

Infections associated with  
percutaneous osseointegrated  
titanium implants for limb  
prostheses

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Cover illustration: Radiograph of implant components with patchy bone loss around fixture, consistent with osteomyelitis. With permission from Dr. Berlin, Centre for Advanced Reconstruction of Extremities, Sahlgrenska University Hospital, Gothenburg.

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## ABSTRACT

Femoral amputation is a devastating event. Percutaneous, bone anchored prosthetic systems reduce problems associated with socket suspended prostheses, but the design is inherently vulnerable to infection. The aims of this thesis were to determine the risk of implant-associated infection, bacterial biofilm properties and the functional impact using this implant treatment regime. Definition of implant related osteomyelitis was based on clinical signs, radiography and positive tissue cultures. In 3-year prospective study 39 patients were evaluated twice for infectious frequency, clinical presentation, and its relation to bacterial flora at the skin-implant interface (*Paper I*). The frequency of implant infection was 5% at inclusion and 18% at follow-up. The most common bacteria in superficial, and deep cultures were *Staphylococcus aureus* and coagulase-negative staphylococci. Despite frequent colonization by potentially virulent bacteria, limited disability, and only one implant removal was found. Phenotypical and genotypical biofilm formation was determined in 13 (7 staphylococcal, 6 enterococcal) osteomyelitis strains (*Paper II*). Antimicrobial resistance was tested with a novel combination of the Calgary biofilm MBEC device, and a custom-made susceptibility MIC plate. The majority of the strains produced biofilm with increased antimicrobial resistance, compared to their planktonic counterparts. Slime producing strains tolerated higher antimicrobial concentrations compared to non-producers. All staphylococcal strains carried *ica* genes. The long-term risk of implant-associated infection, and its relation to patient and method specific factors was determined in a 20-year retrospective analysis of the first 96 femoral implant patients (102 implants) (*Paper III*). A 10-year cumulative risk of 20% for developing osteomyelitis (16 patients), and a 10-year cumulative risk of 9 % for implant extraction due to osteomyelitis (10 patients) was found. Antibiotic treatment (median 3.5 months) and selective minor debridement, with retained implants, cured 7 out of 18 patients at the 24-month follow-up (*Paper IV*). Six patients were cured after implant extraction, and 5 had chronic low-grade infections with stable implants, but variable use of the external prosthetic leg. The most common pathogens were *S. aureus* and *E. faecalis*. C-reactive protein serum levels were significantly higher in patients with osteomyelitis caused by *S. aureus* than other pathogens. It is concluded that the finding of an increased risk of osteomyelitis with time using this implant system calls for; i) careful patient selection and information of long term risks, ii) further studies on infection control, iii) consideration of biofilm in treatment, and iv) improved diagnostics, and antibiotic delivery.

**Keywords:** *amputation, osseointegration, osteomyelitis, clinical presentation, long-term risk, biofilm*

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# SAMMANFATTNING PÅ SVENSKA

Osseointegration innebär en direkt ben-implantatförankring som tillåter överföring av laster. Framgångsrika tillämpningar utgörs av orala implantat, hörapparater, och ansikts- och extremitetsproteser. En del lårbensamputerade patienter kan inte använda hylsprotos på grund av hudproblem orsakade av proteshylsan eller för kort/malformerad stump. Benförankrade implantat som fäste för en yttre protes kan då vara ett alternativ. Forskning visar ökad funktion, komfort och livskvalitet jämfört med hylsprotos. Metoden omfattar två kirurgiska ingrepp. Först sätts en gängad titancyylinder in i rörbenets mörghåla varefter huden sluts. Efter en inläkningsperiod ansluts ett hudpenetrerande fäste till vilket protesen kan kopplas. Alla implantat, i synnerhet de som bryter kroppens naturliga barriärer medför en ökad risk för infektion. Vid kronisk infektion, framförallt i närvaro av främmande material, bildar bakterier så kallad biofilm, motståndskraftiga samhällen, som gör infektionerna svårbehandlade. Akuta skelettinfektioner orsakas främst av *Staphylococcus aureus* som har en mycket god förmåga att kolonisera skadad hud och infektera underliggande vävnad. De koagulasnegativa stafylokockerna utgör en viktig del av hudfloran, och är trots ringa sjukdomsalstrande förmåga, vanligt förekommande vid implantat-infektioner till följd av god förmåga att vidhäfta och bilda biofilm. Kolonisation av implantat och vävnader kan ske i samband med operation, via hudöppningen eller blodburet. I denna avhandling har för första gången infektioner studerats i anslutning till osseointegrerade implantat för behandling av lårbensamputerade patienter. Förekomst av implantatassocierad osteomyelit (benröta) har studerats prospektivt (39 patienter) och retrospektivt (96 patienter). Infektionsförekomsten ökade från 5 till 18 % hos patienter som följdes över en 3 årsperiod. Risken för osteomyelit och att behöva avlägsna implantatet inom en 10-årsperiod till följd av detta var 20 % respektive 9 %. Infektioner orsakades oftast av *Staphylococcus aureus* men också av enterokocker och koagulasnegativa stafylokocker. En majoritet av bakterierna bildade biofilm med kraftigt förhöjd motståndskraft mot antibiotika. En ny metod för att studera antibiotikakänslighet hos de isolerade bakterierna testades. Konservativ behandling med enbart antibiotika botade ca 1/3 av patienterna. Trots risken för infektioner bedöms metoden vara till fördel för många av patienterna. Den ökade risken för osteomyelit över tid kräver dock i) noggrant patienturval och patientinformation om långtidsrisken, ii) fler studier av infektionskontroll, iii) förbättrad diagnostik och behandling med hänsyn till biofilm, och iv) lokal administration av antibiotika som sannolikt är möjligt med denna implantatbehandlingsmetod.





# LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. Tillander J, Hagberg K, Hagberg L, Brånemark R. **Osseointegrated Titanium Implants for Limb Prostheses Attachments: Infectious Complications.** *Clin Orthop Relat Res* 2010. 468: 2781. DOI 10.1007/s11999-010-1370-0
- II. Zaborowska, M, Tillander, J, Brånemark, R, Hagberg, L, Thomsen, P, Trobos, M 2016. **Biofilm formation and antimicrobial susceptibility of staphylococci and enterococci from osteomyelitis associated with percutaneous orthopaedic implants.** *J Biomed Mater Res Part B* 2016:00B:000–000.
- III. Tillander J, Hagberg K, Berlin Ö, Hagberg L, Brånemark R. **Osteomyelitis Risk in Patients with Transfemoral Amputations Treated with Osseointegration Prostheses.** *Clin Orthop Relat Res* 2017. DOI 10.1007/s11999-017-5507-2
- IV. Tillander J, Hagberg L, Brånemark R **Possible to cure osteomyelitis with retention of osseointegrated femoral implants.** *Submitted 2017.*

# CONTENT

ABBREVIATIONS .....	IV
1 INTRODUCTION.....	1
1.1 Osseointegration.....	1
1.1.1 Osseointegration following femoral amputation.....	2
1.2 Tissue response to bio-implants.....	5
1.2.1 Bone and its response to implants.....	6
1.3 Bone response to titanium.....	7
1.4 Implant associated infections.....	8
1.4.1 Experimental implant infections in bone.....	9
1.4.2 Orthopaedic implant infections.....	9
1.4.3 Basics of osteomyelitis.....	13
1.4.4 Infection in percutaneous osseointegration.....	14
1.4.5 Non-infectious complications in percutaneous femoral osseointegration.....	16
1.5 The Bacteria.....	17
1.5.1 Basic properties.....	18
1.5.2 Modes of growth.....	18
1.5.3 Staphylococci.....	20
1.5.4 Enterococci and other streptococci.....	22
1.5.5 Other bacteria.....	22
1.6 Management of bone-implant infections.....	24
1.6.1 Diagnostics.....	25
1.6.2 MIC and MBEC.....	28
1.6.3 Antimicrobials.....	28
2 AIM.....	34
3 PATIENTS, MATERIALS & METHODS .....	35
3.1 Implant design.....	35
3.2 Surgical method and rehabilitation .....	35
3.3 Definitions of infection.....	36

3.4	<i>Patients</i> .....	38
3.5	<i>Culture and antibiotic susceptibility testing</i> .....	41
3.6	<i>Biofilm analyses (paper II)</i> .....	42
3.7	<i>Statistics</i> .....	44
4	RESULTS .....	45
4.1	<i>Paper I</i> .....	45
4.2	<i>Paper II</i> .....	45
4.3	<i>Paper III</i> .....	46
4.4	<i>Paper IV</i> .....	49
5	DISCUSSION .....	50
5.1	<i>Composition of patient cohorts (I - IV)</i> .....	50
5.2	<i>Defining and diagnosing infection</i> .....	51
5.2.1	<i>The skin stoma</i> .....	52
5.3	<i>Clinical presentation</i> .....	53
5.4	<i>Risk and risk factors</i> .....	55
5.5	<i>Bacteria in femoral implant osteomyelitis</i> .....	56
5.6	<i>Diagnostic possibilities</i> .....	57
5.7	<i>Biofilm considerations</i> .....	58
5.8	<i>Treatment options</i> .....	58
5.9	<i>Acceptable level of infectious complications in this novel method</i> ....	59
6	CONCLUSION .....	60
7	FUTURE PERSPECTIVES.....	61
	ACKNOWLEDGEMENTS.....	62
	REFERENCES.....	64

# ABBREVIATIONS

AGR	Accessory Gene Regulator
b.i.d	Twice daily
CFU	Colony Forming Units
CoNS	Coagulase Negative Staphylococci
CRA	Congo Red Agar
CRP	C-Reactive Protein
CV	Crystal violet
DAIR	Debridement, antibiotics, irrigation, and retention
ESR	Erythrocyte Sedimentation Rate
ICA	Intercellular Adhesin
IL-6	Interleukin-6
MBEC	Minimum Biofilm Eradicating Concentration
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
PCR	Polymerase chain reaction
PIA	Polysaccharide Intercellular Adhesin
PJI	Prosthetic Joint Infection
t.i.d.	Thrice daily

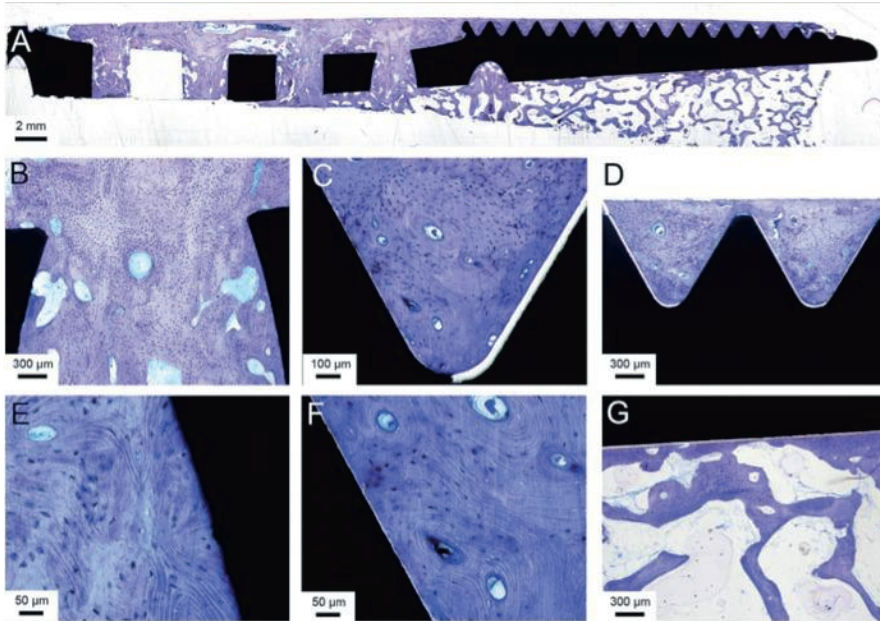
# 1 INTRODUCTION

In this thesis infections in percutaneous osseointegration are described, and discussed. Due to the shortage of previous research in this particular area of orthopaedic reconstruction, there are frequent comparisons to infections in arthroplasty, and to medical applications involving penetration of skin, and mucus membranes.

## 1.1 Osseointegration

Osseointegration is presently defined as the approximation of an implant titanium oxide surface and bone tissue, with no interposition of fibrous tissue or inflammatory cells [1]. The original discovery was made in 1952 by P I Brånemark, in an *in vivo* rabbit model of bone marrow circulation, where a titanium chamber allowing ingrowth of bone, and blood vessels was used [2]. Osseointegration has to date been in clinical practice for more than 30 years in tooth replacement [3, 4]. Later applications include bone-anchored hearing aids [5], cosmetic craniofacial prostheses [6] and, osseointegrated percutaneous residual limb implants [7]. The latter method, was introduced in the 1990s at the Sahlgrenska University Hospital, giving limb amputees a stable attachment site for an external prosthesis. Histologically, stability is achieved by mature compact bone in direct contact with the surface of a load bearing implant [8] (Figure 1). The basic mechanism in fracture healing, and implant incorporation alike, is the recruitment, and maturation of progenitor cells through staged callus formation, eventually giving rise to mineralized bone. Functionally, this corresponds to an implant-tissue interface withstanding the biomechanical forces to which it is subjected in gait [9, 10]. Bone healing relies on primary stability until a hard callus has replaced non-viable bone tissues from trauma during implant insertion. Consequently, implant failure could be defined as the inadequacy of the host tissue to establish or maintain osseointegration. The inflammatory response is more thoroughly described below.

*Figure 1. Light microscopic images of tissue implant integration. A. Implant and bone tissue overview. B. Closer view of one of the transversal holes in the implant, connecting outer and inner surfaces of the implant cylinder. C and D. Mature bone filling the thread of the implant. E and F. Closer view with multiple osteocytes close to the implant surface. G. Trabecular bone in the hollow centre condensing at the implant surface. With permission from Palmquist and co-workers, 2014.*

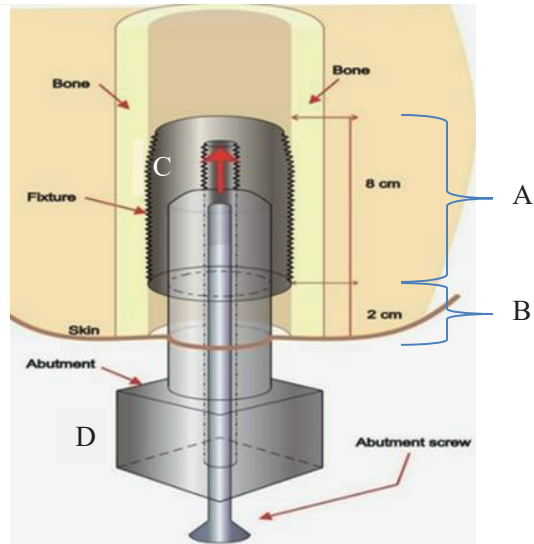


### 1.1.1 Osseointegration following femoral amputation

A limb amputation is a traumatic event with both physical and psychosocial sequelae [11]. The underlying reason for amputation greatly influence the short- and long-term morbidity and mortality following the procedure. Major lower limb amputations (tibial or femoral) for vascular reasons, the leading cause worldwide, [12] carries the highest mortality rates [13, 14], especially in the elderly, and diabetic populations [15]. Post-operative wound infections occur in at least 10 % [14, 16, 17], negatively influencing these rates. Trauma and neoplasms are responsible for a small proportion of lower limb amputations in high-income countries [18]. In contrast, amputation following trauma is frequent among people without underlying co-morbidities in

military conflict areas [19]. Amputation is sometimes a last resort in prosthetic knee joint infection unresponsive to other treatments [20]. The conventional way of restoring lower limb mobility, is through socket suspended prostheses, which result in discomfort, impaired range of motion or skin problems for many patients [21]. A short or malformed residual limb might entirely rule out the use of a socket. Circumventing these problems, through a bone anchored

*Figure 2. Schematic drawing of implant components and sur-rounding tissues. A. Zone referred to in osteomyelitis. B. Zone referred to in distal osteitis. C. Threaded intra-medullary implant cylinder to which bone grows in. D. Outer implant component which can be replaced when damaged; attachment site for external prosthesis. With permission from Cecilia Berlin, Chalmers Institute of Technology.*



transcutaneous prosthetic system, has been desired for decades. Unfortunately, past attempts have not been successful, because of mechanical, and infectious complications [22, 23]. After the discovery of osseointegration, this research field has been rejuvenated. Detailed biomechanical studies in rat, rabbit, dog and humans was carried out by Rickard Brånemark and co-workers before, and during the introduction of a load-bearing percutaneous osseointegrated prosthetic system (Figure 2). These demonstrated an elastic behaviour of the bone-implant interface, similar to surrounding bone, when subjected to pull-out or lateral loading tests [24-27]. The long-term success, and relative absence of stress shielding in dental implants, further supported this novel application. Work on surface topography, biomechanics and peri-implant histology has shown that interface strength can be increased by nano-structured surface modifications [28]. Although sustained osseointegration was observed in an immunological arthritis model in rabbit [27], no infection models have preceded introduction of the treatment. Similar concepts have been developed at other centres, and clinical trials of press-fitted intramedullary implants with macro-porous surfaces are ongoing in Lubeck, Germany [29], and Australia [30]. Some research groups argue that there must

be a dermal or subdermal seal preventing ascending infection, before moving on to human application. A few small animal studies have indicated that a porous flange increases skin attachment and reduce infection [31] [32]. Similarly to osteocytes, [33], keratinocyte attachment and growth appear facilitated *in vitro* by a smooth implant surface topography [34]. Proper surface roughness on the other hand induces increased osteoblast differentiation, matrix mineralization and production of growth factors leading to a stronger interface [35]. This method (Figure 3) requires two separate surgical procedures described below. A systematic treatment protocol started in January 1999, and outcomes are evaluated and continuously published. Follow-up studies reveal considerable advantages in daily life compared to conventional socket prostheses [8, 36], and a prospective two-year study reports daily prosthetic use in 40 out of 45 patients [37]. Osseointegration is not exclusive to above described method, but can be stimulated in porous [38, 39], or hydroxyapatite [40] covered prosthetic components. It has in fact been argued that any inert metal introduced in a suitable bone forming environment, can ultimately result in osseointegration [41]. Firm bone-implant bonding, with no or minimal fibrous encapsulation, is the pre-requisite for long term prosthetic function. It prevents micro-motion and wear particle induced osteolysis and subsequent loosening [42, 43].

