several experiments have been performed *in vitro* and *in vivo* to investigate the role of chemical composition and the adherence of bacteria. Polymethylmethacrylate (PMMA) usually appears as the material most prone to infection. Metals, such as titanium and cobalt-chromium are the materials most resistant to infection. The number of adherent bacteria that bind to the surface of a certain material is dependent upon the strain of the bacteria. According to most studies, *Staphylococcus epidermidis* preferably adheres to polymers and *Staphylococcus aureus* to metals.
Petty et al. (Petty 1985) studied in a dog model infection by peroperative contamination with *Staphylococcus epidermidis, Staphylococcus aureus,* or *Escherichia coli* of stainless steel, cobalt-chromium alloys, high-density polyethylene or PMMA. PMMA was found to be

infected easier than all other implants with *Escherichia coli* and *Staphylococcus epidermidis*. All other implants were significantly more likely to be infected with *Staphylococcus aureus*. Oga et al. (Oga 1988) demonstrated in an *in vitro* study that PMMA was colonised by *Staphylococcus epidermidis* in higher numbers as compared to stainless steel and alumina ceramic. Barth et al. (Barth 1989) investigated *in vitro* and *in vivo* in a subcutaneous rabbit model the susceptibility to infection of PMMA, ultra-high molecular weight polyethylene (UHMWP) and a titanium alloy (Ti6Al4V). *Staphylococcus epidermidis* preferentially colonised pure polymers (PMMA), while *Staphylococcus aureus* preferentially colonised titanium alloy. According to Oga et al. (Oga 1993), *in vitro* adherence of *Staphylococcus epidermidis* was higher to sintered hydroxyapatite than to stainless steel, Ti6Al4V and hydroxyapatite coated Ti6Al4V. Adherence to the HA-coated Ti6Al4V was slightly higher as compared to stainless steel or titanium alloy. Verheyen et al. (Verheyen 1993) evaluated in an *in vitro* study bacterial adherence on stainless steel, a polymer poly(L-lactide) (PLLA) and a composite hydroxyapatite/poly(L-lactide) (HA-PLLA), both as smooth and sandblasted specimens. All materials were challenged with *Staphylococcus aureus* or *Staphylococcus epidermidis* (Figure 2.4). *Staphylococcus aureus* showed a preference for the stainless steel and HA-PLLA. For *Staphylococcus epidermidis* no clear preference was found for one of the investigated materials. However, Chang and Merritt found in a comparative study that the *in vitro* adherence of *Staphylococcus epidermidis* was highest for stainless steel, followed by PMMA and pure titanium. In *in vivo* studies of subcutaneous implantation in Syrian hamsters, the same pattern was found (Chang 1994). Arens et al. (Arens 1996) investigated *in vivo* the susceptibility to infection of plates of either stainless steel or titanium in rabbit tibiae. A strain of *Staphylococcus aureus* was inoculated percutaneously. The rate of infection for steel plates was significantly higher than that for titanium plates. Cordero performed various *in vivo* experiments on implant infections using rabbits. In one study, he demonstrated that polished-surface cobalt-chrome cylinders required slightly less bacteria to become infected than polished-surface Ti6Al4V cylinders (Cordero 1994). Gracia et al (Gracia 1997) tested in an *in vitro* study

the adherence of *Staphylococcus aureus* to PMMA, fresh bone, stainless steel and titanium alloys. The lowest overall adherence was found to PMMA and bone, and the highest to stainless steel and titanium alloy. No differences were found between adherence of *Staphylococcus aureus* to stainless steel and titanium alloy. Hauke et al. (Hauke 1997) observed the rate of infection in a rabbit model where solid nails of stainless steel or titanium were implanted in the medullary cavity of the tibia and contaminated with *Staphylococcus aureus*. In contradiction to the findings of Gracia et al. (Gracia 1997), they found a difference of infection rate between stainless steel and titanium: the rate was higher for stainless steel than for titanium.

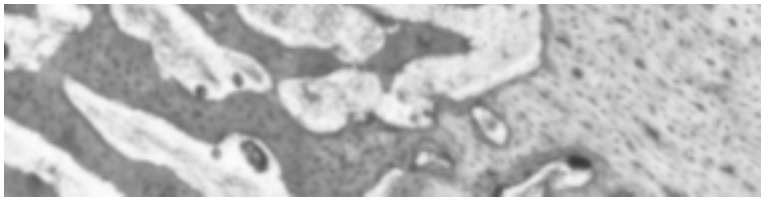


Figure 2.4
Scanning electron micrograph, showing a hydroxyapatite coating covered with Staphylococcus epidermidis. Bar = 10 µm.

Roughness of the material

Surface roughness is determined by measurement of the distance between peak and valley part on a material surface, and it should be realised that it does not represent the actual morphology of the surface. Several studies indicate that increased surface roughness promotes bacterial adhesion (McAllister 1993; Quirynen 1993;

Verheyen 1993; Cordero 1994). One of the explanations for this phenomenon is that a rougher surface has a higher surface area and the depressions in the roughened surfaces provide more favourable sites for colonisation.

Porosity of the material

The physical configuration or porosity of a material surface is considered as a morphological description of the pattern of a material surface. It can be evaluated by scanning electron microscopy. Merrit et al. (Merritt 1979) and Locci et al. (Locci 1981) found that implant site infection rates are obviously different between porous and dense dental materials and are much higher for porous materials. This implies bacteria adhere and colonise the porous surface preferentially. This can be explained by the much greater surface that the porous-coated implants offer for bacterial adherence, but also by a lower accessibility for host defence systems.

Physicochemical characteristics of material: surface hydrophobicity or wettability

Polymers such as UHMWP or Teflon have a low surface energy and are less electrostatically charged and hydrophobic. Metal surfaces have a high surface energy, are negatively charged and hydrophilic (An 1993). Bacteria adhere differently to materials with different hydrophobicities, depending on the hydrophobicity of bacteria as well as material surfaces (Hogt 1983; Satou 1988b). Hydrophobic materials are more prone to bacterial adhesion than hydrophilic materials (Hogt 1983; Ludwicka 1984; Pringle 1986). Coating substrata surfaces with proteins, such as bovine serum albumin (BSA), bovine glycoprotein, or fatty-acid free BSA decrease surface hydrophobicity, leading to a decrease in bacterial adhesion to the surfaces (Fletcher 1982).

2.7.3. Environmental aspects

General environment

Several factors in the general environment, such as temperature, duration of exposure, bacterial concentration ("load"), chemi-

cal treatment, and the presence of antibiotics, have shown to affect bacterial adhesion.

It was found that adhesion of *Streptococcus faecium* increased with increasing temperature (up to 50°C) (Orstavik 1977). The number of bacteria adhering to a material increased with time until they reached a saturation level which was specific for that type of material (Orstavik 1977; Satou 1988b). Adhesion of bacteria increases with increasing cell concentration (Orstavik 1977). Hogt et al. found that treatment of *Staphylococcus epidermidis* with pepsin or extraction with aqueous phenol resulted in a decreased adhesion on fluorinated poly(ethylenepropylene), a biomaterial used in various biomedical applications (Hogt 1983). A culture of *Staphylococcus epidermidis* in subinhibitory concentrations of cephalothin, clindamycin and vancomycin resulted in a 30 to 80 per cent reduction in adhesion (Pascual 1986).

Electrolyte concentration (Orstavik 1977; Abbott 1983), CO_2 (Denyer 1990) and changes in pH value (Orstavik 1977; Gordon 1981) influence the nature of the bacterial surface and with that the process of bacterial adhesion. All of these factors may influence bacterial adhesion by changing surface characteristics of bacteria or materials.

Serum or tissue proteins

Several investigations have been performed to study the effect of serum and tissue proteins, such as albumin, fibronectin, laminin and denatured collagen, on bacterial adhesion to material surfaces (Fletcher 1976; Vaudaux 1984a; Vaudaux 1984b; Kuusela 1985; Pratt-Terpstra 1987; Muller 1991; McDowell 1995; An 1996a). Adhesion of bacteria is promoted or inhibited by serum and tissue proteins, either by binding to material surfaces, binding to the bacterial surface, or being present in the surrounding medium during the adhesion process. When present in surrounding medium, most proteins inhibit bacterial adhesion (Fletcher 1976; Brokke 1991), possibly affecting bacterial adhesion by their association with bacterial cell surface, the material surface, or both. Most of the associations between bacteria and proteins are specific ligand- and receptor-like interactions. Also the adherent behaviour of bacteria

may be changed by these proteins, by changing bacterial surface physicochemical characteristics (Miorner 1980; Reynolds 1983; Pascual 1986).

Whole serum

The adhesion of various CNS onto plasma coated fluorinated poly(ethylenepropylene) (FEP) was studied by Hogt et al. (Hogt 1985). The adhesion of all strains onto plasma coated FEP appeared to be much lower than onto the untreated control surface. Pascual et al. found that preincubation of Teflon catheters in human serum caused an 80 to 90 per cent reduction of adhesion of *Staphylococcus epidermidis* (Pascual 1986). Preincubation of *Staphylococcus epidermidis* in serum similarly decreased adhesion. This effect of serum was mainly due to albumin, while IgG and fibronectin were less effective (Pascual 1986). Similar effects were also found when polymers were preincubated with plasma or albumin (Paulsson 1993).

Fibronectin

Fibronectin is a glycoprotein found in human sera and extracellular matrix. In addition to its ability to mediate surface adhesion of eukaryotic cells, it has also been shown to bind to *Staphylococcus aureus* (Kuusela 1978). Staphylococci can be saturated with fibronectin to a level that suggests the presence of specific receptors (staphylococcal fibronectin-binding molecules) on bacterial cells (Espersen 1982; Ryden 1983; Maxe 1986). A *Staphylococcus aureus* binding domain of fibronectin was indeed found in the fibronectin molecule (Mosher 1980; Bozzini 1992). Therefore, fibronectin clearly promotes *Staphylococcus aureus* adhesion to the substratum surface (Vaudaux 1984a; Vaudaux 1984b; Kuusela 1985; Maxe 1986) and plays an important role in foreign body infections (Vaudaux 1984a). Although strain specific, adherence of clinical staphylococcal isolates to intravenous device (Herrmann 1988) or orthopaedic biomaterial (Delmi 1994) was significantly increased by fibronectin, suggesting the possible contribution to infection.

However there are controversies regarding the fibronectin effect on *Staphylococcus epidermidis* adhesion to material surfaces. Herrmann et al. found that fibronectin promoted the adherence of all

Staphylococcus aureus strains, but only in 4 out of 19 strains of *Staphylococcus epidermidis* (Herrmann 1988), while Naylor and colleagues found that fibronectin can inhibit *Staphylococcus epidermidis* adherence to cobalt-chrome alloy and PMMA surface with 90 per cent (Naylor 1989b).

Albumin

Albumin adsorbed on surfaces of polymer (Hogt 1985; Pascual 1986; Paulsson 1993), ceramic (Gibbons 1983) and metal (McDowell 1995; An 1996a; An 1996b) showed inhibition of bacterial adhesion. It is thought that most of the proteins reduce adhesion through adsorption to the substrata surface, while serum albumin also inhibits the adhesion by means of binding to the bacterial cells (Brokke 1991). The mechanism of the inhibiting effect of albumin bound to either surface is not clear. Bacterial adhesion may be reduced by albumin by changing substratum surface hydrophobicity, because in the presence of dissolved and absorbed bovine serum albumin, the substrata surface became much less hydrophobic (Reynolds 1983).
An et al., reported that a cross-linked albumin coating applied to commercially pure titanium implants reduced the prosthetic infection rate in rabbits. It was concluded that a crosslinked albumin coated prosthesis may represent a new method for preventing prosthetic infection (An 1997).

Fibrinogen

Fibrinogen also mediates bacterial adhesion onto biomaterials and host tissues. Most studies showed that especially for staphylococci, adsorbed fibrinogen promotes adherence to biomaterials (Herrmann 1988; Flemming 1993). However, fibrinogen markedly promotes adherence of all *Staphylococcus aureus* strains but only a few coagulase-negative strains (Herrmann 1988). According to Vaudaux et al. fibrinogen is less active in promoting *Staphylococcus aureus* adherence to intravascular catheters than fibronectin (Vaudaux 1993).

Laminin

Laminin is a major component of basement membranes. It was shown to bind to some strains of *Streptococcus pyogenes* (Switalski

1984). Laminin has a promoting effect on the adhesion of *Staphylococcus aureus* and coagulase-negative strains to PMMA coverslips but to a lesser extent compared to the effects of fibronectin and fibrinogen (Herrmann 1988).

Other proteins or factors

Other proteins that influence attachment or adhesion of bacteria to a material surface are gelatin (Fletcher 1976) and pepsin (Fletcher 1976; Pascual 1986). They impaired the adherence of bacteria. Basic proteins such as histone and poly-L-lysin facilitate adherence of *Streptococcus mutans,* whereas acidic proteins such as, phosvitin, b-lactoglobulin and poly-L-glutamate have been shown to inhibit adhesion (Reynolds 1983).

Specific adhesion by bacterial adhesins to host receptors

The selective binding between bacterial adhesins (a specific molecular component on the bacterial surface) and the substratum receptor (a specific component on the material surface) is defined as specific adhesion. It is less affected by many common environmental factors such as electrolytes, pH, or temperature. Specific adhesion of bacteria by polysaccharide adhesins (Tojo 1988), proteinaceous adhesins (Timmerman 1991) and hemagglutinins (Rupp 1992) is believed to play a major role in the pathogenesis of prosthetic infections.

A capsular polysaccharide adhesin from *Staphylococcus epidermidis* (strain RP-62A) was isolated by Tojo et al. (Tojo 1988). Timmerman et al. found a proteinaceous adhesin of a strain of *Staphylococcus epidermidis* (strain 354) that mediated the adhesion of this bacterium to polystyrene (Timmerman 1991). Rupp and Archer showed that hemagglutinins either play a direct role in adherence to polymers and thus prosthetic-device infection or serve as an easily demonstrable marker for adherence-prone isolates (Rupp 1992).

Morris and McBride demonstrated that saliva-coated hydroxyapatite has two specific receptors for *Streptococcus sanguis* (Morris 1984). Specific adhesion was also demonstrated by Bayer et al. in 1983 by studying the process of *Clostridium thermocellum* adhesion to cellulose (Bayer 1983).

2.7.4. The process of bacterial adhesion

Bacteria as well as tissue cells prefer to grow on available surfaces rather than in the surrounding aqueous phase. Adhesion of bacteria to the surface of a material can be distinguished in two phases (Figure 2.5): firstly an immediate and reversible phase of physicochemical interaction between bacteria and material, and secondly a time dependent and irreversible phase of molecular and cellular interactions between bacteria and material surfaces (Gristina 1987a).

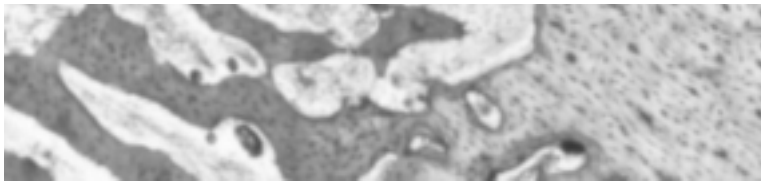


Figure 2.5
Molecular sequences in bacterial (B) attachment, adhesion and aggregation to substratum (From Coombs 1989, reprinted with permission).

Physicochemical interaction between bacteria and material

Physical forces, such as Brownian motion, van der Waals attraction forces, gravitational forces, the effect of surface electrostatic charge and hydrophobic interactions are responsible for the movement of bacteria to the surface of a material. Based upon the distance between the micro-organism and the substrate, these physical interactions are classified, as long-range interactions and short-range interactions (An 1998).

The long-range interactions are non-specific and described by mutual forces, which are a function of the distance and free energy.

Short-range interactions can be separated into chemical bonds (such as hydrogen bonding), ionic and dipole interactions and hydrophobic interactions. In the first phase of bacterial adhesion, transport of the bacteria by long-range interactions leads to attachment to the surface. When bacteria and material surface come in closer contact, short-range interactions become more important. The initial attachment of bacteria to material surfaces is the first point of the process of adhesion and makes the subsequent molecular or cellular second phase of adhesion possible.

Molecular and cellular interaction between bacteria and material

In the second phase of adhesion, molecular reactions between bacterial surface and substratum surface become predominant. Bacterial surface polymeric structures, such as capsules, fimbriae and fibrillae, result in a firmer adhesion of bacteria to a surface. In fact, the functional part of these structures are the adhesins that are parts of these structures, especially when the substrata are host tissues (An 1998).

2.8. Closing remarks

Despite preventive measurements, because of an ageing population and a steady increase in the frequency of total joint replacement operations, infection of an orthopaedic implant still is a source of considerable morbidity.

It can be difficult to make the correct diagnosis of infected joint arthroplasty with reasonable certainty, and such a diagnosis can be reached with an acceptable certainty only by combining investigations. In most cases, an infection can be diagnosed or excluded on the basis of a carefully obtained clinical history and the measurement of the sedimentation rate and the C-reactive protein level. Other preoperative investigations, such as radiography, arthrography, radionuclide imaging and aspiration of the joint, and peroperative investigations, such as frozen sections, gram stains and cultures, may provide additional evidence. Peroperative cultures are most

accurate; however, their usefulness is offset by false-positive and false-negative results.

The choice of treatment is dependent on the health of the patient, bone stock, quality of the soft tissues, virulence of the infecting micro-organism and the mental state of the patient. Beside the antibiotic treatment, other treatment options are debridement without removal of the prosthesis, one-stage exchange arthroplasty, two-stage exchange arthroplasty and the excision arthroplasty. Less frequently used treatments are arthrodesis or disarticulation.

Adhesion between cell and surfaces of materials, which involve both bacteria and tissue cells, are critical, interrelated and based on similar molecular mechanisms. Biomaterial surfaces represent available, unbonded energy sites or potential receptor sites for colonisation by bacteria or by normal tissue. At the moment of implantation, a biomaterial or allograft surface presents the available free energy sites which await physical or chemical bonding, first from ambient protein molecules and subsequently, or concomitantly, from available cells.

The theory of the "race for the surface" suggests competition between tissue and bacterial cells for implant material surfaces (Gristina 1988). Successful colonisation by one cell type favours dominance and exclusion of later arrivals. If tissue cells arrive first at a biomaterial surface and a secure bond is established, bacteria will be confronted by living, integrated cells. If not traumatised or altered, an integrated tissue cell is resistant to bacterial colonisation by virtue of its viability, intact cell membranes, extramembranous polysaccharides and host defence mechanisms. If however, bacteria arrive at the surface first and colonise the biomaterial, an infection will develop. Such biomaterial infections almost always prevent successful integration of the implant.

Biomaterials are susceptible to infection because they are not well integrated for the most part. Well-integrated devices are infrequently infected unless an unfavourable situation is created by perturbed host defences, trauma or exposure to massive infecting inoculi.

The Outcome of Therapy of Osteomyelitis in Children. A Retrospective Study of 28 Patients

3.1. Introduction

Osteomyelitis is a serious disease, seen in patients of every age. The bone is infected by bacteria either directly or, more frequently, via haematogenous contamination. *Staphylococcus aureus* is the primary pathogen, responsible for most cases. Any bone can be involved, although it is most frequently seen in the distal femur and proximal tibia. Deterioration to chronic osteomyelitis is usually associated with a delay in treatment. Spontaneous evacuation of pus to an adjacent joint may lead to a septic arthritis, with serious damage of this joint as result. Also local extension into subcutaneous tissue, sometimes with spontaneous discharge of pus through the skin, may occur. The process of bone destruction may lead to weakening of the bone and a pathologic fracture. Disturbances of growth can be expected when the growth plate is involved.

Clinically, osteomyelitis is suggested by the history and symptoms of the patient. In children, there is usually an acute presentation with high fever and local signs of inflammation, three weeks or less in duration. The affected area is painful, warm, red, swollen and frequently there is diminished function. Infection parameters such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and white blood-cell count (WBC) are usually increased. However, in up to 50 percent of children the presentation is reported to be more chronic, with vague complaints, for example limb pain of 1 to 3 months in duration. In contrast to children, in adults the presentation is usually more chronic. Both in adults and children with chronic osteomyelitis, clinical signs are less pronounced and the infection parameters may be normal.

Radiographs may show swelling of the soft tissues at an early stage of osteomyelitis, but it usually takes 2 to 3 weeks after the clinical onset of disease for typical radiographic changes, such as bone destruction and subperiostal bone formation, to appear. If the diagnosis is unclear, bone scintigraphy, CT-scan and especially MRI may be helpful.

There is no consensus in the literature about the optimal treatment of osteomyelitis, especially with respect to the decision on surgical intervention. Some do not advocate surgical treatment in an early

stage. According to our protocol we perform surgery in every child, including drainage and obtaining samples for microbiologic determination. The range of duration of antibiotic therapy as recommended in the literature is fairly wide, namely 2 to 12 weeks, but is usually 4 to 6 weeks. In our hospital we adhere to a protocol of 2 to 3 weeks intravenous therapy followed by 3 weeks oral antibiotics.
The purpose of this retrospective study was the evaluation of the outcome of therapy in children with osteomyelitis. Special emphasis was given to cure and to recurrence.

3.2. Materials and Methods

We reviewed patient records of children admitted to the Wilhelmina Children's Hospital between 1985 and 1995 with the diagnosis of osteomyelitis. Children who were primarily treated in other hospitals were not included in this study. Also, the children diagnosed with spondylitis or with concomitant septic arthritis were not included. Consequently a total of 28 children were reviewed.
The records were screened for history, clinical presentation and diagnostic work-up. According to definitions proposed in the orthopaedic literature (Crenshaw 1992), patients were separated as having either an acute or chronic haematogenous osteomyelitis and this separation was primarily based on clinical criteria as defined in Table 3.1. Subsequently, treatment and outcome were evaluated.

	Acute Osteomyelitis	Chronic Osteomyelitis
Pain	Severe	Mild
Illness	Fever, malaise	No
Loss of Function	Marked	None or minimal
Prior Antibiotics	Occasional	30-40%
Initial X-ray	Bone normal	Frequently abnormal
WBC	Frequently elevated	Frequently normal
ESR	Frequently elevated	Frequently elevated

Table 3.1.
Diagnosis of acute and chronic osteomyelitis. WBC = white blood-cell count; ESR = erythrocyte sedimentation rate.

3.3. Results

The main results of our retrospective study are described in Table 3.2. Twenty-eight children with a haematogenous osteomyelitis were identified. We distinguished 12 patients with an acute and 16 patients with a chronic osteomyelitis. In both groups, the sexes were almost equally represented. The mean age of the children with an acute osteomyelitis was 5.5 (range: 1.2-16.3) years and with a chronic osteomyelitis 5.9 (0.8-13.6) years. The mean duration from onset of symptoms until surgery was 8 (2-28) days for the acute patients. For the chronic osteomyelitis patients this mean duration was 128 (14-640) days.

In 13 children the osteomyelitis was located in the femur (n=7) and tibia (n=6). In 6 children the infection was located in the calcaneus. Other localisations were fibula, talus, scapula, rib, humerus, radius, ulna and in a finger.

At admission, the mean temperature in the patients with an acute osteomyelitis was 38.4°C and in those with a chronic osteomyelitis 37.3°C. In spite of the acute onset of symptoms in acute osteomyelitis there were 3 patients with a temperature below 38°C, 5 patients with a temperature between 38°C and 39°C and 3 patients with a temperature above 39°C. In the group of chronic osteomyelitis all but one patients had a body temperature below 38°C.

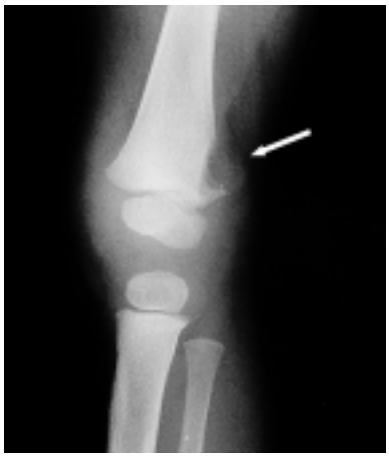
The laboratory values in the patients with an acute osteomyelitis differed substantially from those with a chronic osteomyelitis. The mean ESR, CRP and WBC in patients with an acute osteomyelitis were 49 mm/h, 111 mg/l and 16.2×10^9/l, respectively. In patients with a chronic osteomyelitis these values were 24 mm/h, 33 mg/l and 10.0×10^9/l, respectively. In this last group of patients the mean CRP was biased by a patient who also suffered from chronic renal insufficiency needing dialysis. If we exclude this patient the mean value of the CRP is decreased from 33 to 12 mg/l.

On plain radiographs of the affected bone no abnormalities were seen in the patients with acute osteomyelitis, but in 50 per cent of these cases a high density of the soft tissue was visible. Abnormalities of the bone were seen in all patients with a chronic

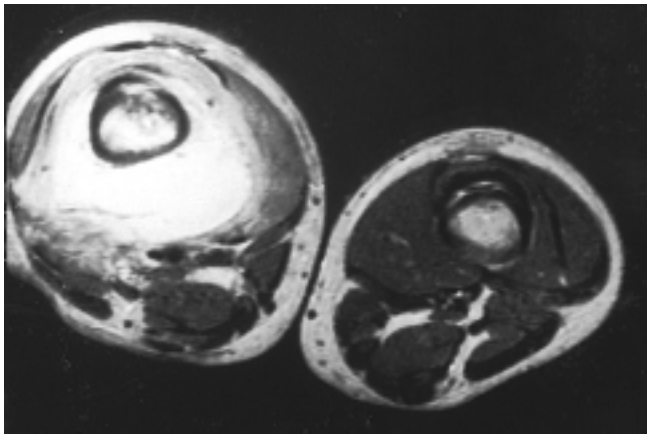
Table 3.2.
Results of treatment. i.v. = intravenous; AB = antibiotics; Hosp. stay = hospital stay; f.u. = follow-up; decr. funct. = decreased function.

Name	Sex	Age (yrs)	Localisation	Duration symptoms (days)	Prior AB	Scinti-graphy	Culture	i.v. AB (days)	Oral AB (days)	Hosp. stay	f.u. (yrs)	Recur-rence	compli-cations
Acute osteomyelitis													
S.	f	1.2	digiti 3	5	-		-	21	21	21	8.3	-	decr. funct.
P.	m	6.6	radius	3	-		*Fusobacterium*	30	21	32	0.7	-	decr. funct.
D.	m	2.8	tibia	2	-		-	16	26	20	1.6	-	-
M.	f	1.4	tibia	6	+		*H. influenzoe*	18	21	21	6.4	-	-
Z.	f	13.3	femur	14	+	pos.	*S. aureus*	29	21	32	2.1	-	-
de H.	m	1.6	ulna	3	-		gr. A streptococcus	17	21	19	4.0	-	-
vd M.	m	3.7	tibia	4	-		*S. aureus*	21	21	22	3.7	-	-
D.	f	1.6	humerus	3	+		*H. influenzoe*	21	21	22	2.0	-	-
S.	m	8.8	scapula	7	-	pos.	*S. aureus*	14	28	14	1.6	-	-
M.	m	16.3	costa 1	7	-	pos.	*S. aureus*	20	22	20	3.9	-	-
K.	f	6.8	femur	14	-	pos.	-	14	21	32	7.8	-	-
de W.	f	2.1	femur	28	+		gr. A streptococcus	21	21	22	2.6	-	-
Chronic osteomyelitis													
S.	m	5.3	calcaneus	21	-		-	9	33	12	0.4	-	-
H.	m	7.5	calcaneus	28	+		-	21	21	25	1.4	-	-
Z.	m	2.4	femur	14	-		-	17	21	19	3.0	-	-
S.	m	0.8	tibia	28	-	pos.	-	19	21	19	3.0	-	-
A.	f	6.3	calcaneus	42	+		*S. aureus*	32	21	32	6.2	-	ankylosis
H.	m	7.4	calcaneus	28	+		-	21	21	25	1.5	-	-
M.	f	11.3	talus	55	+	neg.	*S. aureus*	28	n.a.	30	5.3	+	-
V.	m	4.7	costa 5	28	-	pos.	*S. aureus*	8	21	14	0.8	-	-
E.	m	13.6	fibula	150	+	pos.	*S. epidermidis*	21	42	31	1.8	-	-
W.	f	10.9	calcaneus	120	+		*Propionibacterium*	21	45	21	2.3	-	-
L.	m	2.5	femur	60	-	pos.	-	21	21	22	4.3	-	-
el H.	m	3.6	femur	640	+	neg.	-	21	21	27	7.6	-	-
Z.	f	1.4	femur	60	-	pos.	-	14	21	18	0.9	-	-
F.	f	10.5	calcaneus	180	-	pos.	S. aureus	21	21	23	0.8	-	-
V.	f	5.5	tibia	120	-	pos.	-	21	21	32	2.3	-	-
M.	f	2.1	tibia	480	+		*S. epidermidis*	21	21	22	1.9	-	-

osteomyelitis. A bone scintigram was performed in 4 patients with an acute osteomyelitis and was positive in all cases. Seven out of 9 bone scintigrams were positive in the chronic cases.
Despite examinations such as radiographs and nuclear bone scintigraphy, differentiation between an osteomyelitis and a bone tumour was sometimes difficult. In these cases a CT-scan or MRI was helpful. (Figures 3.1 and 3.2)



a

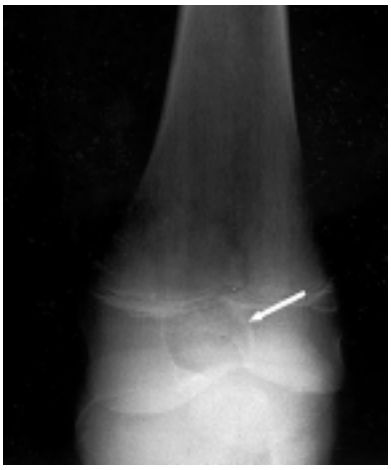


b

Figure 3.1. *X-ray and MRI of a 13 years old girl that presented with pain and swelling of her right leg since 14 days, just above the knee. She was ill and had fever. ESR and WBC were increased.*
a. *X-ray with radiolucency of the metaphysis (arrow).*
b. *MRI showing oedema of the soft tissue and bone.*

Only 3 out of 16 chronic osteomyelitis cases reported clinical signs of a previous acute osteomyelitis. Delay in diagnosis and inadequate treatment presumably had resulted in a chronic osteomyelitis. The

remaining chronic osteomyelitis patients, including all patients with an osteomyelitis of the calcaneus, initially presented with less pronounced signs, suggesting that the osteomyelitis was chronic from the beginning.



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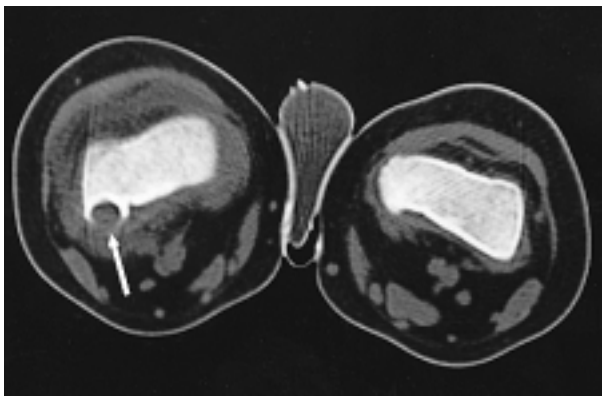

b

Figure 3.2.
X-ray and CT-scan of a 2 years old girl. She did not use her right leg for several months. At examination she was not ill, there was a local swelling just above the knee.
a. *X-ray showing a cyst in the distal metaphysis of the femur (arrow).*
b. *CT-scan with a cyst in the cortex of the bone indicative for a Brodies abscess (arrow).*

According to protocol all patients underwent surgical treatment. In the patients with acute osteomyelitis decompression was achieved by drilling holes or making a small window in the bone at the most painful site. Samples for microbiologic examination were obtained. In the patients with a chronic osteomyelitis, the bone was opened,

followed by saucerisation of the bone and excision of sequestra and abnormal tissue. After obtaining samples for microbiologic examination, in 9 out of 16 cases gentamicin beads were left. Systemic antibiotic treatment was started immediately after obtaining the samples.

Nine out of 12 patients with an acute osteomyelitis had positive cultures, whereas 7 out of 16 positive cultures were seen in the chronic osteomyelitis patients. Only 2 out of 7 blood cultures were positive in patients diagnosed as acute osteomyelitis. Eight times *Staphylococcus aureus* was cultured: 4 times in both acute osteomyelitis and chronic osteomyelitis patients. The remaining positive cultures in the patients with an acute osteomyelitis patients were group A streptococci (n=2), *Haemophilus influenzae* (n=2) and *Fusobacterium* (n=1). In the patients with a chronic osteomyelitis we cultured also *Staphylococcus epidermidis* (n=2) and *Propionibacterium* (n=1).

Four patients had received antibiotics prior to obtaining material for culture in the acute osteomyelitis group and this was also the case in 8 patients with chronic osteomyelitis. The average time of use was 4 (1-7) and 27 (4-56) days, respectively.

The duration of treatment with intravenous antibiotics varied between 14 and 30 days (mean 20) for patients with acute osteomyelitis; and between 8 and 32 days (mean 20) for patients with chronic osteomyelitis. Subsequently, oral antibiotics were prescribed for an average of 22 (21-28) days in patients with acute osteomyelitis and 25 (21-45) days for patients with chronic osteomyelitis.

In the majority of the patients intravenous antibiotic therapy was started with flucloxacilin and gentamicin. The definitive results of the cultures sometimes obliged a change in the choice of antibiotics. The main oral antibiotics prescribed were flucloxacillin, clindamycin or amoxycillin/clavulanate.

There was no difference in hospital stay between the two groups. The children with acute osteomyelitis stayed 14 to 32 days (mean 23) and with chronic osteomyelitis 12 to 32 (mean 23) days in hospital.

The mean follow-up of the patients with acute osteomyelitis was

3.7 (0.7-8.3) years. In this group no patients had a recurrence. In two patients decreased function was observed, one patient with osteomyelitis of the proximal phalanx of a finger and a second with osteomyelitis of the proximal radius. The mean follow-up of the patients with chronic osteomyelitis was 2.7 (0.4-7.6) years. One patient, with known renal insufficiency and multiple other related problems, had a recurrence of osteomyelitis in different other parts of the body. One patient with an infection of the calcaneus developed a subtalair ankylosis.

3.4. Discussion

There is no universally accepted classification system for osteomyelitis (Levine 1993). In this study the 28 children were grouped as having either an acute (n=12) or a chronic (n=16) osteomyelitis according to the clinical criteria as mentioned in Table 3.1. We realise that such a classification is entirely arbitrary, but other classifications, for example based on the time elapsed between onset of symptoms and diagnosis, are similarly inconclusive. Moreover, not every patient has the typical symptoms of an acute or a chronic osteomyelitis, which may further complicate classification (Dich 1975; Wedgewood 1982). It should be noted, however, that our finding that about half of the patients present with a more chronic picture is in agreement with some other data from the literature (Mader 1995).

At examination, the clinical symptoms were more prominent in patients with an acute osteomyelitis compared to patients with a chronic osteomyelitis. Also, the infectious parameters, such as ESR, CRP and WBC, were markedly increased in the acute osteomyeltis group. In the chronic osteomyelitis group, the mean CRP was biased by a patient who also suffered from a chronic renal insufficiency needing dialysis.

Despite history, clinical examination, radiographs and laboratory findings, the diagnosis of osteomyelitis can be difficult. In such situations a technetium bone scintigram may be a useful investigation (Gillday 1975; Howie 1983; Schauwecker 1992). Also in our study,

the technetium bone scintigram proved to be a reliable instrument. In 13 patients in our study bone scintigraphy was performed when the diagnosis osteomyelitis was not clear at fist sight. The 4 bone scintigrams in patients with an acute osteomyeltis were all positive. In the group of chronic osteomyelitis 7 out of 9 bone scintigrams were positive.

In 6 out of 16 patients with a chronic infection, an osteomyelitis of the calcaneus was diagnosed. As Wang et al. described, the clinical symptoms in these cases are less dramatic compared to the symptoms of an osteomyelitis of the long bones (Wang 1992). This is in accordance with our study. Heel pain and swelling were the symptoms in all cases. Bone cultures were positive in only 50 per cent. In the remaining cases the diagnosis of osteomyelitis was made by the pathologist. All children in our study with an osteomyelitis of the calcaneus recovered completely.

At surgery, samples were obtained for microbiological examination by Gram stain and culture. As expected, in most positive samples *Staphylococcus aureus* was cultured. In 9 patients of the acute osteomyelitis group and 7 patients of the chronic osteomyeltis group the cultures were positive. To eight of these 16 culture-positive patients antibiotics had been administered previously. These data are in contradiction to the suggestion by Morrey et al. that pre-administration of antibiotics affect the organism retrieval rates (Morrey 1975).

Especially in acute osteomyelitis, the role of surgery has always been controversial (Nade 1983; Dagan 1993). With Scott et al., we believe that early decompression, by drilling holes in the bone cortex, contributes to the healing of the osteomyelitis (Scott 1990). It may interrupt progression of the infection-pressure-necrosis chain of events. Also samples for Gram stain and culture can be taken and in chronic osteomyelitis cases, gentamicin beads can be left behind.

The duration of antibiotic therapy is controversial. There is a great variability in managing osteomyelitis according to different experts (Ross 1985; Scott 1990; Dagan 1993; Frederiksen 1993; Mader 1993). There are no controlled studies that have critically assessed the type of administration and duration of antibiotic treatment of osteomyelitis. Our results suggest that there is no need for extend-

ing antibiotic therapy in either group beyond 6 weeks. Currently we start treatment by administering antibiotics intravenously for at least 7 days. If the clinical signs improve sufficiently rapidly, i.e. within one week, and the infectious parameters have returned to normal values within the same period, we switch to oral antibiotic therapy, given for another 3 weeks.

Hip Prosthesis Infections. A Retrospective Study of 47 Patients

4.1. Introduction

A deep infection of a total joint replacement is a devastating complication that often results in severe morbidity and may occasionally be fatal. In addition, it may have disastrous consequences for the patient, i.e. causing disruption of his or her social life. One to 5 percent of prosthetic joints become infected, causing tremendous health care costs, due to the time-consuming and intensive treatment that is necessary. It will be evident that it has a particularly large economic impact for hospitals that treat these patients (Sculco 1993; Barrack 1995).

Diagnosis of infection following a total hip arthroplasty is a considerable challenge. No test is 100 per cent sensitive or specific. The diagnosis relies on the judgement of the surgeon of the clinical presentation, the findings on physical examination and the interpretation of the results of investigations (Spangehl 1998). Only recently an attempt was made to formulate criteria for the microbiologic diagnosis of these infections (Atkins 1998).

Depending on the mode of infection of the prosthesis, sometimes the infection can successfully be eradicated by a debridement only. However in most cases the prosthesis has to be removed. A new prosthesis can be inserted immediately or later. However, more than once a replacement cannot be performed and it is decided to leave a Girdlestone resection arthroplasty. Always additional antibiotic treatment is necessary (Antti-Poika 1990; Masterson 1998).

We investigated retrospectively a population of patients who were admitted to our hospital with a clinical diagnosis of infected hip prosthesis, focusing at results of diagnostic studies, management of treatment and ultimately functional outcome of the various treatment categories.

4.2. Materials and Methods

We reviewed 47 patients who were treated because of a deep infection of a hip arthroplasty at our hospital between 1988 and 1998. All patients, in whom a clinical diagnosis of prosthetic hip

infection was made, were included in the study. The parameters analysed were: primary preoperative diagnosis, previous surgical procedures, risk factors for infection, clinical presentation and diagnostic work-up, the infecting micro-organism, surgical and antibiotic therapy, and outcome of therapy. The treatment modalities were divided in three groups: (1) debridement, (2) one- or two-stage exchange procedure and (3) resection of the prosthesis eventually followed by one or more debridement procedures. In addition to these modalities, all patients were treated with antibiotics. The choice of antibiotics was guided preferably by the results of pre- and perioperative cultures. Outcome of therapy was assessed by evaluating recurrent signs of infection and functional clinical studies. Thirty-four out of 35 patients alive at the time the study was initiated, answered 5 items of a questionnaire developed by Dawson et al., evaluating level of hip pain, walking time, limping, work interference due to pain and use of walking aids (Dawson 1996). In addition, they were asked to score their pain on a visual analogue scale (VAS) (Huskisson 1974). Because of neurological problems the answers of 3 patients were excluded.

4.3. Results

The patient characteristics are presented in Table 4.1. Of the 47 patients included in the study, 35 were female and 12 were male. In 25 cases the right hip was infected and in 22 cases the left hip. The series included 39 total hip arthroplasties and 8 hemi-arthroplasties. Twenty-nine total hip arthroplasties were cemented, 5 were cementless and 5 were hybrid. The hemi-arthroplasties were all cemented. The primary preoperative diagnosis was osteoarthritis in 22 hips, rheumatoid arthritis and subcapital hip fractures in 7, avascular necrosis in 5 and congenital dysplasia of the hip, M. Perthes, septic coxitis, psoriatic coxitis or M. Bechterew in 6 hips. The mean age at the time of infection was 68 (range: 40-88) years. The mean interval from the implantation of the hip arthroplasty to infection was 43 (0.1-263.9) months. In 12 patients the infection was diagnosed within 6 weeks; in 2 patients within 3 months and in

7 patients within one year. In 26 patients the infected prosthesis was inserted more than one year prior to infection. The follow-up after onset of treatment for infection was 39.4 (6.3-119.5) months.

Table 4.1. Overview of patient characteristics of the 47 patients involved in the study.

Sex		**Signs**	
males	12	none	2
females	35	pain	44
Age		fever	12
years	68	pus	31
Side		sinus	8
left	22	**Risk factors**	
right	25	none	16
Primary diagnosis		diabetes mellitus	7
osteoarthritis	22	rheumatoid arthritis	7
fracture	7	renal insufficiency	1
rheumatoid arthritis	7	use of steroids	9
osteonecrosis	5	previous operations	16
congenital hip dysplasia	2	**Previously operated before THP**	
M. Perthes	1	no.	17
M. Bechterew	1	**Haematogenous infection**	
coxitis	1	yes	5
psoriatic arthritis	1	possible	9
Implant		unlikely	20
hemi-arthroplasty	8	direct infection	13
total hip replacement	39	**Died**	
Cemented vs. cementless		yes	12
cemented	37	no	35
hybride	5	**Follow-up**	
cementless	5	mean (mth)	39
First symptoms of infection			
<6 wks	12		
<3 mth	2		
<1 yr	7		
>1 yr	26		

Eighteen patients were suffering from rheumatoid arthritis (n=7), diabetic mellitus (n=7) or renal insufficiency (n=1), or were using corticosteroids (n=9). Twelve patients had been operated on the same hip once before; 5 patients had been operated more than one time before.

	ESR n=41	CRP n=18	WBC n=32
mean	59.3	86.1	10.3
range	7-140	3.5-305.0	5.1-25.6
stdev	34.6	90.1	4.5

Table 4.2.
Laboratory data obtained at time of presentation of the patients.
ESR = erythrocyte sedimentation rate (mm/hr), CRP = C-reactive protein (mg/l) and WBC = white blood-cell count (x10⁹/l).

Micro-organism	No.	%
Gram positive		
Coagulase-negative staphylococci	15	25.9
Staphylococcus aureus	14	24.1
Beta-haemolytic streptococci	3	5.2
- group B streptococci (n=2)		
- group G streptococci (n=1)		
Viridans streptococci	3	5.2
- *Streptococcus oralis* (n=1)		
- *Streptococcus bovis* (n=1)		
- non-typable (n=1)		
Pepto-streptococcus sp.	1	1.7
Enterococcus sp.	10	17.2
Micrococcus sp.	1	1.7
Gram negative		
Proteus mirabilis	1	1.7
Pseudomonas aeruginosa	1	1.7
Escherichia coli	3	5.2
Moraxella catarrhalis	1	1.7
Enterobacter cloacae	1	1.7
Anaerobes		
Propionibacterium sp.	1	1.7
Clostridium perfringens	1	1.7
Bacteroides fragilis	1	1.7
Other		
Mycobacterium tuberculosis	1	1.7
Negative	2	

Table 4.3.
Micro-organisms cultured pre- and peroperatively of 45 patients. Of two patients the cultures remained negative.

Pain was the presenting symptom in the great majority of patients (44 out of 47, i.e. 94%). Twelve patients (26%) had fever and from 31 patients (66%) pus was obtained from the hip joint. Eight patients had a draining sinus tract. The laboratory parameters (Table 4.2) showed a mean erythrocyte sedimentation rate (ESR) of 59.3 mm/hr and a mean C-reactive protein value (CRP) of 86.1 mg/l. The mean white blood-cell count (WBC) was 10.3×10^9/l.From 18 patients the radiographs showed abnormalities, such as radiolucency around the prosthesis or periosteal reaction. Twelve of the 13 scintigraphic investigations (93%) performed were abnormal. In 8 cases these scintigraphic abnormalities were compatible with an infection of the prosthesis.