*Figure 3 A-B. A. Radiograph of early, modular design with collar. B. Photo of an attached prosthetic leg, equipped with a guard device to prevent high rotational force propagation to the implant. With permission from Centre for advanced reconstruction of extremities, Sahlgrenska University Hospital.*



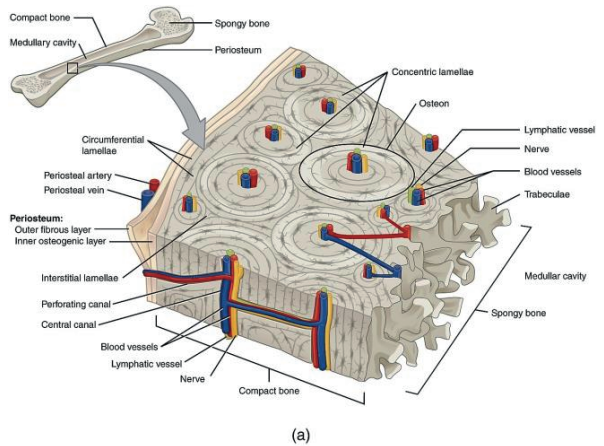


## 1.2 Tissue response to bio-implants

Bio-implants and bio-devices are commonly used in medicine to support function, replace tissues, and provide artificial accesses to anatomical compartments, i.e. pacemakers, joint prostheses and venous catheters. If the implant is intended for long-term use, the demand for high durability, functionality, and bio-tolerability is increased. Adverse tissue responses to implants can be divided into inflammatory, allergic, toxic and carcinogenic, of which the three latter are very uncommon with present implant compositions. Type 4 delayed hypersensitivity may occur with some biomaterials, but is only anecdotally described for titanium [44]. There is some evidence of cytotoxicity in human cell cultures from wear particles of common bio-materials including titanium [45]. This brief summary focuses on inflammatory responses i.e. foreign body reactions. Somewhat arbitrarily, the time from implant insertion can be divided into four phases, i) protein adsorption, ii) acute inflammation, and activation of the coagulation systems, iii) chronic inflammation and neovascularization and, iv) fibrous encapsulation. Within milliseconds upon insertion of a device it is coated with a film of glycoproteins and proteins, such as albumin, fibrin, fibrinogen, vitronectin, fibronectin, and immunoglobulins by van der Waals, hydrophobic, electrostatic, and hydrogen bonds [46]. Adsorption is influenced by both implant surface characteristics, and protein composition. Acute inflammation is dominated by polymorphonuclear cells, mainly neutrophils, followed and replaced (phagocytized) by macrophages within 48-72 hours. Macrophages guide further tissue repair through phenotypical changes. The presence of a bioimplant cause macrophage dysfunction, resulting in compromised healing [47], and a weakened defence against microbes. Deposition of fibrin and other proteins in the implant-tissue interface, provide a scaffold for fibroblast migration leading to encapsulation of the foreign body. Surface modifications improving biomaterial performance, through appropriate tissue responses (i.e. biocompatibility), is presently a very active area of research and development [48]. Of equal importance, as infection limits the usefulness of many bioimplants, is antibacterial surface research, which includes various coatings (e.g. antibiotics, silver), chemical or nanostructure modifications. Toxicities, and biocompatibilities are for the most part not sufficiently investigated for clinical use [49].

## 1.2.1 Bone and its response to implants

*Figure 4. A cross-sectional drawing a) of the basic structural unit of compact bone, the osteon and (b) a light micrograph (x40) of the central canal and concentric lamellae. Permission by OpenStax College CC BY 3.0, Wikimedia Commons*



Bone is a rigid, and highly vascularised tissue of bone cells, collagenous fibres and calcium rich hydroxyapatite,  $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ . The collagen matrix constitutes some 30 % of the bone mass, and the mineral components 65 %. Bone has a lamellar substructure, and is either compact (cortical), or trabecular (Figure 4). It is under constant remodelling

by bone resorbing cells (osteoclasts), and matrix forming cells (osteoblasts) and therefore capable of close to complete regeneration following trauma, or tissue grafting [50]. Furthermore, according to Wolff's law, bone grows stronger when loaded, and weakens when unloaded [51]. As in soft tissues, the introduction of any non-self, non-viable material induces a foreign body reaction, leading to the formation of a protective fibrous capsule. The host response is dependent on implant biocompatibility [52], a very complex interaction with immune, and tissue cells. Implant-adsorbed macro-molecules orchestrate downstream immune reactions, coagulation [53], and serve as ligands to bacterial receptors and/or host cells [54]. Intense or protracted inflammation might jeopardise implant survival. In biocompatible implants, acute inflammation beyond three weeks indicate infection [55]. The bone-implant interface is not static after primary integration, but undergoes radiologically visible remodelling in both arthroplasty [56, 57] and percutaneous bone-anchored implants [58]. Aseptic loosening is the most common cause for revision of hip ( $\approx 70\%$ ), and knee arthroplasties ( $\approx 40\%$ ).

Around 1/3 of prosthetic hip joints fulfil radiological criteria for aseptic loosening after 10 years, not corresponding to the actual revision rates. Current plausible theories include inflammatory responses to wear particles, endotoxins, and micromotions, possibly enhanced by high joint fluid pressure and/or genetic vulnerability [59]. A replacement joint or femoral implant is inevitably subjected to shear, and compressive frictions when used, leading to release of wear debris. The wear debris particles stimulate macrophages, which together with recruited osteoclasts increase osteolysis [60, 61], reducing implant stability. In a murine model, osteolysis was more pronounced when lipopolysaccharide was bound to titanium wear particles [62]. In large register studies, combined antibiotic prophylaxis (systemic + implant cement) has been shown to reduce the risk of revision rates for both infection, and aseptic loosening, compared to systemic antibiotics only [63], and gentamicin in cement reduces the rate of aseptic loosening, compared to cement without [64]. Thus, an ideal orthopaedic implant has selective protein adhesion; stimulates early osteoblast attachment and differentiation, and prevents fibroblast and bacterial adhesion [65, 66]. Most joint prostheses are attached to the surrounding bone by a firm fibrotic capsule, rather than through osseointegration, for reasons discussed above. Properly osseointegrated femoral implants on the other hand, are to a high degree in a direct contact with mineralized bone, thereby forming a stronger anchorage. This is for example illustrated by the laborious trephination required to extract fractured implants, and that partially integrated implants can withstand rotational forces above 12 Nm applied in stability testing.

### **1.3 Bone response to titanium**

Pure titanium has a very high strength to density ratio, and is extremely corrosion resistant [67] due to the oxide ( $\text{TiO}_2$ ) coating, passively formed upon air-contact. From a material point of view, those are excellent properties for long-term load-bearing implants. Equally important, titanium oxide provokes less inflammation, than many other bio-metals, minimizing fibrous encapsulation [68], and allowing early and intimate bone ingrowth translating into interface strength, especially in surface modified implants [69]. Abundant research has made it clear that the thickness of the  $\text{TiO}_2$  layer, and other topographical, or electrochemical properties of the implant surface influence bio-compatibility, and bone formation. Efforts are made to further add

resistance to bacterial adhesion, by nano-modifications reducing bacterial contact area [70], and by coatings including silver, and other noble metals with bactericidal effects [71].

## 1.4 Implant associated infections

Post-operative soft tissue infection, and hematogenous seeding may lead to implant associated infection in all orthopedic implants but most commonly, colonization occurs during implant surgery by direct inoculation or sedimentation of air borne bacteria-laden particles into the wound [62]. Advanced medical care comes at a cost of an increased risk of hospital acquired infections. It is estimated that 65-80 % of nosocomial infections are associated with some type of implanted medical device, primarily in the intensive care setting, with devices breaching natural barriers [72, 73]. Surgical implant infection is less common but more difficult to handle. Implant colonization during surgical procedures is virtually unavoidable. Most colonized implants however, do not become infected. If sufficient bacterial colonization precedes tissue regeneration, local host defenses are unable to prevent a persistent infection. In a classic human experiment, the infective intradermal dose of *Staphylococcus aureus* (*S. aureus*) was around  $10^6$  cells, and in the presence of a suture as low as  $10^2$  bacterial cells [74], demonstrating the vulnerability to foreign body associated infections. Implant size and design, anatomical site, surgical technique, host defenses, and bacterial virulence determine further infectious development. Implant infections typically involve biofilm, a concept described below. Virtually any device may be a platform for such infections. U.S. estimates of surgical implant infections in prosthetic heart valves, pacemaker-defibrillators, ventricular shunts, artificial vascular grafts, and fracture fixation devices are 4-6 % [75]. Annually, five million medical devices or implants are inserted in the United States. Implant associated infection is ultimately diagnosed by typical clinical manifestations, intraoperative signs of infection, and the growth of pathogens in cultures of peri-implant tissues.

### 1.4.1 Experimental implant infections in bone

There is a paucity of useful human clinical trials of implant-associated osteomyelitis due to low incidence rates, population heterogeneity, varying treatment approaches, and the broad range of pathogens, and virulence patterns [76]. To a degree, clinical practice may be guided by animal models investigating pathogenesis, diagnostic tools, prophylactic regimens, and therapeutic outcomes. *S. aureus* osteomyelitis has been studied in fracture fixation, one-stage prosthetic, and hematogenous models, mainly involving small animals, but no unified definition exists [77]. Most *in vivo* osteomyelitis models focus on the influence of various surface-materials, or treatment outcomes, while early phase histopathology is insufficiently understood. In the presence of bacteria, porous implant surfaces are shown to promote infection, compared to smooth [78], influencing the design of functional surfaces. Lower infection rates in (unintegrated) titanium, compared to stainless steel, have been demonstrated for fracture fixation plates (35 % vs. 75 %) [79], and intramedullary nails (59% vs. 75%) [80] in rabbit models. Local treatment with hydroxyapatite (HA)-vancomycin or HA-gentamycin bone substitutes, has repeatedly been shown to safely and effectively resolve infection [81, 82], a modality now clinically applied [83]. In a murine model of early soft tissue infection, *S. epidermidis* was found in lower levels on coated titanium discs, compared with in the interface exudate. Continuous cell-death and expression of pro-inflammatory mediators was seen in contrast to control sites [84]. There are very few *in vivo* models of infection in percutaneous load bearing implants. An American research group has performed a series of studies with the ultimate goal of an infection-free one-stage implant system. Based on the proposed need for an antler-skin type seal [32], infectious frequencies were compared in customized titanium implants in sheep, with either smooth, or porous subdermal surfaces. Skin-interface infection was confirmed by culture and histology in 2/9 in the smooth surface group, and in 0/14 in the porous surface group at the 9-month endpoint. There was no growth in marrow cultures, and no radiological evidence of osteomyelitis [31].

### 1.4.2 Orthopaedic implant infections

Orthopaedic trauma and/or surgery result in an extensive breach of the skin barrier, tissue damage, and haemorrhaging. Both wound site, and implant

becomes colonised with patient, and hospital flora. Factors influencing subsequent infection include wound care, host defences, extent of trauma, microbial properties, and very importantly, the use of fixation, or prosthetic devices. Primary arthroplasty is by design highly vulnerable to bacterial colonization until wound closure. Devices penetrating skin (e.g. fracture fixation pins, and bone anchored prosthetics), are continuously exposed to the external microflora. This translates into various frequencies of implant associated infection. For example, pin site infections range between 0-100 % [85] and, despite antibiotic prophylaxis, infection rates after open fracture fixation may reach 30 % [86]. Joint prostheses constitute a large proportion of all orthopaedic implants. For instance, it is prognosticated that more than 0.5, and 3 million total hip, and knee arthroplasties respectively, will be performed in the United States within 15 years [87]. Corresponding projection for Swedish patients above 40 years of age, is 20 000 hip replacements in the year 2030 [88]. Overall implant survival rates in primary hip, and knee replacements approach 95 %, in the first 10-year period [89]. Important to note is that clinical failure rates (i.e. disability), are almost twice as high as revision rates in hip arthroplasties [90]. Overall rates of prosthetic joint infection (PJI) is currently estimated to about 1-2%, greatly decreased since the introduction of perioperative antibiotics, antibiotic loaded cement, and ultra clean air in operating theatres [91] [92-94] [95, 96]. Infection causes 25.9 % of all revisions during the two first postoperative years, and 2.9% after 10 years [97]. According to the Swedish hip replacement register, the share of infection as reason for first revision is 7.5 %, and 20.9 % if more than 2 previous revisions have been carried out [97]. There is some evidence however, that these rates might be increasing again [98, 99], and somewhat alarming is the almost five-fold relative risk increase between 1995-1999, and 2005-2009 of early (0-3 months) postoperative PJI, reported in a large Nordic register study [100]. Increased co-morbidities, and improved diagnostics partly explain these findings. Whereas minor infection in temporary implants is reasonably easy to handle, the management of orthopaedic large-implant infections in general, and prosthetic joint infections in particular, is costly and time consuming. A French study approximated the direct cost of revising one hip PJI to €32000, almost four times the cost of the primary procedure [101]. Of greater concern is the additional suffering and mortality. Mean hospitalisation, is prolonged by 2 and 3 weeks in deep infection after open fracture fixation, and arthroplasty respectively [102]. A number of studies report a 2.5-5-fold increase in 90-day mortality in PJI [102-104]. Independent risk factors for PJI are listed in Table 1. Major modifiable factors include body mass index  $\geq 40$  kg/m<sup>2</sup>, protracted operating time, and nasal carriage of *S. aureus* [105]. It is likely, but untested, that these apply to other orthopaedic implant procedures

as well. One big data (N=83,011) multivariable analysis identified several comorbidities (e.g. congestive heart failure, renal disease) that discreetly increase risk (adjusted HRs 1.13 to 1.59) for knee PJI [106]. Oftentimes, the arthroplasty patient suffers from a number of these conditions.

*Table 1. Selected independent risk factors for infection in arthroplasty.*

	Pulido et al. 2008. N=9245 [107]	Berbari et al. 1998. N=462 [108]	Sousa et al. 2014. N=2497 [109]
Postop. wound infection		OR 35.9 (CI 8.3–154) p<0.01	
ASA- score' > 2	OR 1.95 (CI 1.0–3.7) p=0.04		
ASA- score ≥ 3			OR 2.12 (CI 0.91-4.95) p=0.08
BMI > 40 kg/m <sup>2</sup>	OR 3.23 (CI 1.6–6.5) p<0.001		
NNIS <sup>#</sup> score 2		OR 3.9 (CI 2.0–7.5) p<0.01	
Prior joint arthroplasty		OR 2.0 (CI 1.4–3.0) p<0.01	
Postop. myocardial infarct.	OR 20.4 (CI 2.1–199) p=0.009		
Postop. atrial fibrillation	OR 6.22 (CI 1.4–28.5) p=0.02		
Knee arthroplasty	OR 2.85 (CI 1.5–5.6) p=0.002		OR 1.39 (CI 1.11-1.72) p<0.003
Metastatic cancer		OR 3.1 (CI 1.3–7.2) p<0.01	
Postoperative urinary tract infection	OR 5.45 (CI 1.0–8.7) p=0.04		OR 6.64 (CI 1.24-35.64) p<0.001

‘ASA-score: American Society of Anaesthesiologists’ 6-grade physical status classification.

<sup>#</sup>National Nosocomial Infection Surveillance Score: A 0-3 score compiled by ASA 3-5 ± contaminated/infected operation ± prolonged operation.

Redness, local swelling, and continuous wound secretion is typical in postoperative prosthetic joint infection, while later onset is characterised by loading and resting pain, and sometimes fistula formation. Two thirds of all

prosthetic joint infections are caused by staphylococci, with little variations over time and setting, followed by streptococci including enterococci [110]. Reported frequencies of polymicrobial infection vary between studies (Table 2). Similar presentations are seen in infections associated with internal fixation devices (i.e. intramedullary nails, screws and plates), but often compounded by the initial trauma. The share of *S. aureus* and enteric rods appear higher [111] compared to PJI, and is influenced by perioperative antibiotic regimens [112].

Table 2. Common culture results in revision for PJI at any time point following primary arthroplasty. The two rightmost columns show US and European microbial patterns in two arthroplasty clinics.