Specimens for microbiological analysis were obtained prior to

Table 4.4.

Suggestive and probable haematogenous infections. In 5 patients a distant infectious focus with identical bacterial species was detected. In 9 patients a haematogenous contamination was probable, but could not be proven by culturing identical micro-organisms from both foci. DM = diabetes mellitus, RA = rheumatoid arthritis, S = use of steroids.

	DM/RA/S		Micro-organism
Suggestive	S	ulcer (ankle)	*S. aureus*
	RA, S	ulcer (cruris)	CNS
	DM	ulcer (foot)	*S. aureus*
	DM	ulcer (foot)	*S. aureus*
	-	cholecystitis	*Cl. perfringens*
Probably	-	operation abd. aneurysm, pos. blood cult.	*S. aureus, E. coli*
	RA	renal insufficiency, dialysis	*S. aureus,* group G streptococcus
	-	*M. tuberculosis* cultured	*M. tuberculosis, E. coli*
	-	necrotising fasciitis	*Pepto-streptococcus sp.*
	-	pulmonary infection	unknown
	DM	paraplegia	*Enterococcus faecalis*
	DM	decubitus	*Enterococcus spp.*
	-	urinary tract infection.	*E. coli*
	-	suprapubic catheter	Group B streptococcus

surgery by aspiration and/or at the time of operation (Table 4.3). The majority of the isolated micro-organisms (47 out of 58, i.e. 81%) were Gram-positive, while Gram-negative bacilli were isolated 7 times (12%) and anaerobic micro-organisms 3 times (5%). In one case *Mycobacterium tuberculosis* was isolated. Thirty-three out of 47 arthroplasties (70%) were infected with a single micro-organism and 12 (26%) with two or more different micro-organisms. Two cultures remained negative, although the hip was clinically infected in both cases.
In 13 patients the prosthesis was infected by direct contamination. In five patients a distant infectious focus with the same bacterial species was detected, suggesting a haematogenous spread of infec-

Table 4.5.
Overview of therapy and final outcome of all patients. Thirty-five patients were still alive at the time of the study. Two patients died because of the infection of the hip prosthesis.

	debridement n=9	resection arthroplasty n=22	one-stage revision n=4	two-stage revision n=12
Infection remaining after initial therapy				
no	5	16	4	12
yes	4	4	-	-
unknown	-	1	-	-
n.a	-	1	-	-
Final outcome				
THP	6	-	4	11
girdlestone	2	20	-	1
arthrodesis	-	1	-	-
died because of infection	1	1	-	-
Draining sinus				
no	6	16	4	12
yes	2	4	-	-
unknown	-	1	-	-
n.a.	1	1	-	-
Died				
no	8	14	4	9
yes	1	8	-	3

tion. In 9 patients a haematogenous contamination was probable, but could not be proven by same micro-organisms cultured from both foci (Table 4.4). In 20 patients a haematogenous contamination of the prosthesis could be possible, but no signs of a haematogenous infection could be found.

Treatment choice and outcome of the initial treatment are described in Table 4.5. Of the 47 infected hips, 9 were initially treated by debridement (group 1). The average follow-up period of these patients was 28 (15-46) months. The mean age at treatment of the infection was 66 (55-84) years. In 4 patients the infection was not eradicated. One patient died as a result of the infection. Removal of the prosthesis was not performed initially, because of the already bad health of the patient. Although in a second stage the prosthesis was removed, the patient became septic after the operation and died. Because of a persistent infection in 2 patients a resection arthroplasty was performed at a later stage. Two patients still have a draining sinus tract: 1 patient without and 1 patient with a prosthesis *in situ*.

Sixteen infected hips were treated by an exchange procedure (group 2). Four patients underwent a one-stage, and 12 patients a two-stage procedure. The average follow-up period of these patients was 37 (28-47) and 50 (8-119) months, respectively. The average age at the beginning of the treatment was 75 (61-88) and 65 (41-79) years, respectively. The average time until reimplantation of a new prosthesis in the patients who underwent a two-stage exchange procedure was 6 months (7-866 days). Seven out of 16 reimplanted hips were cemented, 3 cementless and 6 hybrid total hip arthroplasties. At the time of reimplantation gentamicin-impregnated cement was used in all cases. In 1 patient the infection could not be eradicated by the exchange procedure and removal of the prosthesis was performed successfully. At the time of the investigation 13 patients were still alive.

In 22 patients, a prosthesis was not reimplanted after removal of the infected hip prosthesis (Girdlestone procedure; group 3). The average follow-up period in this group was 38 (6-113) months and the average age was 69 (40-88) years. One patient died; it is not known if this patient died because of infection. One patient died of an

adult respiratory distress syndrome because of a sepsis after removal of the infected prosthesis. This patient was already in a poor physical condition as a result of major surgery (aneurysm of abdominal aorta) some weeks before removal of the prosthesis. Because of pain, in one patient an arthrodesis was performed at a later stage. In 4 patients a draining sinus tract remained. Only 14 out of 22 patients were still alive at the time of the evaluation.

A total of 132 operations requiring anaesthesia were necessary (Table 4.6). On average, for patients in group 1 2.3 operations, in group 2 2.8 operations and in group 3 3.1 operations were necessary. Patients treated by a one-stage revision underwent 1.3 operations; those treated by a two-stage revision procedure 3.7 operations. The average hospital stay in group 1 was 75 days. In group 2 the average hospital stay was 75 days, and in group 3 89 days. Hospitalisation of the patients who underwent a one-stage revision was 75 days and those who underwent a two-stage procedure 94 days.

Treatment	No. of patients	Hospital stay (days)	No. of operations
debridement	9	75 (25-186)	2.3 (1-5)
resection arthroplasty	22	75 (8-231)	2.8 (1-6)
one-stage revision	4	75 (51-122)	1.3 (1-2)
two-stage revision	12	94 (23-303)	3.7 (2-7)

Table 4.6. Hospitalisation and number of operations (mean and range). In total, 132 operations were performed.

In general, intravenous broad-spectrum antibiotics were started as soon as preoperative cultures yielded micro-organisms, or when these cultures remained negative, after collection of specimens for culture during operation. Antibiotic therapy was changed according to the susceptibility of the isolates. Subsequently, therapy was switched to oral antibiotics whenever possible.

The results of the questionnaire from 31 patients are shown in Table 4.7. From these patients, 18 had a total hip arthroplasty, 12 a resection arthroplasty and 1 an arthrodesis of the hip. Thirty-three per cent of the Girdlestone patients had severe pain against only 6 per cent of the arthroplasty patients. Thirty-three per cent of the

Table 4.7. Functional outcome of 31 out of 35 still living patients at the time of study. VAS = Visual Analogue Scale from 0 (no pain) to 10 (intolerable pain).

	THP n=18	girdlestone n=12	arthrodesis n=1
Initial therapy			
debridement	6	1	-
girdlestone	-	11	1
revision, 1 tempo	4	-	-
revision, 2 tempi	8	-	-
Residual sinus			
no	17	11	1
yes	1	1	-
Rheumatoid arthritis			
no	16	10	-
yes	2	2	-
Usual level of hip pain			
none	4	3	-
very mild	6	1	-
mild	4	3	1
moderate	3	1	-
severe	1	4	-
Pain (VAS 0-10)			
score±stdev	3.0±3.0	4.4±3.5	7.8
Work interference due to pain			
not at all	4	2	-
a little bit	10	-	1
moderately	1	1	-
greatly	2	3	-
totally	1	6	-
Walking without severe hip pain			
>30 minutes	4	-	-
16-30 minutes	4	-	1
5-15 minutes	1	-	-
around the house only	6	7	-
not at all	3	5	-
Limping when walking			
rarely/never	2	-	-
sometimes	6	1	1
often	4	-	-
most of the time	2	-	-
all of the time	4	11	-
Use of walking aids			
never	7	-	-
sometimes	-	-	-
often	1	-	-
most of the time	-	-	-
all of the time	10	12	1

Girdlestone patients had none or very mild pain, while of the patients with an arthroplasty 56 per cent had none or very mild pain. The patients with a Girdlestone scored their pain as 4.4 on a scale of 0 (no pain) to 10 (intolerable pain), whereas patients with an arthroplasty scored their pain as 3.0. Fifty per cent of the patients with a Girdlestone said that pain of the hip interfered totally their usual daily activities, in contrast to only 1 (i.e. 6%) of the arthroplasty patients. Pain interfered only slightly or not at all with daily activities in 78 per cent of the patients with a hip arthroplasty. All patients with a Girdlestone could not walk at all or only around the house without severe pain. In the patients with an arthroplasty this percentage was 33 per cent, while 44 per cent could walk more then 15 minutes. Ninety-two per cent of the Girdlestone patients were limping all of the time when walking. All these patients used walking aids. Thirty-three per cent of the patients with an arthroplasty were limping most or all over the time, while 44 per cent were limping sometimes or never. Of this group 56 per cent used walking aids all of the time.

4.4. Discussion

This study retrospectively reviewed patients with infected total hip arthroplasties, in an attempt to evaluate the results of diagnostic procedures and the outcome of the various management strategies. In a period of 10 years 47 patients were treated by either debridement only, removal of the prosthesis and debridement, or a one- or two-stage exchange procedure. Beside surgical intervention antibiotic therapy was given to all patients.

Infection of an arthroplasty is diagnosed by clinical symptoms, laboratory parameters, radiological investigations and cultures taken pre- and/or peroperatively. In accordance with the literature pain is the major presenting symptom in patients with a deep infection (Fitzgerald 1985). This was in concordance with this study, in which almost all patients had pain. Other symptoms observed less frequently were fever and a draining sinus tract. Laboratory parameters suggestive of an infection, like a raised ESR and/or raised

CRP were found in the majority of patients.
Changes on plain X-rays are of limited value as an investigative tool for the diagnosis of infection. Besides that diagnostic findings such as loosening, osteolysis or endostal scalloping are common in both septic and aseptic failure (Tigges 1994b), they are not always present (Tigges 1994a). The findings of the present study agree with these data from the literature, because only a limited number of patients showed radiological changes compatible with infection.
Additional nuclide imaging proved to be only of limited value in diagnosing deep implant infection in the present study. Eight out of 13 nuclear investigations showed signs compatible with infection, whereas 4 investigations showed signs of loosening without infection. These findings are in accordance with the literature. Spangehl et al. already mentioned the limited role of scintigraphy by the inability of the scans to yield consistently acceptable levels of sensitivity and specificity (Spangehl 1998).
Gram-positive micro-organisms were isolated from the majority of patients. Coagulase negative staphylococci (CNS) and *Staphylococcus aureus* were isolated in 26 respectively 24 per cent of the cultured micro-organisms. This is in agreement with other reports which described staphylococci as the most common causative agents of prosthetic joint infections (Fitzgerald 1973; Fitzgerald 1985; Fitzgerald 1989; Ostendorf 1998).
A higher rate of deep infection of a prosthesis after previous operations has been reported (Fitzgerald 1977; Poss 1984; Wilson 1990; Hanssen 1999). Other published risk factors for a deep infection are rheumatoid arthritis (Charnley 1972; Ahlberg 1978; Stinchfield 1980; Glynn 1983; Ainscow 1984; Poss 1984; Bengtson 1987; Maderazo 1988; Wilson 1990), diabetes mellitus (England 1990; Papagelopoulos 1996) and use of steroids (Wilson 1990). In this study 66 per cent had one or more of these risk factors for a deep prosthetic infection.
Although it is difficult to link a preceding bacteremia to a prosthesis-related infection, late postoperative infections are usually considered to result from haematogenous spread of bacteria from a distant focus, which sometimes may become manifest weeks or months later. It has been postulated that the most common sources for

haematogenous total joint infection are skin and soft tissue. Other important sources are mouth, respiratory tract and urinary tract (Ainscow 1984; Maderazo 1988; Deacon 1996). The percentage "suggestive" (i.e. those infections in which a distant focus was present with the same bacterial species) haematogenous infections (11%) is in agreement with the literature (Salvati 1982b; Ainscow 1984). Another 19 per cent of the infections in this study were considered as probably haematogenous. According to Ainscow and Denham transient bacteremia is not likely to infect a replaced joint in otherwise healthy patients. Patients with rheumatoid arthritis are at greater risk than those with osteoarthritis (Ainscow 1984). Four out of 5 patients with a proven haematogenous infection in this study were suffering from diabetes mellitus, rheumatoid arthritis or used steroids.

Depending on the time of onset of infection, the condition of the patient as well as the local condition around the hip, several methods of treatment are described (Carlsson 1980; Härle 1989; Gillespie 1990; Garvin 1995; Hanssen 1999). Debridement without removal of the prosthesis can be performed within 6 weeks of insertion of the infected prosthesis or in the case of an acute haematogenous infection. As reported previously by Crockarell et al. (Crockarell 1998) and confirmed in the present study, the success of debridement is limited: in 4 out of 9 of our patients the infection was not eradicated. One patient died as a result of the infection. Because of a persistent infection, in 2 patients an excision arthroplasty was performed at a later stage. In 3 patients, a debridement was performed despite the fact that the prosthesis was inserted longer than 6 weeks ago. In 2 out of these 3 patients the infection was never eradicated.

An resection or Girdlestone arthroplasty is proposed as the most appropriate treatment for patients who are psychologically or medically unfit to have an additional reconstructive procedure and those who are mentally impaired or may be unable to cooperate with the postoperative restrictions and rehabilitation protocols after a complex reconstruction. Also a severe deficiency of bone stock and poor quality of local soft tissue is an indication for a resection arthroplasty (Ahlgren 1980; Bourne 1984; Forgon 1990; de Laat 1991; Lieberman 1994). According to the literature the infection is

brought under control in a high percentage of cases (Grauer 1989; Castellanos 1998). Pain is less compared with the situation before the operation, but most of the time pain remains to some extent (Ahlgren 1980; Bittar 1982; Bourne 1984; Grauer 1989; de Laat 1991; Castellanos 1998). The functional outcome is poor. After resection there is a shortening of the leg on the operated side, varying from 3 to 11 cm (Ahlgren 1980; Bourne 1984; Grauer 1989; Castellanos 1998). Almost in every case, patients use some type of walking aids (Ahlgren 1980; Bourne 1984; Grauer 1989; de Laat 1991; Castellanos 1998) and walking distance is diminished (Grauer 1989; de Laat 1991). The results of the present study in this category of patients with resection arthroplasty were in accordance with the literature. All these patients were medically or psychologically unfit to undergo a procedure for a new prosthesis. Only 14 out of these 22 patients were alive at the time of the present study was initiated. In only 1 of these 14 patients the infection was not eradicated; this patient still has a draining sinus tract. Although in this category of patients the hip was less painful than prior to removal, pain was reported frequently. Pain scores were higher as compared to patients where the prosthesis was left in place or was revised. In addition, walking distance was diminished in this group, however the ability to walk varied also with the in general diminished health of the patients. Walking aids were used to some extent by all patients who were able to walk.

A one- or two-stage exchange arthroplasty gives a better functional result and remains a better alternative in some cases (Carlsson 1980; Bittar 1982). Usually, and especially in the Anglo-Saxon countries, a two-stage exchange procedure is performed (Härle 1989; Antti-Poika 1990). As was also the case in our patients, sometimes when preoperative cultures were negative and the prosthesis was revised, cultures obtained during surgery appeared to be positive. Thus, the revision can be considered as a one-stage procedure. However, there are groups which perform a one-stage exchange procedure even when an infection of the prosthesis is evident (Buchholz 1972; Elson 1994).

In conclusion, the eradication of an infected arthroplasty of the hip by only surgical debridement and antibiotics is poor. Removal of

the prosthesis gives better results with regard to eradication of the infection. In case the prosthesis cannot be replaced, the functional outcome of the so-called Girdlestone situation is poor. For better functional results, an exchange of the prosthesis, when medically and psychologically possible, is advised. In the current study, several prosthesis infections were likely to be haematogenous infections. It is therefore of great importance to get a better insight into the mechanisms of these infections and to improve the prophylactic and therapeutic options.

Haematogenous Infection of a Total Hip Prosthesis by *Clostridum perfringens*. A Case Report

5

5.1. Introduction

In orthopaedic surgery, an infection of a prosthesis is a very serious, sometimes life-threatening, complication. In the majority of cases the prosthesis has to be removed, followed by prolonged treatment with intravenous antibiotics. In some situations a new prosthesis can be inserted at once, but mostly it is inserted later in a second stage.

A three-stage classification system of hip prosthesis infection is described by Coventry (Coventry 1975) and later Fitzgerald et al (Fitzgerald 1977). This system is based on the time of the presentation of infection. First stage infections occur in the immediate post-operative period. These early post-operative infections are usually caused by direct peroperative contamination. Second stage infections are also believed to originate at the time of the operation, but because of a small inoculum or low virulence of the organism, the onset of the symptoms is usually delayed until 6 to 24 months after the operative procedure. Stage three infections are caused by haematogenous or lymphatic spread to a previous asymptomatic hip. These type of infections start usually 2 years or more after the operative procedure. Early diagnosis may allow salvage of the joint by means of complete debridement. A delay in the diagnosis may necessitate a one or two-stage exchange procedure. The prevalence of haematogenous prosthesis infections is estimated at 0.3 to 1 per cent (Charnley 1969; Ahlberg 1978; Gristina 1983; Jaspers 1985; Schutzer 1988; Nelson 1990), and the most common foci are skin and soft tissue (46%), dental (15%) and urinary tract (13%) infections (Maderazo 1988). For the skin, soft tissue and dental region, *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common pathogens, while for the urinary tract, *Escherichia coli* is the most common pathogen. The relationship between a staphylococcal bacteremia and a distant prosthesis infection has also been demonstrated in animal experiments by Blomgren (Blomgren 1981) and Vogely (Vogely 1996).

This report describes for the first time an unusual presentation of a late haematogenous infection of a total hip prosthesis with *Clostridium perfringens* following an acute cholecystitis.

5.2. Case report

A female patient (52 years old) with primary osteoarthritis of the hip joint underwent in 1982 an intertrochanter osteotomy. Because of persisting pain in 1983, a TARA prosthesis (Total Articular Replacement Arthroplasty) was inserted. Eleven years later, in 1994, the TARA prosthesis was removed because of aseptic loosening and was replaced by a cemented (gentamycin containing bone cement) Müller total hip prosthesis. Antibiotic prophylaxis with cephalothin was given during five days. Before and during this exchange procedure there were no signs of infection. Cultures of aspiration before the operation and samples taken peroperatively were negative. The patient recovered well from surgery and had no postoperative complaints. Ten months later, she was admitted to our hospital because of abdominal pain with high temperature and shaking chills for several days. At examination she had signs of an acute cholecystitis. Blood tests revealed an elevation of white blood-cell count (WBC) and erythrocyte sedimentation rate

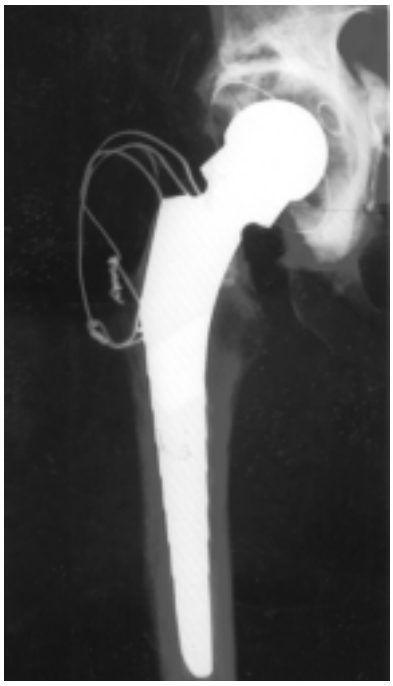

Figure 5.1.
X-ray of total hip prosthesis, six months after the cholecystectomy. There are no signs of loosening or of gas accumulation around the hip.

(ESR). A sonography showed a thickened wall of the gallbladder. Under the diagnosis of an acute cholecystitis, an open cholecystectomy was done. Because of the infection, the wound was not closed. Postoperatively, the patient was treated with intravenous and later with oral amoxicillin and clavulanic acid. Cultures of the gallbladder yielded *Clostridium perfringens*. The postoperative period was uncomplicated. Eleven days after admission the patient was discharged. The wound closed spontaneously. After six months, the patient developed a progressive tenderness of the right groin. At investigation she had a right limp. ESR and WBC were 55 mm/hr and 9.9×10^9/l, respectively. X-ray examination showed no signs of loosening of the prosthesis or signs of gas around the hip (Figure 5.1). However, a technetium bone scan was suggestive for an infection of the prosthesis (Figure 5.2).

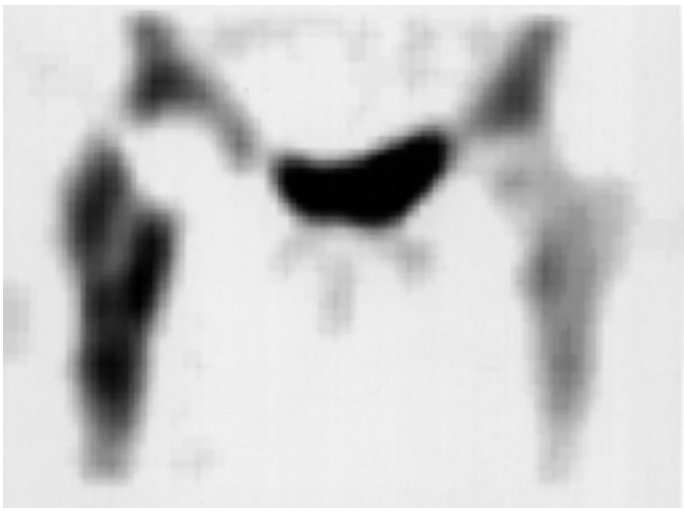

Figure 5.2. *Technetium bone scintigram. An increased uptake is shown around the implant, suggestive for an infection of the prosthesis.*

At aspiration of the joint, pus was obtained and *Clostridium perfringens* was cultured. Because of fear of a clostridial sepsis, the patient was treated with oral penicillin several days before the prosthesis was removed. At removal of the implant, the bacteriological cultures were negative. Postoperatively, the patient was treated with intravenous penicillin and subsequently with oral clindamycin. Ten

days after removing of the prosthesis a surgical debridement was done. Bacteriological cultures obtained at this last procedure remained negative. Five weeks after removal of the implant the patient was temporarily discharged from our hospital and nine weeks later she was admitted to our hospital again for a new total hip prosthesis. A Müller total hip prosthesis was inserted with gentamicin containing bone cement. Antibiotic prophylaxis with intravenous cephalothin was given. Again, samples obtained peroperatively for bacteriological culture remained negative. The patient recovered well and 26 days after she received the new prosthesis she was discharged in good condition. One year after the operation she has only slight complaints of pain and she walks with only one crutch outside. Clinically there are no signs of infection. X-ray examinations show no signs of loosening and blood tests remain normal.

5.3. Discussion

Clostridia are spore forming bacilli and normal inhabitants of the gastrointestinal tract, the biliary system and the genitourinary tract. *Clostridium perfringens* is an anaerobic Gram-positive bacillus that produces toxic proteolytic enzymes. It produces a haemolytic and necrotising alpha-toxin and also lecithinases which can lyse erythrocytes, leukocytes and platelets. In addition to the well-known clostridial myonecrosis of open compound fractures, gas gangrene has been documented without open contamination (Finegold 1985).

Clostridial infections must be treated aggressively. Extensive surgical debridement combined with antibiotic therapy is essential for adequate treatment (Finegold 1996). The antibiotic mostly used is penicillin G. However resistance to beta-lactam antibiotics by *Clostridium perfringens* has been reported (Nord 1990). Other reported antibiotics for treatment of *Clostridium perfringens* infections are metronidazole (Finegold 1996), imipenem (Finegold 1996), clindamycin (Finegold 1996), cefoxitin (Finegold 1996), chloramphenicol (Moehring 1988; Finegold 1996), tetracycline (Chetta 1982) and

erythromycin (Darke 1977). Hyperbaric oxygen therapy may be of value only in selected circumstances, such as in cases of gas gangrene. However, there has never been clear-cut clinical evidence that hyperbaric oxygen therapy is significantly beneficial and one should never delay necessary surgical therapy to administer hyperbaric oxygen therapy (Finegold 1996).

In orthopaedic surgery, infections with *Clostridium perfringens* have been described incidentally. For patients without a prosthesis, several authors described a septic arthritis with *Clostridium perfringens* (Fauser 1988; Lluberas-Acosta 1989; McAllister 1989). For patients with a total joint prosthesis, Stern (Stern 1988) and Rush (Rush 1976) describe the possibility of a direct infection through a disturbed wound healing or local contamination with *Clostridium perfringens*.

An early (stage I) infection of a total knee prosthesis after an acute cholecystitis was described by Wilde (Wilde 1988). This supports the postulation of Blomgren that bacteremia poses a greater risk of infection of an implant in the early postoperative period than it does in the late postoperative period (Blomgren 1980).

Two previous cases were described of a late prosthesis infection with *Clostridium perfringens*, for which a haematogenous spread of bacteria from a distant focus might have been the cause. Kibbler (Kibbler 1991) reported a prosthesis infection in a patient after a period of diarrhoea and vomiting, and Maniloff described a late infection of a total knee prosthesis in a patient with a common bile duct with stones (Maniloff 1987). However, since no positive cultures from the possible primary focus were available in these studies, a direct relationship between focus and prosthesis infection could yet not be made.

The patient in the current paper developed a so-called "stage three" infection of her hip prosthesis, according to the previously mentioned classification (Coventry 1975; Fitzgerald 1977). To our knowledge, this is the first case that confirms how a relative common gastrointestinal infection followed by surgical intervention, results in an infection of a hip prosthesis with *Clostridium perfringens* through a haematogenous pathway. This paper demonstrates that patients with an orthopaedic endoprosthesis *in situ* are at risk to develop a late infection of this implant with *Clostridium perfringens* after a gallbladder infection.

Conclusions from Literature and Clinical Studies. Aims of Experimental Studies

The investigations in this thesis were initiated by the conviction that infections in orthopaedics, in particular prosthesis-related infections, are difficult to understand, to treat and to prevent. Clinical studies are performed with the aim of getting a better insight of the management of infection. Experimental studies focusing at the underlying mechanisms of infection in orthopaedics are important. It is hoped that these studies give us better tools for reliable diagnosis, the optimal therapy and more effective prophylactic measurements. First, in Chapter 2, the current literature of hip prosthesis-related infections has been summarised, and in the subsequent Chapters 3, 4, and 5, various clinical studies of orthopaedic infections were described.

In *Chapter 2,* the current knowledge of prosthesis-related infections was summarised based upon the present literature. The epidemiology and classification of these infections was described, as well as diagnostic examinations and treatment options. In addition, current understanding of the underlying pathogenesis of implant-related infections was presented.

In *Chapter 3*, the haematogenous route as an important pathway for orthopaedic infections was described through a retrospective analysis of 28 children with a diagnosis of osteomyelitis. After surgical intervention, it appeared that six weeks of antibiotic therapy was sufficient to eradicate acute as well as chronic osteomyelitis in children. However, there is a great variability in managing osteomyelitis between different treating physicians. No controlled studies of the type and duration of antibiotic treatment of osteomyelitis exist.

Chapters 4 and 5 refer to various clinical aspects of prosthesis-related infections. In *Chapter 4*, the results of a retrospective analysis of 47 patients with a prosthetic hip infection were described. The specific goals of the analysis were how diagnosis was reached, the results of the cultures, the management, and the infectious and functional outcome of treatment. The conclusion was that the eradication of an infected arthroplasty of the hip by only

surgical debridement and antibiotics is insufficient. Removal of the prosthesis clearly results in more complete eradication of the infection. In case the prosthesis cannot be replaced, the functional outcome of the so-called Girdlestone procedure is unsatisfactory. The population described in this chapter were all patients treated at one hospital over a period of 9 years. Still this population showed that management was far from uniform and contained many variables. This emphasises the need for studies in an experimental, and therefore much better controlled setting.

In *Chapter 5,* an insidious complication of an abdominal infectious process was described, emphasising the significance of the haematogenous route for prosthesis infections. It was postulated that if patients with joint prosthesis develop bacterial infections at distant sites, they should be treated immediately and aggressively with appropriate antibiotics to prevent haematogenous spread to the prosthetic joint.

From the previous chapters it can be concluded that there is a great lack in knowledge related to the pathogenesis of orthopaedic infections, their course and treatment. Further research is necessary, especially since the problem is extremely complex, but also since the problem is growing as a result of the rapid increase of the use of biomaterials in medicine, in particular in orthopaedics. For instance, questions can be raised that are related to the use of more biocompatible materials, such as hydroxyapatite coated implants, and how these may affect the infection susceptibility of an implant. The so-called "race for the surface" theory as postulated by Gristina (see Chapter 2) implies that when cells arrive first at a material surface after implantation, the implant will not be accessible for bacterial adhesion any more, while when bacteria arrive first at the surface, tissue integration cannot take place. In addition, will a local infection influence osseointegration of a bioactive implant or not? Therefore, in *Chapter 7*, we address our aim *to determine whether there is a difference in infection susceptibility of two common orthopaedic implant surfaces with a different biocompatibility (hydroxyapatite coated and noncoated titanium),* and *how the response of the bone surrounding these*

implants is characterised in case of infection. Another question relates to haematogenous infections in orthopaedics, that form a substantial part of all infections. However, the pathogenesis of these infections is not very well understood. This lack in basic and clinical knowledge on the pathogenesis of haematogenous orthopaedic implant infections is one of the motives for further study, and as a first step, in *Chapter 8,* we address our aim *to develop an experimental animal model to study haematogenous implant infections*.

Effects of Hydroxyapatite Coating on Ti6Al4V Implant Site Infection in a Rabbit Tibial Model. Microbiological, Histological and Histomorphometrical Studies

CHAPTER 7

7.1. Introduction

Implant infections in orthopaedic surgery are a serious clinical problem leading to an increase in complicated and costly revision arthroplasties with a high mortality. To date, this infection rate is about 1 to 2 per cent for a total hip arthroplasty and 4 per cent for a total knee arthroplasty (Walenkamp 1990; Sanderson 1991). Since the number of patients receiving an arthroplasty is still increasing (Okhuijsen 1998), it is obvious that the problem of implant-related infections will further increase in the future. Most infections are caused by *Staphylococcus aureus* and *Staphylococcus epidermidis* (Ostendorf 1998). Other frequently seen micro-organisms are the Gram-negative bacilli, such as *Escherichia coli, Proteus, Pseudomonas* and *Klebsiella* (Maderazo 1988).

Many new biomaterials, coatings and surfaces were developed in the last decade to improve noncemented fixation of an implant. Noncemented implants are especially indicated in younger patients with severe osteoarthritis (Engh 1987; D'Antonio 1992; Malchau 1993; Geesink 1995; Tonino 1995). Noncemented fixation potentially leads to a permanent bond between prosthesis and bone (Engh 1987; Geesink 1987; Geesink 1988; Soballe 1990; Hardy 1991; Soballe 1993; Ang 1997; Overgaard 1997; Dhert 1998; Tonino 1999). One of the most widely used materials for noncemented joint prostheses is titanium, a biocompatible material that permits bone to grow in close proximity to the implant surface (contact osteogenesis) (Engh 1989; Mulliken 1996). To promote implant-bone contact even more, bioactive materials such as hydroxyapatite, a calcium phosphate ceramic, have been applied to implant surfaces as a coating. Several prosthetic designs using hydroxyapatite coated titanium have proven to be successful after medium term follow-up and are subject of constant evaluation (D'Antonio 1992; Geesink 1995; Tonino 1995; Vedantam 1996; Tonino 2000). Thus, noncemented implants derive their success from direct bone-to-implant contact, bone ingrowth into pores, or from biological bonding between bone and a bioactive prosthesis surface. A long lasting fixation will then be achieved by functional integration of the prosthesis with the surrounding bone. For the

process of bony integration, the early healing phase (days-weeks after implantation) is especially of importance, and is characterised by a cascade of various cellular events at the implant surface (Dhert 1998). Since the functionality of these implants is material or surface dependent, a logical question would be how the (optimal) characteristics for tissue integration relate to the susceptibility for bacterial colonisation and infection. Is a highly bioactive implant also more susceptible to infection, or is bacterial adhesion and colonisation prevented by a rapid tissue integration, as described in the "race for the surface" theory of Gristina (Gristina 1987a)? Although a bioactive implant surface does not necessarily mean that such a surface is also highly susceptible for bacterial adhesion and colonisation, the relationship between implant bioactivity and infection susceptibility has not yet been investigated.
The purpose of the present study was to investigate the infection susceptibility and osseointegration related to peri-implant infection, of two commonly used surfaces for noncemented fixation of orthopaedic implants: grit-blasted Ti6Al4V as a biocompatible surface and hydroxyapatite plasma sprayed Ti6Al4V as a bioactive surface. We hypothesise that the microbiological and histological behaviour of these two surfaces is different in the presence of bacteria.

7.2. Materials and methods

7.2.1. Design

The evaluation of infection susceptibility of titanium (Ti) and hydroxyapatite (HA) coated titanium implants was carried out in a contaminated implant bed model in the proximal left tibia of the rabbit. A total of 32 rabbits received in both tibiae either HA coated (n=16) or non-coated Ti6Al4V (n=16) implants. Each group was divided into 4 subgroups (n=4) that received different concentrations of bacterial inoculi of *Staphylococcus aureus*. Each right tibia served as control with an identical implant, but without contamination. After 28 days, the animals were killed and both tibiae were excised. Samples of surrounding bone were cultured and

bacteria were counted. The remaining part of bone with implant was prepared for histological and histomorphometrical (semiquantitative scoring of bone response, bone-implant contact, bone area next to implant) analysis. In Figure 7.1, the main steps in the animal experiment are summarised.

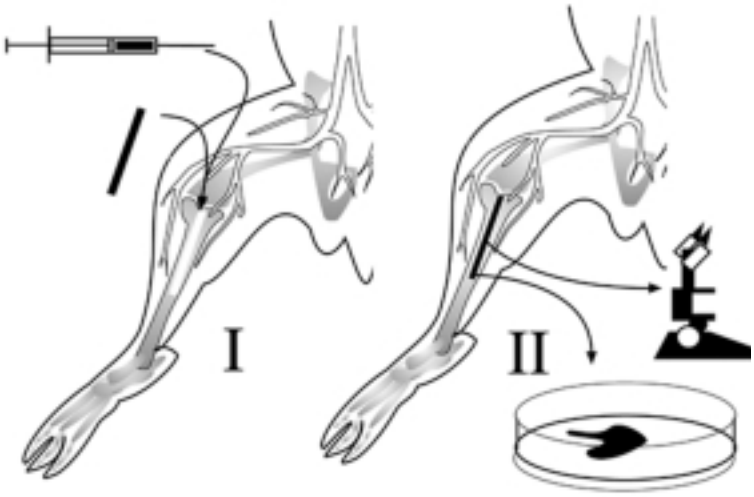

Figure 7.1. Artist's impression of the two steps in the experimental animal model. In step I, an inoculum S. aureus or saline (control) is injected into the medullary canal. Subsequently, a Ti or HA implant is press-fit inserted. In step II, four weeks after surgery, the animal is sacrificed, and samples for bacteriological, histological and histomorphometrical examination are obtained.

7.2.2. Preparation of implants

Cylindrical non-coated, grit-blasted titanium-aluminium (6%) vanadium (4%) (Ti6Al4V) implants and cylindrical hydroxyapatite coated Ti6Al4V implants (length 20 mm and final diameter 3.9 mm) were used in the present experiment (Figure 7.2). The thickness of the hydroxyapatite coating was approximately 60 microns. The coating was applied onto the surface by a vacuum plasma-spray technique and had a mean crystallinity of 75% and a porosity of less than 10%. The implants were sterilised in an autoclave prior to use. The surface roughness of the two implant types was established by a UBM laser profilometer (UBM, Etlingen, Germany). Scanning electron micrographs (Philips 525M, Philips, Eindhoven, The Netherlands) of carbon-coated Ti and HA implants were made to determine the surface morphology of the two implant surfaces.

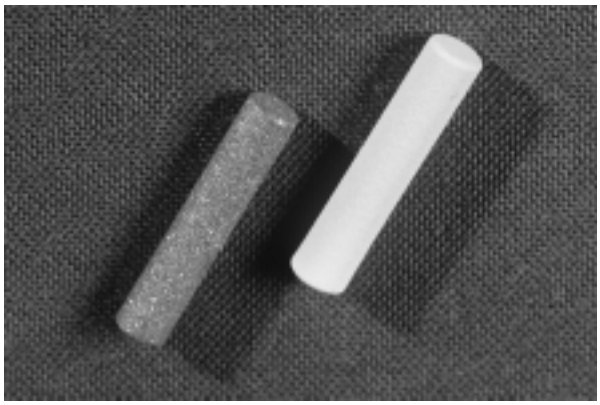

Figure 7.2.
Photograph of Ti6Al4V (left) and HA coated Ti6Al4V (right) implant. Final dimensions of the implants were 20 mm (length) and 3.9 mm (diameter).

7.2.3. Bacteria

Staphylococcus aureus, strain Wood-46 (ATCC 10832) was used throughout the experiment. This strain was described in detail by Delmi and Zimmerli (Zimmerli 1982; Delmi 1994). For preparation of the stock, a colony was grown overnight in tryptic soy broth at 37°C. Overnight cultures were centrifuged and resuspended in sterile saline. Standardised suspensions were frozen with liquid N_2 and held at -70°C until an inoculum was used. At regular intervals a vial was thawed, opened and cultured; the bacteria were counted to verify that the vial still contained the same amount of bacteria as at the time the original suspension was prepared. On the day of use, for preparation of an inoculum, the suspension was thawed and diluted with sterile saline up to concentrations of 10^3, 10^4, 10^5 or 10^6 colony forming units per millilitre (CFU/ml). During surgery the rabbits were inoculated with 0.1 ml of bacterial suspension, resulting in inoculum sizes of 10^2, 10^3, 10^4, and 10^5 CFU. Previous pilot experiments revealed that higher inoculum sizes resulted in severe systemic reactions, such as septicaemia.

7.2.4. Animals and surgery

The experimental design was approved by the institutional review board (Animal Care and Use Committee, Faculty of Medicine, Utrecht University). Female New Zealand White rabbits, weighing from 2.9-3.6 kg (mean 3.4 kg), were obtained from a

professional stock breeder (Ico:NZW, Broekman Instituut B.V., Someren, The Netherlands). In earlier studies this animal proved to be suitable to study arthritis and osteomyelitis (Marks 1980; Blomgren 1981; Eerenberg 1994; Belmatoug 1996; Cordero 1996a; Vogely 1996; Nijhof 1997a). The rabbits were housed individually in cages and fed with 80-100 g daily Hope Farms rabbit diet LKK-20 (Hope Farms Standard Laboratory Diet LKK-20, Hope Farms B.V., Woerden, The Netherlands). Water was given ad libitum. After contamination of the implant, the animals were cared under the same conditions in an infection unit, which is a barrier-separated wing of the animal laboratory.

Surgery was performed under general inhalation anaesthesia and aseptic conditions. The rabbits were premedicated by an intramuscular injection with 4 mg acepromazinemaleate (Vetranquil®, Sanofi Sante B.V., Maassluis, The Netherlands) and 4 mg methadone (methadoneHCl). The anaesthesia was initiated by an intravenous injection of 8-12 mg etomadate (Hypnomidate® , Janssen Pharmaceutica BV, Tilburg, The Netherlands). After intubation, inhalation anaesthesia was followed by a mixture with O_2 and N_2O in a ratio of 1 to 1 and halothane 1% (Albic BV, Maassluis, The Netherlands). Pain-relief was provided by 3 mg nalbufine (Nubain®, Lamepro B.V., Raamsdonkveer, The Netherlands) i.m. immediately postoperative, and subsequently 0.1 mg buprenorphine (Temgesic®, Reckitt and Colman Products, Kingston-upon-Hull, United Kingdom) i.m. if necessary.

After both legs of the rabbit were shaved, the animal was placed in supine position on the operation table. After disinfection with 10% povidone-iodine (Betadine® solution, Dagra, Diemen, The Netherlands) and sterile draping, first the right knee was operated. After a medial parapatellar incision of approximately 3 cm length, the knee joint was opened. Anterior to the insertion of the anterior crucial ligament on the tibia, the medullary canal was opened with an awl, and reamed by hand until a depth of about 25 mm and a diameter of 3.9 mm was obtained. Then, an inoculum of 0.1 ml of saline was injected into the cavity with a small pipette. Subsequently, a Ti or HA implant was press-fit inserted in the medullary canal. The joint capsule was closed with Vicryl 3-0

(Vicryl®, Ethicon GmbH & Co, Norderstedt, Germany) and the skin with steel 4-0 (Ethicon GmbH & Co, Norderstedt, Germany). The same procedure was performed on the left knee, but prior to implant insertion, an inoculum of 0.1 ml of saline containing 10^2, 10^3, 10^4 or 10^5 CFU bacteria was injected into the cavity.
Postoperatively, X-rays were made to check the position of the implants, and the animals were examined daily for activity, eating and wound healing. Weight and body temperature were measured. Blood samples were taken for erythrocyte sedimentation rate (ESR) and white blood-cell count (WBC) preoperatively and 1, 2, 3 and 4 weeks postoperatively. The rabbits were sacrificed four weeks after the operation with 5 ml pentobarbital N_2 (200 mg/ml) (Euthesate®, Apharmo B.V., Arnhem, The Netherlands) intravenously.

7.2.5. Post mortem sample acquisition and evaluation procedures

Immediately after sacrifice, X-rays were made. The X-rays were blindly examined by two investigators and compared with X-rays made immediately after implantation for gross signs of infection of the tibiae, such as periosteal reaction and osteolysis around the implant.
Excision of the tibiae was performed under aseptic conditions. After shaving of the skin, the operative area was disinfected with 10% povidone-iodine and isolated with sterile drapes. Subsequently, the right tibia was excised. Using a high-speed dental drill with a circular metal saw (Minitech electronic, Zeist, The Netherlands), the proximal tibia was divided into an anterior half of bone and a posterior half containing the implant. Hereafter, the same procedure was performed for the left tibia. The bone fragments of the anterior half of each tibia were weighed and ground in a sterile metal mortar. Subsequently, the bone was homogenised in 10.0 ml phosphate buffered saline (PBS, pH 7.4) using a Polytron PT 3100 tissue grinder (Kinetica Benelux B.V., Best, The Netherlands), 3 minutes at 2500 rpm and 5 minutes at 6000 rpm. Serial 10-fold dilutions from these homogenates were then plated on blood agar plates. After an overnight incubation at 37°C the samples were

counted for viable bacteria. For each tibia, the number of viable bacteria per gram of bone was calculated (colony forming units per gram of bone, CFU/g).

The implants with the remaining surrounding bone of the posterior half of the tibiae were fixed in a 4% buffered formaldehyde solution, washed in PBS, and dehydrated by graded series of ethanol. These samples were embedded in a mixture of methylmethacrylate/dibuthylphthalate (Merck-Schuchardt, Hohenbrunn, Germany) in a ratio of 4 to 1. Bis(4-tertiairbutylcyclohexyl) peroxydicarbonate (Perkadox 16®, AKZO NOBEL, Arnhem, The

Table 7.1. Semiquantitative grading system used for histological scoring of infection. Eleven items were scored and a value was assigned to the severity of inflammation.

Medullary Canal:				
leukocytes	no	less	moderate	many
	0	0	2	4
microabscesses	no	less	moderate	many
	0	2	4	6
granulation tissue	no	light	moderate	much
	0	2	4	6
fibrosis	no	slight	moderate	much
	0	0	2	4
Cortex:				
destruction cortex	no	slight	moderate	much
	0	2	4	6
enlarged Haversian canals	no	slight	moderate	much
	0	0	2	4
leukocytes	no	less	moderate	many
	0	0	2	4
microabscesses	no	less	moderate	many
	0	2	4	6
granulation tissue	no	slight	moderate	much
	0	2	4	6
fibrosis	no	slight	moderate	much
	0	0	2	4
Periosteal Reaction:				
quantity	no	slight	moderate	much
	0	2	4	6

Netherlands) was suspended as a catalyst. After polymerisation, nondecalcified sections of approximately 10 μm thick were sawn perpendicular to the length axis of the implant, using a sawing microtome (van der Lubbe 1988; Klein 1994). The sections were stained with basic fuchsin and methylene blue.

For the histological evaluation, proximal and distal sections of each tibia with implant were examined for signs of infection using a light microscope. Semiquantitative scoring of infection was performed on blinded sections, together with a pathologist, according to the scoring system described in Table 7.1. This system was derived from Petty (Petty 1985) and adapted to the current situation of infection around a noncemented implant. Each sign of histological abnormality was scored according to severity in none, slight, moderate and many/much. A value was assigned to the items according to the severity of damage. The sum of values of the proximal and distal section of each tibia was calculated and averaged for each infected and non-infected implant subgroup.