Bacteria	USA 1969- 1991 Hip/Knee Berbari [108]	Sweden 1986-2000 Knee Stefansdottir [113]	Taiwan 2006-2011 Hip/Knee/ Elbow Tsai [114]	USA 2000-2011 Hip/Knee Aggarwal [115]	Germany 2000-2011 Hip/Knee Aggarwal [115]
Number of revised joints	263/199	426	52/88/4	353/419	568/330
	n (%)	n (%)	n (%)	%	%
Staphylococcus aureus	101 (22)	130 (30.5)	28 (19.4)	33/30	14/12
Methicillin resistant <i>S. aureus</i>	-	-	15 (10.4)	48 % of <i>S.au.</i>	13 % of <i>S.au.</i>
CoNS	86 (19)	117 (27.5)	24 (16.7)	18/22	41/37
Enterococcus spp.	6 (1)	33 (7.7)	14 (9.7)	] 9/10	] 13/15
Streptococcus spp.	42 (9)	36 (8.4)	12 (8.3)		
Other Gram-positive aerobes	3 (1)	4 (0.9)	N/A	N/A	N/A
Enteric Gram-negative bacilli	38 (8)	16 (3.6)	12 (8.4)	7/6 incl:	4/5 incl:
<i>Pseudomonas aeruginosa</i>	N/A	9 (2.1)	8 (5.6)	<i>Pseudomonas</i>	<i>Pseudomonas</i>
Other Gram-negative aerobes	N/A	1 (0.2)	11 (7.7)	N/A	N/A
<i>Propionibacterium acnes</i> + sp.	N/A	8 (1.8)	N/A	Prop + other	Prop + other
Other anaerobes	29 (6)	4 (0.9)	N/A	1.5/0.5	13/2
Polymicrobial	88 (19)	27 (6.3)	16 (11.1)	7.5/7.5	3.5/3.5
Other	12 (3)	2 (0.4)	2 (1.4)	10/8	1.51
Culture negative	57 (12)	39 (9.2)	27 (18.8)	15/16	11/25

PJIs can be classified as early, delayed (3-24 months), or late. Acute postoperative (< 3 weeks) infection is dominated by virulent bacteria (*S. aureus*,  $\beta$ -haemolytic streptococci, *Enterobacteriaceae*) inoculated intra-operatively, or through continuous spread from the wound. In delayed, and late prosthetic joint infections coagulase negative staphylococci are the most frequent pathogens, while *S. aureus* accounts for approximately two thirds of community acquired haematogenous infections [113, 116].



### 1.4.3 Basics of osteomyelitis

Healthy mature bone is highly resistant to infection, partly illustrated by peak incidences of osteomyelitis in the very young, elderly and infirm [118]. Directly inoculated or invading bacteria or fungi during trauma or orthopaedic surgery are the most common causes of osteomyelitis, followed by secondary spread from soft tissue infections in vascular insufficiency or the diabetic foot [119].

Haematogenous infection is less common and mostly affect prepubertal children and elderly. Osteomyelitis is mainly affecting the vertebrae, feet or long-bones. Three main factors determine the development of osteomyelitis: i) the presence of ischemic or sequestered bone, ii) the ability of the infecting organism to evade immunological clearance and adhere to bone tissues, and iii) the size of the inoculate. Acute disease evolves over days or weeks, whereas chronic osteomyelitis is a long-standing infection of months and even years. Clinical signs and symptoms are dependent of type, locus and severity of infection. In chronic osteomyelitis pain and loss of function dominates, while fever is uncommon. The long-standing (chronic) infection is characterised by sequestered bone, and a draining sinus is typically formed. The sequester is a nidus on which bacteria may form biofilm and

Table 3. *The Cierny-Mader staging system of osteomyelitis emphasizing the patients' physical status as the most important factor in treatment decisions [117].*

Anatomical type	
Type	Characteristics
I	Medullary
II	Superficial
III	Localized
IV	Diffuse
Physiological class	
Class	Characteristics
A	Good immune system and delivery
B	Compromised locally or systemically
C	Requires suppressive or no treatment Minimal disability Treatment worse than disease Not a surgical candidate

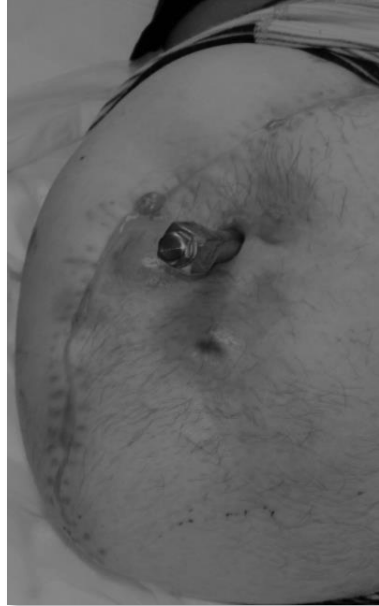
undermine the body's ability to clear infection. Thus, once established, chronic osteomyelitis is not easily resolved, and progresses through continuous inflammatory deterioration of the vascular bed [118]. The Cierny-Mader system (Table 3) [117], which includes the general physical status of the patient, offers a usable clinical staging for treatment guiding and research purposes. Albeit not developed for foreign body osteomyelitis, most of its elements are arguably applicable in implant driven infection. Treatment involves removal of devitalised bone, local and systemic antibiotics. Failure to restore bone cavities, by various forms of bone transplants or osteoconductive scaffolding, often results in relapse infection [83]. One recent concept, combining fast and slow resorbing types of antibiotic loaded calcium phosphates, allows high early release effectively targeting biofilm infection, while forming a porous scaffold offering more prolonged structural stability, and bone ingrowth [120].

#### **1.4.4 Infection in percutaneous osseointegration**

Previously, there have been no clinical studies dedicated to deep infections in percutaneous osseointegrated orthopaedic implants. In fact, there are very few reports specifically addressing below skin infection, in the entire field of percutaneous osseointegration. Clinical research has largely been focused on implant function, implant survival, and skin reactions. Although osteitis appears rare, skin and soft-tissue infection is the most common complication [121]. *S. aureus* is the primary pathogen in these skin infections [122]. In the literature, implant failures in bone anchored hearing aids are chiefly attributed to poor osseointegration or “delayed disintegration”. One Swedish study of 281 mastoid process implants, reports removal due to loss of integration in 9 cases, and mild to moderate skin reactions not leading to removal in 30 %, during an 8-year period [123]. A large meta-analysis of failures in other cranio-facial implants reports a mean 5.5 % probability of failure with variability mainly dependent of bone tissue quality including previous radiotherapy [124]. Possibly, the low degree of relative motion at cranio-facial sites, better preserve the barrier of immune-response cells, described by Holgers and co-workers [125], compared to limb implants. Furthermore, there is a significant difference in bacterial residents, and therefore colonisation patterns, between the scalp (sebaceous skin), groin (moist skin), and femur (dry skin) [126]. In dental osseointegration infectious failure is better characterized, but often without enough stringency in differentiating from

other causes [127]. The role of microorganisms is currently under debate. The weighted mean prevalence of oral peri-implantitis in one meta-analysis of 11 studies was 22 % [128], whereas other studies have demonstrated 5-10-year cumulative case incidences in the range of 1 to >45 % [129, 130]. One study describing the outcome for femoral intramedullary press-fit implants (n=50, mean follow-up: 21.5 months), report 21 instances of non-descript infections requiring antibiotics (i.v. in 5), and soft tissue debridement in 3. No extraction due to infection was reported [131].

*Figure 5. Photograph showing the protruding abutment through an uninflamed stoma, but with a sinus tract directly below. With permission from Centre for advanced reconstruction of extremities, Sahlgrenska University Hospital.*

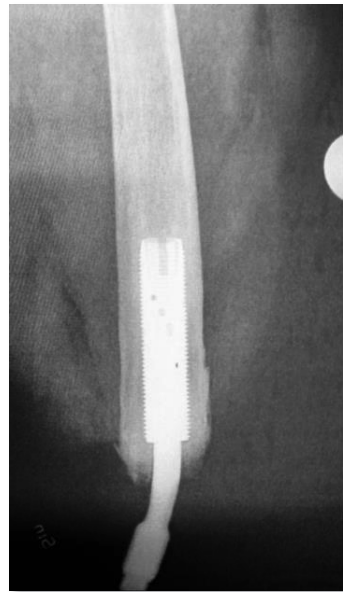


A recent Danish thesis investigating bone mineral density, metabolic factors and implant migration, in femoral osseointegrated implants (N=20), reports that six of the implants were extracted due to aseptic/septic loosening [132]. This frequency of implant loss, is similar to the early outcomes after method introduction in Gothenburg [133]. Skin infection responding to short courses of oral antibiotics is reported in 55 % of patients with femoral implants, over a 2-year period [37]. In the same study, only one implant was lost to infection. A British report with a rehabilitation perspective of the Brånemark method stated that after one year, 2 of the 11 patients had the fixture removed because of infection [134]. A clinical score for skin inflammation has been developed for bone anchored hearing aids; grade 0, no irritation; grade 1, slight redness, responsive to local treatment; grade 2, red, and slightly moist, extra control; grade 3, reddish and moist with granulation, skin revision needed; grade 4, extensive soft tissue reaction requiring implant removal [135]. With modifications, it may be applicable for percutaneous limb implants (Figure 5) as well. However, despite higher frequencies of macroscopic inflammatory signs compared to hearing aids, modification of this scoring system did not correlate with clinical diagnosis, radiological, microbiological or proinflammatory markers in a fairly small (N=30) femoral implant study [136]. Since dermal attachment so far has eluded researchers trying to reduce infections in permanent percutaneous devices, optimization

of the skin-implant zone (i.e. using “skin-friendly” surgical technique, minimizing iterative microtrauma and chemical irritants, promoting commensal and reducing opportunistic microflora by proper hygiene), must be prioritized, and is further discussed below.

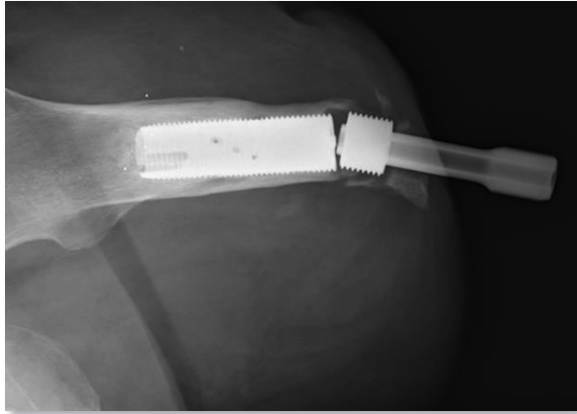
### 1.4.5 Non-infectious complications in percutaneous femoral osseointegration

*Figure 6. Radiograph of present design of the implant, with a bent abutment needing replacement. Reprinted with permission of Drs. Y. Li and R. Brånemark.*



Rehabilitation after the 2-step surgical procedure aims at slowly increasing the load until the interface is strong enough for normal gait, a process often lasting longer than 4 months. Early failure due to non-integration occur in 1-2 % of dental implants, which cannot be attributed to immediate or early loading [33]. In the prospective 2-year outcome study of 51 patients with femoral implants, 3 were extracted due to aseptic loosening and one due to infection [37]. Failure of the implant to integrate with the bone results in movement of the fixture screw and loading pain. The abutment and implant-bone interface is challenged by forces along all axes and especially the long axis [10]. Both overloading and material fatigue can lead to bending (Figure 6) of the abutment or fracture of the fixture (Figure 7). The 6-month healing period after fixture insertion prevents early motion, but whether later loading forces can lead to loosening (e.g. titanium particle induced) is not yet known. Although the method aims for minimal soft tissue movement, relative movement between the skin and abutment may tentatively lead to local inflammation, and formation of a fibrous ring. The extent of bacterial involvement in this process is not known. Epithelial down-growth and pocket formation (marsupialization) may lead to implant exposure and an increased risk of osteitis.

*Figure 7. Radiograph of a fracture of the distal femur and tibia. The combined strength of the implant and thin cortical bone was unable to withstand repeated forward forces in gait. With permission from Centre for advanced reconstruction of extremities, Sahlgrenska University Hospital.*



## 1.5 The Bacteria

Bacteria are single cell organisms with a ubiquitous distribution and indispensable function in nature. Classification is based on shared phenotypical traits, chemical properties and most recently genetic similarities [137]. Bacterial-host coexistence is most often divided into three categories; parasitism, symbiosis and commensalism. Under normal circumstances, the human micro-flora falls under the latter two. However, when the general host susceptibility is increased (malignancy, AIDS, cytostatic treatment etc.), or locally compromised (bio-devices, catheters etc.) bacterial behaviour is shifted towards parasitism. Compared to the mucus membranes of the gastrointestinal, upper respiratory and genitourinary tracts the skin is a hostile environment reflected by the relatively few species permanently residing here. Coagulase negative staphylococci, *Corynebacterium* and *Propionibacterium* species dominate the skin flora in culture-based [138] and genomic [139] studies. Transient colonisers include *S. aureus*, various Streptococci and gram-negative rods and temporal variation appears greatest in areas of greater microbial diversity such as the antecubital fossa and between the fingers [126]. It is also important to recognise that both antibiotics, and hospitalisation may greatly affect composition of the patient microflora [140], and pave the way for infections refractory to antibiotics.

### 1.5.1 Basic properties

Like all living cells, bacteria are separated from the external environment by a lipid bilayer (cytoplasmic membrane), which with only a few exceptions is enveloped by a supportive peptidoglycan mesh (cell wall). The simple Gram stain visualises these structures and can still be usable for initial categorization and treatment decisions [141]. In gram-positive bacteria, the cell wall is thick compared to gram-negative bacteria, in which the outer surface is a second membrane containing potent antigenic structures, mainly LPS. Bacteria lack the intracellular organisation (no organelles) and genomic stability (no nucleus or histones) of eukaryotic cells (animals, plants and fungi), but have in return short generation times, with rapid development of resistances against most environmental stressors including antimicrobial agents. Unregulated use of environmentally persistent and mobile antibiotics in medicine, and agriculture, rapidly expands pre-existing antimicrobial resistances [142].

### 1.5.2 Modes of growth

Bacterial growth is regulated by both intracellular and cell-to-cell interactions [143]. It has long been recognised that most bacteria prefer communal, surface-bound growth, which gives protective and nutritional advantages, compared to planktonic growth [144]. Both gram-positive, and gram-negative bacteria use chemical signalling called quorum sensing, to determine bacterial density, which in turn is crucial to the behaviour of the community [145]. Quorum sensing may be a future target in the treatment of staphylococcal biofilm-driven infections, by specific inhibitor substances [146]. Two common antibiotics, ciprofloxacin and azitromycin, possess such properties against *Pseudomonas aeruginosa* [147]. In times of plentiful nutrients and no competition by other microbes, or immune cells, bacterial growth rates are close to exponential. Such milieus are routinely created in microbiology labs, when detection is focused on single species. However, bacterial growth within a protective community, known as biofilm, is not only found in nature, but is central in many chronic infections; such as native valve endocarditis, otitis media, cystic fibrosis pneumonia, and infections associated with implanted biomaterials [148]. Biofilm formation is multifactorial and is influenced by various environmental stresses, such as limited supply of oxygen, iron [149], or sub-inhibitory concentrations of antibiotics in both gram-negative bacilli, and staphylococci [150] [151] [152, 153]. Biomaterials further promote

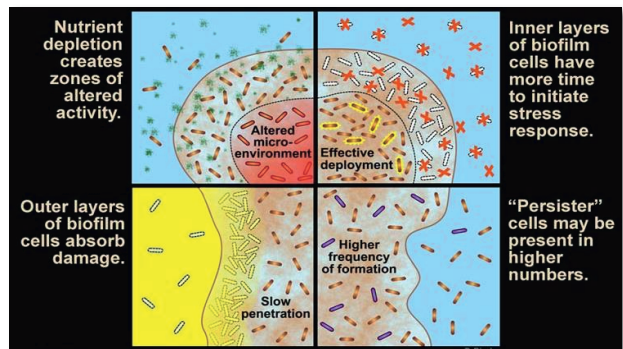
biofilm formation by allowing less virulent bacteria to persist. The first step is bacterial adhesion to implant adsorbed serum proteins, by both unspecific (e.g. surface hydrophobicity) and ligand–receptor mechanisms. The attached bacterial quorum secretes pheromone-like substances, inducing a population-wide genotype switch, which include secretion of extracellular polymers embedding the bacteria (Figure 8).

*Figure 8. Illustration of the main steps in biofilm formation. Reprinted with permission from Centre for Biofilm Engineering (CBE), Montana State University.*



In staphylococci, the polysaccharide intracellular adhesin (PIA), made by the enzyme product of *icaADBC* [154], is the principal biofilm compound. Mature biofilms are complex, often containing more than one bacterial species, and recruited host platelets [155]. The extracellular polymeric substances filter harmful compounds, and channel nutrients, and minerals to deep seated cells [156]. Depending on *in vitro* methodology, a 10- to 1000-fold increase in antimicrobial resistance in biofilm bacteria compared to planktonic bacteria have been demonstrated [157, 158]. Although horizontal transfer of resistance genes and mutation phenomena readily occurs in biofilms [159, 160], resistance mechanisms such as efflux, target alterations, lowered permeability, or enzymatic destruction of antimicrobials are not key features in biofilm resistance. Four main biofilm resistance mechanisms have been described (Figure 9); reduced penetration of antimicrobials, altered microenvironment, slow growth and “persister”-cells [161].

*Figure 9. Illustration of how biofilm multicellularity in an extra-cellular matrix results in heightened bacterial defences. Reprinted with permission from CBE, Montana State Univ.*



In slow growing populations, cell wall synthesis is low, and antibiotics targeting key enzymes, such as  $\beta$ -lactams, will be ineffective [161]. The diffusion of antimicrobials through most biofilms is not as restricted as might be assumed. However, micro-gradients of oxygen, or pH in the biofilm could inactivate certain antimicrobials (e.g. aminoglycosides).

*Table 4. Proposed criteria for biofilm associated infections. Adapted from [162].*

1	Pathogenic bacteria are associated with a surface/ interface of a biomaterial or in chronically infected native tissues.
2	Direct examination of infected tissue or materials demonstrate aggregated cells in cell clusters encased in a matrix, which may be of bacterial and host origin (e.g. fibrin, collagen, fibronectin).
3	Infection is localized to a particular site in the host with occasional systemic signs secondary to the primary locus.
4	Recalcitrance to antibiotic treatment in spite of a demonstrated standard or routine susceptibility testing of the specific bacterium.
5	Culture-negative result despite a clinically high suspicion of infection.
6	Evidence of ineffective host clearance with bacterial aggregates demonstrated by the co-localization of inflammatory cells within discrete areas of the host tissue.

The means of bacterial detachment from the biofilm which may result in disseminated infection is thought to be regulated by quorum sensing and secretion of surfactants in staphylococci [163] (Figure 8). Table 4 underscores typical features in biofilm centered infections that should be considered in both diagnostics and treatment.