Histomorphometry included semiquantitative scoring of the bone response next to the implants by measurements of bone-implant contact and bone area next to implants. The percentage of bone-implant contact and bone area next to the implants was also measured on blinded sections, using a light microscope (bone-implant contact) or a macroscope (bone area), coupled to an IBAS image analysis system (Carl Zeiss, Eching, Germany). Images of the sections were digitised in a resolution of 768 x 512 pixels. From the proximal and distal sections of each tibia, the percentage of bone apposition at the implant surface was measured (Figure 7.3). This was defined as the percentage of implant length with direct bone-implant contact (microscope with objective 1.0 x Optovar 1.6 resulting in a pixel size of 6.1 x 6.5 microns). From the proximal sections only (distal sections were not used for bone area measurements), the percentage of bone area within a radius of 1 mm next to the implant was measured (Figure 7.4). Bone area measurements were performed using a macroscope equipped with a Carl Zeiss Luminar 40 mm objective (Carl Zeiss, Germany) resulting in a pixel size of 10.1 x 10.6 microns.

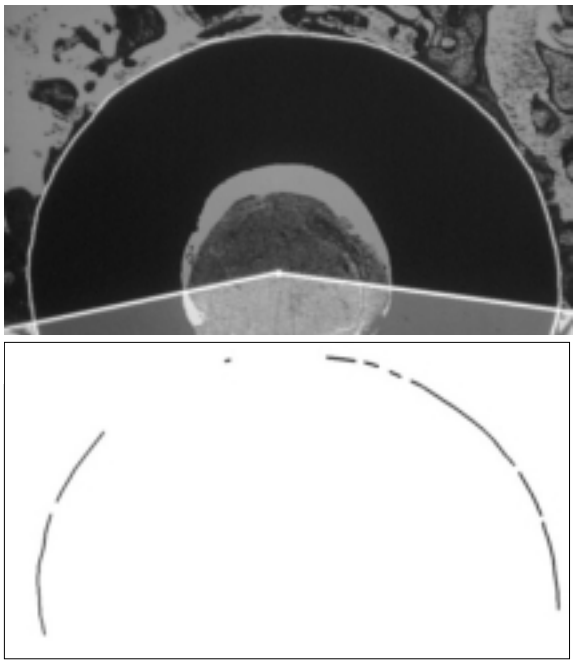

*Figure 7.3. Histomorphometrical measurement of bone apposition at the implant surface. On a digitised micrograph in a specific area (**a**) the percentage at which there was direct bone-implant contact was measured (**b**).*

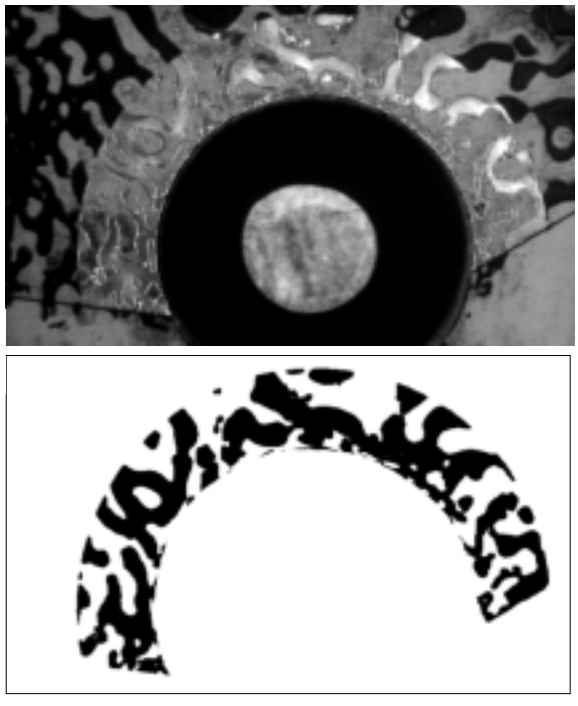

*Figure 7.4. Histomorphometrical measurement of bone area. On a digitised macrograph the percentage of bone area within a radius of 1 mm next to the implant (**a**) was measured (**b**).*

7.2.6. Statistical analysis

The study consisted of two treatment groups (HA and Ti), with 16 rabbits in each group, receiving 10^2, 10^3, 10^4, or 10^5 CFU. In general, statistics was performed using an analysis of variance with various dependent and independent variables ($p \leq 0.05$). We investigated the effect of implant type and dosage group upon the severity of infection (log(CFU) or histopathological score) and upon the bone-implant contact or bone area measurements, for the left and right tibiae separately. In addition, to obtain further insight into the relationship between actual infection and bone response, we performed an analysis of variance on the effect of the presence or absence of infection at sacrifice upon the bone contact and bone area data. Furthermore, we calculated the correlation between log(CFU) with the bone-implant contact and bone area measurements. Statistical calculations were performed using Statview for Macintosh and SPSS/PC.

7.3. Results

7.3.1. General

Two rabbits died due to a septicaemia after several days and were replaced. The first few days after the operation, all animals showed a decrease in appetite, which was more prominent in the animals that had received a higher dose of bacteria. After 28 days the mean loss of body weight was 12.2 per cent and 11.0 per cent for the HA and Ti rabbits respectively. The rabbits that received a higher inoculum dose lost more in body weight. After one week postoperatively, an increase in ESR followed the same pattern: ESR was more elevated in rabbits which received a HA implant. After 4 weeks there was no difference of ESR in relation to implant or inoculum (Table 7.2). With respect to the WBC, after one week only a slight difference was seen between the two different implants after receiving 10^5 CFU *Staphylococcus aureus*: rabbits with a HA implant had higher WBC than rabbits with a Ti implant. Rabbits receiving a lower inoculum had no difference of WBC. After four weeks, the difference of WBC in those two different implant

groups had decreased (Table 7.2).
Because of technical reasons, X-rays of 5 rabbits could not be made after 28 days. In the left tibia of 8 out of 27 rabbits radiological signs of infection, such as periosteal reaction, were seen: in 6 rabbits which received a HA implant and in 2 rabbits which received a Ti implant (see Figure 7.5). Six out of these 8 rabbits received a 10^4 or 10^5 CFU *Staphylococcus aureus*. No radiological signs of infection were seen in the right tibiae. All animals with radiological signs of infection had a positive culture after 28 days.

Table 7.2.
Erythrocyte sedimentation rate (ESR) and white blood cell counts (WBC) (mean ± standard error) before surgery, and at 1, 2, 3 and 4 weeks postoperative.

ESR (mm/hr)

	wk 0		wk 1		wk 2		wk 3		wk 4	
	HA	Ti	HA	Ti	HA	Ti	HA	Ti	HA	Ti
10^2	1.5 ± 0.5	1.3 ± 0.3	5.5 ± 2.9	1.5 ± 0.3	1.0 ± 0.0	1.3 ± 0.3	1.3 ± 0.3	1.0 ± 0.0	1.0 ± 0.0	1.8 ± 0.8
10^3	2.0 ± 0.6	2.0 ± 0.7	11.8 ± 9.4	2.0 ± 0.4	1.3 ± 0.3	4.3 ± 3.3	1.5 ± 0.3	1.8 ± 0.5	1.0 ± 0.0	1.0 ± 0.0
10^4	1.3 ± 0.3	1.0 ± 0.0	24.8 ± 8.8	13.3 ± 9.6	5.3 ± 2.8	4.3 ± 2.6	2.3 ± 0.9	1.5 ± 0.3	2.3 ± 0.6	1.5 ± 0.3
10^5	1.0 ± 0.0	1.3 ± 0.3	34.5 ± 5.7	29.0 ± 10.0	5.0 ± 3.3	4.5 ± 1.0	2.3 ± 1.3	2.0 ± 0.7	1.5 ± 0.3	2.0 ± 0.0
all	1.4 ± 0.2	1.4 ± 0.2	19.1 ± 4.3	11.4 ± 4.2	3.3 ± 1.2	3.6 ± 1.0	1.8 ± 0.4	1.6 ± 0.2	1.4 ± 0.2	1.6 ± 0.2

WBC ($x10^9$/l)

	wk 0		wk 1		wk 2		wk 3		wk 4	
	HA	Ti	HA	Ti	HA	Ti	HA	Ti	HA	Ti
10^2	4.3 ± 1.3	4.3 ± 0.6	9.2 ± 2.5	7.0 ± 1.3	5.1 ± 1.2	4.8 ± 0.4	3.9 ± 1.3	4.2 ± 0.7	4.4 ± 1.0	4.3 ± 0.9
10^3	5.0 ± 0.6	4.9 ± 0.6	6.8 ± 0.8	9.0 ± 3.0	5.8 ± 1.4	6.1 ± 1.7	4.7 ± 0.9	6.1 ± 0.8	4.2 ± 0.7	4.7 ± 1.3
10^4	4.3 ± 0.9	4.3 ± 1.1	8.5 ± 0.7	9.0 ± 0.7	7.6 ± 0.4	7.5 ± 1.6	6.3 ± 0.7	7.4 ± 2.1	5.3 ± 0.3	6.9 ± 0.8
10^5	3.5 ± 0.6	4.0 ± 0.6	12.8 ± 3.9	8.0 ± 1.3	10.4 ± 1.6	7.5 ± 0.6	8.0 ± 0.6	6.4 ± 0.5	6.8 ± 0.8	5.3 ± 0.3
all	4.3 ± 0.4	4.4 ± 0.4	9.3 ± 1.2	8.2 ± 0.8	7.4 ± 0.8	6.5 ± 0.6	5.7 ± 0.6	6.0 ± 0.6	5.2 ± 0.4	5.3 ± 0.5

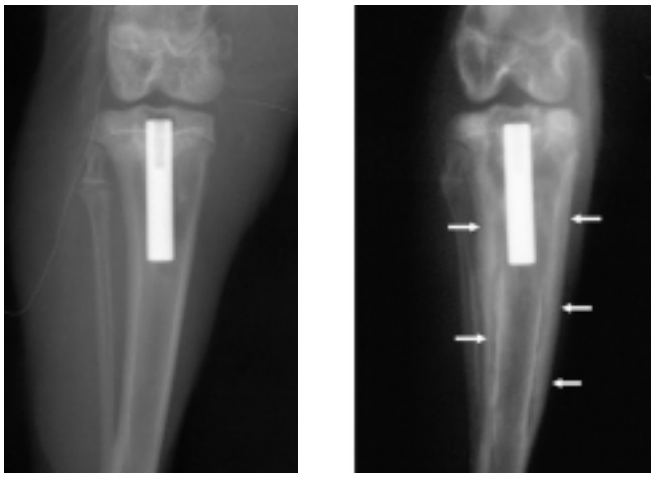

Figure 7.5.
X-ray of left tibia at day 0 (left) and 28 (right). After 28 days a periprosthetic reaction was visible (arrows).

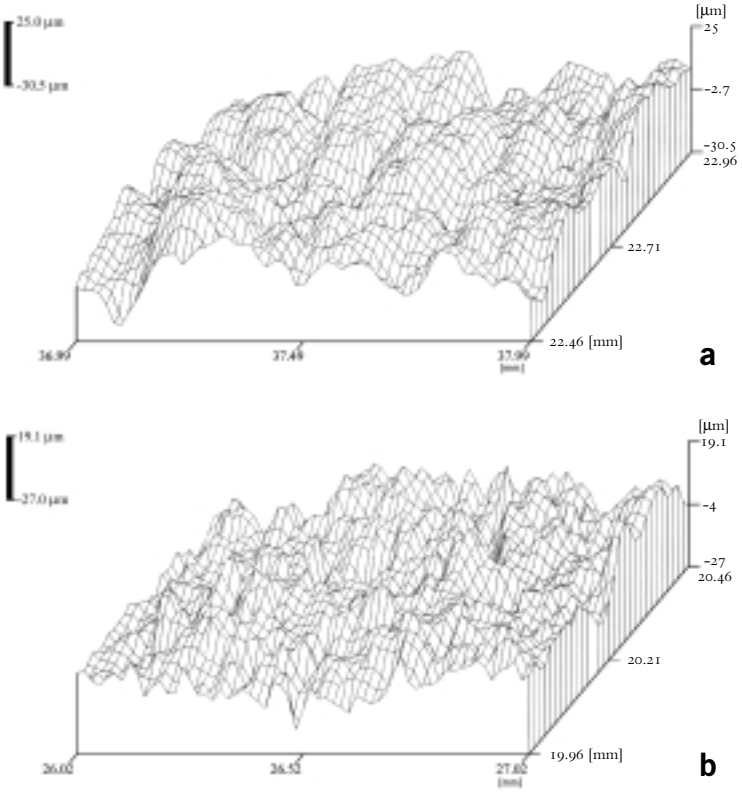

Figure 7.6.
*Laser profilometer surface scans of the HA coated (**a**) and non-coated (**b**) Ti6Al4V implants. The roughness of the HA implant was some what higher (Ra = 3.74) than the Ti6Al4V implant (Ra = 2.96).*

7.3.2. Implant surface characteristics

The surface roughness, as determined by the R_a values, was 2.96 for the Ti implant and 3.74 for the HA implant (Figure 7.6). Scanning electron micrographs of the Ti and HA implants show that there is an actual difference in surface morphology between the two implants (Figure 7.7): the HA implants show a more

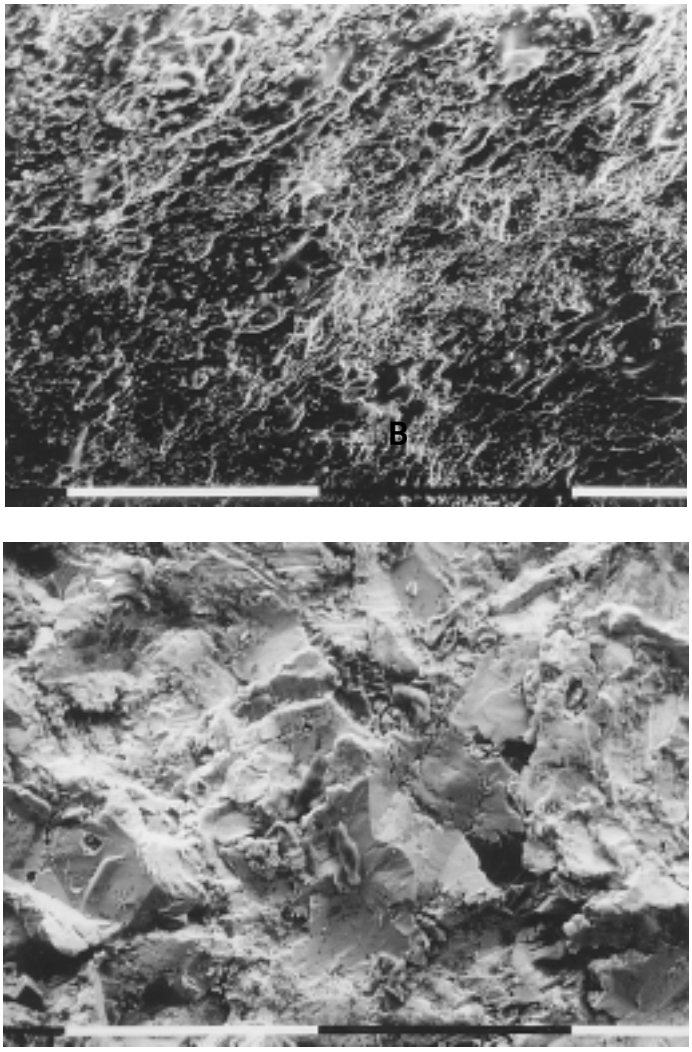

Figure 7.7. Scanning electron micrographs, showing the surface morphology of the HA coated (a) and non-coated (b) Ti6Al4V implants. Bar = 0.1mm.

"rounded/smooth"-like surface as a result of the vacuum plasma-spraying, while on the surface of the Ti implants clearly the sharp edges, as a result of the grit blasting procedure, are visible.

7.3.3. Bacteriology

The results from the bacteriological studies are summarised in Figure 7.8. In 9 out of 16 rabbits with HA implants, and 6 out of 16 rabbits with Ti implants, bacteriological cultures from samples of the left tibiae were positive. The number of colony forming units per gram (CFU/g) increased with higher inoculum doses, as there was a significant effect of inoculum dosage on bacterial counts ($p \leq 0.025$). From the left tibiae with a Ti implant contaminated with an inoculum of 10^2, 10^3, 10^4, and 10^5 CFU on average none, 1.3 ± 1.3, 2.1 ± 1.2 and 3.7 ± 1.3 log CFU/g (mean ± SEM) *Staphylococcus aureus* were retrieved respectively. In the left contaminated tibiae with a HA implant on average 1.6 ± 1.6, 2.0 ± 2.0, 5.6 ± 1.9 and 6.3 ± 0.7 log CFU/g (mean ± SEM) were retrieved respectively. The analysis of variance revealed significantly more bacteria in the HA group than in the Ti group ($p \leq 0.05$). In two rabbits, we also found positive cultures in the right, control tibia (one Ti and one HA implanted rabbit).

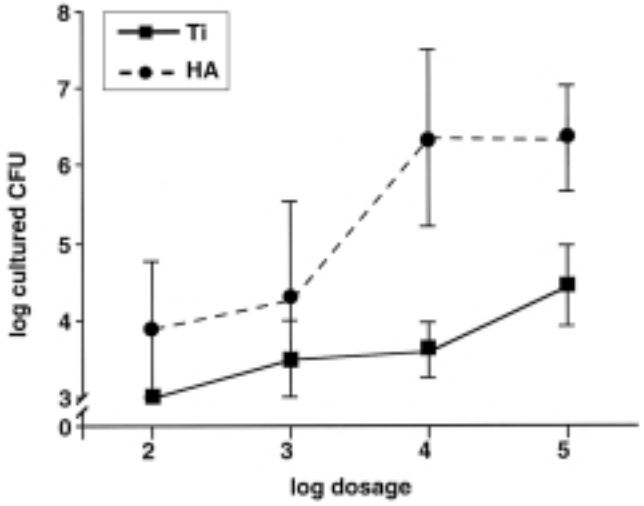

Figure 7.8.
Graphical representation of the results of bacteriological cultures for the various inoculi and implant types. Data are presented as mean (log CFU) ± standard error. At high inoculum dosages, more CFU had grown in the bone next to the HA implants than in the bone next to the Ti implants.

7.3.4. Histology

Histological evaluation of sections of implants not contaminated with bacteria (right tibiae) showed a normal cortex without periosteal reaction and small Haversian canals. Microabscesses, granulation tissue or fibrosis were not seen and only occasionally a few leukocytes were observed in the medullary canal. Especially in sections from left tibiae, which were contaminated with a high inoculum dosage, we saw severe loss of cortical bone. The remaining cortex showed enlarged Haversian canals and an extensive periosteal reaction. Around the implant in the medulla and the cortex many leukocytes were found, sometimes accompanied by the formation of microabscesses and granulation tissue. In some tibiae extensive fibrosis was observed (Figure 7.9).

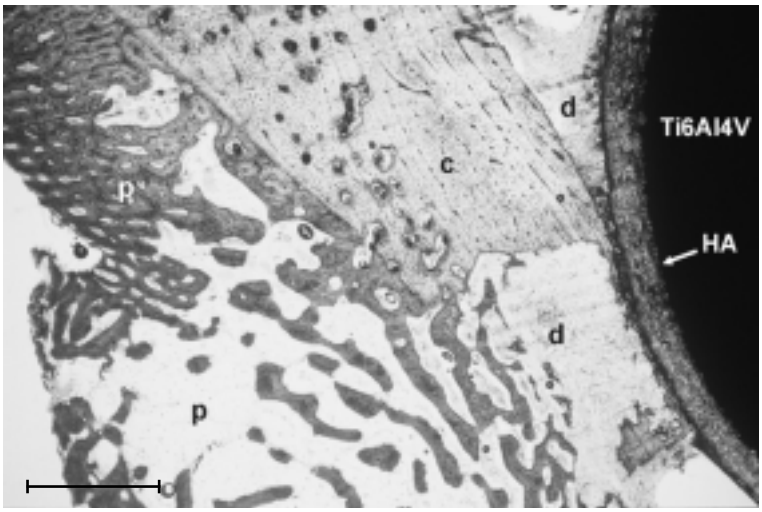

Figure 7.9. Photomicrograph typically representing a HA coated Ti6Al4V implant with a severe infection: remaining destructed cortical bone (c), debris and pus characterised by polymorphonucleair granolocytes, nuclear dust and bacteria (d), and periosteal reaction (p). Bar = 0.5 mm.

The results from the histopathological semiquantitative scoring are summarised in Figure 7.10. From the left HA tibiae, contaminated with 10^2, 10^3, 10^4, or 10^5 CFU, scores (mean ± SEM) were 4.5±1.0, 16.5±9.2, 33.5±8.5 and 49.0±8.7 respectively. In the left Ti tibiae, scores (mean ± SEM) were 4.5±1.0, 11.0±4.0, 21.5±7.3 and 24.0±3.6 respectively. For the right, not contaminated tibiae with a Ti or HA implant, scores were below 5.5. With increasing inoculum dosage, both the infected left tibiae of the HA and Ti

showed an increase in score, as compared to their contralateral control, and for the left, contaminated tibiae, there was a significant effect of inoculum dosage on the histopathological scores ($p \leq 0.0005$). In addition, with increasing inoculum dosage, there was an increasing difference between the HA and Ti implants, resulting in higher scores for the HA implants ($p \leq 0.05$). This indicates that with increasing inoculum concentration, HA implants developed a more severe infection as compared to the Ti implants.

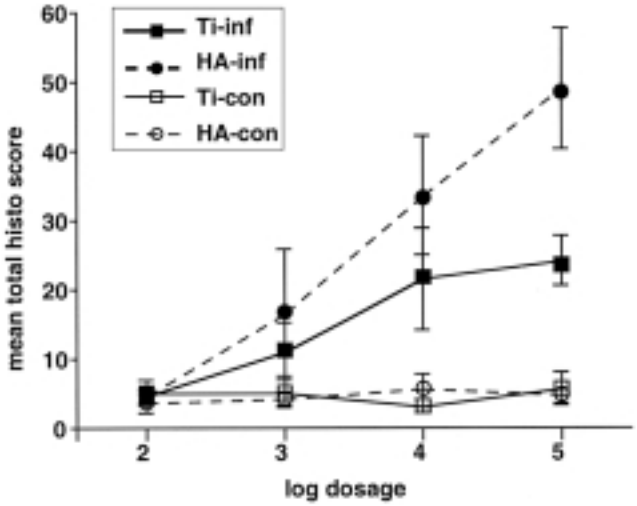

Figure 7.10. Graphical representation of results from semiquantitative scoring of the histology for the various inoculi and implant types. Data are presented as mean of the total histological score ± standard error. In the bone with HA implants, after a higher inoculum dosage a higher score was seen as compared to the Ti implants.

7.3.5. Histomorphometry

In Table 7.3 and 7.4, the results of the bone contact measurements for the proximal and distal sections are presented for the separate inoculum concentrations. The overall mean percentage of bone contact with the proximal part of the implants in the right tibiae was 67.0 for the HA-coated implants and 66.0 for the Ti implants. In the left, contaminated tibiae, these data were 67.6 per cent and 60.0 per cent respectively. In the distal sections, the overall mean percentage of bone contact in the right tibiae was 51.6 for the HA-coated implants and 38.8 for the non-coated titanium. In the left, contaminated tibiae these data were 33.4 per cent and 41.4 per cent respectively. In table 7.5, the results of the bone area measurements for the proximal sections are presented. The overall mean

Table 7.3.
Mean percentage of bone contact ± standard error in the proximal sections, as related to dosegroups for each implant type and each side.

	HA		Ti	
	contaminated	**control**	**contaminated**	**control**
10^2	75.3 ± 7.9	68.4 ± 5.7	64.5 ± 8.1	58.0 ± 8.3
10^3	65.6 ± 10.0	76.6 ± 6.4	70.9 ± 6.9	75.9 ± 10.9
10^4	50.5 ± 11.2	69.5 ± 11.5	56.4 ± 5.0	67.3 ± 18.9
10^5	82.7 ± 5.4	54.2 ± 7.6	48.2 ± 8.4	62.7 ± 0.8

Table 7.4.
Mean percentage of bone contact ± standard error in the distal sections, as related to dosegroups for each implant type and each side.

	HA		Ti	
	contaminated	**control**	**contaminated**	**control**
10^2	59.5 ± 13.0	65.3 ± 12.7	66.8 ± 14.4	55.8 ± 14.3
10^3	50.0 ± 14.7	68.2 ± 3.1	36.2 ± 9.3	21.2 ± 12.4
10^4	21.5 ± 10.8	45.7 ± 5.8	41.4 ± 17.4	36.4 ± 13.6
10^5	2.5 ± 0.0	25.9 ± 9.7	21.3 ± 9.3	41.6 ± 11.0

Table 7.5.
Mean percentage of bone area ± standard error in the proximal sections, as related to dosegroups for each implant type and each side.

	HA		Ti	
	contaminated	**control**	**contaminated**	**control**
10^2	44.1 ± 2.2	43.2 ± 9.1	36.2 ± 4.4	37.8 ± 8.2
10^3	48.7 ± 3.0	40.6 ± 8.4	41.4 ± 6.3	41.4 ± 5.3
10^4	35.1 ± 3.3	35.1 ± 2.3	31.6 ± 7.5	37.2 ± 10.7
10^5	42.4 ± 3.9	35.8 ± 7.4	32.4 ± 7.5	34.1 ± 8.4

percentage of bone area in the right, i.e. not contaminated tibiae was 38.9 for the HA-coated implants and 37.6 for the non-coated implants. For the left, contaminated tibiae, these percentages were 42.6 and 35.4, respectively. Statistical analysis revealed for the HA implants a significant effect of the inoculum concentration (dosage group) on the bone contact in the distal sections ($p \leq 0.05$) and on the bone area next to the proximal part of the implant ($p \leq 0.05$), suggesting a decrease as a result of increasing inoculum concentration. For both the left and right sides separately, there were no significant differences in bone contact or bone area between the two implant types. In order to obtain further insight into the relation-

ship between actual infection and bone response, we present in Figures 7.11 - 7.13 the bone contact and bone area as a function of the presence or absence of infection (i.e. positive or negative culture at sacrifice). In the proximal sections (Figure 7.11), the mean percentage of bone-implant contact was 63.2 for HA implants and 58.5 for Ti implants, when cultures were positive, and 69.1 and 64.2 respectively, when cultures were negative (no significant differences). For the distal sections (Figure 7.12), the mean percentage of bone-implant contact was 13.0 for HA implants and 20.6 for Ti implants in positive cultures, and 56.6 and 45.0 respectively in negative cultures. This difference in bone contact between positive and negative cultures was significant for the HA implants ($p \leq 0.0001$), but not for the Ti implants, although the effect of infection approached significance ($p = 0.0524$). With respect to the bone area (Figure 7.13), we measured in case of an infection mean percentages of 40.2 around the HA implants, and 35.0 around the Ti implants. When the cultures were negative, these data were 41.0 and 36.9 respectively (no significant differences). With respect to the correlation between log(CFU) and the bone contact and bone area measurements, we found a negative relationship for the bone contact in the distal sections (correlation coefficient -0.69; $p \leq 0.0001$). No significant correlations were found for the bone contact and the bone area data in the proximal sections.

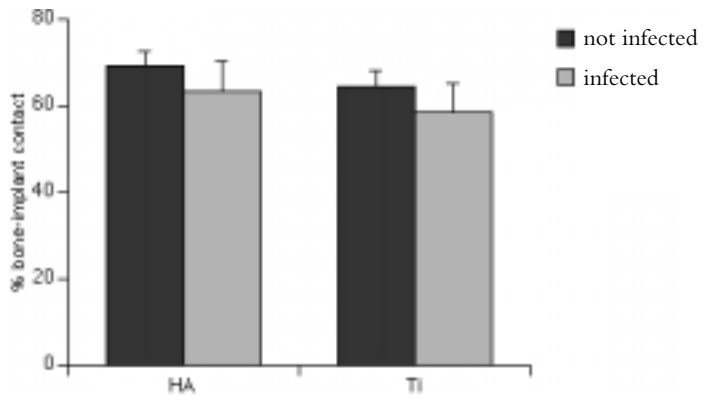

Figure 7.11.
Graphical representation of the bone-implant contact (mean ± standard error) for the infected and not infected HA and Ti implants in the proximal sections. No significant differences in bone implant contact were measured between the infected and not infected HA and Ti implants.

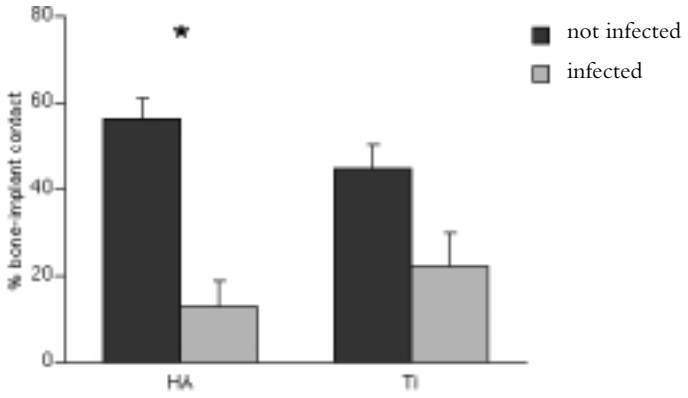

Figure 7.12. Graphical representation of the bone-implant contact (mean ± standard error) for the infected and not infected HA and Ti implants in the distal sections. A significant difference in bone implant contact was measured between the infected and not infected HA implants ($p \leq 0.0001$). The difference between the infected and not infected Ti implants was not significant.*

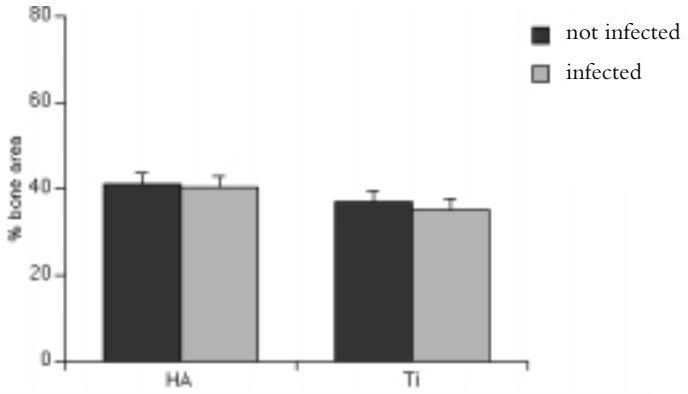

Figure 7.13. Graphical representation of the bone area (mean ± standard error) around the infected and not infected HA and Ti implants in the proximal sections. No significant differences of bone area were measured between the infected and not infected HA and Ti implants.

7.4. Discussion

In the present study, we demonstrated that infections of biocompatible, noncemented implants can develop after direct contamination of the local implant bed, and are related to the dose of the original inoculum. Surprisingly, we also found that bacteria were more likely to grow onto or next to the HA coated implants than on non-coated Ti implants, and resulted in a more severe

histopathological characterisation of peri-implant inflammation and infection. These results are especially of interest, since the outcome of clinical studies on HA coated implants are successful and do not report a higher infection rate (Geesink 1995; Tonino 1995). The histomorphometrical results on bone contact and bone area can be considered as a representation of implant fixation (Dhert 2000). Our results show, in particular for the HA implants, a significant effect of the inoculum concentration and/or the presence or absence of infection on the bone contact (distal implant side) or bone area. These data, in combination with the negative correlation between the number of colony forming units at sacrifice with the distal bone contact, confirm a relationship between implant loosening and infection. We speculate that this was probably not related directly to the implant type, but related to the severity of infection. Since the HA implants revealed more severe infections, as we demonstrated with the semiquantitative scores, this can explain the histomorphometrical results. Wilke et al. demonstrated for HA coated implants good osseous ingrowth behaviour in spite of local infection (Wilke 1993). In our study, in case of infection, the percentage of bone contact around the HA implants in the distal sections had decreased. This can also be explained by the fact that infections were more severe around the distal part of the HA implants (bacteriological cultures could not be differentiated between proximal and distal side).

Various other groups have studied implant-related infections. Cordero demonstrated that the presence of PMMA bone cement required less bacteria to produce an infection in rabbit femora compared to femora without an implant (Cordero 1996a). In another study, he found that PMMA usually appears to be more prone to cause an infection in relation to titanium and cobalt-chromium (Cordero 1996b). Hauke described in a rabbit tibial model for titanium, a lower susceptibility to *Staphylococcus aureus* infection as compared with stainless steel (Hauke 1997). Petty demonstrated in a dog model an early infection of stainless steel and cobalt-chromium alloys, high-density polyethylene, prepolymerised PMMA, and PMMA polymerised in vivo. The implants were inserted in the femoral canal after a suspension of bacteria had been injected.

Bacteriological and histological evaluation demonstrated that all implants were more likely to be associated with infection with *Staphylococcus aureus* than without an implant. He concluded that efforts expended in reduction of bacterial contamination when implants are used are justified (Petty 1985).

A point for discussion in all these studies is how to define implant infection and cope with negative cultures. In these as well as in our studies, bacteriological results were applied by counting the direct culture of bone homogenate, obtained by grinding. This method has a minimal detectable level of ± 1000 CFU/g. Therefore, a negative culture does not necessarily mean that no bacteria were present. This problem is dealt with in most studies by counting the number of infected implants (= positive culture) for each treatment. In the current study, we quantified the number of bacteria that were cultured from the tibiae, as a function of inoculum dose and implant type ("dose-response curve"). Therefore, for our statistical calculations, we assigned an absolute value of 0 CFU/g to each negative culture, which increased the variation of the data and reduced the possibility of demonstrating significant differences. Despite this, we still found significant differences between HA and Ti. To increase the readability the graph that demonstrates this dose-response curve (Figure 7.8), we however assigned the absolute value of 1000 CFU/g (= the minimal detectable level) to each negative culture. The reader should realise that in reality, this value will be somewhere in-between 0 and 1000 CFU/g. Since we also studied the peri-implant regions histologically, we could correspond negative cultures with histopathological signs of infection. In case of a negative culture, in the majority of cases the histopathological scores did not indicate a local inflammatory reaction.

In 1987, Gristina described the "race for the surface" theory (Gristina 1987a): *"The competition between host tissue cells and bacteria to adhere onto an implanted surface is "won" by the organisms that arrive first at the implant surface"*. So a fast tissue integration of a bioactive material (HA coating) could result in less bacteriological colonisation, as compared to a slower tissue integration of a biocompatible surface (Ti6Al4V). HA coatings are bioactive and well known for their favourable bone-bonding properties, as compared to non-

coated Ti6Al4V (Geesink 1987; Geesink 1988; Soballe 1990; Dhert 1991; Dhert 1993). Biocompatibility could be a determining factor in infection susceptibility or in the development of an infection after contamination of different materials. On the other hand, physical parameters such as surface morphology, porosity, charge, or total surface area could also influence bacterial colonisation.

Since our aim was to use the materials as they are applied in the clinical situation, the implant surfaces were not standardised with respect to surface area and porosity. It was previously mentioned that surface roughness may influence bacterial adhesion (McAllister 1993; Quirynen 1993). In addition, according to Naylor et al., the effect of implant surface roughness on infection is also related to differences in colonisation velocity (Naylor 1989a). The HA coating had a porosity of less then 10%, and as seen on Figure 7.7, there are actual differences in surface architecture between the two implant types. The presence of pores can increase the absolute surface area, despite comparable roughnesses. It can also be hypothesised that bacteria (in a liquid milieu) can more easily migrate into the pores than the tissue cells after implantation, and that once the bacteria adhered to the coating surface in the pores, they can be difficult to access by the host defence system (Cordero 1994). These factors should be taken into consideration when interpreting the differences that were found between the HA and Ti implants in the current study.

In the current study, we used concentrations of *Staphylococcus aureus,* ranging from very low (10^2) until relatively high (10^5). It should be realised that especially the higher concentrations are most likely not to occur perioperatively under uncomplicated, standard circumstances. However, the results from our study do indicate that HA coated implants can, in the presence of bacteria (e.g. perioperative contamination) more easily develop a more severe infection than non-coated Ti implants. This suggests that precautions to prevent contamination (sterility) or infection (perioperative antibiotics) are even more important for the highly biocompatible HA coated implant. This is in particular the case for patients with a higher susceptibility to infection.

The Development of an Animal Model to Study the Mechanisms of Haematogenous Infection of Cementless Implant Materials

CHAPTER 8

8.1. Introduction

An infection of a prosthesis in orthopaedic surgery is a very serious, sometimes life-threatening, complication. In the majority of the cases the prosthesis has to be removed, followed by intravenous antibiotic therapy. In some situations a new prosthesis can be inserted, usually at least 6 weeks after removal of the infected prosthesis. However, more than once it is decided to do a Girdlestone resection arthroplasty. Thirty years ago, the first total hip replacements showed infection rates of 7 to 11 per cent (Charnley 1969; Wilson 1972). Improvements in prosthesis design and surgical technique, the recognition of the high-risk patient and the peri-operative use of antibiotic prophylaxis are responsible for a decrease of infection rate as a result of peroperative contamination. Today this infection rate is about 1 to 2 per cent for a total hip arthroplasty and 4 per cent for a total knee arthroplasty (Walenkamp 1990; Sanderson 1991). Nevertheless, this is still a considerable group of patients when realised that in the Netherlands in 1992 more than 20,000 total hip and knee prostheses were implanted and that in the United States up by now almost 2 million patients have been treated with a total hip implant. It is expected that the number of revisions of the implants because of an infection will further increase in the future.

Prosthesis related infections can be split in two groups, based upon their pathogenesis. Firstly, the early postoperative infections, which can be caused peroperatively by direct contamination due to contaminated dust particles in the air, gloves or surgical instruments, or by a disturbed wound healing (wound haematoma and wound necrosis). Secondly, the late postoperative infections, of which the first symptoms appear more than one year after operation. The late postoperative infections are usually a result from haematogenous spread of bacteria from a distant focus. Various authors have estimated the prevalence of haematogenous implant infections to be approximately 0.3 to 1.0 per cent (Charnley 1969; Ahlberg 1978; Gristina 1983; Jaspers 1985; Schutzer 1988; Nelson 1990). However, it is difficult to link a preceding bacteremia to a prosthesis-related infection, which may become manifest weeks or months

later. It has been postulated that the most common sources for haematogenous total joint infection are skin and soft tissue (46%), dental (15%), and urinary tract (13%) infections (Maderazo 1988). Still, the mechanisms, prevention and therapy of late haematogenous infections continue to be a source of discussion (Blackburn 1991). It is therefore of great importance to get a better insight into the pathogenesis of such infections to improve the prophylactic and therapeutic options. In addition, the relationship between loosening of the prosthesis and (low-grade) infection is still unclear. It can even be speculated that interface phenomena such as fibrous encapsulation and micro-motion are not only related with loosening, but also with a lower resistance against infection.
Since no reliable and reproducible animal models are available to study late implant infections, the aim of the current study was to develop an experimental animal model that allows us to study haematogenous implant infections.

8.2. Materials and Methods

A total of 10 New Zealand White rabbits, weighting 3.0-3.5 kgs, were used in the present study. The rabbits were housed individually in cages and fed with 80-100 g Hope Farms rabbit diet LKK-20 (Hope Farms Standard Laboratory Diet LKK-20, Hope Farms B.V., Woerden, The Netherlands). Water was given ad libitum. After contamination of the implant, the animals were cared under the same conditions behind a barrier.
Five cylindrical non-coated, grit-blasted Ti6Al4V implants and 5 cylindrical hydroxyapatite coated Ti6Al4V implants (length 50 mm and final diameter 3.9 mm) were used in the present experiment. The thickness of the hydroxyapatite coating was approximately 50 microns. The coating was applied onto the surface by the plasma-spray technique (HC Implants B.V., Leiden, The Netherlands). The implants were sterilised in an autoclave prior to use.
In Figure 8.1, the various steps in the animal experiment are summarised. In a first surgical procedure, the implants were placed in the medullary canal of the proximal right tibia. The surgery was

performed under general anaesthesia. The rabbits were premedicated with 4 mg acepromazinemaleaat (Vetranquil®, Sanofi Sante B.V., Maassluis, The Netherlands), 4 g methadone and 0.5 mg atropine (Atropinesulfaat, Kombivet, Etten-Leur, The Netherlands). The anaesthesia was initiated by an intravenous injection of 8-12 mg etomadaat (Hypnomidate®, Janssen Pharmaceutica B.V., Tilburg, The Netherlands). After intubation, inhalation anaesthesia was followed by a mixture with O_2 and N_2O in a ratio of 1 to 1 and halothane 1%. Peroperatively, 200 mg amoxycilline (Clamoxyl®, SmithKline Beecham, Rijswijk, The Netherlands) was given as infection prophylaxis. The right leg was shaved and prepared for surgical procedure with 10% povidone-iodine (Betadine® solution, Dagra, Diemen, The Netherlands). After a

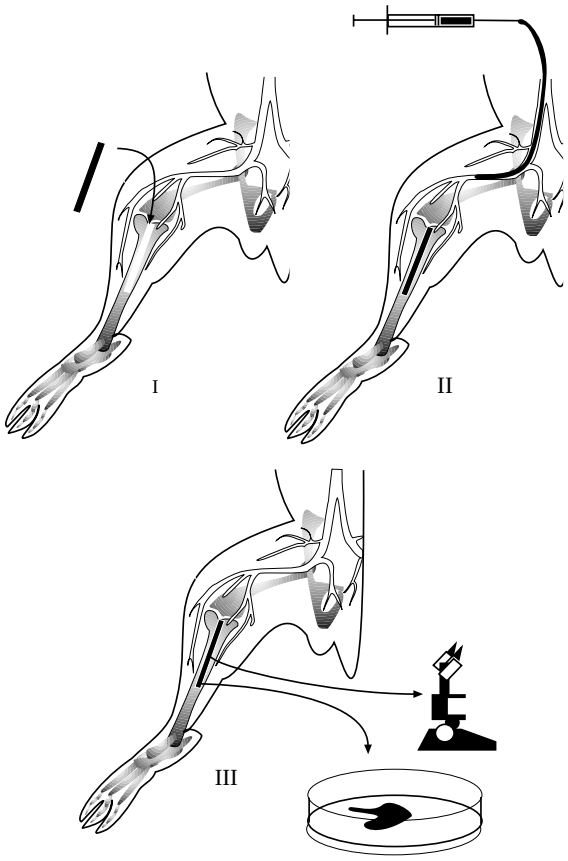

Figure 8.1.
Artist's impression of the various steps in the experimental animal model. In step I, the implant is placed in the medullary canal of the proximal right tibia of a NZW rabbit. In step II, four weeks after the first operation, Staphylococcus aureus bacteria are injected into the local bloodstream through a catheter which is brought in the right carotid artery and moved down until in the right femoral artery. Finally, in step III, the animal is sacrificed, and samples for bacteriological and histological examination are obtained.

parapatellar incision the knee joint was opened. Anterior to the insertion of the anterior cruciate ligament on the tibia, the medullary canal was opened with an awl, and reamed up by hand until a length of at least 50 mm and a diameter of 3.9 mm. Subsequently the implant was press-fit inserted in the tibial medullary canal. The joint capsule and skin were closed in layers with Vicryl (Ethicon, Norderstedt, Germany). Postoperatively, X-rays were made to check the implant position (Figure 8.2). Post-operative pain-relief was reached by a single dose of 0.1 mg buprenorfin (Temgesic®, Reckitt and Colman Products, Kingston-upon-Hull, United Kingdom) i.m. If necessary, a second dose was administered to the animal.

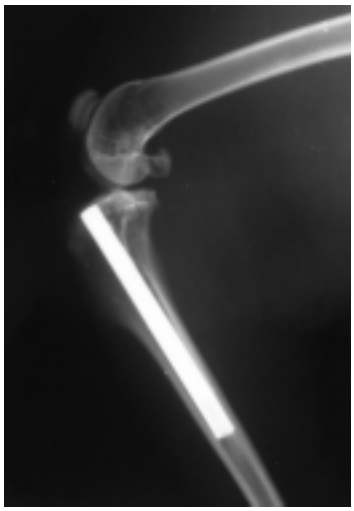

Figure 8.2.
Radiograph showing an implant in the right tibia of a NZW rabbit.