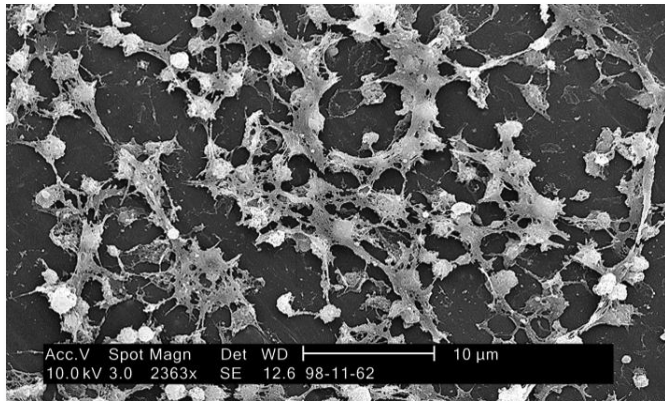
### 1.5.3 Staphylococci

Within the Micrococcaceae family some 50 species of staphylococci have been identified, many of which are colonizing the human skin and mucus membranes. They are broadly divided into two groups based on ability to produce coagulase. The only clinically significant species in the coagulase positive group is the frequent human pathogen *S. aureus*. Its wide arsenal of virulence factors; including 5 cytotoxins, 18 enterotoxins, two exfoliative toxins, several specific adhesins and enzymes translates into frequent invasive disease with high morbidity and mortality [164]. *S. aureus*, especially the small colony variant phenotype can survive intracellularly [165], thereby avoiding oxidative destruction and “extracellular” antibiotics. These strains clearly have a role in persistent and recurring infection. Conversely, the usually commensal coagulase negative staphylococci (CoNS) become pathogenic following disruption of host barriers, and immunological response,



increasingly common in modern medicine. Consequentially, CoNS causes a majority of nosocomial blood stream infections [166]. Central venous catheters are the prime source of these infections [167]. CoNS virulence is centred on cellular attachment, slime formation and suppression of inflammatory response of neutrophils [168]. As an exception *S. lugdunensis* express more potent exotoxin, and exoenzymes [169]. Other *Staphylococcus* spp. isolated in bio-material infection include *S. caprae*, *S. similans* [98], *S. capitis*, *S. warneri* and *S. haemolyticus*, which since the introduction of matrix assisted laser desorption ionization time of flight (MALDI-TOF) spectrometry, can be rapidly and reliably identified [170]. Several staphylococcal virulence factors are regulated by the chromosomal *agr*-locus [171]. A high *agr*-transcription upregulates the synthesis of exotoxins/-enzymes and downregulates the synthesis of surface proteins [172]. Biofilm shedding which is also regulated by the *agr*-gene cassette can cause metastatic infection or thromboembolic events. Staphylococcal attachment, and accumulation is largely mediated by the *ica*A<sub>4</sub>B<sub>3</sub>C<sub>2</sub>D<sub>1</sub>-encoded slime substance PIA (polysaccharide intercellular adhesion) [173] seen in Figure 10. Mutations of these genes lead to biofilm deficiency, reduced virulence and adhesion [174]. Interestingly, sub-inhibitory concentrations of the antimicrobial compounds tetracycline and quinupristin-dalfopristin have been shown to strongly promote *ica*-expression while oxacillin, clindamycin, gentamycin, teicoplanin and ofloxacin did not [153].

Figure 10.  
SEM image of  
an immature  
*S. aureus*  
biofilm  
formed on the  
inner surface  
of a venous  
catheter.  
Photo Credit:  
CDC/Janice  
Carr. Wiki-  
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## 1.5.4 Enterococci and other streptococci

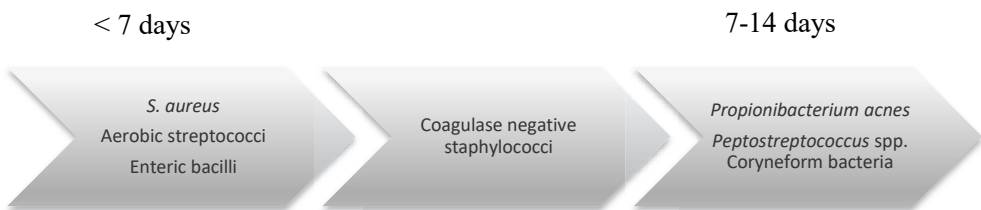
These two genera consist of facultatively anaerobic, Gram-positive, spherical cells arranged in pairs or chains. Enterococci are commensals of the intestinal tract of humans and animals. Antibiotic treatment and/or diarrhoea can result in transient, or long-term colonisation of the skin, especially in the case of vancomycin resistant enterococci [175]. Wounds are frequently colonized, but more seldom infected. Still, enterococci are important pathogens in urinary, bloodstream, heart valve and implant infections. *Enterococcus faecalis* is responsible for 4/5 of all enterococcal infections, and up to 1/3 of all catheter-associated urinary tract infections. The proportion of enterococci as sole causative agent in prosthetic joint infections is low, but may represent an increasing problem. Clinical presentation is similar to other pathogens of lower virulence, with pain as the dominating symptom (90 %) while fever is uncommon (10 %). Two and 5-year survivorship free of treatment failure, in the largest case series of enterococcal PJI (n=50) so far, were 82 and 73 % respectively. Sixteen per cent of the cases required chronic antibiotic suppression, worsening overall outcome. Addition of systemic aminoglycosides did not appear to improve outcome in the same study population [176]. Streptococcal classification is complicated, and traditionally based on blood-agar haemolytic capabilities and cell wall antigens. Many *Enterococcus* spp. are classified as group D streptococci. In the  $\beta$ -haemolytic group (A-C, E-H, K-M, O) some of the most important human pathogens can be found. Group A-streptococci, mainly *S. pyogenes*, commonly colonises mucus membranes and skin, where they can cause a wide range of pyogenic infections, and sometimes disseminated disease in uncompromised hosts. Many potent exotoxins and immune-evasive mechanisms have been identified in these streptococci [177]. Comparatively, streptococci are less prominent pathogens in biomaterial centered infections, except for the oral streptococci on dental implants, and in late prosthetic valve infection [178, 179].

## 1.5.5 Other bacteria

Enteric gram-negative bacilli are uncommon in delayed PJI, but more frequent in acute blood borne infection. In a Swedish study of 426 infected knee arthroplasties the proportion of all haematogenous PJI was 8.2 % [113]. Anaerobes were almost exclusively found in delayed and late infections.

Many bacteria of the human commensal micro-flora previously not identified in clinical specimen are now implicated in bio-device infections. Examples are *Peptostreptococcus* spp. and *Corynebacterium* spp. which together account for some 3 % of PJIs [113, 180]. They pose a challenge to clinicians in distinguishing pathogens from contaminants, often high natural resistances to antimicrobials and limited treatment experience. It is possible or even likely that culture negative infections around prosthetic joints, and other devices to a high degree are caused by fastidious organisms, or biofilm genotypes not easily detected on routine media. *Propionibacterium acnes* is a non-spore-forming, anaerobic, gram-positive commensal bacteria in hair follicles and sebaceous glands of the skin. Previously only recognized as a factor in acne vulgaris and a common contaminant of anaerobic blood cultures [181], it is now accepted as an important pathogen in delayed bio-device infections [182]. In fact, any predisposing bio-device should be taken into account in cases of invasive *P. acnes*. The increasing frequency of such infections is likely explained by prolonged tissue culturing (Figure 11), and other diagnostic improvements (discussed below). In the absence of bio-devices *P. acnes* may be isolated, often in conjunction with other anaerobes, in dental, brain or pleural abscesses. In orthopaedics, *P. acnes* is more frequently isolated in instrumented spinal fusions [183] and shoulder arthroplasties. Possibly, low-grade infection by *P. acnes* is one cause of presumed aseptic prosthetic joint loosening [184]. Despite that many *Propionibacterium acnes* strains readily produce biofilm, there is a high degree of *in vitro* penicillin susceptibility [185].

Figure 11. Laboratory growth times of important bacteria in implant infection [186].



## 1.6 Management of bone-implant infections

Awaiting the introduction of bacterial repellent surfaces, treatment of implant-associated infections must be aimed at eradicating the biofilm central in these processes. Surgery is the primary treatment in deep infection. All affected biomaterials and compromised tissues should be removed. The reduction of bacterial loads, and damaged tissues allow vascular ingrowth potentiating immune responses and antibiotics. Of further importance is the elimination of unprotected tissue surfaces (i.e. bone cavities). Extraction or revision of internal devices such as pacemakers [187], heart valves [188], and joint prostheses [189, 190] carries an increased morbidity and mortality, especially if repeated surgery is needed. One- or two-stage revision arthroplasties are standard in PJI. Short duration of symptoms, or recent primary arthroplasty allows for debridement, irrigation and plastic insert removal in staphylococcal infection [191], but staged revisions have better outcomes ranging between 75-100 % [98]. However, most data originate from single centre retrospective cohorts, sometimes without enough follow-up. Thorough removal of all hardware and cement, paired with aggressive debridement of all affected soft tissues, increases likelihood of a desired outcome. One-stage revisions are favoured in Europe in cases of limited complicating factors. In general, single stage revision could be considered when; i) the implant is stable, ii) soft tissue quality is good, iii) there is no need for bone grafting, and iv) the infection is monomicrobial, and susceptible to effective antibiotics [192]. In two-stage revision, a moulded cement spacer, loaded with antimicrobials, bridge the prosthesis-free period between surgeries. The spacer is however a foreign body inserted into infected tissues, and once depleted of antibiotics, vulnerable to re-emerging infection. Unfortunately, there are no prospective studies comparing the two procedures for superiority, but slightly better success rates in two-stage exchanges are seen in the Norwegian hip arthroplasty register [193]. Importantly, overall cure rates when hard-ware retention is attempted are lower, roughly 55 % in staphylococcal infection [194], although some studies including a recent Swedish register study of infected knee arthroplasties reported a 75 % success rate [195]. Although necessary, revision surgery also cause tissue trauma and increases the risk of complications [196], in a population where comorbidities are common [197]. Current antimicrobial recommendations for staphylococcal PJI, advocate parenteral treatment with a cell wall acting antibiotic for 2-6 weeks, followed by a prolonged per oral regimen of rifampicin and a companion drug. Background data supporting this are from animal implant models [198], and retrospective studies, in which the latter show less consistency in favour of rifampicin [194, 199]. The only RCT

is small, and has a mixed selection of implant types [200]. *In vitro* studies offer little clinical guidance due to conflicting, and/or method-dependent results [201] [202]. Definitions and recommendations can be found in the MSIS consensus documents [203], and the IDSA guidelines [192]. Early switch to oral antibiotic agents require acceptable bone penetration, and high oral bioavailability. The optimal treatment length is however not clarified, and the need for prolonged per oral treatment is challenged. There is mounting data from well-designed retrospective studies, of non-inferiority in 6-week, compared to 12-week treatment in both DAIR and staged exchanges [199] [204] [205], especially when a quinolone is combined with rifampicin against staphylococcal PJI [206]. In infection associated with internal fracture fixation, bone healing rather than microbial eradication is the goal. The two main approaches are; i) switching to external fixation with, or without local antibiotics and bone replacement, ii) suppressive antibiotics until stability is achieved, and extraction of plate or medullary nail. Similarly to PJI, adding rifampicin to the treatment of susceptible staphylococcal infection in the presence of a device is currently recommended [86].

### 1.6.1 Diagnostics

Acute or extensive osteomyelitis seldom requires histological verification, whereas the presence of an implant often complicate diagnostics, especially in cases of low virulence organisms. In peri-implantitis histopathological examination has excellent specificity but variable sensitivity (25–100 %), when acute inflammation is defined as  $\geq 5$  polymorphonuclear leucocytes per high-power field ( $\geq 400 \times$ ) [207]. Histopathological diagnosis is considered the standard, to which other tests should be compared [208]. Analysis of the peri-prosthetic tissues can differentiate non-infectious inflammation (e.g. abrasive, toxic, particle induced) [89], from peri-implant infection [209], but no in-depth information on causative agent or antimicrobial resistance can be obtained. Ideally, all diagnostics of infections involving bone, and associated structures should therefor also include non-contaminated tissue samples for microbial analyses. Joint fluid is less sensitive than tissue culture, but may yield a preoperative diagnosis, and early guidance for antibiotic treatment. A few studies have demonstrated comparable, or better accuracies than tissue cultures, when joint aspirates are cultured in blood flasks [210].

Percutaneous interface biopsies have been proposed to complement preoperative diagnostics in cases of dry-aspirations or negative joint fluid cultures. This procedure is more invasive but has a higher diagnostic accuracy [211] than joint aspirations. Sensitivities and specificities for tissue and joint fluid cultures from two studies are summarised in Table 5. Swab cultures from wound, or fistula are likely to be misrepresentative in deep-seated infection, and should generally be avoided [212]. An exception might be wound cultures in acute postoperative prosthetic joint infection yielding *S. aureus* [213]. Fastidious organisms are relatively more common in biomaterial infections, which emphasizes the need for tissue cultures. Thus, the method of choice for the etiological diagnosis of prosthetic joint infections, is peri-prosthetic tissue culturing. Three to 6 tissue biopsies yielding three, or more indistinguishable microbes, have a > 99% specificity for infection [214], and 2 or more positive samples acceptable accuracies for virulent organisms. The biofilm growth mode often leads to prolonged growth times, and complicates interpretation.

Table 5. Comparison of accuracy in the detection of prosthetic joint infection between different analyses of peri-prosthetic samples.

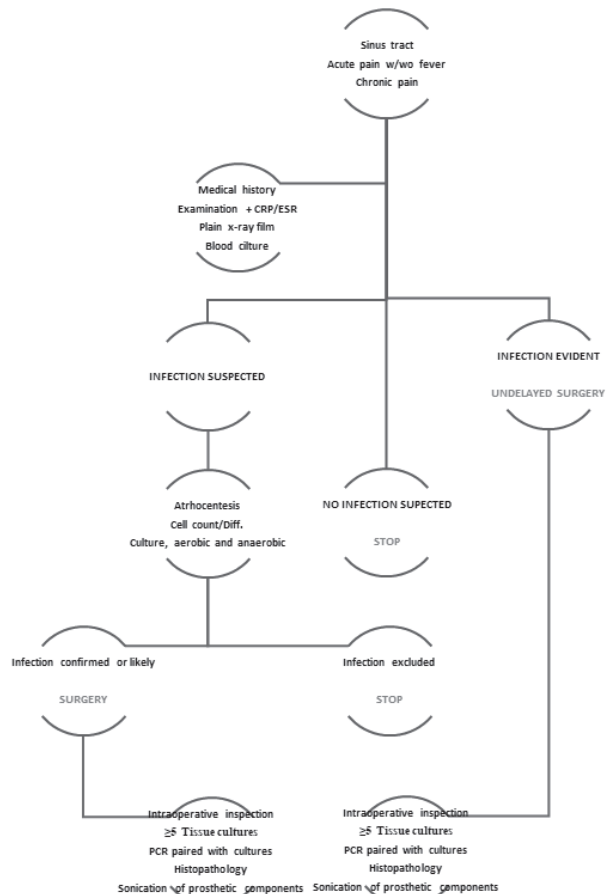
Test	Müller et al 2008. N=50, 37 PJI [215]			Trampuz et al 2007. N=331, 79 PJI [216]		
	Histology	Tissue culture ≥ 1 pos/>'>2'	Joint fluid culture	Tissue culture ≥2 pos	Sonicate-fluid culture ≥ 10 CFU	Joint fluid culture
Sensitivity	0.95	0.78	0.57	60.8	72.2	0.56
Specificity	0.92	0.92	0.5	99.2	98.8	0.98
PPV	0.97	0.96	0.78	96.0	95.0	90.0
NPV	0.86	0.63	0.29	89.0	91.9	88.3
Accuracy	0.94	0.82	0.54	N/A	N/A	N/A

'>2 positive cultures in low virulence organisms.

Figure 12. Diagnostic flow-chart of prosthetic joint infection. Adapted from Osmon 2013 [192].

Culture-matched PCR might enhance detection of fastidious organisms, or if antimicrobials are given prior to sampling. Interestingly, refined culture and PCR methods, are reported to reliably detect bacteria in about 5 % of presumed aseptic failures [99], which appear to be in line with reduced revision rates for aseptic loosening following antibiotic prophylaxis [63]. It is however important to note that PCR-techniques are vulnerable to contamination, which might inflate diagnostic yield. Sonication of prosthetic components, and cement [216] may improve sensitivities, but interpretation is often challenging, especially when poly-microbial.

Various bio-markers have been evaluated in the preoperative diagnosis of PJI. In a large meta-analysis from 2010 comparing IL-6, CRP, ESR and WBC-count, IL-6 had the highest diagnostic accuracy followed by CRP and ESR [217]. Elevated CRP or ESR is a proposed criterion for the definition of prosthetic joint infection [218]. Various studies have shown that CRP above 13.5 to 32 mg/L discriminate septic from aseptic failure [219], even though low virulence infection might go undetected [220]. Furthermore, CRP greater than 120 mg/L has been identified to be an independent marker for treatment failure in early prosthetic joint infection [221]. There is an increasing trend towards multi-disciplinary, and algorithm-driven diagnostics and treatment of prosthetic joint infections [192]. Management in the presence of a bio-implant should aim at (i) high suspicion of infection leading to an



appropriate diagnostic work-up, (ii) intraoperative tissue sampling for reliable treatment decisions, (iii) surgical intervention removing compromised tissues, and implant-components, and iv) treatment with intravenous, or highly bioavailable per-oral antimicrobials. An adapted diagnostic flowchart is shown in Figure 12.