A second surgical procedure was performed at least 4 weeks after the first operation under comparable anaesthesiological circumstances, but now without antibiotic prophylaxis. The neck of the rabbit was shaved and prepared with 10% povidone-iodine. Then a longitudinal incision over the right carotid artery was made. The artery was identified and mobilised. Next a catheter was brought in and under fluoroscopy moved up until in the right femoral artery (Figures 8.3 and 8.4). This artery supplies the local implant bed. Through this catheter the animals subsequently received 1 ml of

saline containing a varying concentration of *Staphylococcus aureus,* strain Wood 46. Since the purpose of the study was to develop the animal model, the number of bacteria was varied between 10^8 and 10^9 to find the optimal challenge dose. This dose range is higher than used for the experiments described in Chapter 7, but in the present experiment, the bacteria are expected to flow through the

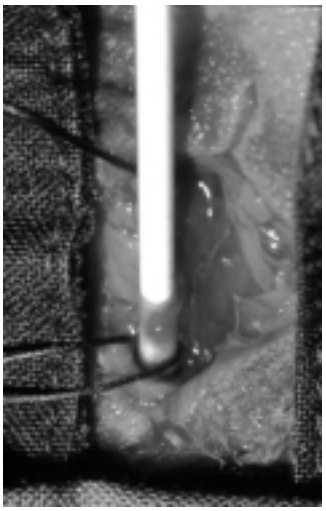

Figure 8.3. *Photograph of the right carotic artery, which is catheterised under general anaesthesia. The catheter is moved up until in the right femoral artery.*

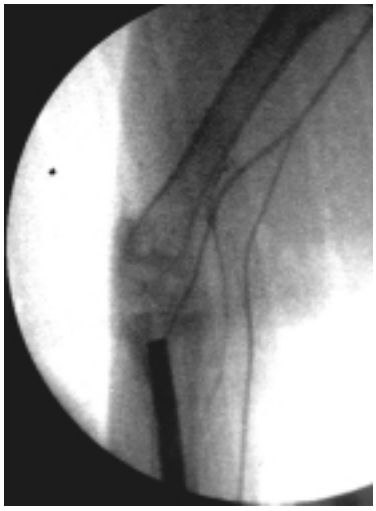

Figure 8.4. *Fluoroscopy of the right hind limb after injection of contrast fluid in the right femoral artery to show the blood supply of the implant bed.*

bloodstream in the implant bed, several weeks after implantation, instead that bacteria are introduced simultaneously with the implant (direct infection). After injection of the bacteria in the local bloodstream, the catheter was removed and the carotid artery was ligated with Vicryl. The skin was also closed with Vicryl. Subsequently, the animals were examined daily. Body temperature was measured and blood samples were taken for erythrocyte sedimentation rate (ESR) and white blood-cell count (WBC).
The rabbits were sacrificed at least one week after the second operation with 5 ml Pentobarbital N_2 (Euthesate®, Apharmo B.V., Arnhem, The Netherlands) (200 mg/ml). After sacrifice, sterile bone and marrow biopsies were taken next to the implant and cultured. Also cultures of liver, spleen, lung and kidney were obtained. The specimens were cultured by rolling them onto blood agar plates. In addition, specimens were suspended in Mueler-Hinton broth (Mueller-Hinton broth, Difco, Detroit, Michigan, USA). Plates and broth were incubated for 48 hours at 37°C and inspected for growth of *Staphylococcus aureus* and possible contamination after 24 and 48 hours. Identification of the bacteria occurred by standard bacteriological dilution and counting methods. The implants with the remaining surrounding bone were fixed in a formaldehyde solution, dehydrated and embedded in PMMA. Thin (10 micron) sections were cut using a sawing microtome and stained with basic fuchsine and methylene blue (van der Lubbe 1988). These sections were examined under a light microscope.

8.3. Results

Most animals passed the first operation without complications. Two rabbits had a disturbed wound healing as a result of biting at the stitches. These wounds healed secondary without additional complications. One rabbit had a spiral fracture of the tibia after inserting the implant. This fracture consolidated without a problem during the first postoperative weeks.
Our first goal was to find an optimal challenge dose, which resulted in a contaminated implant, without serious systemic reactions. The

first rabbit received 10^9 colony-forming units (CFU) *Staphylococcus aureus,* and died after one day due to a sepsis. A second rabbit received 10^8 CFU and did not develop any clinical signs of infection. At sacrifice, after one week, all cultures were negative. The third rabbit was challenged with a dose of 3.3×10^8 CFU. In this animal we saw no clinical signs of illness, but only a slight increase of the WBC for a few days. It was decided not to sacrifice this animal after one week, but to wait and follow the clinical course. When this animal was sacrificed after 12 weeks, there was a positive culture of the bone samples with *Staphylococcus aureus*. All other rabbits (n=7) received a dose of 5×10^8 CFU *Staphylococcus aureus*. In

Table 8.1.

Summary of results showing dosages, results from blood tests and bacteriological analyses and complications. ⋆ *= preliminary death of animal; WBC = white blood cell counts; ESR = erythrocyte sedimentation rate; - = no change;* ± *slight increase during first postoperative days; n.a. = not applicable.*

Animal	Dose	Implant	Follow-up	Blood tests	Bone culture	Organ culture	Remarks
1	10^9	HA	1 day*	WBC: n.a ESR: n.a.	*S. aureus*	*S. aureus*	*S. aureus* sepsis
2	10^8	HA	7 days	WBC: - ESR: -	none	none	no infection
3	3.3×10^8	HA	12 days	WBC: ± ESR: -	*S. aureus*	none	
4	5×10^8	HA	7 days	WBC: ± ESR: ±	*S. aureus*	lung: *S. aureus*	
5	5×10^8	HA	7 days	WBC: ± ESR: ±	*S. aureus*	none	
6	5×10^8	Ti6Al4V	7 days	WBC: ± ESR: ±	*S. aureus*	none	
7	5×10^8	Ti6Al4V	7 days	WBC: - ESR: ±	*S. aureus*	none	
8	5×10^8	Ti6Al4V	2 days*	WBC: n.a. ESR: n.a.	n.a.	*Proteus*	*Proteus* sepsis
9	5×10^8	Ti6Al4V	2 days*	WBC: n.a. ESR: n.a.	*S. aureus*	*Proteus*	*Proteus* sepsis
10	5×10^8	Ti6Al4V	28 days	WBC: - ESR: -	*S. aureus*	none	

these animals, signs of illness were present in the first few days after surgery, as represented by refusal to eat, lethargy and an increase of body temperature. Laboratory tests revealed a slight increase of ESR and WBC during the first postoperative week. Two rabbits died preliminary, after two and three days respectively, due to a *Proteus* infection. Four of the remaining five animals were sacrificed after one week, and one rabbit was sacrificed after four weeks. At sacrifice, all animals showed that cultures of bone and/or marrow along the interface were positive for *Staphylococcus aureus*. In one rabbit there was a positive culture of lung tissue as well, but all other organ tissue cultures were negative for *Staphylococcus aureus*. In Table 8.1, these results are summarised.

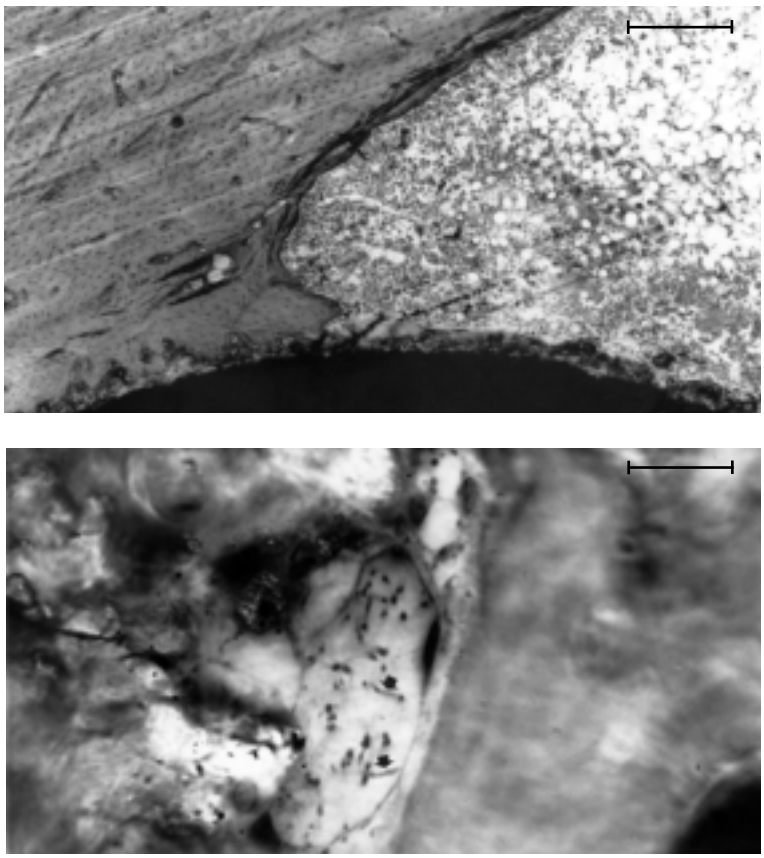

Figure 8.5.
*Histological appearance of a hydroxyapatite coated implant. (**a**) Survey micrograph showing bone and marrow in contact with the implant surface. Bar = 0.25 mm*
*(**b**) Detail of bone-hydroxyapatite coating interface showing strings of cocci between coating and bone (arrows). Bar = 0.01 mm*

The histological analysis revealed that all implants were properly incorporated in the surrounding bone and that especially the hydroxyapatite coated implants showed an intimate bone-implant contact (Figure 8.5a). There were no signs of bone resorption, loosening or fibrous encapsulation. Incidentally a mild, focal inflammation was present along the implant surface, as represented by polymorphonucleair granulocytes and macrophages. Also Gram positive cocci were detected occasionally in remodelling lacunae and blood vessels near the implant surface (Figure 8.5b).

8.4. Discussion

To study implant related infections in orthopaedic surgery, various experimental models have been applied. The relationship of infection of metal implants with *Staphylococcus aureus* has been demonstrated by Barth (Barth 1989). Regarding the implantation in bone, only a few studies relate to implant infection. Petty demonstrated in a dog model the early infection after peroperative contamination of stainless steel and cobalt-chromium with *Staphylococcus aureus* (Petty 1985). In another report on direct implant infection and bony integration, Wilke showed that even under persistent infection with *Staphylococcus aureus,* hydroxyapatite coated implants were able to establish an intimate bone-coating contact (Wilke 1993). In Chapter 7, we described the susceptibility of HA coated and non-coated Ti6Al4V implants to infection. However these studies are based upon peroperative introduction of bacteria to the implant bed and do not address the clinical problem of late haematogenous implant infections. Several attempts have been made to investigate haematogenous implant infections in experimental models through intravenous inoculation of cemented knee (Blomgren 1981) or hip (Southwood 1985) prostheses. However, the systemic challenge with bacteria resulted in a mortality up to 50 per cent due to sepsis. In addition, these studies focused only at the infection rate of cemented implants. Our study was a step-by-step pilot experiment to develop an animal model to study late haematogenous infections of cementless implants, without serious

systemic complications. We chose the New Zealand White rabbit for our model. In earlier studies this animal proved to be suitable to study arthritis and osteomyelitis (Schurman 1978; Marks 1980; Blomgren 1981), and as a model for direct infection of a tibial implant (this thesis). In contrast with previous studies by other investigators, we injected the bacteria through a catheter that was brought in the femoral artery. An advantage of this method is that the bacteria are injected into the local bloodstream, so the fraction of bacteria not passing the implant bed (e.g. the right femoral artery) is minimal. Thus, the dose will be more precise and locally a relative high dose can be given. In our study, two animals died because of a *Proteus* infection. Both animals were operated and injected with *Staphylococcus aureus* on the same day. Since *Proteus* is a commensal micro-organism in the rabbit, and any deterioration in physical condition can cause a *Proteus* infection, we speculate that this complication is a result of the surgical procedure or is related with the infection with *Staphylococcus aureus*.

Histological examination showed occasionally bacteria present near the implant surface without signs of bone resorption and loosening of the implant. According to Wilke (Wilke 1993), the incorporation of the implants in bone is also related with the infection sensitivity of an implant. In the present study the relative short follow-up of one week for most animals was sufficient for the evaluation of the model, but to study the local processes of possible resorption of bone and loosening of the implant after infection, longer follow-up periods will be necessary.

The present study demonstrated that injection of *Staphylococcus aureus* into the local bloodstream of an intramedullary implant in the rabbit tibia can result in a contamination of the implant bed, as demonstrated by positive cultures for bone and/or marrow near the implant. In our study, a dose of 5×10^8 CFU was successful in reaching a local implant bed contamination, but it should be realised that this dose was specific for the current study. Differences in strain, breeder, age of rabbit and even individual differences in resistance against staphylococci, for instance as a result of previous skin (feet) infections that can occur in rabbits that are housed for a longer period in metal cages of an animal lab, can exist. In subsequent

studies, standardisation with respect to age and breeder is necessary, and it is recommended again to vary the number of bacteria in a specific dose range. In the current study, the optimal dose was $5x10^8$ CFU, and by injecting these bacteria in the local bloodstream, the incidence of systemic infection was low. It is concluded that in rabbits, with catheterisation of a femoral artery and subsequent injection of bacteria, it is possible to create a haematogenous infection of a tibial implant, located in the distal bloodstream of the catheterised femoral artery. It is recommended to focus further studies on haematogenous infections with this model at possible effects of implant surface, osseointegration, follow-up, or at various prevention measures such as antibiotic prophylaxis. This falls however beyond the scope of the current thesis.

Summary and Discussion

Infection in orthopaedic surgery is a serious and growing problem with a considerable physical and social impact on the patient. Especially the introduction of implants in orthopaedic surgery has resulted in a new kind of infection, prosthetic joint-related infection. Despite advances in diagnosis, more effective antibiotics and refined surgical techniques infections remain a formidable challenge. Our understanding of the pathogenesis of infection is incomplete and both management and prevention far from optimal.

The author's interest in orthopaedic infections was initiated by the diagnostic difficulties, variability in outcome and above all, the heterogeneity of the problem of orthopaedic infections. It stimulated to a study of the literature (Chapter 2), and several clinical studies (Chapters 3 to 5). The outcome of these investigations, summarised in Chapter 6, led to several questions for experimental studies. These studies are presented in Chapters 7 and 8.

Based upon the currently available literature, in *Chapter 2* the present knowledge of prosthesis-related infections is summarised. A review is given from a clinical, practical as well as from pathogenetical viewpoint. The epidemiology and classification of these infections in orthopaedics is described. Despite preventive measurements, because of an ageing population and increase in the frequency of total joint replacement operations, infection of an orthopaedic implant still is a source of considerable morbidity, and the problem is expected to increase in the years ahead. It can be difficult to make the correct diagnosis of an infected arthroplasty. Only the combination of clinical, laboratory and imaging investigations enables the clinician to reach the diagnosis with an acceptable degree of certainty. In many cases, an infection can be diagnosed or excluded on the basis of a carefully obtained clinical history and the determination of the sedimentation rate and the C-reactive protein level. Other preoperative investigations, such as radiography, arthrography, radionuclide imaging, aspiration of the joint and peroperative investigations, such as frozen sections, Gram stains and cultures, may provide additional proof. Peroperative cultures are most accurate. However, their usefulness is offset by false-positive

and false-negative results. For treatment and prognosis, it is important to know when and how an infection actually started to develop, and in which patient with his or her specific profile. A classification system of infection of orthopaedic implants was proposed by Coventry and later amended by Fitzgerald et al. To facilitate the management of the patients with an infected hip arthroplasty, Estrada described a new classification (Table 2.1). This classification system is based on the moment of diagnosis of infection, and on how the implant was infected. Because also other patient-related factors play an important role in treatment of prosthesis-related infections, this classification has its limits and can therefore only be used as a rough guideline for treatment. A direct infection that appears within 4 weeks after implantation of a primary arthroplasty can be treated by debridement in combination with antibiotics only. Also an acute haematogenous infection can be treated in this way. Longer existing infections are treated by antibiotics only, an exchange arthroplasty (one- or two-stage) or a resection (Girdlestone) arthroplasty. Less frequently used treatments are arthrodesis or disarticulation. The choice of treatment is dependent on the health of the patient, the available bone stock, the quality of the soft tissues, the virulence of the infecting micro-organism and the mental state of the patient. In addition, the underlying pathogenesis of implant-related infections is described in this chapter. The adhesion of bacteria to the surface of human tissue or an implanted biomaterial surface is the step that precedes any orthopaedic infection. At the moment of implantation, a biomaterial surface presents the available free energy sites, which await physical or chemical bonding, first from ambient protein molecules and subsequently, or concomitantly, from available cells. The so-called "race for the surface" theory, as postulated by Gristina, suggests competition between tissue and bacterial cells for implant material surfaces. Successful colonisation by one cell type favours dominance of this cell and exclusion of later arrivals. According to this theory, if tissue cells arrive first at a biomaterial surface and a secure bond is established, bacteria will be confronted by living, integrated cells and unable to colonise. If not traumatised or altered, an integrated tissue is resistant to bacterial colonisation by virtue of its viability,

intact cell membranes, extramembranous polysaccharides and host defence mechanisms. If however, bacteria arrive first at the surface and colonise the biomaterial, an infection will develop. Such biomaterial infections almost always prevent successful integration of the implant. After adherence of some strains of bacteria to an implant surface, a layer of slime is produced, which makes bacteria less accessible to the host defence system and decreases antibiotic susceptibility significantly. These bacteria can then remain "quiet" on the surface of a material for a long period of time. However, when environmental influences change, such as a decreased host immune function or poor tissue ingrowth around a prosthesis, these bacteria can induce a manifest clinical infection. Biomaterials are susceptible to infection because for the most part they are not well integrated. Well-integrated devices are infrequently infected unless an unfavourable situation is created by perturbed host defences, trauma or exposure to massive infecting inoculi.

In *Chapter 3*, the haematogenous route as an important pathway for orthopaedic infections is described through a retrospective analysis of 28 children with an osteomyelitis. The patients were evaluated by chart review for history, clinical presentation, diagnostic work-up, treatment and outcome. The patients had an acute or a chronic osteomyelitis with an average follow-up of 3.7 and 2.7 years, respectively. All children were treated surgically: a cortical window was made, pus was drained and necrotic tissue was excised. Positive cultures were obtained in 9 out 12 and 7 out of 16 children with respectively acute or chronic osteomyelitis. Subsequently, all patients were treated with intravenous and oral antibiotics. After treatment, all but one patients were clinically well and the infection parameters had returned to normal values. Three patients had residual signs (ankylosis, decreased function). In addition to surgical intervention, six weeks of antibiotic therapy appears to be sufficient to eradicate acute as well as chronic osteomyelitis in children.

In *Chapter 4*, the results of a retrospective analysis of 47 patients with a prosthetic hip infection are described. The specific goals of the analysis were how diagnosis was reached, the results of

the cultures, the management of treatment, and the infectious and functional outcome of treatment. Diagnosis was based upon clinical presentation, laboratory investigations and radiological and eventually nuclear imaging studies. Two patients died because of the infection. Most prostheses were infected by direct contamination. In addition, in 5 prostheses (11%) a haematogenous source of infection could be ascertained. On the basis of treatment, the patients were divided into three groups. Group I comprised 9 patients who were managed with debridement and retention of the original components. Group II comprised 22 patients who were managed with resection arthroplasty and group III comprised 16 patients who had removal of the prosthesis and debridement followed by immediate (n = 4) or staged reimplantation (n = 12). The infection was successfully eradicated initially in 35 patients (group I 44%, Group II 73%, Group III 94%). At follow-up, 31 out of 35 patients still being alive answered a questionnaire about pain and functional outcome. Patients with a prosthesis *in situ* had less pain and a better ability to walk. Despite the small population, relative short follow-up and variability of treatment in this population, it is concluded that the eradication of an infected arthroplasty of the hip by only surgical debridement and antibiotics is suboptimal therapy. Removal of the prosthesis is superior for eradication of the infection. In case the prosthesis cannot be replaced, the functional outcome of the so-called Girdlestone situation is poor. For better functional results, an exchange of the prosthesis, when medically and psychologically possible, is advised.

In *Chapter 5,* a treacherous complication of an abdominal infectious process is described, emphasising the significance of the haematogenous route for prosthesis infections. In this chapter, a clinical lesson is presented describing the development and course of a haematogenous hip prosthesis infection after an abdominal infection. A 66-year-old woman, with a total hip prosthesis *in situ*, developed an acute cholecystitis. She was treated by a cholecystectomy and *Clostridium perfringens* was isolated. After several months she developed complaints suggesting an infection of the prosthesis. At aspiration of the hip joint, the same *Clostridium perfringens* was

isolated. She was successfully treated by a two-stage revision procedure of the prosthesis and antibiotic therapy. This case demonstrates that patients with an orthopaedic endoprosthesis *in situ* are at risk to develop a late infection of this implant with the causative agent of a gallbladder infection. If patients with a total joint prosthesis develop bacterial infections at distant sites, they should be treated immediately and aggressively with appropriate antibiotics to prevent haematogenous spread to the prosthetic joint

The review of the literature and clinical investigations as described in chapters 2 to 5 led to several questions, which were the basis for the experimental studies. In *Chapter 6*, the aims for these studies were defined. The basis for further studies in general was the lack in knowledge of the pathogenesis of implant-related infections. One of the questions concerned the relationship between infection susceptibility and biocompatibility of common implant surfaces. The "race for the surface" theory implies that when cells arrive first at a material surface after implantation, the implant will not be accessible for bacterial adhesion any more, while when bacteria arrive first at the surface, tissue integration cannot take place. This was further discussed in Chapter 7. Another problem relates to haematogenous infections in orthopaedics, which form a substantial part of all infections. To gain a better basic and clinical knowledge of the pathogenesis of haematogenous orthopaedic implant infections, appropriate animal models are necessary, but not yet available. Therefore, an experimental animal model to study haematogenous implant infections was developed as a starting point for further studies. The development of this model was described in Chapter 8, as final chapter on experimental studies in this thesis.

In *Chapter 7,* the question of infection susceptibility in relation with implant surface characteristics and biocompatibility is addressed. The main question here was whether there is a difference in infection susceptibility of two common orthopaedic implant surfaces with a different biocompatibility. Will a more biocompatible surface indeed be in favour of the tissue cells, as postu-

lated by Gristina's "race for the surface" theory? This was addressed by studying two clinically used implant surfaces: grit-blasted Ti6Al4V and hydroxyapatite coated Ti6Al4V. Thirty-two New Zealand White (NZW) rabbits were operated on. A hydroxyapatite coated (HA) or non-coated (Ti) Ti6Al4V implant was inserted into both tibiae. Prior to implantation the left tibia was contaminated with increasing concentrations of *Staphylococcus aureus* (10^2-10^5 CFU). Four weeks after implantation, half of the tibial bone adjacent to the implant was harvested for bacteriological examination and quantification. For the histological evaluation, sections of the implant with the remaining tibia were examined by semiquantitative scoring. Histomorphometrical data, which are a representation of implant fixation, were obtained by measuring the part of the circumference of the implant that was in direct contact with bone and by measuring the quantity of bone around the implants (within a radius of 1 mm from the outer diameter of the implants). Bacteriological data showed a significant effect of inoculum dose and implant type on the culture outcome: more bacteria were retrieved from the HA coated implants as compared to the non-coated Ti implants. Histology showed more severe infections for the HA implants as compared to the Ti implants. Histomorphometry revealed in particular for the HA implants, a relationship between the inoculum concentration and/or the presence or absence of infection with the bone contact or bone area. This confirms a relationship between implant loosening and infection. We demonstrated that infections of biocompatible, noncemented implants can occur and are related to the dose of the original inoculum. In addition, the more biocompatible HA implant showed in this study more severe infections as compared to the Ti implants, which at least suggests that the "race for the surface" theory should be reconsidered for the more complex *in vivo* situation.