## 1.6.2 MIC and MBEC

Minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs) are the lowest antimicrobial concentrations that will inhibit visible microbial growth after overnight incubation, and prevent growth on antibiotic-free media respectively. MICs are used to confirm resistance in clinical labs, and to determine *in vitro* activities of new antimicrobials in order to determine MIC breakpoints. Routine antimicrobial susceptibility is determined by disc diffusion tests, whereas MIC require serial liquid broth dilutions, or an antibiotic gradient strip test. Both methods challenge free-living bacteria *in vitro*, disregarding biofilm properties. In acute infection, MIC may add therapeutic guidance, but is likely to be misrepresentative in chronic, or device-related infections involving biofilms [222]. *In vitro* assays for determining minimum biofilm eradication concentrations (MBEC) are developed [158]. Their clinical usefulness has not been evaluated, but MBEC is likely to better reflect the antimicrobial concentrations needed to effectively treat biofilm infections. However, surpassing these concentrations without systemic toxicity could sometimes only be achieved by local drug administration via dissolving or removable beads [223], impregnated cement spacers [224], or bone fillers [83].

## 1.6.3 Antimicrobials

Treatment choices should be guided by both susceptibility patterns, and how well an antimicrobial agent performs in the infected tissue compartment in question. For instance, the anaerobe milieu of deep purulent infection can be expected to markedly reduce efficacy of aminoglycosides [225]. In the fibrous synovial-like tissue often surrounding the joint prosthesis [226], systemic antimicrobials are likely to reach lower concentrations compared to serum and



synovial fluid. In normal cortical and cancellous bone antimicrobial penetration varies.  $\beta$ -lactams and vancomycin concentrations rarely exceed 20 % of serum concentrations while ratios for fluoroquinolones, clindamycin, fusidic acid, metronidazole and rifampicin ranges between 30 - >100 % [227]. Some antimicrobials may reach higher concentrations in infected bone. Accurately measuring antibiotic bone concentrations is difficult, illustrated by substantial study differences, in which sampling and analysis techniques vary. Bone homogenates may inaccurately reflect unbound drug concentrations, or uneven (organic/inorganic or intra-/extracellular) distribution of antimicrobials [228]. In Table 6 mean bone mg/kg to serum mg/L ratios of common antimicrobials are listed. Other aspects of antimicrobial performance such as mode of action, intracellular efficacy and interaction with other drugs need to be considered. Completely resolving implant infections (microbiological resolution) with antimicrobials alone, almost uniformly fails. In circumstances where debridement or removal of the infected implant is not possible (e.g. infirm patients or technical reasons) suppressive antimicrobials should be considered. When mainly penicillin is used, eventless 24-month suppression can be achieved in 60 % of 80-90-year old patients [229].

*Table 6. Selected reports of antimicrobial bone penetration expressed as bone to serum concentration ratio. Adapted and simplified from [228].*

Agent	No. studies	Time range (h) since last dose	Mean bone:serum [mg/L]
Ampicillin	3	0.25-4	0.11-0.71
Azithromycin	2	0.5-6.5 days	2.5-6.3
Cefotaxime	1	0.75-4	0.02-0.28
Cefuroxime	7	0.2-6.5	0.09-0.55
Cefuroxime (infected bone)	1	1	0.04-0.08
Ciprofloxacin	3	0.5-13	0.27-1.2
Ciprofloxacin (infected bone)	1	2-4.5	0.42
Clindamycin	4	1-2	0.21-0.45
Flucloxacillin	3	0.3-3	0.12-1.2
Fusidic acid	1	5-10 days	0.46-0.94
Fusidic acid (infected bone)	2	1-13	0.12-0.33
Levofloxacin	2	0.7-2	0.36-1
Linezolid	2	0.5-1.5	0.4-0.51
Linezolid (infected bone)	1	0.9	0.23
Rifampicin	3	2-14	0.08-0.56
Rifampicin (infected bone)	1	3.5-4.5	0.57
Vancomycin	6	0.7-6	0.05-0.67
Vancomycin (infected bone)	1	1-7	0.27

Several in vitro studies have shown that certain compounds such as rifampicin and quinolones perform better against biofilms [157]. The degree of resistance to antimicrobials within a species varies more in biofilm, than in planktonic growth [230], adding to the outcome unpredictability of implant infections. Other than for rifampicin based regimens in staphylococcal implant infection, there is little high-quality clinical evidence on other antimicrobials for bone infections [231].

#### *Rifampin/Rifampicin*

Rifampicin is a potent staphylococcal antibiotic with high bone penetration, intracellular activity against phagocytized “persisters”, and ability to eradicate the stationary phase bacteria on biomaterials and devitalized bone alike. Intestinal absorption is delayed and incomplete when taken with food, but may also be variable when taken on an empty stomach mainly due to drug formulations [232]. It is mainly eliminated by biliary excretion, but the proportion of renal excretion increases with higher doses. Repeated daily dosing however, results in half-life reduction, probably through metabolic enzyme induction [233], which should be kept in mind as twice daily dosing is currently recommended for staphylococcal PJI [192]. Rifampicin is a very potent inducer of cytochrome p-450 hepatic enzymes and p-glycoproteins, leading to an increased break down and cellular efflux of several compound in important drug classes such as corticosteroids, anti-coagulants, opioids, protease-inhibitors, antibiotics, antifungals, and many more [234]. Common side-effects are mild-moderate liver toxicity, gastro-intestinal discomfort and rashes. Rare, but serious side-effects include severe liver damage, and bone marrow suppression. Even though rifampicin is widely used in treatment of prosthetic joint infections, it is advisable to consider potential drug toxicity, interactions and, if the risk is outweighed by beneficial effects. Below follows a brief description of key features in commonly used antimicrobials for the treatment of orthopaedic device infections.

#### *Fusidic acid*

Fusidic acid is derived from the fungus *Fusidium coccineum* and has been in clinical use since the 1960s. It inhibits protein synthesis by binding elongation factor G, a unique mechanism without cross resistance to other antimicrobials. Primarily active against staphylococci including methicillin resistant, and vancomycin intermediate *S. aureus* (MRSA and VISA), and to a lesser degree against *Corynebacterium* spp, and *Peptostreptococcus* spp. It has excellent bone penetration making it valuable in the treatment of osteomyelitis, septic arthritis and prosthetic joint infection [235]. Fusidic acid is not to be used without a companion antimicrobial agent due to fast emergence of resistance

mutations [236, 237]. Currently, the rate of fusidic acid resistance in Sweden is below 10 %, and well above 10 % in *S. aureus* and coagulase negative staphylococci respectively [238]. Clinical MIC-breakpoints for susceptibility is  $\leq 1$  mg/L. *In vitro* studies of interaction with other antimicrobials, have shown antagonism with fluoroquinolones, and in time-kill measurements synergy with rifampicin [239].

### *Fluoroquinolones*

Modern fluoroquinolones have a wide antibiotic spectrum, high per-oral availability, low toxicity, and good penetration into most tissues including bone [240]. Three fluoroquinolones with documented efficacy in bone, and prosthetic joint infection are approved in Sweden, namely ciprofloxacin, levofloxacin, and moxifloxacin. The fluoroquinolones accumulate intracellularly, and interact with bacterial DNA gyrase and topoisomerase IV essential in DNA replication, transcription, recombination and repair [241]. Chromosomal mutations in these enzymes, and drug efflux are also the main mechanism of fluoroquinolone resistance [242]. Globally fluoroquinolone resistance is much more common in methicillin resistant staphylococci, than in sensitive (MSSA). Based on MIC<sub>90</sub> values, the approximated order of activity against MSSA is moxifloxacin > levofloxacin > ciprofloxacin. Overall activity against *Enterococcus* spp. is weak, especially against *Enterococcus faecium*, where moxifloxacin is more effective than levofloxacin and ciprofloxacin [243]. Ciprofloxacin has the best overall Gram-negative efficacy, and is the only available fluoroquinolone recommended in the treatment of infections caused by *P. aeruginosa*. Absorption is excellent for most fluoroquinolones even when taken with food, but chelation to multi-valent cations in co-administrated drugs reduces uptake. When taken with rifampicin, there is a one third reduction in overall moxifloxacin exposure [234], and to a lesser degree for ciprofloxacin [244]. Elimination is mainly renal except for moxifloxacin. Important class side-effects include QT-prolongation and tendinopathy. Paediatric use is restricted because of developmental cartilage damage seen in animal studies.

### *Cloxacillin/Flucloxacillin*

Cloxacillin (for i.v. administration), and flucloxacillin (p.o.), are the two main isoxazolyl penicillins used in Sweden. They play important roles in the in- and outpatient treatment of acute *S. aureus* bone, and joint infections, although superiority over other regimens has not been tested [245]. The isoxazolyl-penicillins, like other penicillins, are bactericidal against Gram-positive cocci through time dependent inhibition of bacterial cell wall synthesis. Against penicillin G susceptible bacteria, they are however less effective than

penicillin G. Penetration into normal bone and synovia is poor, but higher concentrations can be expected in inflamed tissues. The required time above MIC varies, depending on drug, pathogen and site of infection, but is generally 40–50% of the dosing interval, while outcome in severe or complicated infection may be improved by frequent, or continuous infusion [246]. Toxicity is low, and reduced dosing in severe renal deficiency is seldom necessary.

#### *Gentamicin/Tobramycin*

Belonging to the aminoglycosides, gentamicin and tobramycin inhibits bacterial growth by binding the 30S subunit of bacterial ribosomes, which causes misreading of the genetic code and blocks protein synthesis. Against Gram-negative bacteria they also interfere with cell membrane integrity. Aminoglycosides exhibit concentration dependent bactericidal affect against aerobic Gram-negative bacilli and staphylococci. Reports from recent years state, that some two thirds of CoNS strains isolated from PJI are insusceptible [247], and that aminoglycoside resistance is more common in MRSA than in methicillin sensitive *S. aureus* [248]. In many cases this is likely overcome by high peri-prosthetic concentrations from impregnated cement, and other local vehicles. Aminoglycosides are not intestinally absorbed, but bioavailability after intramuscular injection is close to 90 %. Normal half-life is about 2 hours, but may in severe renal failure be prolonged to 40–50 hours. Gentamicin penetrates well into synovial fluid, but bone to serum concentration ratios range from 0.057 to 0.75 at 1–12 hours after of 1mg/kg, and tobramycin mean bone to serum concentrations is 0.13 at 0.3 hours, and 0.091 at 14.3 hours [228]. Nefro- and ototoxicity are the most important side effects.

#### *Vancomycin*

Vancomycin is a large glycopeptide with complex pharmacokinetics, an approximately 90 % renal elimination, and variable tissue penetration. It is not absorbed intestinally [249]. Vancomycin is routinely administered following revision surgery in low virulence prosthetic joint infection, and often part of the initial treatment in culture negative PJI [250]. Although widely used against methicillin-resistant infection, the effectiveness of vancomycin has come into question. MIC values  $\geq 2$  mg/L are associated with increased overall treatment failure and mortality in blood stream infections [251]. Achieving sufficient through concentrations, important for efficacy without renal toxicity, is challenging. Considering poor bone penetration and performance in high inoculum situations it should be reserved when other options are inferior.

### *Clindamycin*

Clindamycin inhibits protein synthesis by binding to the 50S subunit of the ribosome, and is primarily bacteriostatic against most Gram-positive aerobic bacteria (an important exception is *E. faecalis*), and many anaerobes. Due to high per oral absorption, and good penetration into abscesses, and bone it is a widely used option in the treatment continuation phase of bone and joint infections [245]. Higher bone penetration, and faster tissue sterilization than beta-lactams, have convincingly been demonstrated in animal models [252]. Clindamycin is chiefly eliminated by hepatic mechanisms. Common side effects are related to fungal, and bacterial overgrowth. Co-administration of Linezolid, and other antibiotics that bind to the same ribosomal subunit should be avoided.

### *Linezolid*

Belong to new class of bacterial protein synthesis inhibitors. High bacteriostatic efficacy against staphylococci and enterococci with MIC<sub>90</sub> - values are 4 and 2 mg/L respectively. No cross-resistance to other antimicrobials has so far been demonstrated. Complete intestinal absorption and bone tissue concentrations are around 40 % of the plasma concentrations [253]. Elimination is mainly renal, and a 10 – fold increased exposure to main metabolites has been measured in renal impairment requiring hemodialysis. Reversible bone marrow suppression is seen in some 7 % of patients on prolonged courses, and a proposed mechanism is through vitamin B<sub>6</sub>-depletion. Linezolid-induced peripheral neuropathy may be irreversible, but appear uncommon [254]. Linezolid has weak and reversible monoamine oxidase inhibitory effects, which may cause serotonin syndrome if co-administered with drugs that inhibits serotonin reuptake [255]. Linezolid has demonstrated poor efficacies in rat/murine models of *S. aureus* osteomyelitis, and soft tissue infection respectively [256]. In a guinea pig model of biomaterial infection linezolid, and linezolid + rifampicin was both inferior to levofloxacin + rifampicin [257]. Interaction with rifampicin has been demonstrated reducing the concentration-time curve of linezolid by one third [258]. Observational efficacy in osteomyelitis range between 55-75 % in small retrospective case series where often Linezolid has been given as a last resort (e.g. multi drug resistance, previous failures etc.). In two prospective series in patients with bone and joint infections without prosthetic materials, success rates were 80-100 % [259], and in PJI (DAIR in 78 %) salvage treatment with linezolid + rifampicin had a 69 % remission rate at the 2-year follow up [260].

## **2 AIM**

The general aim of this thesis was to define, and quantify infections involving bone tissues around percutaneous titanium implants, used in the treatment of femoral amputees.

The specific aims of the included studies were:

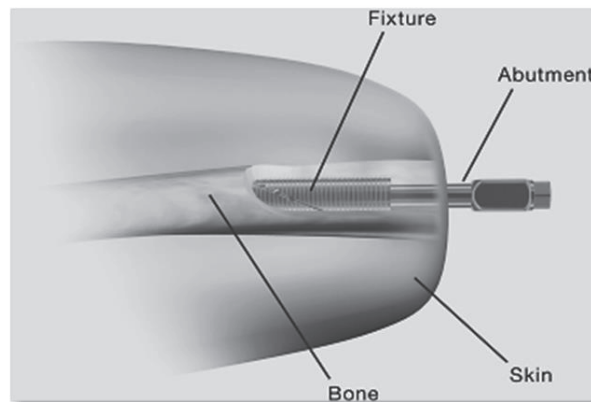
- To describe the bacterial flora, the clinical presentation and the frequency of superficial and deep infection.
- To evaluate biofilm formation, antimicrobial resistance, and clinical outcome in femoral implant associated osteomyelitis.
- To quantify the risk, and characterize the impact of osteomyelitis in patients with transfemoral amputations treated with osseointegrated titanium implants.
- To describe conservative treatment outcomes in femoral implant associated osteomyelitis.

## 3 PATIENTS, MATERIALS & METHODS

### 3.1 Implant design

The main components of the implant system are the threaded fixture, the abutment, and the abutment screw (Figure 13). The fixture threads engage the inner aspect of the cortical bone. The skin-penetrating abutment is interference-fitted into the distal part of the fixture, and secured by an abutment screw. If bent, or fractured it can be replaced. The fixture chamber is separated from the marrow cavity by an air-tight screw, which can be removed when diagnostic marrow sampling is indicated.

*Figure 13. Drawing of implant components and their relation to the residual femoral shaft and surrounding soft tissues. Reprinted with permission of Drs. Y. Li and R. Brånemark.*



### 3.2 Surgical method and rehabilitation

Following introduction in 1990, several surgical improvements have been made. For femoral implants, surgical technique was standardized in 1998. Drawing from experience with bone anchored hearing aids, all hair follicles around the abutment is removed, and skin is directly attached to the end of the underlying femoral shaft. This optimizes the dermal milieu and reduce skin movement, otherwise increasing the risk for local skin inflammation or infection. Furthermore, a 20-mm embedment of the fixture is now routine to

reduce the risk of implant exposure from occasional resorption of distal cortical bone [261]. All patients in this work were fitted with the complete implant system by two separate surgical procedures. The fixture is inserted into the marrow cavity of the residual bone. Intimate contact between fixture threads and inner cortex is considered a prerequisite for good osseointegration. Early loading is avoided not to jeopardize healing, excess micromotion has been shown to be incompatible with osseointegration [262]. A cylindrical iliac crest bone graft can be used in cases of inadequate residual bone length, or distal osteopenia from previous lack of skeletal loading. The implant is left to integrate with bone tissue, currently for six months, before a replaceable percutaneous extension (abutment) is inserted. In all but a few cases, antibiotic prophylaxis at first, and second surgeries was i.v. cefuroxime 1.5 grams t.i.d for one day, followed by cefadroxil 0.5 grams b.i.d. until skin healing.

### 3.3 Definitions of infection

There are no previously accepted definitions of superficial, or deep infection in this novel treatment method. Classifications of pin tract infection [263] are not usable. For research purposes, we established culture based criteria adapted from definitions of prosthetic joint infections, further discussed below. Bacteria were considered belonging to the same strain, if indistinguishable by standard methods and antibiograms. This might lead to misclassification for common skin colonizers with similar antibiotic susceptibilities, mainly within the CoNS group. Our definition of implant associated osteomyelitis (Table 7) does not account for grade of infection, or overall physical condition of the patient, as in the Cierny-Mader osteomyelitis classification. The main reason for this is the limited site, and the good comparative health of the patient cohort. In *papers III* and *IV* osteomyelitis were grouped according to our definitions, but were jointly included in survival and outcome analyses to avoid underestimation. Semi-stratification is reasonable, given that accuracy of method-specific sampling is not known. Radiographic evidence of osteomyelitis includes osteolysis with, or without periosteal sclerosis around a previously integrated implant (Figure 14). Since plain x-ray has a low sensitivity in early bone infection, osteomyelitis was considered definite in the few patients with acute symptoms and positive tissue cultures. The accuracy of marrow cultures alone in diagnosing



osteomyelitis, need histopathological verification. Possibly a distinction between cases with, and without proven bone pathology should be made, and studied regarding treatment outcome. Since early diagnosis, and limited tissue involvement likely lead to better treatment outcome, an operational algorithm should aim at early identification.