The development of an experimental animal model to study haematogenous infections of cementless implants is described in *Chapter 8*. Ten New Zealand White rabbits were subjected to a two-stage surgical procedure. A cylindrical Ti6Al4V or hydroxyapatite coated Ti6Al4V implant was inserted into the right

tibia. After a period of at least 4 weeks, the implants were selectively contaminated with varying doses of *Staphylococcus aureus,* via the right femoral artery. During the postoperative period, the animals were examined daily and blood samples were taken. After at least 1 week, the animals were sacrificed and biopsies of bone, marrow, liver, spleen, lung and kidney were cultured. The remaining implant with surrounding bone was prepared for histological examination. Injection of $5x10^8$ colony forming units caused positive cultures in all cases and minimal systemic reactions. The histological sections showed minimal numbers of bacteria and signs of only a mild, focal inflammation were seen. It was concluded that this animal model was useful for further studies of haematogenous implant infections on, for instance the effect of implant surface, osseointegration, follow-up, and various prevention measures such as antibiotic prophylaxis.

The investigations presented in this thesis do not provide all the answers to the many questions related to pathogenesis of orthopaedic infections, their course, and management. Further research is necessary, especially since the problem is very complex, but also since the problem is growing as a result of the rapid increase in the use of biomaterials in orthopaedic surgery. Clinical studies are necessary, but have their limitations. Many factors, such as microbiological, patient and even emotional factors, play a role in these infections. The number of patients in clinical studies is usually too small to permit sound conclusions. Therefore experimental studies remain important for answering a number of specific questions in the field of orthopaedic infections. More knowledge is required about the pathogenesis of these infections. Also more reliable and faster diagnostic tools (polymerase chain reaction, imaging) is urgently needed and should be developed. And lastly, improvements and standardisation of treatment modalities should be the goals for the immediate future. The animal models described in this thesis, and the results of the specific experiments we performed using these models will hopefully be a small but significant step towards a better understanding of these infections, and provide a basis for new experimental studies.

Samenvatting en Discussie

Infecties zijn binnen de orthopaedie een ernstig en groeiend probleem met een belangrijke fysieke en sociale impact op de patiënt. Met name de introductie van implantaten heeft geresulteerd in een nieuw soort infectie, de gewrichtsprothese gerelateerde infectie. Ondanks de vooruitgang in diagnostiek, de beschikbaarheid van meer effectieve antibiotica en verbeterde chirurgische technieken, blijven infecties een enorme uitdaging. Onze kennis van de pathogenese van infecties schiet tekort; zowel therapie als preventie zijn bij lange na optimaal.

De belangstelling van de auteur voor de infecties binnen de orthopaedie werd gewekt door de diagnostische problemen, de wisselende resultaten van behandeling en met name de heterogeniteit van het probleem van de orthopaedische infecties. Het leidde tot een bestudering van de literatuur (Hoofdstuk 2) en een aantal klinische studies (Hoofdstuk 3 t/m 5). De resultaten van deze studies, samengevat in Hoofdstuk 6, leidden tot een aantal vraagstellingen voor experimentele studies. Deze studies worden gepresenteer in Hoofdstuk 7 en 8.

Gebaseerd op de thans beschikbare literatuur wordt in *Hoofdstuk 2* een samenvatting gegeven van de huidige kennis over de prothese gerelateerde infecties. Een overzicht wordt gegeven vanuit zowel een klinisch, praktisch en pathogenetisch gezichtspunt. Ook de epidemiologie en classificatie van deze infecties worden beschreven. Ondanks preventieve maatregelen neemt, vanwege een ouder wordende populatie en een daarmee samengaande stijging van het aantal totale heupprothese operaties, de morbiditeit van infectie van orthopaedisch implantaten toe. Dit zal naar verwachting de komende jaren nog verder toenemen. Het kan moeilijk zijn om tot de juiste diagnose van een geïnfecteerde gewrichtsprothese te komen. Alleen de combinatie van klinisch, laboratorium en beeldvormend onderzoek stelt de clinicus in staat om tot de juiste diagnose te komen binnen acceptabele grenzen van zekerheid. In veel gevallen kan een infectie al worden gediagnostiseerd of uitgesloten op basis van een zorgvuldig klinisch onderzoek en bepaling van BSE en/of CRP. Radiologisch onderzoek, nucle-

air geneeskundig onderzoek, aspiratie van vocht rondom het gewricht, evenals peroperatief verrichte onderzoeken, zoals beoordeling van vriescoupes, Grampreparaten en kweken, kunnen aanvullende zekerheid geven. Peroperatief afgenomen kweken zijn het meest nauwkeurig, hoewel ze soms ook fout-positieve of fout-negatieve resultaten kunnen geven. Voor de behandeling en prognose is het van belang te weten wanneer en hoe een infectie zich openbaarde, en bij welke patiënt met zijn of haar specifieke kenmerken. Een classificatiesysteem voor infecties van orthopaedische implantaten werd opgesteld door Coventry en later verbeterd door Fitzgerald. Om de behandeling van de patiënten met een geïnfecteerde heupprothese te vergemakkelijken, ontwikkelde Estrada een nieuwe classificatie (Tabel 2.1). Dit classificatiesysteem is gebaseerd op het tijdstip dat een infectie wordt gediagnostiseerd en hoe het implantaat is geïnfecteerd. Omdat ook andere patiënt-gerelateerde factoren een rol spelen bij de behandeling van infecties van prothesen, heeft deze classificatie zijn beperkingen en moet daarom alleen worden gezien als een grove richtlijn voor de behandeling. Een directe infectie die zich binnen 4 weken na het inbrengen van een primaire heupprothese openbaart, dient te worden behandeld met nettoyage en antibiotica. Ook een acute hematogene infectie dient op deze wijze te worden behandeld. Langer bestaande infecties kunnen worden behandeld met alleen antibiotica, een wisseling van de prothese (in een of twee tempi) of een resectie (Girdlestone) arthroplastiek. Minder vaak gebruikte behandelingen zijn een arthrodese of een disarticulatie. De keuze van behandeling is afhankelijk van de gezondheid van de patiënt, de nog aanwezige "bone stock", de kwaliteit van de weke delen, de virulentie van het infecterende micro-organisme en de mentale toestand van de patiënt. In dit hoofdstuk wordt eveneens de onderliggende pathogenese van infecties van implantaten besproken. De adhesie van bacteriën aan het oppervlak van humaan weefsel of aan het oppervlak van een geïmplanteerd biomateriaal is een fase die aan elke orthopaedische infectie vooraf gaat. Op het moment van implantatie zijn er op het oppervlak van een biomateriaal vrije, energetisch geladen plaatsen beschikbaar waar een fysische of chemische binding kan plaatsvinden met omringende eiwitmoleculen en vervolgens of tegelijkertijd

met cellen. De zogenaamde "race for the surface" theorie, opgesteld door Gristina, suggereert een competitie tussen weefselcellen en bacteriën voor binding aan het oppervlak van een implantaat. Succesvolle kolonisatie door een bepaald celtype leidt tot een dominantie van dit celtype en tot uitsluiting van andere cellen. Volgens deze theorie zou, als weefselcellen het eerst het oppervlak van een biomateriaal bereiken en een stevige binding hiermee aangaan, bacteriën worden geconfronteerd met levende geïntegreerde cellen en niet meer in staat zijn om het implantaat te koloniseren. Indien niet getraumatiseerd of veranderd, biedt dit geïntegreerde weefsel een zekere weerstand tegen bacteriële kolonisatie. Als echter eerst bacteriën het oppervlak bereiken en het biomateriaal koloniseren, dan zal er een infectie ontstaan. Een infectie van het biomateriaal voorkomt bijna altijd een succesvolle ingroei van het implantaat. Sommige bacteriestammen produceren na adhesie aan het oppervlak van een implantaat een slijmlaag, die hen minder toegankelijk maakt voor de afweer van de gastheer en hun gevoeligheid voor antibiotica aanzienlijk vermindert. Deze bacteriën kunnen dan voor een lange tijd "slapend" blijven op het oppervlak van een biomateriaal. Echter, wanneer omgevingsfactoren veranderen, zoals bij een verminderde weerstand, of bij een slechte ingroei van weefsel rondom een prothese, dan kunnen deze bacteriën een manifeste infectie veroorzaken. Biomaterialen zijn gevoelig voor een infectie wanneer zij niet goed in het weefsel van de patiënt zijn geïntegreerd. Goed geïntegreerde implantaten infecteren minder frequent tenzij er een minder gunstige situatie ontstaat door een verstoorde weerstand, trauma of blootstelling aan een grote hoeveelheid microorganismen.

In *Hoofdstuk 3* wordt de hematogene verspreiding als een belangrijk mechanisme voor orthopaedische infecties benadrukt door een retrospectieve analyse van 28 kinderen met een osteomyelitis. De patiënten werden geëvalueerd door een analyse van statussen, waarbij werd gekeken naar de voorgeschiedenis, anamnese, klinische presentatie, diagnostiek, behandeling en het resultaat. De patiënten hadden een acute of chronische osteomyelitis met een gemiddelde follow-up van respectievelijk 3,7 en 2,7 jaar. Alle kin-

deren werden chirurgisch behandeld: een corticaal luik werd gemaakt, pus werd gedraineerd en necrotisch weefsel werd verwijderd. Positieve kweken werden verkregen in 9 van de 12 en 7 van de 16 kinderen met een acute, respectievelijk chronische osteomyelitis. Na afname van de kweken werden alle patiënten behandeld met intraveneuze, gevolgd door orale antibiotica. Na behandeling waren op één na alle patiënten klinisch genezen en waren de infectie parameters genormaliseerd. Drie patiënten hielden restverschijnselen (ankylose, verminderde functie). Naast chirurgische behandeling blijkt 6 weken antibiotische behandeling voldoende te zijn voor het genezen van zowel een acute als een chronische infectie bij kinderen.

In *Hoofdstuk 4* worden de resultaten van een retrospectieve analyse van 47 patiënten met een geïnfecteerde heupprothese beschreven. De specifieke aandachtspunten van deze analyse waren hoe men tot de diagnose kwam, de resultaten van de kweken, de methode van behandeling en de infectieuze en functionele resultaten van deze behandeling. De diagnose werd gesteld op basis van de klinische presentatie, laboratorium onderzoek, radiologisch onderzoek en eventueel nucleair geneeskundig onderzoek. Twee patiënten overleden als direct gevolg van de infectie. De meeste prothesen werden geïnfecteerd via een directe contaminatie. Bij 5 prothesen (11%) kon een hematogene bron van infectie worden vastgesteld. Op basis van de behandeling werden de patiënten onderverdeeld in 3 groepen. Groep I bevatte 9 patiënten die waren behandeld met een nettoyage en waarbij de prothese niet werd verwijderd. Groep II bevatte 22 patiënten die waren behandeld met een resectiearthroplastiek en groep III bevatte 16 patiënten waarbij de prothese was verwijderd, een nettoyage was verricht en waarbij de behandeling direct (n = 4), dan wel in een latere fase (n = 12) werd gevolgd door een reïmplantatie. Alle patiënten kregen antibiotica. De infectie werd primair succesvol behandeld bij 35 patiënten (groep I 44%, Groep II 73%, Groep III 94%). Ten tijde van het onderzoek beantwoordden 31 van de 35 nog levende patiënten een vragenlijst over pijn en functie. Patiënten met een prothese *in situ* hadden minder pijn en een grotere actieradius. Ondanks de kleine

populatie, de relatieve korte follow-up en de verschillende wijzen van behandeling van deze populatie, werd geconcludeerd dat de behandeling van een geïnfecteerde heupprothese met uitsluitend het debrideren en toedienen van antibiotica een niet optimale behandeling is. Het verwijderen van de prothese heeft de voorkeur in het kader van de behandeling van de infectie. In het geval dat een nieuwe prothese niet kan worden ingebracht, is het functionele resultaat van deze zogenaamde Girdlestone-situatie slecht. Voor een beter functioneel resultaat is een wisseling van de prothese, indien medisch en psychisch mogelijk, aan te raden.

Een verraderlijke complicatie van een abdominaal infectieus proces, die het bestaan van een hematogeen mechanisme voor een prothese infectie onderschrijft, wordt beschreven in *Hoofdstuk 5*. In dit hoofdstuk wordt in een klinische les de ontwikkeling en het beloop van een hematogeen geïnfecteerde heupprothese beschreven na een abdomidale infectie. Bij een 66-jarige vrouw met een totale heupprothese openbaarde zich een acute cholecystitis. Zij werd behandeld door middel van een cholecystectomie waarbij een *Clostridium perfringens* werd gekweekt. Na enkele maanden ontwikkelde zij klachten die een infectie van de heupprothese suggereerden. Uit geaspireerd vocht rondom de heup werd eveneens een *Clostridium perfringens* geïsoleerd. Zij werd met succes behandeld met een "two-stage" revisie procedure van de prothese en antibiotica. Deze casus demonstreerde dat een patiënt met een gewrichtsprothese *in situ* het risico loopt op het ontwikkelen van een late infectie van deze prothese met een micro-organisme vanuit een geïnfecteerde galblaas. Wanneer patiënten met een gewrichtsprothese een bacteriële infectie ontwikkelen in een op afstand gelegen focus, dienen zij onverwijld behandeld te worden met de juiste antibiotica ter voorkoming van een hematogene verspreiding van bacteriën naar de gewrichtsprothese.

Het literatuuroverzicht en de klinische studies zoals beschreven in de hoofdstukken 2 t/m 5, leidden tot een aantal vragen die de basis vormden voor experimenteel onderzoek. In *Hoofdstuk 6* worden de doelstellingen voor deze studies geformuleerd. De basis

voor verder onderzoek in het algemeen was het gebrek aan fundamentele kennis over de pathogenese van implantaatinfecties. Zo is het de vraag of er een verschil bestaat in infectiegevoeligheid tussen de verschillende oppervlakken van gangbare implantaten en hoe zich dat verhoudt tot de biocompatibiliteit. De "race for the surface" theorie impliceert immers dat wanneer cellen het eerst het oppervlak van een biomaterial bereiken na implantatie, het implantaat niet toegankelijk meer is voor bacteriën, terwijl wanneer bacteriën het oppervlak eerder bereiken, weefselintegratie niet meer kan plaatsvinden. Dit werd verder onderzocht in Hoofdstuk 7. Een ander probleem betrof de hematogene infecties, welke een substantieel deel vormen van alle implantaatinfecties. Om meer basaal en klinisch inzicht in de pathogenese van deze infecties te verkrijgen, zijn diermodellen noodzakelijk. Derhalve werd in het onderzoek zoals beschreven in Hoofdstuk 8, een experimenteel diermodel ontwikkeld waarmee hematogene infecties van implantaten nader kunnen worden bestudeerd.

In *Hoofdstuk 7* wordt de vraag betreffende de infectiegevoeligheid van een implantaat in relatie tot de specifieke karakteristieken en biocompatibiliteit van het oppervlak bestudeerd. De belangrijkste vraag was of er een verschil is in infectiegevoeligheid tussen twee gangbare oppervlakken van orthopaedische implantaten, beiden met een verschillende biocompatibiliteit. Is een meer biocompatibel oppervlak inderdaad minder gevoelig voor het ontwikkelen van een infectie, zoals gepostuleerd in de "race for de surface" theorie van Gristina? Hiervoor werden twee klinisch veel gebruikte materialen nader bestudeerd: het ongecoate gezandstraalde Ti6Al4V en het met hydroxyapatiet gecoate Ti6Al4V. Tweeëndertig "New Zealand White" konijnen werden geopereerd. Een met hydroxapatiet gecoate (HA) of een ongecoat (Ti) Ti6Al4V implantaat werd in beide tibiae van het konijn gebracht. Voor implantatie werd het linker pootje gecontamineerd met een opklimmende concentratie *Staphylococcus aureus* (10^2-10^5 "kolonie vormende eenheden" (CFU)). Vier weken na implantatie werd de helft van het bot naast het implantaat verwijderd voor bacteriologisch onderzoek en telling van het aantal bacteriën. Voor de histologische en histomorfo-

metrische evaluatie werden preparaten van het implantaat met het overblijvende omringende bot semi-kwantitatief histologisch beoordeeld, en werd de hoeveelheid bot in contact met de implantaten, alsmede de hoeveelheid bot rond de implantaten gemeten. De bacteriologische resultaten toonden een significant effect van de inoculum dosis en het implantaattype op de hoeveelheid teruggekweekte bacteriën: meer bacteriën werden teruggekweekt bij de HA implantaten in vergelijking met de ongecoate Ti implantaten. De histologie liet meer infectie zien bij de HA implantaten dan bij de Ti implantaten. De metingen van botcontact en botoppervlak tonden verder aan, in het bijzonder voor de HA implantaten, dat er een relatie bestaat tussen infectie en verlies van botcontact en botoppervlak, dus een relatie tussen infectie en loslating van de prothese. Infecties van biocompatibele, ongecementeerde implantaten zijn gerelateerd aan de dosis van het ingebrachte inoculum. Voorts lieten de meer biocompatibele HA implantaten in deze studie een ernstiger infectie in vergelijking met de Ti implantaten zien. Dit suggereert in ieder geval dat de "race for the surface" theorie heroverwogen zou moeten worden voor de meer complexe *in vivo* situatie.

De ontwikkeling van een experimenteel diermodel om hematogene infecties van ongecementeerde implantaten te bestuderen wordt beschreven in *Hoofdstuk 8*. Tien "New Zealand White" konijnen ondergingen een chirurgische procedure in 2 tempi. Een cylindrisch Ti6Al4V of hydroxyapatiet gecoate Ti6Al4V implantaat werd in de rechter tibia ingebracht. Na een periode van minimaal 4 weken werden de implantaten via de arteria femoralis selectief gecontamineerd met een wisselende dosis *Staphylococcus aureus*. Gedurende de postoperatieve periode werden de dieren dagelijks gecontroleerd en werden er bloedmonsters afgenomen. Na minimaal 1 week werden de dieren opgeofferd en werden er biopten van bot, beenmerg, lever, milt, long en nier gekweekt. Het overblijvende implantaat met omliggend bot werd bewerkt voor histologisch onderzoek. Injectie van $5x10^8$ CFU veroorzaakte in alle gevallen positieve kweken, met minimale systemische reacties. De histologische preparaten lieten alleen kleine hoeveelheden bacteriën

zien met slechts een milde lokale ontsteking. Er werd geconcludeerd dat dit diermodel bruikbaar is voor verdere bestudering van hematogene infecties, bijvoorbeeld naar het effect van implantaatoppervlak, integratie in bot, follow-up, en verschillende preventieve maatregelen, zoals antibioticaprofylaxe.

De in dit proefschrift beschreven studies leiden niet tot alle antwoorden op de vele vragen over de pathogenese, het beloop en de behandeling van orthopaedische infecties. Verder onderzoek is noodzakelijk, met name omdat dit probleem zo complex is, maar ook omdat het een groeiend probleem is als gevolg van een toenemend gebruik van biomaterialen binnen de orthopaedie. Klinische studies zijn noodzakelijk, maar hebben hun beperkingen. Vele factoren, zoals microbiologische, patiënt gebonden en zelfs emotionele factoren, spelen een rol bij deze infecties. Het aantal patiënten in de klinische studies is vaak te klein om tot betrouwbare conclusies te komen. Daarom blijven experimentele studies belangrijk om een antwoord te kunnen geven op een aantal specifieke vragen binnen het gebied van de orthopaedische infecties. Meer kennis van de pathogenese van de infecties is noodzakelijk. Ook snellere en meer betrouwbare diagnostische instrumenten ("polymerase chain reaction", beeldvormende instrumenten) zijn nodig en zullen moeten worden ontwikkeld. Tenslotte zal het ontwikkelen van verbeterde en meer gestandaardiseerde behandelingsmodaliteiten het doel moeten zijn voor de directe toekomst. De diermodellen die in dit proefschrift werden beschreven en de specifieke experimenten die wij met deze modellen verrichtten zullen een kleine maar hopelijk belangrijke bijdrage leveren aan een beter begrip van deze infecties en de basis zijn voor nieuwe experimentele studies.

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Curriculum vitae

Henri Charles Vogely werd op 12 mei 1957 in Utrecht geboren. In 1976 behaalde hij het diploma Atheneum-B op het College Blaucapel te Utrecht. Na een jaar biologie gestudeerd te hebben, werd vervolgens aangevangen met de studie geneeskunde. In 1985 werd het artsexamen behaald. Na een periode werkzaam te zijn geweest als consultatiebureau-arts voor kleuters en zuigelingen, was hij enkele jaren werkzaam als AGNIO in de vakgebieden chirurgie, urologie en orthopaedie. In 1990 werd begonnen met de opleiding tot orthopaedisch chirurg. De vooropleiding algemene heelkunde werd gedaan in het Medisch Spectrum Twente te Enschede (opleider Dr. I.J. Hoogendam). Na een korte periode weer als AGNIO orthopaedie te hebben gewerkt, kon de opleiding in het Academisch Ziekenhuis Utrecht worden voortgezet (opleider Prof. Dr. A.J. Verbout). De perifere stages werden gedaan in het Medisch Centrum Alkmaar (opleider Dr. W.J. Willems) en het Onze Lieve Vrouwe Gasthuis te Amsterdam (opleider Prof. Dr. J.W. van der Eyken). Op 4 juni 1997 volgde de inschrijving in het specialisten register als Orthopaedisch Chirurg. Sindsdien is hij werkzaam in het huidige Universitair Medisch Centrum en het Centraal Militair Hospitaal te Utrecht, alwaar hij een fellow-ship kinderorthopaedie deed, en tevens de voet- en enkelchirurgie en infecties van het houdings- en bewegingsapparaat als aandachtsgebied heeft. De auteur is gehuwd met Marilous Chaillet. Zij zijn in het gelukkige bezit van drie dochters: Frédérique, Clementine en Claire.