*Table 7. Definitions of osteomyelitis around the implant system*

Type of infection	Signs and symptoms	Tissue cultures	Positive radiograph
Definite implant infection	Yes	$\geq 2/5$ Positive	Yes
Probable implant infection	Yes	$< 2/5$ Positive	Yes
Possible implant infection	Yes	Negative	Yes

*Figure 14 A-B. A. Plain x-ray showing several osteolytic zones (arrows). B. Computed tomography image showing extensive osteolytic zones, patchy osteosclerosis and cortical destruction (arrowhead).*



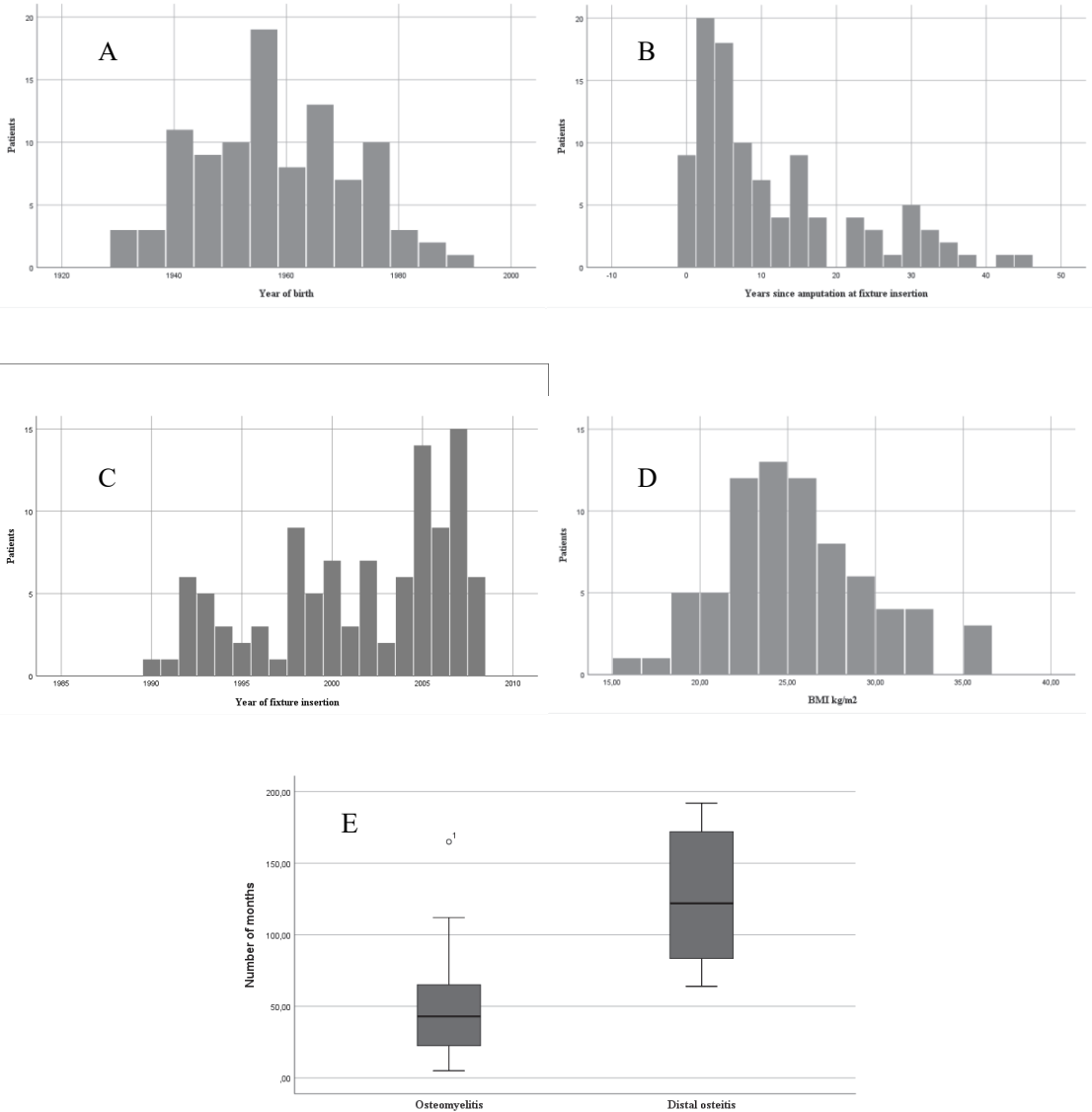
### 3.4 Patients

The total number of patients in the four papers is 106 (39 women). Per-paper, and all-patient demographics are presented in Table 8, and Figure 15 A-E respectively. Study designs, and cohort overlaps are shown in Figure 16. 107 patients were transfemorally amputated, and the remaining seven were upper extremity (3 humeral, 3 lower arm), and one tibial.

*Table 8. Patient demographics and selected outcomes of papers I-IV.*

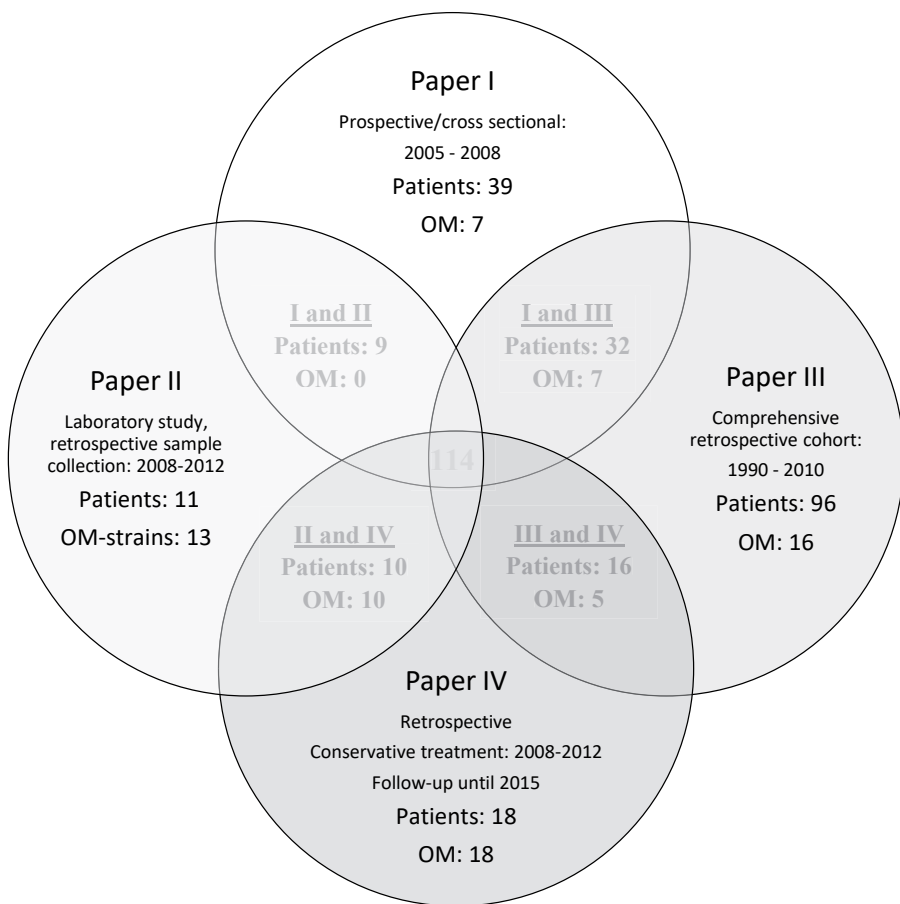
	Paper I	Paper II	Paper III	Paper IV
No. of patients (women/men)	39 (18/21)	11 (3/8)	96 (36/60)	18 (7/11)
No. implants	45 (2 bilateral)	11	102 (6 bilateral)	18
End of study median age in years (range)	52 (31-77)	42 (22-71)	67 (21-79)	48 (22-71)
Limb amputated; femoral/other	33/12	11/0	102/0	18/0
Amp. reason: trauma/tumor/infection/other	27/10/2	10/1/0	71/20/5/6	7/6/5
Femoral stump length; short/medium/long	N/A	3/7/1	34/60/8	5/12/1
Median no. months since insertion (range)	54 (3-132)	47 (2-143)	74.5 (18-235)	3.75 (2.5-12)
Osteomyelitis; definitive/prob./possible	2, 7 (follow-up)	8/2/1	12/3/1	13/5/-
Median no. months on antibiotics (range)	N/A	4 (1.5-8)	N/A	4.4 (0.5-12)
Median years to osteomyelitis from insertion	N/A	N/A	2.6 (0.3-13.8)	5 (0.3-13.8)
Implant extraction due to osteomyelitis	1	4	10	6
Other cause implant extraction	-	1	5	-

Figure 15 A-E. **A.** Distributions of birth years. **B.** Distribution of durations between amputation, and implant surgeries. **C.** Variation of number of implant surgeries performed between 1990 and 2008. **D.** Distribution of body mass indexes at the time of implant surgery (mean  $\text{kg/m}^2$  25.5,  $SD$  4.3). **E.** Comparison of time distributions from implant surgery to diagnosis of definitive/probable osteomyelitis, and distal osteitis respectively ( $p < 0.001$ ).



In papers II-IV, only patients treated at the femoral level were included. Reasons for amputations varied, but trauma and tumour dominated. There were fewer co-morbidities, and better overall health in the patient cohort compared to the average prosthetic joint recipient [197], because of lower mean age and preoperative selection. In the first study, patients were included prospectively and consecutively. In the second and fourth retrospective studies, the patient cohorts included all eligible patients treated from method introduction until the start of data retrieval. In the third study, patient selection was dictated by retrievable freeze-dried tissue cultures, from the hospital microbiology lab.

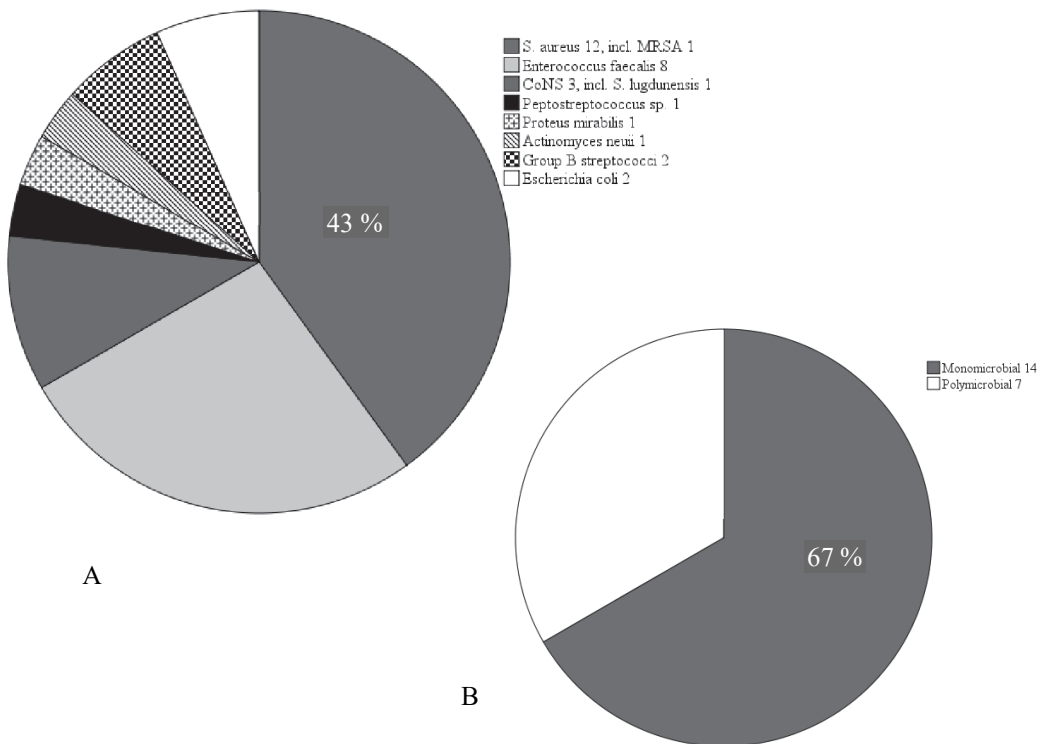
Figure 16. Diagram of study design and population overlaps in papers I-IV



### 3.5 Culture and antibiotic susceptibility testing

In *paper I, III, and IV* susceptibilities were determined by standard disc diffusion test, after culturing on solid, and liquid routine media. For swab cultures, solid plate media was used. All culturing was performed by experienced lab technicians at the clinical microbiology lab at Sahlgrenska University Hospital. By these methods, growth of indistinguishable bacteria in 2 or more intraoperatively obtained bone or bone-marrow samples, were required for diagnosis of definitive osteomyelitis (Figure 17). Although often yielding the same results, intraoperative swabs were disregarded.

*Figure 17 A-B. A. Pie chart of the distribution of bacteria causing definitive or probable osteomyelitis associated to 1 humeral and 20 femoral implants. B. Proportion of infections caused by 2 or 3 organisms.*



## 3.6 Biofilm analyses (paper II)

Biofilm formation by staphylococci and other bacteria is described above. Due to variability in protein expression, detection of genes governing these processes does not fully explain biofilm properties and behaviour. Complementary phenotypic methods suffer from analytical limitations and inaccuracy in detecting bacterial adherence. In *paper II* the methods outlined below were applied to determine biofilm properties of clinical strains from osteomyelitis in percutaneous femoral implants. Bacterial prerequisites for biofilm formation are discussed above. There are a number of assays, in which biofilm can be grown and detected [264]. In *paper II*, staphylococcal, and enterococcal biofilms were cultured in a microtiter plate assay without continuous supply of nutrients or air. Such static assays produce early-stage biofilms suitable for investigating adherence, colony forming and chemical signals involved those processes [265]. For the production of mature biofilms continuous-flow, or supplementation systems are needed. In the microtiter staining assays of *paper II*, strains were cultured on 5% horse blood Columbia agar overnight. Incubation temperatures were 37°C in all assays. Tryptic soy broth (Trek Diagnostic, East Grinstead, UK) stem solutions, were adjusted to  $10^8 \times \text{ml}^{-1}$ , by species-specific absorbance measurements, and diluted to assay inoculum concentration of  $10^5 \text{ CFU} \times \text{mL}^{-1}$ . Absorbances was measured in a FLUOstar Omega plate reader, (BMG Labtech, Offenburg, Germany). Assays were repeated three times to detect inconsistencies. All assays used in *paper II* are briefly described below.

### *Calgary biofilm device assay*

The Calgary biofilm device assay (CBD), is a commercially available assay for growing biofilms on the pegs of a 96-pegged lid, which fits into a 96-well plate containing bacterial colony suspensions. A tilt table provide the shear force required for biofilm growth during 24-hour incubation. The biofilms were further incubated with gentamicin, clindamycin, vancomycin, linezolid, ciprofloxacin, oxacillin, fusidic acid, ampicillin, trimethoprim/ sulfamethoxazole, and rifampicin in a custom-made panel (Sensititre<sup>®</sup>, TREK<sup>®</sup>, Cleveland, OH, USA) in common MIC-ranges, and a few extreme concentrations. Biofilms were dislodged by sonication, and re-incubated in a recovery plate. The minimal biofilm eradication concentrations (MBEC), were finally determined by ocular and optical density measurements.

### *Crystal violet assay*

The crystal violet assay (CV) is a rapid and easy staining method to quantify early biofilm accumulation in a microtiter plate. Four x 200 mL of the strain solutions were incubated in flat-bottom 96-well plates (Nunc, Thermo Fisher, Gothenburg, Sweden). The plate was inverted, rinsed, and stained with 0.1% crystal violet solution (Scharlau, Sentmenat, Spain) for 10 min, then rinsed and eluted in 95% ethanol. The cutoff value (OD<sub>c</sub>) was defined as three standard deviations above the mean OD of the blank (TSB). The strains were classified as previously described by Christensen et al. [266], and further categorized by our own biofilm biomass scoring (range 0–3).

### *Syto9 assay*

Syto9, a green-fluorescent nucleic acid stain, emits a markedly enhanced signal when bound to nucleic acid. The signal strength is similar for live and dead cells [267]. It was used to target bacterial cells within the biofilm. The strains were cultured under the same conditions and concentrations as for the microtiter plate assay. From a working solution of Syto9: saline (3:1000) (Molecular Probes, Eugene, OR, USA), 200 mL was added to each biofilm grown on the bottom of a 96 microtiter well plate and incubated in the dark at room temperature for 30 min. The wells were rinsed in a water bath and read in a plate reader for fluorescence top reading, using an excitation filter of 485 nm and an emission filter of 520 nm.

### *Congo red agar method*

The Congo Red Agar (CRA) method, is a quick, and easy detection-test for biofilm formation, based on the Congo Red stain mixed in solid medium. Biofilm-producing test strains form black colonies. Compared to other common detection methods, studies on nosocomial infection strains report lower sensitivity, and comparable specificity (90-100 %) for CRA [268, 269]. CRA plates were prepared by adding 0.8 g of Congo red (Sigma) and 36 g of saccharose (Sigma-Aldrich, St. Louis, MD, USA) to 1 L of brain heart infusion agar (Oxoid, Basingstoke, Hampshire, UK). Strains were cultured on CRA plates and incubated for 24 and 48 h at 37°C. A six-colour reference scale was used in assigning colonies biofilm formation or not.

### *Detection of *icaA* and *icaD* genes*

The products of the chromosomal intercellular adhesion genes (*icaADBC*) synthesize polysaccharide intercellular adhesin (PIA), essential for biofilm formation, especially when *icaA*, and *icaD* is expressed simultaneously [269]. Bacterial DNA was extracted by a commercial DNA-elution kit (Sigma, St.

Louis, MO, USA), following centrifugation of individual colony suspensions ( $10^8$  CFU  $3 \text{ mL}^{-1}$ ). The products of a commercial multiplex-PCR (Qiagen GmbH, Hilden, Germany) was identified in an 2.2% agarose gel electrophoresis (Lonza, Rockland, ME, USA).

### 3.7 Statistics

In *papers I and IV*, descriptive statistics were used for clinical outcome, and inflammatory markers. In *paper II*, comparative statistics were performed per species, and jointly. MIC versus MBEC per species was analysed with one-way ANOVA. Differences between MBEC/MIC ratios and biofilm score, biomass score and slime score respectively, were compared by nonparametric ANOVA, and Mann–Whitney. A number of bivariate correlations between various biofilm and clinical outcome scores were also undertaken. In *paper III*, statistical end-points were first implant osteomyelitis, and first implant extraction due to osteomyelitis. The Kaplan-Meier estimator was used to calculate the risk of osteomyelitis and extraction with time. Based on data at the time of implant insertion, risk factor correlation was performed with the Cox proportional hazard model. A hazard ratio for cumulative abutment replacements was obtained through a time-modified Cox analysis. Alongside analyses in the papers a few additional cross-tabulations were run for risk factor associations. In all tests differences were considered significant at a probability less than 0.05. In *paper I, II, and IV* SPSS Statistics 21 (IBM Corporation, USA), and in *paper III* GraphPad Prism 6 (GraphPad Software Inc, San Diego, CA, USA), and SAS (SAS Institute Inc, Cary, NC, USA) statistical software were used to compute the data.



## 4 RESULTS

### 4.1 Paper I

Colonizing patterns, and infection frequencies were not known in this percutaneous implant method, presumably vulnerable to infection. This first paper was an initial survey to that end. The frequencies of possible/probable and definite implant associated deep infection (i.e. osteomyelitis) were 5 % at inclusion, and 18 % after a mean 3- year follow-up. In this initial work, no differentiation between infection involving bone in proximity to the skin stoma, and osteomyelitis was made. However, the former was subjected to debridement and prolonged antibiotics, strongly supporting significant bacterial involvement. Standardized sampling at the skin/implant interface yielded similar patterns at the two time-points, with *S. aureus* in 44 %, and 63 % respectively. There was an association (Chi square;  $r=6.62$ ,  $p=0.01$ ) between redness at the skin stoma, and growth of *S. aureus* vs. no growth or other bacteria (not included in paper). Furthermore, abundant growth was more common in *S. aureus* than in coagulase negative staphylococci, the second most common colonizer. Faecal flora was foremost represented by group B streptococci, while *Enterococcus spp.* and enteric rods were less common.

### 4.2 Paper II

It has been established that biofilm is central in most biomaterial promoted infections [222]. Informed treatment decisions on antimicrobial, dosing, administration, and duration in such infections will improve cure rates, and reduce morbidity. To that end, a fast, reliable and inexpensive clinical tool is ultimately needed. Biofilm formation has not previously been demonstrated in clinical strains from osteomyelitis associated with percutaneous femoral implants. Antibiotic resistance in biofilm assays is often increased, sometimes 1000-fold, but varies substantially. This is not solely attributable to the amount of biofilm formed [222, 270]. Given that biofilm decreases the likelihood of

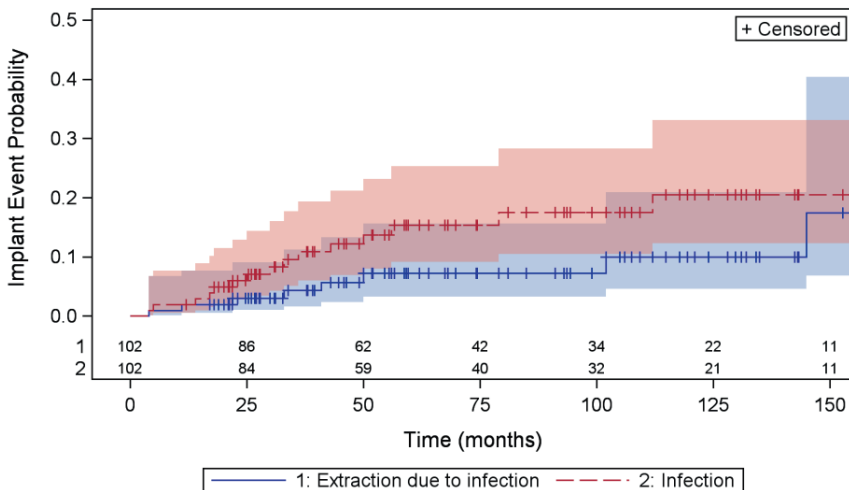
treatment success, and clinical biofilm strains display different degrees of antimicrobial resistance, it is likely that clinical outcome correlates to measurable levels of MBEC. Furthermore, MBEC antibiograms do not proportionally correspond to MIC, which also support MBEC-testing prior to antibiotic treatment in appropriate cases. A novel combination of the Calgary Biofilm Device [158], and a commercial susceptibility MIC plate (Sensititre®) was tested, on the most common bacterial isolates (7 staphylococcal and 6 enterococcal) in femoral percutaneous implant osteomyelitis. Biofilm mass, biofilm cell content, and slime production was quantified by crystal violet, fluorescence, and by Congo red agar assays respectively, to produce a compound biofilm score (0-5), which was related to MBEC/MIC ratios, and treatment outcome. Eleven out of thirteen clinical strains isolated from bone, bone-marrow and implant pseudo-membranes had biofilm scores > 2, which were correlated to higher MBEC/MIC ratios for vancomycin, linezolid, ciprofloxacin, ampicillin, and rifampicin ( $p < 0.0001$ ), by Kruskal-Wallis nonparametric analysis of variance. All enterococcal strains had uniformly high MBECs, and higher MBEC/MIC ratios than staphylococcal strains. Nineteen percent of the strains were below clinical MIC-breakpoints for vancomycin, ciprofloxacin, linezolid, and rifampicin, whereas 77% were unsusceptible according to MBEC. Treatment failure was qualitatively associated with high MBECs for treatment antibiotics, but the small numbers available did not allow for any statistical relationship to be determined.

### 4.3 Paper III

To date there has been no long-term analysis of implant survivorship free from femoral osteomyelitis with or without implant removal. Previously, a 2-year prospective study of 51 patients treated 1999-2007, reports 4 implant losses (1 infection, 3 aseptic) [37]. The main purpose of this paper was therefore to estimate the long-term risk of osteomyelitis based on the complete patient cohort treated in Gothenburg. The retrospective design allowed for inclusion of all but a few patients ( $n=96$ ) treated with a femoral implant during a 19.5-year period. During retrieval of clinical data, it became evident that infections could be roughly divided into two categories. Firstly, an infection around and/or above the implant with positive cultures from bone marrow or bone (osteomyelitis), and secondly an infection distal to the implant where

radiologically evident bone attrition often is accompanied by local skin inflammation and fibrous transformation. Identification of the latter, termed *distal osteitis*, was based on symptoms, radiographs, and swab cultures of the skin-abutment interface, rather than tissue cultures. By 10 years the estimated risk of osteomyelitis reached 20% (95% CI, 0.12–0.33), displayed graphically in Figure 18. The median time from implantation to osteomyelitis was 2.6 years (range, 0.3–13.8). In 10 instances osteomyelitis led to extraction of the implant corresponding to a 10-year cumulative risk of 9% (95% CI, 0.04–0.20). Seven of these patients were treated before the OPRA (Osseointegrated Prostheses for the Rehabilitation of Amputees) protocol. Although method developments likely have affected infection frequencies in this time span, eliminating early treatments might have led to underestimation.

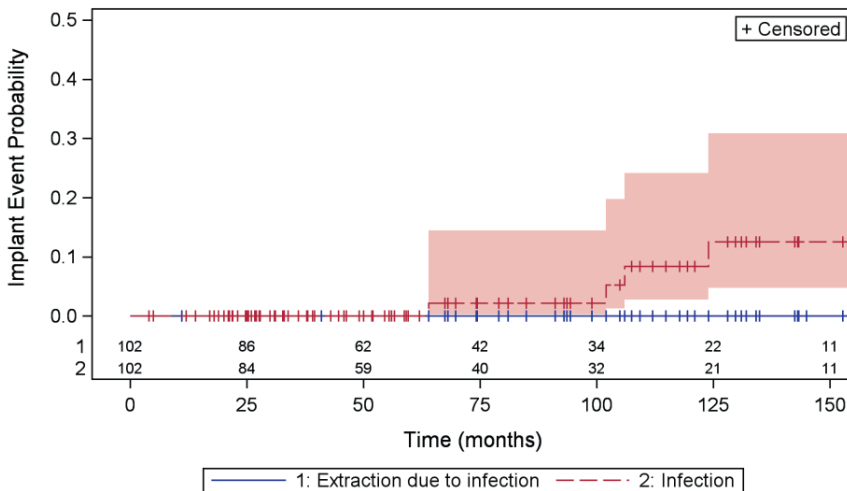
Figure 18. Kaplan-Meier plot of the probability, with 95 % confidence intervals, of osteomyelitis, and extraction due to osteomyelitis with time.



Six patients (bilateral implants in one), without definable osteomyelitis, had signs of distal osteitis, translating into an 8% (95% CI, 0.02–0.24) risk, at 10 years of implant use (Figure 19). The median time from implant insertion to osteomyelitis (n=16) was 2.6 years (range, 0.3–13.8), and for distal osteitis (n=6) the corresponding figure was 10.5 years (range, 5.5–16). The mean observation time was 60.5 months in the remaining 80 patients. The natural course of distal osteitis was interrupted by more short-course antibiotics, than

in osteomyelitis patients. In patients not diagnosed with osteomyelitis or distal osteitis, antibiotic consumption was much lower. More than 2/3 of the patients in this group were prescribed short course antibiotics, mostly flucloxacillin, less than five times during a mean study period of 6.7 years (SD 4.5). This average frequency of less than one antibiotic treatment per year is similar to that of an unpublished conference report by Sooriakumaran in 2004, describing infection patterns in transfemoral osseointegration in Britain.

Figure 19. Kaplan-Meier plot of the probability, with 95 % confidence intervals, of distal osteitis, and extraction due to distal osteitis with time.

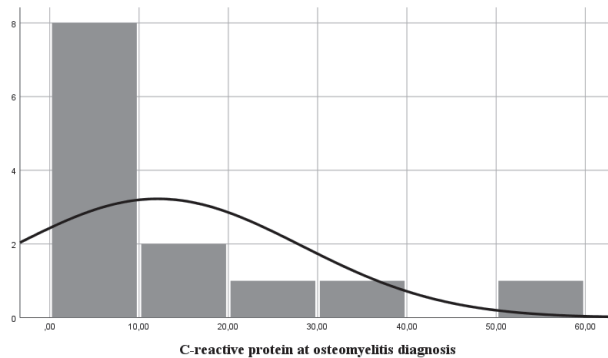


Distal osteitis per se has a milder clinical course not leading to extraction or the need for long term antibiotics. Microbial involvement was suspected in most cases, especially when accompanied by recurring skin infections, but has only seldom been confirmed by tissue cultures. Clinical presentation in osteomyelitis is dominated by loading pain, and impaired function. Only 4 out of 22 patients in paper 2 were diagnosed within 3 months of the debut of local symptoms. Plain x-ray film seldom showed clear osteomyelitic signs, and inflammatory markers (CRP, ESR) were sometimes not elevated despite positive marrow cultures. Common risk factors for PJI were analysed for association to implant osteomyelitis but the study was greatly underpowered to detect any relationships with obesity, smoking, high age, or uncomplicated diabetes.

## 4.4 Paper IV

*Paper IV* describes conservative treatment outcomes 2008-2012, in an 18-patient osteomyelitis cohort. Prior to 2008, rifampicin, was not used against these infections. Implant retention in infected tissues have lower success rates, compared to revision treatments, as demonstrated in prosthetic joint infections [194]. Furthermore, conservative treatment with no, or minimal debridement most often demand suppressive antibiotics [229]. Acknowledging the robustness of the well-integrated femoral fixture, attempts of resolving osteomyelitis by antibiotics alone, and sometimes with minor debridement, have been made. Conservatively treated osteomyelitis was identified in 18 patients. Minor debridement of infected bone was performed in 4 of 7 resolved, and in 1 of 11 failed cases ( $p < 0.05$ ). In 7 patients, there were no clinical relapse 24 months after discontinued antibiotics.

*Figure 20.  
Histogram  
showing the low  
CRP-levels at  
the time of  
diagnosis in 13  
patients with  
implant  
osteomyelitis*



In six out of eleven treatment failures implants were subsequently extracted followed by prolonged antibiotics, and cure of infection in all patients. At diagnosis, patients with osteomyelitis caused by *S. aureus* displayed higher C-reactive protein (CRP) serum concentrations (mean 29 mg/L, range 5-54) than patients with other bacteria (mean 3.6 mg/L; range 1-14,  $p < 0.01$ ). CRPs at diagnosis were low (Figure 20), and not significantly higher in the treatment failure group than in the cured group ( $p = 0.34$ ). Rifampicin had no obvious impact on treatment outcomes (4/9 vs. 3/9 resolved), but numbers were too small, and groups poorly matched to draw any conclusions.

## 5 DISCUSSION

Identifying and reporting adverse events, when introducing a novel treatment is paramount. Regarding medical implants, infection is arguably the most important complication to detect, quantify, and ultimately control. In all, 21 cases of definitive, or probable osteomyelitis (femur 20, and humerus 1) was identified, leading to extraction of the implant in 10 patients. Reimplantation was performed in 13 patients (13 implants), and successful in 3 out of 6, where implants previously had been extracted due to osteomyelitis.

### 5.1 Composition of patient cohorts (I - IV)

In *paper I*, patients were included consecutively, in the order of scheduled postoperative visits, during a 6-month period in 2005. In *paper II*, the patients corresponded to all retrievable freeze-dried clinical specimens, from 2008-2012. The cohort of *paper III* included all patients fitted with femoral implants between 1990 until 2010, and *paper IV* patients where conservative treatment of osteomyelitis was attempted 2008-2012. Osteomyelitis was throughout identified by easily evaluated, dichotomised criteria (Table 7), found in clinical praxis, and therefore easily accessed from medical records. Difficulties in correctly defining implant osteomyelitis was anticipated, why three levels (definite, probable and possible) were used. To further avoid misclassification, all cases in *paper III*, were twice examined by all authors to exclude non-infectious reasons for ambiguous pain, or x-ray findings. Twelve out of 16 cases were found definitive, so the impact of non-definitive cases was low. With the risk of slight overestimation, all levels were included in the final analyses. In *paper I*, cultures at the skin-implant interface were collected prospectively, while tissue cultures for osteomyelitis were retrieved retrospectively in all papers.

## 5.2 Defining and diagnosing infection

The understanding of infection, associated with these implants has evolved during the work on this thesis. The original working definition, derived from diagnostic PJI criteria, has for present study purposes appeared useful. Its validation has not been the aim of the thesis. Between *papers I*, and *II* minor changes in criteria was made; for definitive osteomyelitis 3/5 positive cultures was changed to  $\geq 2/5$ , and radiographic evidence was not required in cases of acute infection. In the larger patent cohort of *paper III*, biopsy cultures had not always been carried out in a standardized way, and adhering to the original definition would have underestimated the osteomyelitis number. In addition to percutaneous, or open biopsies, this method offers a complimentary way of obtaining tissue samples. At the proximal end of the hollow fixture, a removable airtight screw allows for bone marrow aspirations, and minor tissue biopsies. Furthermore, our definition does not clearly differentiate between osteomyelitis, and distal osteitis. For example, the dermal interface is not characterized with respect to histology and inflammation, and tissue sampling is here at greater risk of contamination with skin or gut flora. Bone juxtapositioned to the skin-implant interface often display radiological changes indicating inflammatory bone remodelling, with similarities to aggressive periodontitis [271]. Possibly this is provoked by micromotions in the interface, but bacteria are likely involved. Histological analysis of clinically inflamed interface-skin in craniofacial implants, and in sheep indicate foreign body reactions with superimposed infection [31, 125]. Due to fear of introducing pathogens, and/or inducing progressive implant loosening, debridement and percutaneous tissue sampling of this region have only been carried out exceptionally, why this remains to be corroborated. Although it is counterintuitive not to assume local bacterial spread to proximal parts of the implant, the evidence so far indicates that distal osteitis *per se*, does not constitute a continuum with osteomyelitis. It has become clear, that infected bone surrounding the fixture should be treated, and that resolution without implant extraction, or suppressive antibiotics is attainable in some cases. Whether early management of distal osteitis may prevent ascending infection must be prospectively studied. Repeated skin infection, often associated to underlying osteitis, was treated with short courses of per-oral antibiotics, and sometimes excisions of skin granulations. Despite the shortage of direct evidence, it must be assumed that the two main ways of bacterial entry to the peri-implant bone, is during insertion procedures, and through the skin stoma. Indirect evidence of this is that simultaneous bacteraemia is only confirmed in one case (*S. aureus*), low CRP levels during infection with virulent species (*S.*

*aureus*, beta haemolytic streptococci and *Escherichia coli*, and the similar proportions of different bacteria in skin swabs, and deep cultures alike. Furthermore, infection caused by enterococci appear more common than in prosthetic hip or knee joint infection, especially in monomicrobial infection (table 1)[110], despite shared proximity to the perianal region. Early infectious failure as indicated by painful rehabilitation reinforce the assumption that primary osteointegration offers protection against deep infection.

## 5.2.1 The skin stoma

Skin integration to the outer implant component (abutment) does not develop. The histology of the skin and bone tissue surrounding the abutment has yet to be described in normal condition, mechanical stress and infection. Visible skin reactions vary. A fibrous dermal ring is sometimes formed around the abutment. Given the biological inertness of titanium, this is primarily believed to be a response to skin movement, or continuous microbial irritation. Care of the skin-abutment region should reduce invasion prone bacteria without damaging the skin. One 24-week study in rabbit, show a 75% reduction of percutaneous pin infection when a topical antimicrobial (pexiganan acetate) was applied daily [272]. Preventive measures to avoid pin tract infection, e.g. using antibacterial coatings, could be tested on the part of the abutment residing in bone tissue [273]. New insights in using *Lactobacillus* sp. in chronic wound care, and to reduce biofilm formation by *S. aureus*, and *P. aeruginosa* [274] present an interesting approach. No systematic evaluation of different hygiene regimens in this application has been performed, hence no proper recommendations can be made. Although skin infections were not within the scope of this thesis, based on clinical experience and indirect study observations some recommendations can be made:

### *Recommendations for management of skin infections around the abutment*

- Grade extent; measure redness, apply modified Holger score
- Obtain a swab culture from the skin pocket after saline cleaning.
- A 5-7-day course of flucloxacillin 1 g, t.i.d. is likely sufficient. Change if culture yield unsusceptible *virulent* organism.



- Assume distal osteitis if recurring skin or soft tissue infection.
- Assume osteomyelitis if deep pain is associated with skin infection. If possible, withhold antibiotics until deep cultures.
- Assume osteomyelitis if purulent secretion without obvious skin inflammation. Withhold antibiotics until deep cultures.

In *paper I*, the microflora was categorised twice, with a 3 years interval. Staphylococci were the most common bacteria and grew abundantly (semi-quantified) at both times while the combined flora of enteric rods, and streptococcal species was variable, and better reflect the transient colonisation seen in the dermal micro-flora [138].

## 5.3 Clinical presentation

### *Infectious, and other complications after implant surgeries*

There were few postoperative complications following fixture insertion in the *paper III* cohort. Out of 96 patients, 71 had no, or negligible problems, and in 15 there was insufficient information. In the remaining patients; severe pain in 3, episode of fever with negative cultures in 3, and minor infection in skin or hematoma in 4. Following second surgery (abutment connection) complications were more frequent; various degree of skin flap necrosis with, or without infection in 14, pyelonephritis in 1, revision of surplus skin in 2, and other wound-related problems in 9.

### *Osteomyelitis*

Like in other bio-device infection, the virulence of causative organism(s) affect clinical presentation. However, compared to bigger, and non-osseointegrated orthopaedic devices, systemic illness has very rarely been observed. The most common clinical presentation was loading pain. In *paper III*, 8/16 patients had acute/subacute debut of symptoms, in 7 cases caused by *S. aureus* (MRSA in one). Median time from implant insertion was 31.5 months (range 5-112) which was not different from chronic presentations of osteomyelitis (median 36). *S. aureus*-bacteraemia was only documented in one case, and was considered secondary. This is likely attributable to the low mean age, overall physical fitness, and low prevalence of factors (e.g. chronic

ulcers, severe diabetes, malignancy) predisposing virulent bacteraemia. However, blood cultures have not been performed in patients with body temperature below 38.5° C. Late haematogenous PJI caused by *S. aureus* is more common than early, or delayed [275]. Logistic regression showed no association (n=9, p=0.33) between *S. aureus* osteomyelitis, and duration from implant insertion, which does not contradict the assumption that late infection is not commonly blood borne in this patient group. Three patients with chronic osteomyelitis developed sinus tracts, which were all associated with polymicrobial growth in bone marrow cultures. Radiologically, lesions were mostly limited to segments of the bone-implant interface, but in several instances investigations of chronic pain revealed staphylococcal growth in tissue samples without x-ray findings, or elevated CRP. Applying the Cierny-Mader grading, almost all cases were IIIB, i.e. localized osteomyelitis in locally compromised patients. Although uncommon, even in long standing infection, fistulation occurred to both the skin surrounding the abutment and horizontally through soft tissues.

#### *Distal osteitis*

In *paper I*, all bone involvement was considered deep infection. With the sufficient patient numbers in paper III, it became evident that bone attrition at the distal end of the residual femur (Figure 2), had less associated disability, and no long-term implant removal. Since antibiotics were prescribed much more frequently in this group, and various skin reactions often was present, microbial involvement is likely. In one patient, long-term antibiotic suppression for bilateral osteitis was issued. Fibrous transformation of the irritated skin is often seen, sometimes demanding minor revision for unrestricted prosthetic use. Further, being a continuous process, only diagnosed when radiographical changes were evident, a considerable diagnostic delay can be assumed.

#### *Skin infection*

In *paper I*, the incidence rate of skin infection was 0.36, and 0.63 cases/person-year at inclusion, and follow-up respectively. This contrasts skin reactions in craniofacial implants, which are reported to decrease over time [123]. A histological comparison between interface tissues in orthopaedic, and craniofacial implants showed less inflammatory cells in the former [276]. Although not evident from a foreign body reaction point of view, the low degree of relative motion at head and neck sites, possibly help preserve the barrier of immune-response cells, described by Holgers and co-workers [135]. Almost all infections responded well to short courses of flucloxacillin, or clindamycin in doses recommended for skin infection.

## 5.4 Risk and risk factors

Both two-year [37], and 5-year (Brånemark et al, unpublished data) cumulative success rates are 92 % in prospective analyses, with implant removal as endpoint. By comparison, in *paper III*, osteomyelitis-free survivorship was 80 % by 10 years. Twenty-seven patients were treated before the start of the systematic treatment OPRA protocol, used in aforementioned studies. The risk of implant osteomyelitis increased most rapidly during the first 5 years, and thereafter by roughly the same rate, although the numbers available made predictions beyond 15 years highly uncertain. In the group treated before 1990-1998 (before OPRA), 8 implants were extracted due to osteomyelitis, and 1999-2012, 4 implants. Taken together this might indicate that the surgical procedure, rehabilitation, and general care standardization indeed have reduced the risk of osteomyelitis. However, there is an impression that the actual number of infected patients was higher. Several error sources can be identified; i) estimations are based on non-validated criteria, ii) complete medical charts have been inaccessible for roughly 5 % of the patients, iii) a mismatch between symptoms, and diagnostics to fulfil study definitions in several cases. Furthermore, the survivorship analysis only took into account first occurrence of osteomyelitis, and extraction. In six cases re-implantation were undertaken with subsequent infectious failure in 3 (extraction 2, chronic 1). To more accurately know the true width of the problem, a prospective registration of osteomyelitis based on standardized tissue sampling for paired culture and histology should be undertaken. Soft tissue infection (Chi Square;  $r=0.26$ ,  $p=0.25$ ), or tumour ( $r=0.26$ ,  $p=0.60$ ) as cause of amputation, could not be associated to later osteomyelitis with available patient numbers. However, if bone tissues have been involved in prior infection, culture of bone biopsies should be included in the preoperative evaluation. The chosen patient factors (diabetes, obesity, smoking, and old age) could not be tied to risk for infection in *paper III*. Previous studies have identified obesity as an independent risk-factor for PJI. None of the other independent risk factors for PJI listed above (Table 1), was identified in the present patient cohorts in enough numbers to analyse. The interpretation is that preoperative selection reduces age-related and other risk-factors below the level of detection in this medium sized cohort. Intended for life long prosthetic support, future co-morbidities must be taken into consideration before surgery, and ASA-score association to osteomyelitis should be prospectively studied for long term treatment recommendations. Although abutment changes could not be statistically related to osteomyelitis (HR 1.13,  $p=0.16$ ), the temporal association in a few previously uninfected patients calls for aseptic technique during exchange and prospective surveillance. There was

a moderate association (Chi-Square;  $c=0.313$ ,  $p=0.008$ ) between soft tissue complications at second surgery and later osteomyelitis, mainly chronic polymicrobial infections, with unclear time of onset. Antibiotic use is high in this patient group, with increased risk of unwanted drug effects, and negative impact on antibiotic resistances. From the crude antibiotic consumption data gathered in *paper III*, average short courses alone (7 courses per 7.9 years x 10 days per course), surpasses the average outpatient use in Sweden [277]. Furthermore, current postoperative antibiotic prophylaxis (cefadroxil until skin healing) is not evidence based, and select for enterococci and CoNS. From the comprehensive review of patient records, the distinct impression despite not calculated, is that diagnosis is often delayed in both osteomyelitis, and distal osteitis. If so, both doctors delay, and lack of early distinct symptoms from the bone-implant interface play a part. Early diagnosis does not decrease the frequency of osteomyelitis, but likely improve treatment outcome, and shorten patient suffering.

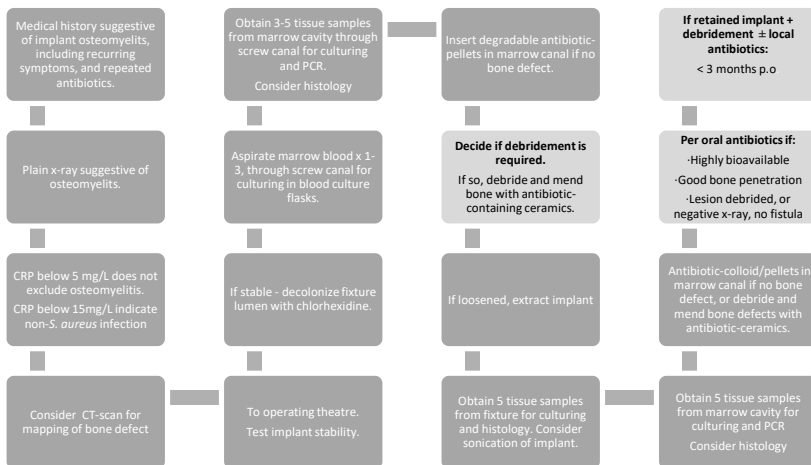
## 5.5 Bacteria in femoral implant osteomyelitis

The overall composition of colonizing, and infecting bacteria was similar. *S. aureus* constituted 43 % of isolated bacterial species, and was found in 60 % of osteomyelitis cases. In *paper I*, but not in *paper III*, concordance between colonization of *S. aureus* at the skin stoma, and in deep infection was poor, likely due to long sampling intervals, and a small patient number in the former. Swab cultures have reduced overall sensitivity [212], and lack specificity for deep infection when obtained from sinus tracts [278]. Since osteomyelitis is poly-microbial in approximately one out of three cases, and colonization by *S. aureus* is frequent, superficial cultures will be unreliable. The comparatively high rate of polymicrobial osteomyelitis to other elective implant procedures, is consistent with the permanent skin penetration. Enterococci are the second most common bacteria in femoral bone tissue cultures, often alongside a *Staphylococcus* sp. So far, all enterococci have been susceptible to ampicillin. In at least three instances relapse infection has been caused by enterococci following *S. aureus* treatment. Group B streptococci, increasingly recognized as an important pathogen in PJI [279], frequently colonize the skin stoma, while the enteric rods are less common. In one case a *Peptostreptococcus* sp. was involved in a relapsing infection with sinus tract formation. The only MRSA infection led to implant removal prior to second surgery.

## 5.6 Diagnostic possibilities

The implant design with replaceable components, and a hollow centre allows for minimal-invasive tissue sampling through the implant, valuable in diagnosing deep infection, without disrupting the bone interface. Furthermore, avoiding percutaneous sampling decreases the risk of skin commensals in the sample. Marrow blood is easily accessible through aspiration. A guide device reduces the risk of contact contamination. Bone biopsies are more complicated but performable. The optimal number of marrow aspirations for acceptable diagnostic accuracy is not known. Theoretically, culture sensitivity will only increase marginally in multiple small marrow blood aspirations compared to one large, as bacteria are distributed in a liquid phase. However, there is presumably a risk for contamination, which supports a current practice of 3 separate aspirations. Below (Figure 21) is a suggested step-by step plan of action in cases of suspected osteomyelitis.

*Figure 21. A proposed diagnostic/therapeutic algorithm to be used in implant osteomyelitis with or without extraction.*



## 5.7 Biofilm considerations

Similar to teeth, the external aspect of the percutaneous implant is accessible to biofilm preventive measures. Most of the dental biofilm can be removed by tooth-brushing, but build-up starts anew from the high concentration of microorganisms in saliva [280]. The man made breach of percutaneous microenvironment of femoral implant differs in several aspects; the microflora originates from skin and fecal commensals, and the antibacterial proteins (e.g. lysozyme, immunoglobulins, lactoferrins) of saliva [281], are not present. Many of the microbes found at the skin stoma readily adhere, and form biofilm at the proximal portion of the abutment [136]. Similarly, to cleaning of teeth however, reduction of bacterial load is likely to reduce infection rates. Atraumatic cleaning tools, and non-irritating antiseptics should therefore be part of the postoperative maintenance, and systematically evaluated. As demonstrated in *paper II*, peri-implant staphylococcal, and enterococcal isolates produced biofilm of varying strength *in vitro*, and although underpowered there was a trend of high biofilm scores in complicated treatments. Combining the results of *papers II* and *IV*, there is cause for prospective evaluation of MBEC guided treatment, and optimizing drug delivery in conservative treatments.

## 5.8 Treatment options

Due to limited tissue involvement, stable implants and good overall health, antibiotic treatment has mostly been per oral, sometimes following short intravenous administration. To a lesser extent, because of technical difficulties mainly, local administration of gentamicin-containing colloids has been used. Local delivery of antibiotics is preferable for two main reasons, namely the manifold increase in tissue concentrations resulting in potent anti-microbial efficacy in compromised tissues and biofilm, and lower systemic toxicity for the patient. Even if penetration of antimicrobials to the bone-implant interface is not known, histological sections of the peri-implant tissues indicate vasculature similar to mature bone presumably granting sufficient transport of bioactive molecules to the tissue-implant interface. In non-implant osteomyelitis, there are promising treatment results when thorough debridement is followed by antibiotic loaded hydroxyapatite/calcium-sulphate

bone replacement [83]. One clinical trial with a 12 month follow-up in 2-stage uncemented PJI-revision, showed a 95 % clinical and radiological success when the re-implanted joint had been covered in antibiotic loaded bi-phasic calcium-sulphate [282]. Focal cortical destructions in an otherwise unaffected bone-implant interface appear well suited for this treatment modality. Bone replacement might further prevent the defect from becoming a platform for re-emerging infection by reforming a protective interface with the titanium oxide implant surface. Weak electrical currents (25-2000  $\mu\text{A}$ ) have *in vitro* been demonstrated to hinder [283], or disrupt [284] staphylococcal biofilms and enhance antimicrobial efficacy [285]. In a small goat model, *S. epidermidis* pin tract infections were to a high degree prevented by a 100  $\mu\text{A}$  current compared to control pins [286]. The implant design similarly allows non-invasive electrode attachment. Currents of approximately 1 mA generate a barely perceptible tingling, why any peri-implant tissue damage most likely will not occur if controlled attempts are performed with this implant system. Although not conclusive, treatment success appears more likely when minor debridement is performed. This is in line with well-established treatment superiority of surgical intervention in long bone osteomyelitis [287]. Furthermore, despite many-fold higher rates of implant osteomyelitis (20 % vs. 1-2 %) compared to major joint arthroplasties, treatment success rates with retainment of implant compare better (30 % vs. 50-55 %).

## **5.9 Acceptable level of infectious complications in this novel method**

Given the results in this thesis, infections are too common, and not sufficiently resolvable without discomfort, and potential side effects, to recommend this treatment without restrictions. Three main factors need to be weighed against the quantified risk of infectious implant failure; i) The degree to which lost walking ability and quality of life may be restored, ii) the degree of inability to achieve sufficient mobility by other (i.e. socket-based prosthetics) means, and iii) lasting patient understanding of the specific vulnerability to infection and how to take preventive measures. If 20 patients out of 100 develop infection within 10 years, and successful treatment with retained implants can be achieved in some 30 %, it seems reasonable to assume high prosthetic use, and minor need for antibiotics in the remaining patients.

## 6 CONCLUSION

Implant-associated osteomyelitis is more common than anticipated when the treatment was initiated. Furthermore, the cumulative risk of osteomyelitis, and secondary implant removal increases with time. This jeopardizes long-term treatment success, and a reduction of infection rates must be achieved before the indication can be expanded. Presently, from an infection point of view, this treatment should be reserved for patients with no other prosthetic options, and very limited co-morbidities. It is evident that there is less functional impairment during implant osteomyelitis compared to infection in large arthroplasties. The explanation appears two-fold. Firstly, good primary osseointegration limits infectious progression and prevent instability of the implant. Secondly, the small implant size reduces soft tissue and systemic inflammatory load. Systematic monitoring of infections, when introducing a medical procedure or treatment is paramount. Several research questions have been raised pertaining to prevention, reliable diagnostics, and treatment regimens.



## 7 FUTURE PERSPECTIVES

Alongside the four papers included in this thesis, clinical experience has illuminated areas of particular interest for future investigation namely:

Applying the long-term risk for implant osteomyelitis in preoperative selection.

Tailoring treatment through full compliance to sampling protocols, and prospectively analysing MBEC-based treatment choices.

Developing early indicator schemes for osteomyelitis, and improving diagnostic algorithms utilising the unique sampling possibilities inherent to this implant system.

Developing method adapted local drug delivery systems, and evaluating local anti-microbial treatment for; i) diffuse osteomyelitis by insertion of resorbable antimicrobial vehicles through the proximal fixture canal, and ii) localised osteomyelitis through minor debridement and deposition of antibiotic loaded bi-phasic calcium-phosphate.

Introducing standardised preventive measures at the skin-implant interface, including antiseptic abutment coatings and cleaning, non-irritant skin hygiene routines, and possibly the investigation of probiotics in prevention of local biofilm infection and chronic microbial irritation.

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